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Coral reefs in a time of change – case studies to understand potential biogeochemical consequences of phase shifts from corals to benthic algae

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Zusammenfassung

Es wird angenommen, dass der weltweite Klimawandel zusammen mit direkten anthropogenen Einflüssen, insbesondere Eutrophierung und Überfischung, dazu führt, dass riffbildenden Korallen von Algen als dominante benthische Organismen abgelöst werden. Diese sogenannten „phase shifts“ wurden in jüngster Zeit weltweit dokumentiert. Dabei haben Studien gezeigt, dass gerade Korallen als wichtige Ingenieure des gesamten Ökosystems fungieren. Sie bilden komplexe 3-dimensionale Riffstrukturen, die zur Habitat- und Artenvielfalt beitragen und dienen als Lieferant für biogene Riffsande, welche als biokatalytisches Filtersystem ein effizientes Recycling der Nährstoffe ermöglichen. Unter den extrem nährstoffarmen Bedingungen, die in den meisten Warmwasserkorallenriffen herrschen, ist jedoch der wohl beachtlichste Beitrag dieser Ökosystem-Ingenieure die Abgabe von organischem Material in Form von Schleimen. Dieses organische Material ist offensichtlich in der Lage, biogeochemische Stoffkreisläufe zu initiieren und durch das Einfangen von Partikel aus der Wassersäule wichtige Nährstoffe für das Ökosystem Korallenriff bereitzustellen. Andererseits können aber auch benthische Algen, die Begünstigten der meisten „phase shifts“, ihre Umgebung auf verschiedene Weise beeinflussen. Einige Algen, wie zum Beispiel koralline Rotalgen, können die Struktur ihrer Umgebung verändern, oder wie die Grünalge *Halimeda*, zur Herstellung von biogenen Sanden beitragen. Die meisten Algen aber können ihre unmittelbare Umgebung durch die Abgabe von organischem Material beeinflussen. Welche Rolle jedoch dieses von riffassoziierten Algen abgegebene organische Material in den biogeochemischen Kreisläufen des Riff-Ökosystems spielt, ist bis heute noch weitgehend unbekannt.

Das Hauptziel der vorliegenden Arbeit ist es deshalb, die ökologische Relevanz des von Algen abgegebenen organischen Materials umfassend zu untersuchen. Kapitel 1 und 2 der Arbeit befassen sich unmittelbar mit Interaktionen zwischen Korallen und Algen im Hauptuntersuchungsgebiet der vorliegenden Arbeit, dem nördlichen Roten Meer, und den Auswirkungen von saisonalen Umweltveränderungen auf diese Interaktionen. Im Anschluss liegt in Kapitel 3 bis 7 der Schwerpunkt auf vergleichenden Untersuchungen von durch riffbildende Korallen und benthische Algen abgegebenem organischem Material. Ergänzend dazu wird die mikrobielle Abbaubarkeit der jeweiligen Exsudate analysiert (Kapitel 8 und 9). Die letzten Kapitel (10 – 12) beschäftigen sich schließlich mit der in-situ Relevanz der bisher erworbenen Erkenntnisse und vergleichen darüber hinaus zwei verschiedene Riffsysteme im Roten Meer und in der Karibik.

Kapitel 1 der vorliegenden Arbeit gibt einen allgemeinen Überblick über die benthische Gemeinschaftsstruktur, sowie das Auftreten von Interaktionen zwischen Algen und Korallen in einem Saumriff des nördlichen Roten Meeres, welches grundsätzlich als Hauptuntersuchungsgebiet für die vorliegende Arbeit diente. Die Studie ergab, dass sich während des Winters mehr als 30 % aller riffbildenden Korallen in Interaktion mit benthischen Algen befanden, wohingegen im Sommer nur 17 % der Korallen in solche Interaktionen involviert waren. Dies deutet darauf hin, dass der Verlauf dieser Interaktionen zwischen Korallen und Algen in-situ stark von der Jahreszeit beeinflusst wurde. So zeigte sich im Herbst ein schneller Überwuchs von Korallen durch die konkurrierenden benthischen Algen, auf den ein Gleichgewichtszustand beider Organismengruppen im Sommer folgte. Obwohl die zeitgleich gemessenen Umweltparameter (Temperatur, Lichtintensität, Konzentrationen anorganischer Nährstoffe) saisonal stark variierten, konnte kein kontrollierender Schlüsselparameter eindeutig identifiziert werden.

Um mehr über mögliche Umweltfaktoren zu erfahren, die solche Korallen-Algen Interaktionen beeinflussen, beschäftigt sich **Kapitel 2** der Arbeit mit den Auswirkungen erhöhter anorganischer bzw. organischer Nährstoffkonzentrationen auf den direkten Wettbewerb zwischen riffbildenden Korallen und benthischen Algen im Untersuchungsgebiet. Ein Langzeit-Experiment mit Korallen der Gattung *Acropora* und einer typischen Gemeinschaft benthischer Turfalgen in Mesokosmen zeigte, dass erhöhte Ammonium- und Glukosekonzentrationen das Algenwachstum stimulierten, wohingegen sich die Gewebepigmente der Korallen und deren Chlorophyll a-Gehalte signifikant verringerten. Dabei waren alle gemessenen Auswirkungen der Konkurrenz mit Algen auf Korallen durch die erhöhten Glukosekonzentrationen im Umgebungswasser noch signifikant verstärkt. Zusätzliche O_2-Konzentrationsmessungen in einer hohen zeitlichen Auflösung wiesen darüber hinaus in den Becken mit Glukosezugabe eine, im Gegensatz zu allen anderen Behandlungsformen, deutlich niedrigere O_2-Konzentration im Wasser auf, was Auswirkungen auf die mikrobielle Aktivität im Umgebungswasser nahe legt. Diese Beobachtungen deuten somit auf einen negativ verstärkenden Effekt auf Interaktionsprozesse zwischen Korallen und Algen durch die Zufuhr von gelöstem organischem Kohlenstoff hin.

Um die Wirkungsweisen solcher Effekte besser zu verstehen, sind zunächst grundlegende Untersuchungen der chemischen Eigenschaften des von den Interaktionspartnern abgegebenen organischen Materials notwendig. **Kapitel 3** eröffnet deshalb eine Reihe von aufeinander aufbauenden Analysen der Zusammensetzung der von den jeweiligen Organismusgruppen abgegebenen Exsudate. Inkubationsexperimente mit zwei, in

Korallenriffen häufig vorkommenden, grünen Makroalgen, *Halimeda opuntia* und *Caulerpa serrulata*, zeigten, dass von diesen Algen abgegebenes organisches Material im Wesentlichen aus gelösten Kohlehydraten und Proteinen bestand. Spuren von Fettsäuren und Chlorophyll a traten nur in vernachlässigbaren Mengen auf (< 1 %). Besonders interessant war das Ergebnis, dass Glukose einen Anteil von 77 ± 8 % aller Kohlehydraten und sogar 42 ± 8 % des gesamten abgegebenen Kohlenstoffs darstellte. Die hohen Glukose- und Proteingehalte deuten auf eine gute mikrobielle Abbaubarkeit der Algenexsudate hin. Dies war sehr wahrscheinlich der Hauptgrund für den beobachteten starken Anstieg des mikrobiellen O_2-Verbrauchs im Umgebungswasser.

Aber nicht nur die chemische Zusammensetzung, sondern auch die Menge der abgegebenen Exsudate kann offensichtlich nachfolgende Prozesse wie die mikrobielle Aktivität maßgeblich beeinflussen. **Kapitel 4** der vorliegenden Arbeit präsentiert erste umfassende Studien zu Abgaberaten organischen Materials durch Algen im Roten Meer in saisonaler Auflösung. Hierbei wurden die Abgabe von gelöstem organischem Kohlenstoff sowie partikulärem organischem Kohlenstoff und Stickstoff durch dominante, korallenriffassoziierte Algen bestimmt. Außerdem wurden die Abgaberaten einer Grünalge der Gattung *Caulerpa* in verschiedenen Wassertiefen gemessen sowie auch saisonal auftretende Algen in diese Studie mit aufgenommen. Dabei konnte gezeigt werden, dass alle untersuchten Algen organischen Kohlenstoff in erheblichen Mengen und hauptsächlich in gelöster Form abgaben. Um die Analysen zu vervollständigen und mögliche Umwelteinflüsse auf die Abgabe von organischem Material durch Algen festzustellen, wurden einige Umweltparameter (Temperatur, Lichtintensität, Konzentrationen anorganischer Nährstoffe) zeitgleich bestimmt. Die Menge des abgegebenen organischen Materials variierte je nach Jahreszeit und Wassertiefe und wies dadurch Temperatur- und, bis zu einem gewissen Grad, auch Lichtabhängigkeit auf. Es zeigte sich weiter, dass die Abgaberaten von organischem Material wohl mehr durch funktionale Eigenschaften der Algen (Wachstumsform, -strategie) beeinflusst wurden, als durch ihre taxonomische Zugehörigkeit. Somit liefert dieses Kapitel erste umfassende Informationen über einen möglichen Zusammenhang zwischen verschiedenen benthischen Riffalgen und Stoffkreisläufen im Ökosystem Korallenriff. Außerdem werden Schlüsselfaktoren (Temperatur und Lichtintensität) aufgezeigt, welche die Abgabe von organischem Material durch korallenriffassoziierte Algen beeinflussen.

Um die Untersuchungen über Einflüsse benthischer Algen auf organische Stoffkreisläufe in ihrem Ökosystem weiter zu vertiefen, behandelt **Kapitel 5** einen speziellen Fall der Abgabe organischen Materials durch Algen: die konzertierte Abgabe von Sexualprodukten. Hierbei

wurde die Rolle einiger wesentlicher Umweltfaktoren als möglicher Auslöser für diese Abgabe von Sexualprodukten bei zwei Arten der Gattung *Caulerpa*, *C. taxifolia* und *C. serrulata*, untersucht. Zudem beinhaltet dieser Abschnitt eine Quantifizierung der allgemeinen Abgaberaten von partikulärem organischem Material als Reaktion auf veränderte Temperaturen und Salzgehalte des Umgebungswassers. In weiteren Inkubationsexperimenten fand schließlich eine Untersuchung der mikrobiellen Abbaubarkeit des abgegebenen organischen Materials statt. Während durch einen verringerten Salzgehalt bei beiden *Caulerpa*-Arten eine Gametogenese (Differenzierung der Keimzellen) induziert wurde, zeigten Veränderungen der Wassertemperatur nur geringe, artspezifische Auswirkungen. Zwischen Gametogenese und einem Anstieg der Menge des abgegebenen organischen Materials konnte eine signifikante Beziehung beobachtet werden. Auch korrelierte die mikrobielle Aktivität mit der Menge des von Algen abgegebenen organischen Materials. Das führt zu der Annahme, dass plötzliche Änderungen von Umweltparametern (zum Beispiel starke Regenfälle) eine konzertierte Abgabe von Sexualprodukten bei Algen auslösen können, welche wiederum eine Stimulation der mikrobiellen Aktivität nach sich zieht.

Um die ökologische Bedeutung der Ergebnisse aus den letzten 3 Kapitel beurteilen zu können, waren nun korrespondierende Untersuchungen über die Abgabe von organischem Material durch die zweite Organismengruppe, die riffbildenden Korallen, notwendig. Kapitel 3 entsprechend wurde in **Kapitel 6** die Zusammensetzung der Kohlehydrate in organischem Material, das von weltweit verbreiteten Warm- und Kaltwasserkorallen gesammelt wurde, analysiert. Hierbei wurde offensichtlich, dass Exsudate von Korallen eine weitaus kompliziertere Zusammensetzung aufwiesen als die der untersuchten benthischen Algen. Darüber hinaus zeigte sich, dass in den jeweiligen Schleimproben unterschiedlicher Korallenarten abweichende Zusammensetzungen von Glycosylen zu finden waren, was auf eine gattungsspezifische Komposition des Korallenschleims hinweist. Der Glucosegehalt der Korallenexsudate war - außer in einer Schleimprobe der Koralle der Gattung *Stylophora* - signifikant niedriger als der von Algenexsudaten. Da Glukose den meisten Organismen als universelle Energiequelle dient und sie darüber hinaus einen hervorragenden Nährboden für heterotrophe Bakterien in marinen Ökosystemen darstellt, wiesen diese Ergebnisse darauf hin, dass Algenexsudate, verglichen mit Korallenexsudaten, ein relativ schnell abzubauendes Material liefern.

Kapitel 7 vervollständigt die Reihe vergleichender Untersuchungen von Algen- und Korallenexsudaten mit einer quantitativen und qualitativen Analyse des von dominanten Korallen des nördlichen Roten Meeres abgegebenen organischen Materials. Übereinstimmend

mit Kapitel 4 wurde die Abgabe von gelöstem organischem Kohlenstoff, partikulärem organischen Kohlenstoff und partikulärem Stickstoff durch riffbildende Korallen bestimmt. Die Versuche fanden an sechs, im Untersuchungsgebiet des nördlichen Roten Meeres dominanten, Korallengattungen in saisonaler Auflösung statt wobei der Einfluss von Umweltfaktoren auf die gleiche Weise bestimmt wurde wie bereits in Kapitel 4 für Algen beschrieben. Die Ergebnisse wiesen gattungsspezifische Unterschiede bei der Abgabe von partikulärem organischem Material auf, die aber keine signifikanten saisonalen Variationen zeigten. Die gemittelten Abgaberaten von partikulärem organischem Kohlenstoff und Stickstoff korrelierten jedoch mit den Veränderungen der Wassertemperatur, der Lichtstärke und der Nitratkonzentrationen im Umgebungswasser. Hohe C:N Verhältnisse wurden, gattungsübergreifend, bei allen Korallenexsudaten gefunden. Lediglich bei 50 % der untersuchten Korallen konnte eine Abgabe von gelöstem organischem Material festgestellt werden, die wiederum signifikant geringer war als bei den dominanten Algengattungen im Untersuchungsgebiet. Der umfangreiche Datensatz aus den Kapitel 3 - 7 bildet eine wichtige Grundlage für ein besseres Verständnis von Stoffkreisläufen im Ökosystem Korallenriff und den beeinflussenden Umweltfaktoren.

In den nun folgenden Kapiteln werden die Erkenntnisse der bisherigen Studien auf ihre ökosystemare Relevanz geprüft. **Kapitel 8** verbindet die vorangegangenen Ergebnisse über die Abgabe von organischem Material durch Algen bzw. Korallen und vervollständigt sie durch Untersuchungen über dessen mikrobielle Abbaubarkeit. Die Resultate werden vor allem in Hinblick auf die sich verändernden Korallenriff-Gemeinschaften in Zeiten des Klimawandels diskutiert. Die daraus resultierenden deutlichen Unterschiede in Abgaberaten und Abbaubarkeit des jeweils von Algen und Korallen entlassenen Materials weisen auf weitreichenden Auswirkungen eines „phase shifts“ auf die biogeochemischen Kreisläufe im Ökosystem Korallenriff hin.

Kapitel 9 knüpft an die Untersuchungen aus Kapitel 8 an und testet zusätzlich deren in-situ Relevanz. Präsentiert wird der gesammelte Datensatz über die Abgabe organischen Materials durch die dominanten benthischen Organismen des nördlichen Roten Meeres, einschließlich Stein- und Feuerkorallen, benthischen Quallen und Algen (n = 273). Die Auswirkungen des abgegebenen Materials auf die mikrobielle Aktivität im Inkubationswasser wurden untersucht und deren in-situ Relevanz durch hochauflösende O_2-Konzentrationsmessungen im Umgebungswasser von Algen- bzw. Korallendominierten Riffabschnitten bestimmt. Dabei wurde deutlich, dass die Abgabe von gelöstem organischem Kohlenstoff durch Algen signifikant höher war als die von Korallen oder benthischen Quallen. Auch wurde die

mikrobielle Aktivität im Umgebungswasser durch Algenexsudate signifikant mehr stimuliert als durch die Exsudate der untersuchten Stein- und Feuerkorallen. Infolgedessen wiesen die O_2-Konzentrationsmessungen in der Wassersäule direkt über Korallendominierten Riffabschnitten (< 10 cm) signifikant höhere Konzentrationen auf als über Algendominierten Standorten, was die in-situ Relevanz der vorangegangenen Studien unterstrich. Darüber hinaus bestätigten diese Befunde, dass benthische, riffassoziierte Algen die Verfügbarkeit von O_2 im Umgebungswasser für andere Rifforganismen durch die Abgabe von schnell durch planktonische Mikroorganismen abbaubarem organischem Material beeinträchtigen.

Diese erstmalig durchgeführten in-situ Messungen der Auswirkung benthischer Gemeinschaftszusammensetzungen auf O_2-Konzentrationen im Wasser werden in **Kapitel 10** noch detaillierter beschrieben. Die vergleichenden Studien ergaben, dass sowohl die Untergrenze der O_2-Konzentrationen in der umgebenden Wassersäule als auch deren Variabilität während des Tagesganges signifikant mit dem Grad der benthischen Bedeckung durch Algen korrelierte, während kein Zusammenhang zum Grad der Bedeckung durch Korallen nachzuweisen war. Die Ergebnisse deuteten darauf hin, dass eine Verschiebung des Gleichgewichtes von Korallen- zu Algendominanz möglicherweise Auswirkungen auf die O_2-Verfügbarkeit und die Höhe der Konzentrationsschwankungen im Tagesgang hat und sich somit auf alle sauerstoffabhängigen Rifforganismen auswirkt.

Mit der Untersuchung eines weiteren Abbauortes organischen Materials vervollständigt **Kapitel 11** die Studien über organische Stoffkreisläufe in dem Hauptuntersuchungsgebiet, einem Saumriff des nördlichen Roten Meeres. Biogene Riffsande wurden schon früher als wichtige Orte des Abbaus und Recyclings von organischem Material beschrieben. Deshalb wurde die O_2-Aufnahme und die benthisch-pelagische Kopplung in diesem Saumriff während unterschiedlicher Jahreszeiten mit Hilfe von benthischen Rührkammern und Sedimentfallen untersucht. Die Ergebnisse wiesen auf ein limitiertes Angebot von schnell abbaubarem organischem Material in den Riffsanden hin, was eine mögliche Konsequenz des effizienten Recyclings von Nährstoffen in der darüberliegenden Wassersäule sein könnte. Darüber hinaus wurde ein Anstieg der sedimentären O_2-Aufnahme mit zunehmender Wassertiefe in der Lagune, nicht aber in den Riffsanden festgestellt. Dieses Resultat deutete eher auf einen lateralen Transport von leicht abbaubaren Partikeln, die wie oben beschrieben von den Rifforganismen abgegeben werden, hin als auf ein Absinken der Produkte aus der Wassersäule. Dies unterstreicht einmal mehr die Existenz von kurzgeschlossenen Stoffkreisläufen in Korallenriffökosystemen.

Kapitel 12 beendet die vorliegende Arbeit durch eine geographische Ausweitung der bisher aus dem Roten Meer erlangten Erkenntnisse auf ein Lagunensystem des nördlichen mesoamerikanischen Barriereriffs. Methodisch identisch zu den Studien am Roten Meer wurden in diesem Kapitel vergleichende Analysen über die Quantität und die Zusammensetzung von partikulärem und gelöstem organischem Material erstellt, das von den dominanten benthischen Primärproduzenten einer Rifflagune vor Puerto Morelos im mexikanischen Teil der Karibik abgegeben wurde. Auch wurde die mikrobielle Abbaubarkeit des abgegebenen organischen Materials untersucht und Tagesgänge von O_2-Konzentrationen in-situ in Lagunenabschnitten, die von unterschiedlichen Primärproduzenten dominiert waren, aufgezeichnet. Auch hier konnte nachgewiesen werden, dass von Algen abgegebenes organisches Material den planktonischen mikrobiellen O_2-Verbrauch signifikant stärker erhöhte als das anderer benthischer Primärproduzenten. Ebenso übereinstimmend mit Untersuchungen aus dem Saumriff des Roten Meeres, zeigten die in-situ O_2-Logger signifikant niedrigere gemittelte O_2-Konzentrationen in der Nähe von Algendominierten Standorten als in den übrigen Teilen der Lagune. Dieses abschließende Kapitel bestätigt einen Großteil der Ergebnisse aus dem Hauptuntersuchungsgebiet der vorliegenden Studie und vergrößert somit ihre räumliche Gültigkeit.

Zusammenfassend weist die vorliegende Arbeit auf weitreichende Konsequenzen für biogeochemische Kreisläufe im Ökosystem Korallenriff hin, die durch Veränderungen in der Gemeinschaftsstruktur von Algen zu Korallen als dominanten benthischen Organismen hervorgerufen werden können. Dieser Befund sollte, gerade in den Zeiten des globalen Klimawandels und „phase shifts“, die von vielen Korallenriffstandorten weltweit gemeldet werden, mit besonderer Aufmerksamkeit wahrgenommen werden

Summary

It is generally assumed that global climate change along with direct anthropogenic factors, e.g. eutrophication and overfishing, leads to a gradual replacement of reef building corals by benthic algae, i.e. phase shifts, in many modern reef locations around the world. Previous studies showed that corals can act as important engineers of their entire ecosystem. They construct complex reef frameworks that contribute to habitat and species diversity and supply calcareous reef sands which act as biocatalytic filter systems for efficient nutrient recycling. In highly oligotrophic environments such as coral reefs, however, the most remarkable feature of this particular ecosystem engineer may be the release of organic matter in the form of coral mucus, which initiates element cycles and has the ability to conserve essential nutrients for the ecosystem via particle trapping.

The beneficiaries of most phase shifts, benthic algae, have also been described to influence their surroundings. Some algae may contribute to alter the physical environment (e.g. crustaceous coralline red algae) or to generate permeable reef sands (e.g. the green algae *Halimeda*). But many benthic algae may influence their ambient surroundings primarily by the release of organic matter. The role of coral reef associated algae-derived organic matter for biogeochemical processes in reef ecosystems is however largely unexplored.

Main goal of the present thesis therefore was to elucidate the ecological relevance of macroalgae-derived organic matter. Within the chapters 1 and 2 of this thesis, the dynamics of coral-algae interactions along with the potentially relevant environmental factors are investigated. In the following chapters 3 to 7, the main focus lies on comparative analyses (i.e. quantity and chemical composition) of organic matter released by either hermatypic corals or benthic macroalgae. Subsequently the microbial degradability of the exudates is discussed, with the final chapters highlighting the in-situ relevance of these findings and comparing these between two different reef locations (Red Sea versus Caribbean Sea), thereby expanding their spatial validity.

Chapter 1 of this thesis "Seasonal in-situ monitoring of coral-algae interaction stability in fringing reefs of the Northern Red Sea" presents a general overview of the benthic community structure and coral algae interaction occurrence within a Red Sea fringing reef, where the majority of the presented work was conducted. This study shows that more than 30 % of all hermatypic corals were involved in interactions with benthic reef algae during winter as compared to 17 % during summer. It also demonstrates that the character of competition between corals and algae in natural assemblages is highly variable between seasons, displaying fast overgrowth of corals by benthic reef algae in autumn that follows close to equilibrium between both groups of organisms in summer. Although the simultaneously assessed environmental parameters (temperature, light availability, inorganic nutrient concentrations) were highly variable between the seasons, no singular determining environmental factor controlling the stability of coral-algae interactions could be identified.

To learn more about potential factors determining coral-algae interaction processes, **Chapter 2** examines the "Effects of inorganic and organic nutrient addition on direct competition between hermatypic corals and benthic reef algae in the Northern Red Sea". A long term mesocosm experiment with branching corals of the genus *Acropora* and a typical consortium of benthic turf algae revealed that elevated ammonium and glucose concentrations stimulated algal growth, while coral tissue pigmentation and chlorophyll a content were significantly decreased. However, only in the elevated glucose treatments were all effects on corals significantly pronounced when assembled with benthic turf algae. Supplementary logger measurements in a high temporal resolution also revealed significantly lower O_2 water concentrations in the elevated glucose mesocosm compared to all other treatments, confirming side-effects on microbial activity. These findings indicate reinforcing effects of supplementary dissolved organic carbon (DOC) input onto coral-algae interaction processes.

To elucidate the mechanisms of such effects, basic investigations on the chemical nature of organic matter released by the interaction partners are necessary. Therefore **Chapter 3** "Composition analysis of coral reef associated green algae exudates" starts this series of related investigations on the chemical composition of exudates derived from the respective groups of organisms. Incubation experiments with two cosmopolitan coral reef associated green macroalge, *Halimeda opuntia* and *Caulerpa serrulata* revealed that organic matter released from both algae predominately consists of dissolved carbohydrates and proteins. Traces of fatty acids and Chlorophyll a were found in a quantitatively negligible amount. Of particular interest was that glucose accounted for 77 ± 8 % of the carbohydrate fraction and 42 ± 8 % of total organic matter released by the investigated reef algae. High glucose and

protein contents found in this study further indicate high microbial degradability of algae exudates with ensuing effects on microbial O_2 consumption rates.

However not only the chemical composition, but also the quantity of the released exudates may control subsequent processes such as microbial activity. **Chapter 4** "Organic matter release by coral reef associated benthic algae in the Northern Red Sea" presents first comprehensive studies on algal organic matter release rates in a seasonal resolution in the Red Sea. DOC and particulate organic carbon (POC) and nitrogen (PON) release by dominant reef associated benthic algae were quantified. Additionally, seasonal blooming algae were included in these investigations, and the green algae *Caulerpa* was studied at different simulated water depths. To complete these investigations, environmental parameters (temperature, light availability, inorganic nutrient concentrations) were monitored simultaneously to assess potential environmental influences on algal organic matter release. This chapter shows that all investigated algae exuded organic carbon in significant amounts and primarily in dissolved form. It also indicates that organic matter release rates are rather influenced by functional properties (growth form, life strategy) of algae than by their taxonomic affiliation. Quantities of organic matter release showed seasonal and water depth-mediated variations and were positively correlated with temperature and, until a certain threshold, light availability. The chapter provides first comprehensive information about the potential contribution of different benthic reef algae to cycles of matter in fringing reef ecosystems. It also describes environmental key factors influencing organic matter release by coral reef associated benthic algae.

To further cover influences of benthic algae on cycles of matter in their ecosystem, the following **Chapter 5** investigates a special case of algal organic matter release, i.e. sexual products released during algae spawning. "Salinity and temperature effects on release of sexual products and other organic matter by coral reef-associated green algae of the genus *Caulerpa*" investigates the role of some key environmental parameters as potential trigger for spawning in two *Caulerpa* species, *C. taxifolia* and *C. serrulata*. The chapter also presents quantification of particulate organic matter release rates in response to changes in temperature and salinity conditions. Finally, microbial degradability of the released organic matter was assessed in subsequent incubation experiments. Gametogenesis was induced in both *Caulerpa* species by decreased salinity, whereas changes in temperature had only species-specific, minor effects. A strong relationship between gametogenesis and increases in organic matter release was found and microbial activity positively correlated to the quantity of the algae-derived organic matter. This excursion to the phenomenon of concerted sexual reproduction

confirms that sudden changes in environmental parameters may trigger algae spawning events, which in turn stimulate microbial activity through the release of labile organic matter.

To evaluate the ecological significance of results presented in the last three chapters, corresponding investigations on organic matter released by the other interaction partner, the hermatypic coral, were necessary. **Chapter 6** therefore starts with complementary studies on coral exudates by analyzing the "Carbohydrate composition of mucus released by scleractinian warm and cold water reef corals". This study presents, congruent to Chapter 3, carbohydrate composition analyses in mucus collected from dominant cosmopolitan warm and cold water corals. These investigations revealed that hermatypic coral exudates present a more complex carbohydrate matrix than coral reef associated macroalgae released matter. No single common glycosyl could be detected in all mucus samples, which indicates coral genus-specific carbohydrate composition. Compared to algae exudates, glucose content was significantly lower in all mucus samples except for one sample derived from Stylophora. As glucose is used as universal energy source in most organisms and represents a substantial substratum for heterotrophic bacterial production in marine environments, this indicates that algae-derived exudates may provide a more rapidly degradable material than coral-derived exudates.

Chapter 7 completes the series of comparative investigations with a quantitative and qualitative analysis of "Organic matter release by the dominant hermatypic corals of the Northern Red Sea". Again congruent to chapter 4, dissolved organic carbon and particulate organic carbon and nitrogen release by 6 dominant hermatypic coral genera of the main study area in the Gulf of Aqaba were measured in a seasonal resolution. The influence of environmental factors on coral organic matter release rates was assessed in the same way as for algae incubations. Results showed genus-specific variations in POM release with no significant seasonal variations. However, average values of POC and PON release rates correlated with water temperature, light availability and ambient nitrate concentrations. Genera-wide high C:N ratio of the released POM was found. DOC release was detectable only for 50 % of the investigated coral genera and generally lower when compared to the dominant algae species in the study area. The comprehensive data set of Chapter 3, 4, 6 and 7 provides an important basis for the understanding of coral reef organic matter dynamics and relevant environmental factors.

The following chapters scale-up results from these investigations to the ecosystem level. **Chapter 8** entitled "Phase shifts in coral reefs – comparative investigation of corals and benthic algae as ecosystem engineers" combines previous findings on organic matter release

by hermatypic corals and benthic algae, complementing them with studies on its benthic and planktonic microbial degradability and further examines the results in the context of shifting reef communities in times of global climate change. Outcomes show clear differences between organic matter release by corals and algae, thus suggesting effects of phase shifts onto reef biogeochemical cycles.

Chapter 9 intensifies the studies of chapter 8 and additionally investigates "Organic matter release by benthic coral reef organisms in the Red Sea – its effect on planktonic microbial activity and potential implication for in-situ O_2 availability". The complete dataset (273 reef organisms separately incubated within 54 independent experiments during 4 seasonal expeditions) of organic matter release by the dominant benthic organisms from the Northern Red Sea is presented here, including scleractinian and fire corals, upside-down jellyfish and reef-associated algae. Effects of the released exudates on microbial activity in incubation waters were determined and in-situ relevance was tested in high resolution O_2 concentration measurements within reef environments dominated by corals or algae. It became clear that DOC release of reef algae was significantly higher compared to corals and jellyfish. Microbial activity in the adjacent water was also stimulated significantly more by algae-derived organic matter than by organic matter released by the investigated scleractinian and fire corals. Consequently, in-situ O_2 concentration measurements in the water directly above the reef (< 10 cm) revealed significantly higher O_2 concentrations at coral-dominated compared to algae-dominated sites, thereby emphasizing in-situ relevance of the previous findings. This confirms that benthic reef algae decrease O_2 availability in waters close to reef environments via the release of labile organic matter and its subsequent fast microbial degradation.

These first in-situ measurements of benthic community-specific O_2 water concentrations are further elaborated in **Chapter 10**, which shows that "Benthic community composition affects O_2 availability and variability in a Northern Red Sea fringing reef". The chapter comparatively investigates potential in-situ effects of different benthic cover by reef macroalgae and scleractinian corals on water column O_2 concentrations in the study area. It becomes evident that minimum O_2 concentrations and variability in the overlying water column were significantly correlated to increasing benthic cover by algae, while no correlation with coral cover was found. These results indicate that shifts from corals to benthic algae may likely affect both in-situ O_2 availability and variability with potential O_2-mediated effects on reef organisms.

Chapter 11 completes the studies on organic matter cycles in the investigated Red Sea fringing reef by highlighting another compartment of organic matter degradation. Calcareous

sands are known to be major sites for recycling of organic matter in coral reef ecosystems. Therefore "Coral sand O_2 uptake and benthic-pelagic coupling in a subtropical fringing reef, Aqaba, Red Sea" was studied during several seasons using benthic chambers and sediment traps. Results indicate a limited supply of quickly degradable organic material to the calcareous reef sands as a possible consequence of efficient recycling processes in the reef-overlying water column. Further, an increase of sedimentary O_2 uptake with water depth in lagoon, but not in reef sands was detected. This indicates lateral transport of labile organic particles, derived from reef organisms as described above, rather than sedimentation of water column production.

"Organic matter release by the dominant primary producers in the Puerto Morelos reef lagoon, Mexican Caribbean - implication for in-situ O_2 availability" finalizes the present thesis by geographically extending the studies conducted in the Red Sea to a coral reef lagoon system of the Mesoamerican Reef. Congruent to the studies conducted in the Red Sea, the final **Chapter 12** comparatively investigates quantity and chemical composition of particulate and dissolved organic matter released by the dominant benthic primary producers from the coral reef lagoon of Puerto Morelos, Mexican Caribbean. As in the main study area, microbial degradability of the released organic matter was determined along with diurnal in-situ measurements of O_2 concentrations at lagoon sites dominated by different primary producers. It became again apparent that benthic algae-derived organic matter stimulated planktonic microbial O_2 consumption significantly more as compared to other benthic primary producer-derived organic matter. Congruent to the fringing reef environment, in-situ O_2 loggers revealed significantly lower average O_2 concentrations at algae-dominated sites compared to other benthic lagoon environments. This final chapter supports findings from the main study area in the Red Sea and gives them a more extensive geographical validity.

Over all, the presented study suggests far reaching consequences for biogeochemical cycles in reef ecosystems caused by shifts in benthic community structure towards algal dominance. This may be of particular interest in times of global climate change and the concomitant phenomenon of "phase shifts" in benthic community structure reported for many reef ecosystems around the world.

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Seasonal monitoring of coral–algae interactions in fringing reefs of the Gulf of Aqaba, Northern Red Sea

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Abstract

This paper presents seasonal in situ monitoring data on benthic coverage and coral–algae interactions in high-latitude fringing reefs of the Northern Red Sea over a period of 19 months. More than 30% of all hermatypic corals were involved in interaction with benthic reef algae during winter compared to 17% during summer, but significant correlation between the occurrence of coral–algae interactions and monitored environmental factors such as temperature and inorganic nutrient availability was not detected. Between 5 and 10-m water depth, the macroalgae *Caulerpa serrulata, Peyssonnelia capensis* and filamentous turf algae represented almost 100% of the benthic algae involved in interaction with corals. Turf algae were most frequently (between 77 and 90% of all interactions) involved in interactions with hermatypic corals and caused most tissue damage to them. Maximum coral tissue loss of 0.75% day^{-1} was observed for *Acropora*-turf algae interaction during fall, while an equilibrium between both groups of organisms appeared during summer. Slow-growing massive corals were more resistant against negative algal influence than fast-growing branching corals. Branching corals of the genus *Acropora* partly exhibited a newly observed phenotypic plasticity mechanism, by development of a bulge towards the competing organism, when in interaction with algae. These findings may contribute to understand the dynamics of phase shifts in coral reefs by providing seasonally resolved in situ monitoring data on the abundance and the competitive dynamic of coral–algae interactions.

Introduction

Coral reefs are highly endangered ecosystems. Since 1950, 19% of all coral reefs world-wide have already been lost and another 35% are endangered by anthropogenic activities such as overfishing, marine pollution, eutrophication, tourism and by global climate change (Wilkinson 2008). Global climate change and the related increase of atmospheric CO_2 concentration can lead, through warming and acidification of the oceans, to an increased frequency of coral bleaching events (Hoegh-Guldberg 1999), and reduced calcification rates (Gattuso et al. 1999; Buddemeier and Gattuso 2000), thereby increasing the vulnerability of coral reefs. Temperature-induced coral bleaching may kill, or at least weaken hermatypic corals in such a way that they cannot compete with reef associated benthic algae. Substantial impacts on community structure have been observed in coral reefs during periods of warmer than normal sea temperatures (Walther et al. 2002). The impact of thermal stress on reefs can be dramatic, with eventually extensive removal of corals (Brown 1997; Spencer et al. 2000; Mumby et al. 2001).

On the other hand, algae growth may be facilitated by an enhanced supply of inorganic nutrients and the reduction of herbivore fishes as a result of overfishing (McCook *1999*; Smith et al. *2001*; Littler and Littler *2006*). It is generally assumed that the combination of these different factors results in ecological change, in particular, a gradual replacement of hermatypic corals by other benthic invertebrates such as soft corals, hydrozoans, sponges and tunicates (Bak et al. 1996; Maliao et al. 2008), or, as most often reported, by benthic reef algae and cyanobacteria (Done 1992; McCook 1999).

There have been numerous reef locations affected by such ecological shifts, especially since the extensive coral bleaching events in 1998 and 2002 (Gardner et al. 2003; Bruno and Selig 2007). Owing to its high latitude, the northern Red Sea has remained relatively unaffected by these temperature-induced bleaching events (Sotka and Thacker 2005). Heiss et al. (1999), however, showed that sea surface temperatures in the northern Gulf of Aqaba had increased by at least 1.3°C since the early nineteenth century. As a possible consequence of these elevated temperatures Loya (2004) described for the first time sporadic coral bleaching during the summers of 2002 and 2003. Additional pressures from urban, industrial, port and tourism developments may thus result in deterioration of the coral reefs in the northern Red Sea to a macroalgal-dominated ecosystem (Kotb et al. 2004).

Competition is a key process determining the composition and structural changes of benthic communities on coral reefs. In particular, interaction between hermatypic corals and benthic algae is considered to fundamentally determine community structure of coral reefs (McCook et al. 2001). McCook et al. (2001) described a variability of interaction mechanisms whereby algae and corals potentially have direct effect on each other. These mechanisms by which algae are able to directly compete with corals for the limiting factors space and light (e.g., overgrowth, abrasion, allelopathy, recruitment barrier and epithelial sloughing) may further be accompanied by indirect effects like shading inhibition of particle-capture rates and sediment trapping, potentially increasing the effects of the direct interaction mechanisms (McCook et al. 2001). Most studies investigating such coral–algae interactions reported a decline in live coral cover and a respective increase in macro and turf algae abundance (Tanner 1995; River and Edmunds 2001; Jompa and McCook 2002a, b; Nugues and Bak 2006; Mumby et al. 2007).

However, it is hypothesised that different species of coral and algae are differently susceptible to the variability of interaction mechanisms also depending on the environmental factors to which they are exposed (McCook et al. 2001). Studies describing the effects of environmental factors on coral–algae interactions were previously conducted in the context of ecological

disturbances such as hurricanes (Hughes 1989; Rogers et al. 1997), El Niño events (McClanahan et al. 2001), changes in the population of herbivores (Lewis 1985; Smith et al. 2001; Thacker et al. 2001) and eutrophication (Goreau 1992; McCook 1999; Costa et al. 2000; Fabricius 2005). Such investigations were carried out in tropical reefs, mainly the Caribbean and the Great Barrier Reef, but not in the northern Red Sea, which is generally under-investigated in the context of coral–algae interactions and phase shifts. However, because of its high-latitude location, reefs of the northern Red Sea are subjected to pronounced seasonal variations in environmental factors such as water inorganic nutrient concentrations and temperatures (Manasrah et al. 2006). These variations in nutrient availability (Fishelson 1973) and temperature (Ateweberhan et al. 2006) have already been suggested to influence the benthic community structure, and primarily benthic algal abundance, in annually recurring patterns (Benayahu and Loya 1977). This provides the opportunity to monitor the development of species-specific coral–algae interactions in relation to alterations of environmental variables in their natural environment.

The present study therefore aimed to provide first in situ monitoring data in a seasonal resolution on the abundance and the competitive dynamics of coral–algae interactions, i.e., growth and tissue mortality of the participating organisms, in the northern Red Sea. Data were collected with identical methodology during each of four seasonal expeditions and are discussed in relation to species-specificity and simultaneously monitored environmental factors.

Materials and methods

Study site

The surveys presented in this study were mainly conducted at the Marine Science Station (MSS) in Aqaba, Jordan (29°27′N, 34°58′E) between November 2006 and May 2008. Four different field expeditions were carried out to cover each season (Fall: 7 November to 12 December 2006; Summer: 9 August to 13 September 2007; Winter: 11 February to 19 March 2008; Spring: 6 May to 28 May 2008). The MSS is located at a marine reserve including a fringing reef in the northern Gulf of Aqaba that comprises a 170-km long, 14–26-km wide and more than 1,800-m deep segment of the Red Sea (Klinker et al. 1978) (Fig. 1a). Line point intercept (LPI) transects were carried out along the whole stretch of the MSS seafloor in order to characterise benthic community structure. Interaction observations and belt transects were conducted on the reef slope just west of the MSS at a water depth of 5–9 m. Additional interaction observations were conducted for comparison at a second study site at the Sinai coast close to Dahab, Egypt, (28°25′–28°34′N, 34°27′–34°32′E) between 7 and 23 May 2007 (Fig. 1b).

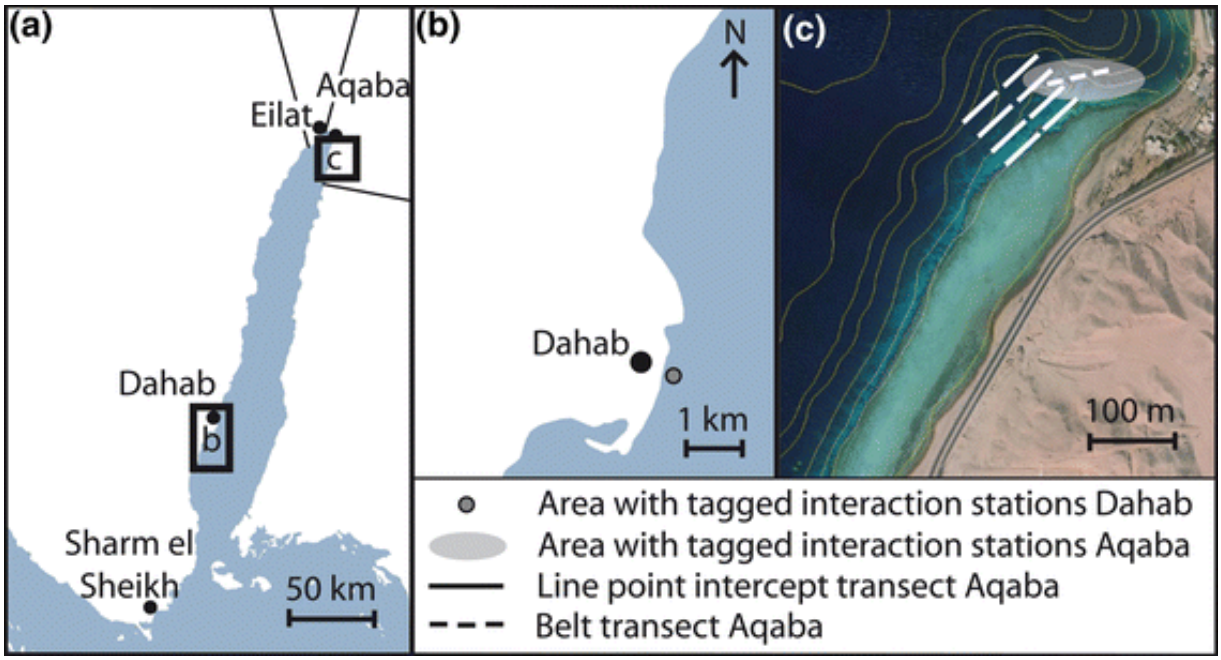

Fig. 1 *Study sites in the Gulf of Aqaba.* ***a*** *Northern part of the Red Sea;* ***b*** *location of interaction observations conducted in Dahab, Egypt;* ***c*** *area of tagged interaction stations, LPI and belt transects conducted in the fringing reef in front of the MSS in Aqaba, Jordan (photograph provided by ASEZA GIS Unit)*

Coral and benthic algae distribution in the study area

To determine the relative seafloor cover by hermatypic corals, benthic macroalgae and turf algae (Fig. 1c), a range of LPI—transects as described in Hodgson et al. (2004) were conducted. Two 50-m LPI—transects were carried out from specific starting points in water depths of 1, 5, 10 and 20 m during each expedition using SCUBA. Data for specific benthic algae and cyanobacteria seafloor coverage during fall had to be excluded, as categories for benthic algae cover were improved and specified for all surveys conducted during the other three seasons. Specific categories for benthic cover, including all occurring hermatypic coral, along with their growth form, and benthic macroalgae genera, turf algae, sand, bare coral rock and other benthic cover, were monitored at 0.5-m intervals directly below the measurement point, and recorded on a dive slate (=100 recorded data points per LPI transect). Each hermatypic coral observed during the LPI transects was carefully inspected for the occurrence of benthic algae in direct contact with the coral tissue. If this was the case, identity of algae was determined and marked as coral–algae interaction. Abundance of specific benthic algae and hermatypic coral genera, as well as respective interactions, were in the following analysed by counting their occurrences directly under the transect line according to Roelfsema et al. (2006) and calculating the percentage seafloor coverage for the respective categories.

Abundance and competitive dynamics of coral–algae interactions

During each expedition, a 50-m belt transect (water depth 5.5–7.0 m), as described in English et al. (1994), was conducted in the selected part of the MSS study area, in order to identify the associated organisms photographically and to specifically quantify the coral–algae interactions relative to the abundance of the single organisms. For this belt transect, a quadrate (1 m²) was placed 50 times consecutively left and right along a defined line indicated in Fig. 1c, thereby creating a checkerboard pattern. Photographs were taken from directly above each quadrate using a *Sony Cybershot* digital camera (resolution: 5.1 megapixels)

within underwater housing. In addition, close-up pictures from each coral–algae interaction within each quadrate were taken. Later, the total number of unaffected corals and number and kind of coral–algae interactions per area were determined using the digital image processing software *Image J*. This procedure was repeated during each expedition.

In situ monitoring of direct coral–algae interactions

In total, 29 different in situ locations of direct interactions between hermatypic corals and benthic algae were randomly selected at the beginning of the study period in November 2006 in water depths of 5–9 m using SCUBA. Interaction was thereby defined as a status where coral and algae had direct contact. All locations were tagged with small, submerged marker buoys and mapped. In August 2007, 10 additional direct interactions along with 15 coral–algae transplants and 15 healthy coral colonies serving as controls were additionally included in the monitoring. Turf algae used for transplants were taken from algae growing on fragments of dead coral skeletons. Turf algae fragments, defined as densely packed, filamentous algae or cyanobacteria rising less than one centimetre above the substratum, were collected from one dead coral colony and placed next to healthy coral colonies, thereby avoiding physical damage of the coral tissue. Algae fragments were fixed to the ground or to adjoining reef structure allowing a maximum of 0.5 cm distance relative to the living coral tissue. By February 2008, the first 29 locations were terminated and additionally 10 interaction and 10 transplant locations were established. Table 1 gives an overview of replication and the identity of involved coral and algae. Transplants on massive growing corals of the genus *Goniastrea* could not be observed on a seasonally resolved scale owing to difficulties of precisely placing algae transplants close to the typically dome-shaped corals without causing direct tissue damage.

Table 1 *Overview (n) of all tagged and monitored in situ interactions at the two study sites*

	Aqaba			**Dahab**	
	Acropora	***Stylophora***	**Massive**	***Acropora***	***Pocillopora***
Turf	17	15	/	7	9
Peyssonnelia	6	5	/	4	/
Caulerpa	/	/	6	/	/
Turf transplant	10	9	4	/	/
Control	4	3	5	5	5

The interaction locations were photographed directly from above at the start of each monitoring period using the *Sony Cybershot* digital camera and a ruler with millimeter scale as reference for the image plane. Similar to the methodology used by McCook (2001), all interaction locations were re-photographed after a defined time, as described earlier, in order to gain information about the short-term competitive dynamics of the investigated coral–algae interactions. To get further information about the temporary changes in competitive dynamics, this test procedure was replicated at the beginning and end of each expedition over a total period of 19 months. Tissue loss was then calculated by subtraction of the initial dead coral area from the dead coral area at respective observation times.

All photographs were processed using the digital image software *Image J*, allowing determination of the total projected area of living and dead coral surfaces. From each data point (site on a certain day), two pictures were used and every parameter on each picture was determined twice to minimise measurement error, which accounted for approximately 1%.
In May 2007, in Dahab, Egypt, the same methodology was used to determine the short term competitive dynamics of coral–algae interactions at a second study site in the northern Red Sea. Here, 30 hermatypic coral colonies (see Table 1), situated in a water depth of 7 m at the House Reef of the INMO hotel (28°29′N, 34°30′E), were monitored over a period of 13 days. Twenty of these colonies were involved in direct interactions with benthic algae, whereas ten healthy colonies were again monitored in parallel as controls.

Monitoring of water temperature and inorganic nutrients

In situ water temperatures were recorded every 60 s by ONSET HOBO® Pro v2 Water Temperature Data Loggers, permanently deployed in a water depth of 10 m at the study area in Aqaba. Inorganic nutrient concentrations (ammonium, nitrate, nitrite and phosphate) were measured monthly in the study area according to the methodology described by Grasshoff et al. (1999) and provided by Dr. Al-Zibdah and Dr. Rasheed, MSS Aqaba.

Statistical analyses

Normal distribution with homogenous variances allowed for all data to be analysed using analysis of variance (ANOVA). As transect data were not independent owing to repeated measurement, a one-way repeated measure design was applied. Homogeneity of variance was tested with a Levene test for every ANOVA. Coral tissue loss data were pooled for each genus-specific interaction in the respective season and assigned to groups with one or more factors influencing coral tissue loss. For comparison of groups with more than one factor having potential influence on the measured parameters, least significant difference (LSD) post hoc tests were conducted. To test the possible influences on the developing of the surveyed interactions, a Pearson product–moment correlation was used, as two normally distributed variables were measured on a continuous scale (Dytham 1999).

Results

Coral and benthic algae distribution in the study area

Average live hermatypic coral coverage at the surveyed locations along the Gulf of Aqaba was between 27 and 49%. The maximum coverage (55–65%) along the reef crest in Aqaba rapidly changed to less than 10% in the sand-dominated 5 m zone, but gradually increased from there with increasing water depth (Table 2). The LPI—transects revealed a gradual mean live hermatypic coral cover decline over the entire study period of 19 months from 41 to 36% in the MSS study area. Congruent with the observations from the LPI transects, the seasonal resolved belt transects revealed a gradual hermatypic coral cover decrease from 14.6 colonies m^{-2} in fall 2006 to 13.4 colonies m^{-2} in summer 2007 and 12.4 colonies m^{-2} in winter and spring 2008.

Table 2 *Seasonal and vertical distribution of benthic algae and hermatypic coral coverage at the study site in the northern Gulf of Aqaba (NM not measured, SE standard error)*

Season	Date	Depth	Coral cover (% ± SE)	Algae cover (% ± SE)
Fall 2006	12.11–08.12.2006	1	65 ± 1	NM
		5	6 ± 2	NM
		10	42 ± 2	NM
		20	58 ± 2	NM
Summer 2007	15.08–07.09.2007	1	59 ± 8	34 ± 10
		5	7 ± 2	7 ± 1
		10	41 ± 5	19 ± 1
		20	58 ± 1	17 ± 8
Winter 2008	15.02–14.03.2008	1	55 ± 5	37 ± 3
		5	9 ± 1	9 ± 5
		10	35 ± 5	15 ± 1
		20	55 ± 3	26 ± 1
Spring 2008	09.05–23.05.2008	1	57 ± 8	34 ± 6
		5	10 ± 5	7 ± 5
		10	30 ± 1	13 ± 1
		20	50 ± 6	24 ± 1

However, as changes in hermatypic coral cover varied between different water depths, this temporal decline was not significant. Benthic algae and cyanobacteria covered ca. 20% of the MSS fringing reef seafloor and showed, unlike the hermatypic coral cover, significant variations (Oneway repeated measure ANOVA; $n = 16$; $p = 0.040$) with 22% average algae cover during winter compared to 20% in spring and 19% in summer (Table 2). There was no significant correlation between inorganic nutrient availability and algae cover.

The most dominant hermatypic coral genera found in the MSS study area were *Acropora* and *Stylophora* accounting for 7 and 4% of the seafloor coverage, respectively (Fig. 2a). The dominant algae genera were the green algae *Caulerpa serrulata*, the red algae *Peyssonnelia capensis*, and different assemblages of turf algae. The turf algae assemblages were predominantly composed of green algae of the genus *Cladophora*, red algae of the genus *Gelidium* and cyanobacteria, which are known to be often assembled with turf algae (Paul et al. 2005). Turf algae alone accounted for more than 90% of the total benthic algae coverage. All three mentioned algae usually accounted for almost 100% of benthic reef algae found at the MSS study site, but benthic blooms of the green algae *Enteromorpha flexuosa* were observed during late winter and early spring. These blooms coincided with the lowest seasonal water temperature and the highest seasonal concentration of inorganic nutrients in the study area.

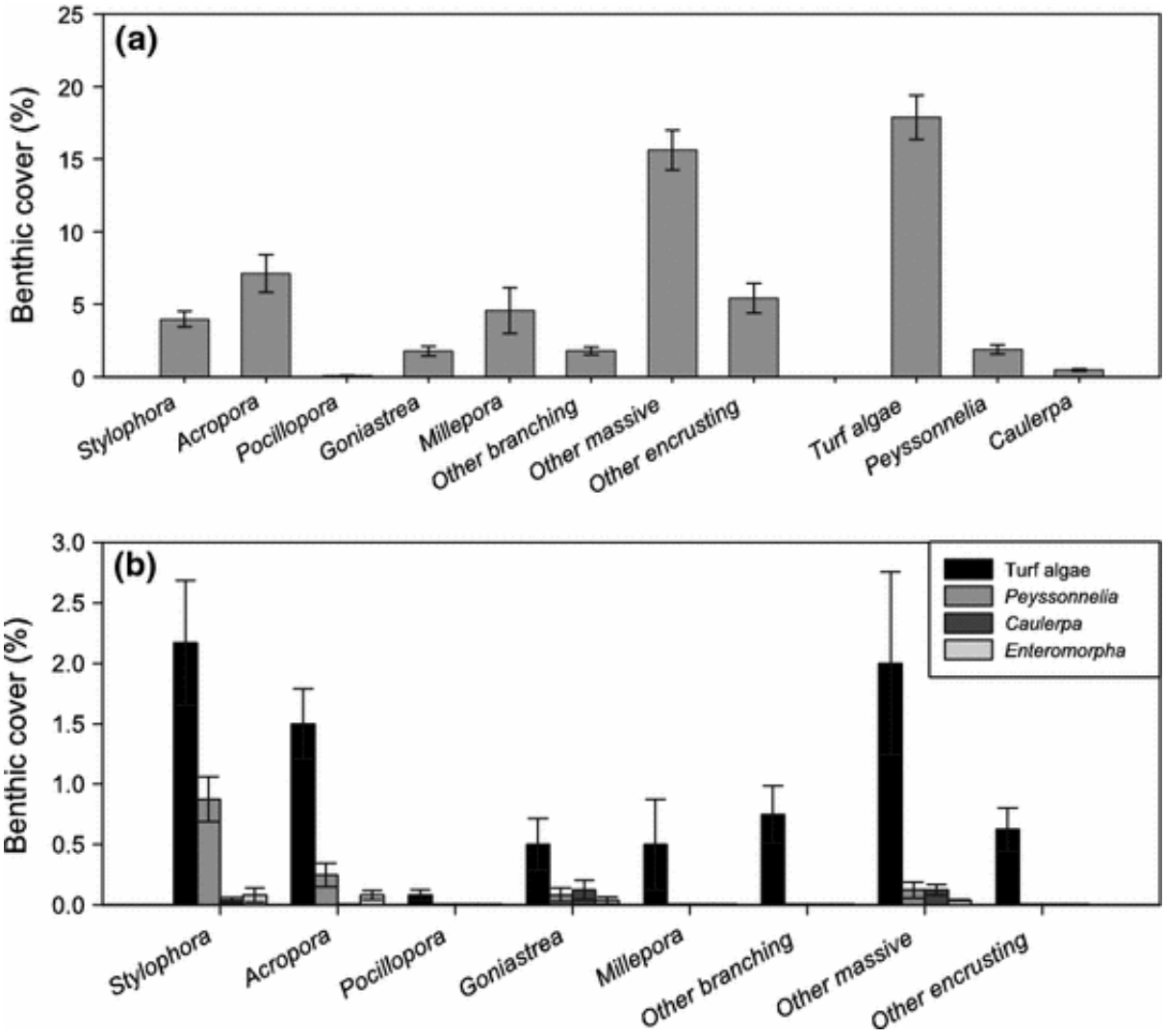

Fig. 2 a *Benthic coverage of hermatypic coral and benthic alga genera in the study area in Aqaba,* ***b*** *benthic coverage of hermatypic corals found in direct interaction with benthic algae. The single coral genera are subdivided according to the respective algal interaction partner*

Abundance and variability of coral–algae interactions

Belt transects revealed that the number of hermatypic corals in interaction with benthic reef algae varied from 3.8 in winter to 2.1 m^{-2} during summer (Fig. 3). This difference was significant (one-way repeated measure ANOVA; $n = 50$; $p < 0.001$). Turf algae were the most frequent algae representatives, being involved in 77–90% of all coral–algae interactions, followed by *Peyssonnelia* and *Caulerpa* (Fig. 2b).

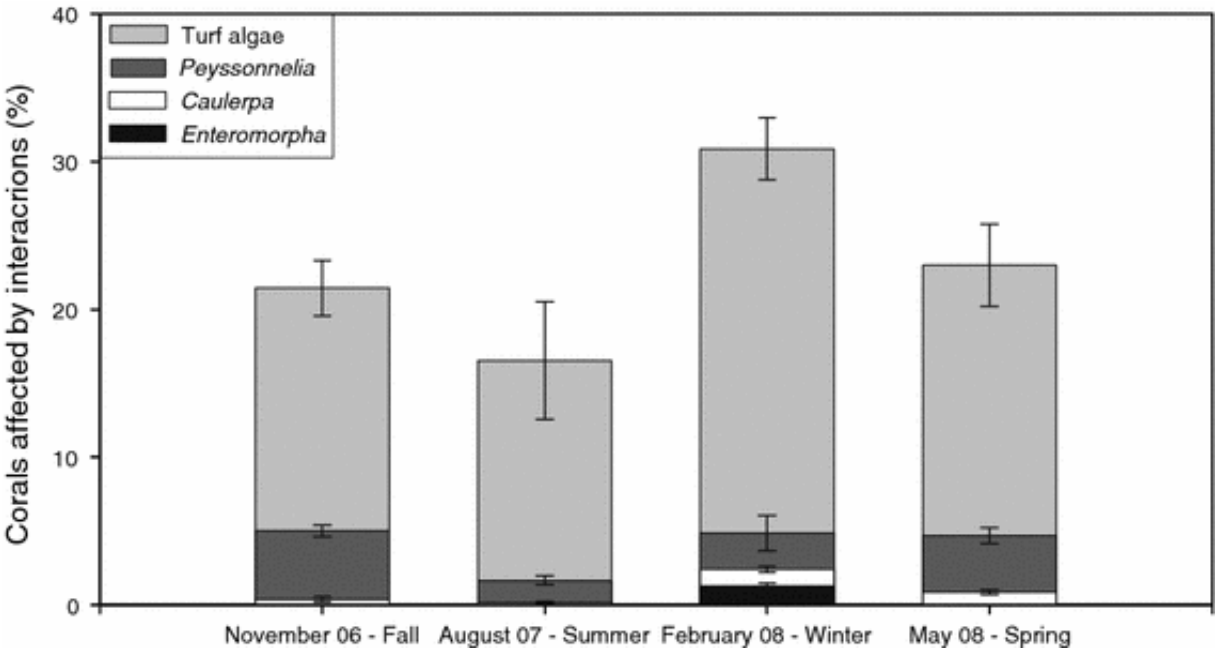

Fig. 3 *Proportion of corals in direct coral–algae interactions at the study site in Aqaba (mean ± standard error), subdivided into participating algae species*

Line point intercept—transects revealed that turf algae were involved in 81–90% of all observed coral–algae interactions. A temporary benthic bloom of the filamentous green algae *Enteromorpha* could be recorded in February 2008, rapidly forming an up to 30-cm thick canopy at various reef locations, covering about 2% in the 5–10-m benthic observation zone of the MSS reef. Even though this canopy vanished within 3 months, bleaching and tissue lesion occurred on multiple corals, which is likely caused by algae overgrowth.

Among the surveyed hermatypic corals, *Stylophora* was, relative to abundance, disproportionately often in direct interaction with algae (Fig. 2b). Though branching corals were more often affected by interactions than massive corals (21% of all branching compared to 15% of all massive), massive growing corals were involved in 82% of all interactions with the green algae *Caulerpa*.

Variations in interaction dynamics

Over the entire study period in Aqaba, a significant loss of living coral tissue could be observed for all investigated natural coral–algae interactions and transplants compared to the control corals (ANOVA with water depth as covariate, $p = 0.008$) (Fig. 4a). There was no significant tissue-loss differences in natural coral–algae interactions compared to the transplant interactions, indicating that the contact with algae was responsible for the observed tissue loss. Interaction with turf algae resulted in the highest mean tissue loss of corals, but no significant differences between the specific participating branching coral and benthic algae species (Oneway ANOVA, $p = 0.361$) were observed. This may have resulted from the high standard deviation owing to individual cases of corals overgrowing neighbouring algae. However, a significant difference in tissue loss of different coral growth forms was observed (Oneway ANOVA, $p = 0.047$), with massive corals displaying about twofold slower tissue loss (0.07% day^{-1}) compared to branching corals (0.15% day^{-1}).

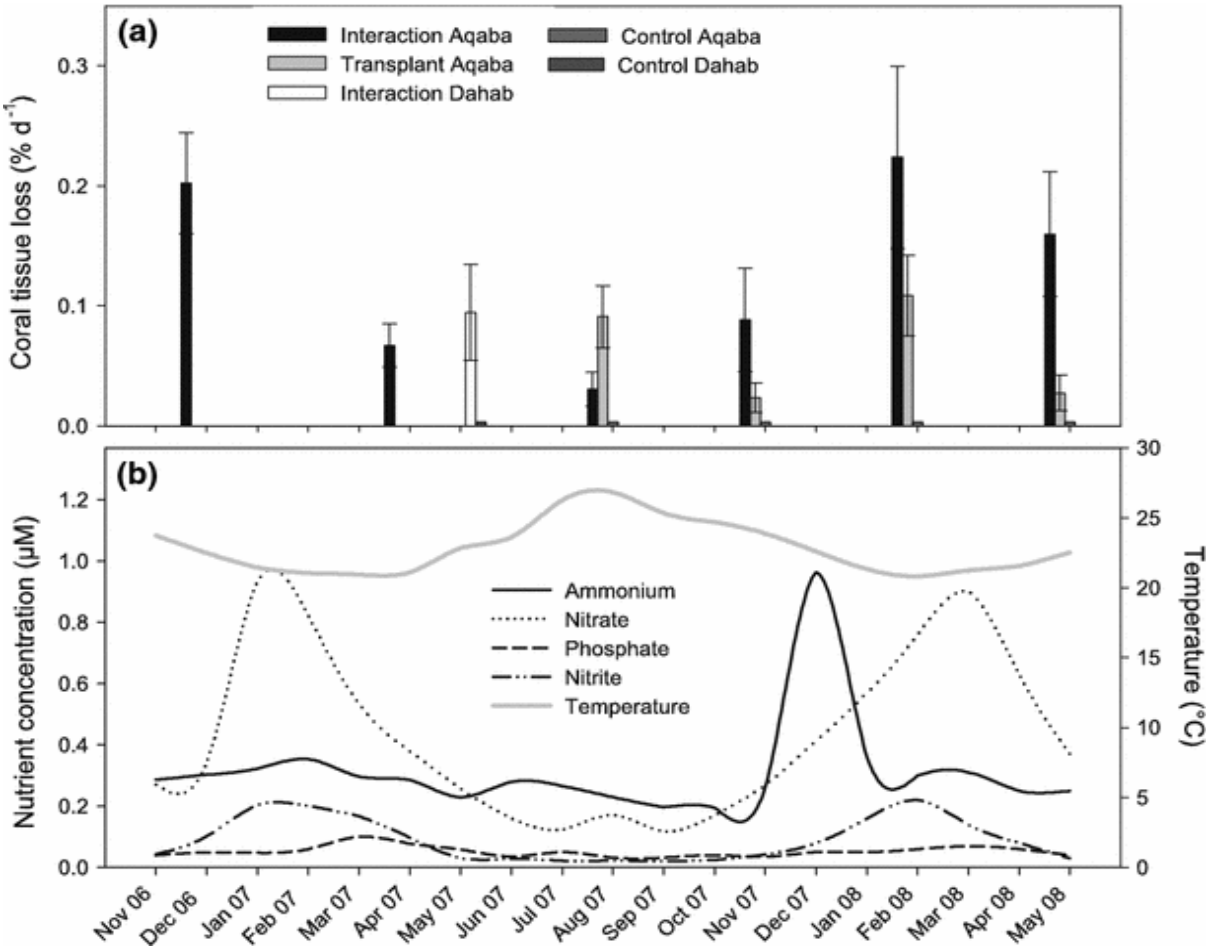

Fig. 4a *Seasonal resolved coral tissue loss (mean ± standard error) of natural coral–algae interactions, coral–algae transplants and control corals. Values of coral represent mean between proximate survey dates.* ***b*** *Water temperature and inorganic nutrient concentrations in the study area*

Significant correlation between water depth and observed coral tissue loss as a result of algae interaction was not detectable, but there was a significant difference in tissue loss of corals in interaction with algae between the seasonal resolved study periods (Oneway ANOVA, $p = 0.001$), as shown in Table 3.

No significant correlation could be found between single inorganic nutrient concentrations or temperature (Fig. 4b), and the temporary changes in coral tissue loss as a result of algae interactions over the whole study period (Pearson Correlation, Nitrate $p = 0.094$, Temp $p = 0.058$). There was a positive correlation ($R^2 = 0.55$) of overall high inorganic nutrient concentrations and highest coral tissue loss during winter months compared to the lowest nutrient concentrations accompanied by the lowest coral tissue loss during summer (Fig. 5).

About 10% of the observed hermatypic corals in direct interaction with algae died during the observation period and were henceforth not included in the statistics. Another 10% of the hermatypic corals involved in interaction with algae showed a visible interaction area, which was indicated by a bleached, not yet algae-overgrown zone. This occurrence was almost exclusively found for interactions between *Acropora* and turf algae. Approximately 12% of the involved *Acropora* colonies developed a bulge towards the adjoining algae (Fig. 6). Such bulge formation resulted in equilibrium between corals and algae, as no growth of any of the groups of organisms was subsequently detected.

Table 3 *Single p test values of Oneway ANOVA with LSD post hoc test. Difference in tissue loss of corals in interaction with algae between the respective observation periods (P1 – P6) are plotted against each other. *The mean difference is significant at the 0.05 level*

Observation period	**P1 (12.11.06–08.12.06)**	**P2 (09.12.06–15.08.07)**	**P3 (16.08.07–07.09.07)**	**P4 (08.09.07–18.02.08)**	**P5 (19.02.08–14.03.08)**
P6 (15.03.08–09.05.08)	0.245	0.136	0.027*	0.315	0.325
P5 (19.02.08–14.03.08)	0.976	0.015*	0.002*	0.064	
P4 (08.09.07–18.02.08)	0.036*	0.770	0.386		
P3 (16.08.07–07.09.07)	<0.001*	0.501			
P2 (09.12.06–15.08.07)	0.004*				

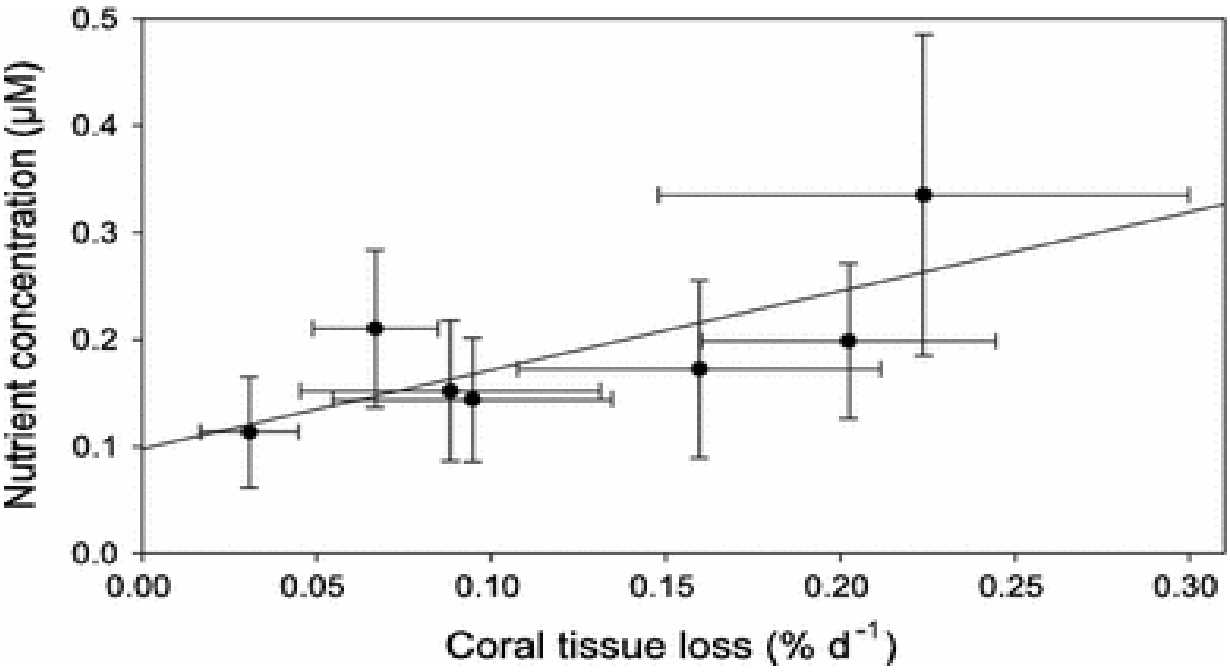

Fig. 5 *Nutrient concentration in the study area (mean of all assessed nutrients ± standard error) versus coral tissue loss (mean ± standard error) in the respective study period*

Tissue loss of hermatypic corals involved in interaction with algae at the study site in Dahab, Egypt, during spring 2007, was also significantly higher compared to the control corals (Oneway ANOVA, $p = 0.042$), and the surveyed corals showed similar percental rates of tissue loss as during this time period compared to the study area in Aqaba (Fig. 4a). This extends the spatial validity of the findings from Aqaba.

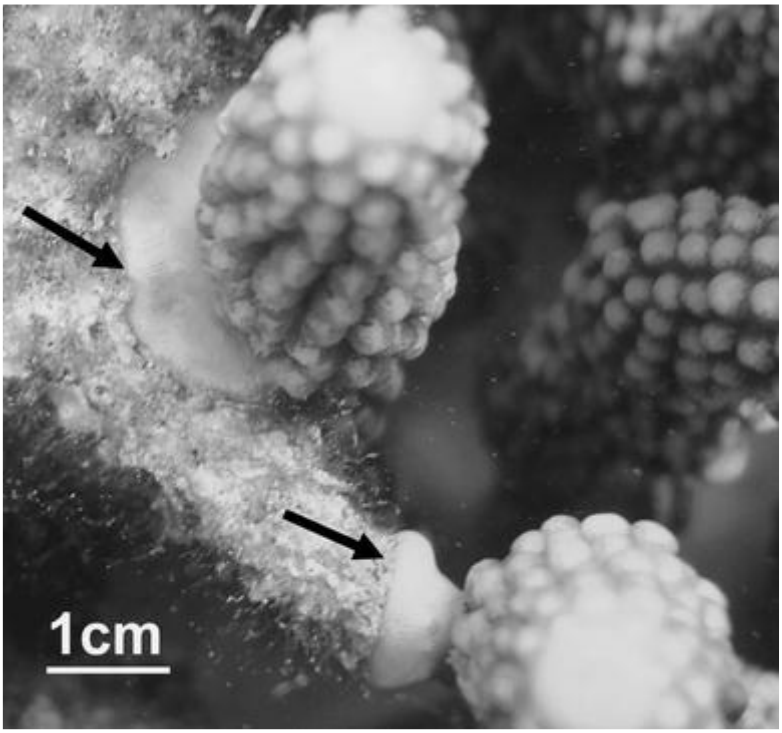

Fig. 6 *Phenotypic response (bulge generation, see arrows) of Acropora branch in direct interaction with filamentous turf algae*

Discussion

Abundance of coral, algae and coral–algae interactions in the Gulf of Aqaba

The present study identified hermatypic corals and benthic algae as the two major kinds of benthic organisms in competition for space at the investigated fringing reefs in the Gulf of Aqaba. Absolute coverage was 27–49% for hermatypic corals and 19–57% for benthic algae.

Benayahu and Loya (1977) already reported that for the Gulf of Aqaba hermatypic corals (24 ± 11%) and benthic reef algae (3–75%) were the main benthic macro-organisms in the observed fringing reefs. These similar abundances may indicate that competition dynamics for the limited substratum between hermatypic corals and benthic reef algae have remained rather stable for the last 30 years. Yet, differences in transect methodology and lack of further historical data do not permit a direct comparison of benthic community dynamics over such a long period.

Although significant variations in benthic algal cover and coral–algae interaction abundances were found, the conducted belt and LPI transects revealed a loss in coral cover within the MSS reef over the observation period of 19 months.

Benthic algae abundances in coral reefs are known to be a highly dynamic component of the reef ecosystem (Lapointe 1997; Lirman and Biber 2000). The determining factors, however, have not yet been established (Diaz-Pulido and Garzón-Ferreira 2002). In order to evaluate these temporary variations, possible relevant species-specific differences have to be considered along with environmental factors (Jompa and McCook 2002a). This will therefore be discussed later.

Species specificity of coral–algae interaction

Both survey techniques showed that turf algae assemblages involving cyanobacteria and the red algae *Peyssonnelia capensis* can have negative influence on neighbouring hermatypic corals. These findings are supported by prior results from Titlyanov et al. (2007), who demonstrated that the blue-green turf algae *Lyngbya bouillonii* acts as a poison against hermatypic corals and is able to kill live coral tissue. Tanner (1995) also observed that *Peyssonnelia* actively overgrew hermatypic corals in the direct vicinity. The combination of high occurrence of coral–turf algae interactions and concomitant coral damage indicates that the turf algae consortia are the most deleterious algae opponent against hermatypic corals in the Gulf of Aqaba. However, turf algae differ in their features (height, density, amount of accumulated sediment) and taxonomic composition, which may lead to variation of potential allelochemical interactions (Jompa and McCook 2003) so that findings of this study cannot be generalised for other coral reef ecosystems. The finding that algae transplants initially caused higher coral tissue loss compared to the natural coral–algae interactions may be explained by the reciprocal inhibition of algae growth by the coral (Jompa and McCook 2002b).

The present study also showed that temporary blooming algae had negative influence on hermatypic corals. The observed bloom of *Enteromorpha* covered an extensive area of the MSS reef during winter and spring. This space monopolisation (Lirman and Biber 2000) lasted for 3 months, and the benthic algae bloom was able to kill several hermatypic corals. But the present study also showed that the observed algae blooms, commonly known in this area (Genin et al. 1995; Lapointe et al. 1997; Morand and Merceron 2005) did not significantly alter benthic community structure.

However, not only the identity of algae, but also that of hermatypic corals involved in coral–algae interactions was shown to have effect on the outcome of this competition (Nugues and Bak 2006). The present study did not reveal any significant differences in susceptibility to algal influence between the different branching coral genera, but between the different coral growth forms. These findings are supported by Lirman (2001), who suggested that coral morphology has an important influence on the outcome of coral–algae competition. Littler and Littler (1997) described the entanglement of, in particular, branching corals by algae, often resulting in necrosis and death of the polyps. This could point towards an ecological trade off, as branching corals usually exhibit higher growth rates (Huston 1985; James et al. 2005) and are therefore likely unable to dedicate the same amount of resources for defence compared to slow growing, massive corals.

The observed development of a bulge towards the adjoining algae, potentially functioning as a physical barrier to restrain the competitor, was only found for branching corals of the genus *Acropora*. Such phenotypic plasticity, representing interaction between the developmental programme of an organism and the influencing ecological factors (Schlichting 1989), is commonly known among hermatypic corals (Bruno and Edmunds 1997; Todd 2008), particularly of the genus *Acropora* (Potts 1978). Morphologic adaptations to competition by changes of growth direction (Lang and Chornesky 1990; Romano 1990) and rate (Zilberberg and Edmunds 2001) or development of defence mechanisms such as sweeper tentacles (Nugues et al. 2004) have already been described in the scientific literature. The observations of the present study may hint to a new mechanism for *Acropora* to reduce negative algae influence by a specific growth modification. Such species-specific differences in competitive ability of hermatypic corals can play a significant role in structuring entire reef communities (Nugues and Bak 2006).

Influence of environmental parameters on coral–algae interactions

Coles (1988) and Ateweberhan et al. (2006) described temperature as one of the principal factors controlling coral and macroalgae growth in high-latitude reefs, because algal growth was stimulated during low water temperatures in winter. However, higher tissue loss of corals

in interactions with benthic algae at lower temperature could not be demonstrated by the present study. This could potentially result from microbial involvement in coral–algae interactions (Kline et al. 2006; Smith et al. 2006) as lower temperatures of the ambient water result in higher O_2 solubility and lower microbial activity (Shiah and Ducklow 1994), thus preventing O_2 depletion as observed by Kuntz et al. (2005), Kline et al. (2006) and Smith et al. (2006). Another reason may be decreased release of bacteria-derived toxic secondary metabolites (Littler and Littler 1997; Jompa and McCook 2003; Nugues et al. 2004).

A combination of low temperatures and higher nutrient availability through seasonal upwelling (Labiosa et al. 2003) and water column mixing (Manasrah et al. 2006) is typical for the northern Red Sea, resulting in a fivefold higher concentration of inorganic nutrients during the winter months (Badran et al. 2005).

The current discussion on factors influencing coral–algae interactions and ensuing phase shifts refers to bottom-up factors such as nutrient availability as one of the determining ecological parameters influencing community structures in coral reefs. Yet, the present study could not identify significant correlation between seasonal changes in availability of inorganic nutrients (bottom-up factor) and coral tissue loss in interaction with benthic reef algae. This indicates the possible simultaneous involvement of other factors.

Besides the determining bottom-up factors, Jompa and McCook (2002a) pointed out that nutrient influence on algal growth only led to competitive advantages over corals when herbivory was insufficient to consume excess algal growth. Although not assessed in the present study, Rilov and Benayahu (1998) in this context demonstrated lower grazing on benthic algae during winter in the study area. This combination of algae growth facilitating factors may explain the finding of the present study that abundance and harmfulness of coral–algae interactions was significantly increased during winter. This study can thus not identify one single factor controlling the competitive dynamics in coral–algae interactions, but suggests that several factors act synergistically as described by other studies (Smith et al. 2001; Burkepile and Hay 2006; McManus and Polsenberg 2004).

Ecological implications

The present study suggests the importance of both seasonality and species-specificity as factors influencing the competitive dynamics of coral–algae interactions in the northern Red Sea. As no single determining environmental factor could be established, it becomes apparent that a combination of seasonally varying environmental parameters can cause temporal changes in benthic cover in the northern Red Sea. However, these temporal changes in benthic cover are reversible owing to higher competitive ability of the corals and a seasonal dieback in algae blooms during the summer months. These seasonal phase shifts have already been described for other reefs not subjected to such extreme seasonality (Diaz-Pulido et al. 2009). This study also indicates the potentially important role of the summer season (with its low nutrient concentrations owing to water column stratification) for the recovery of hermatypic corals in interaction with benthic algae. Disturbances like coral bleaching events during summer, recently described for the northern Red Sea (Loya 2004), or increased tourist abundance as predicted by Al-Halasah and Ammary (2007) could lead to non-reversibility of the observed temporal phase shifts and to a permanent benthic community structure alteration in fringing reefs of the northern Gulf of Aqaba.

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2

Effect of inorganic and organic nutrient addition on coral–algae assemblages from the Northern Red Sea

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Abstract

Previous studies in fringing reefs of the Northern Red Sea demonstrated that the in-situ competition of corals and algae in natural assemblages is highly variable between seasons displaying fast overgrowth of corals by benthic reef algae in fall that follows close to equilibrium between both groups of organisms in summer. This may be caused by up to 5-fold higher inorganic nutrient and 6-fold higher organic nutrient concentrations in fall and winter, thereby potentially promoting algae and cyanobacteria growth with concomitant phase shift. A long term mesocosm experiment (duration: 90 days) was conducted in order to study the effect of dissolved inorganic (ammonium, phosphate, nitrate, and mix of all three) and organic (glucose) nutrient addition onto the competitive process in the dominant coral–algae assemblages of the Northern Red Sea involving branching corals of the genus *Acropora* and a typical consortium of benthic turf algae. Nutrients were added in 3-fold higher concentrations compared to the annual averages, and the parameters algal growth, extension of bleached area on corals, tissue colour change and chlorophyll a concentrations were monitored at regular intervals over experimental duration. This revealed that elevated ammonium concentrations and elevated organic nutrient concentrations stimulate algal growth, while coral tissue pigmentation and chlorophyll a content were significantly decreased. But only in the elevated organic nutrient treatment all effects on corals were significantly pronounced when assembled with benthic turf algae. Supplementary logger measurements revealed that O_2 water concentrations were significantly lower in the elevated organic nutrient mesocosm compared to all other treatments, confirming side-effects on microbial activity. These findings indicate that organic nutrient input into coral reefs can affect physiology and metabolism of both corals and benthic turf algae. Reinforcing interaction between both groups of organisms along with involvement of microbes may facilitate phase shifts in coral reef ecosystems.

Introduction

Coral reef ecosystems are hotspots of biodiversity, but have always been subjected to natural disturbance (Nyström et al. 2000). Yet, the acceleration of anthropogenic disturbances, with far-reaching consequences for the resilience of these ecosystems, i.e. the ability to absorb environmental stressors and regenerate thereafter, may lead to unpredictable synergistic effects, which substantially alter the structure of coral reef communities (Hatcher et al. 1989; Bellwood et al. 2004). The last decades have witnessed an increasing concern about the effect of human disturbances on coral reefs (Hoegh-Guldberg 1999; Wilkinson 1999).

A major focus of this attention is now aimed at phase shifts, whereby an ecosystem dominated by hermatypic corals changes to one dominated by benthic algae (Done 1992; McCook 1999; Pandolfi et al. 2005). The competition of corals and benthic reef algae for the limited substratum does widely occur in many tropical coral reefs (Tanner 1995; Lapointe et al. 1997; McCook 2001). Two main factors have been established as causatives for the phase shift phenomenon (McCook 1999; Bell et al. 2006): the reduction of herbivore pressure ("top down") on benthic algae by overfishing (Jompa and McCook 2002; Hughes et al. 2007), and the enhancement of algal growth facilitated by eutrophication ("bottom up") due to nutrient input by pollution and terrestrial run offs (Costa et al. 2000; Fabricius 2005). However, the factor ultimately influencing the competitive balance between corals and algae is still not resolved, but various studies have shown that both top down as well as bottom up factors can independently and interactively facilitate phase shifts (Smith et al. 2001), but may also act synergistic on the degradation of coral reefs (Lapointe 1997).

Several studies have been conducted to elucidate the role of nutrient enrichment and poor water quality on coral mortality, (reviewed in Fabricius 2005). While, for example, Costa et al. (2000) found that enhanced nutrient availability affected the community structure of coral reefs by favouring fast growing turf and macroalgae, Jompa and McCook (2002a) suggested in contrast, that nutrient influence on algal growth only led to competitive advantages over corals when abundance of herbivorous fish and invertebrates was insufficient to consume excess algal growth.

In the context of nutrient effects on corals, Kline et al. (2006) experimentally showed that inorganic nutrients, such as the routinely measured components of water quality (nitrate, phosphate, ammonia) did not cause coral mortality, whereas dissolved organic nutrients,

rarely considered in water quality monitoring, significantly did. Although various studies concerning the direct effects of organic and inorganic nutrients on hermatypic corals and benthic algae have been carried out (Wittenberg and Hunte 1992; Kline et al. 2006; Coles 2007), only some regarded the influence of changes in inorganic nutrient concentrations onto the direct competitive processes between hermatypic corals and benthic algae (Miller and Hay 1996; McCook 2001; McCook et al. 2001; Jompa and McCook 2002a) and none investigated the effects of organic nutrient addition on coral–algae assemblages.

Recent studies in the Northern Gulf of Aqaba revealed that the in-situ competition within natural coral–algae assemblages was highly variable with fast overgrowth of coral by algae in fall, and an equilibrium between both groups of organisms in summer (Haas et al., unpublished data). This may be due to 5-fold higher inorganic nutrient (Badran et al. 2005) and 6-fold higher organic nutrient (Wild et al., unpublished data) concentrations in fall and winter, thereby potentially accelerating algae as well as cyanobacteria growth and a concomitant phase shift. The pronounced seasonal variations of nutrient concentrations in this high latitude reef may thus affect the competitive processes between hermatypic corals and benthic algae. For this purpose, a long term mesocosm experiment (duration: 90 days) with typical corals and benthic algae of the Northern Red Sea was carried out and effects of both inorganic (ammonium, phosphate, nitrate) and organic (glucose) nutrient addition on coral–algae assemblages along with associated O_2 dynamics were monitored.

Materials and methods

Study site and collection of corals and benthic reef algae

This study was conducted from February 22 to May 21, 2008 at the Marine Science Station (MSS) in Aqaba, Jordan (29° 27′ N, 34° 58′ E), located at a marine reserve including a fringing reef, in the northern Gulf of Aqaba. Preliminary transect work identified a hermatypic coral of the genus *Acropora* and a typical consortium of benthic turf algae growing on dead coral skeletons as the dominating coral and benthic reef algae species, respectively (Haas et al., submitted). Both of these groups of organisms consequently were also most often observed in direct contact to each other. Hence, specimens of these organisms were collected from a water depth of 5 m from the reef located directly southwest of the MSS using SCUBA. Fragments of both organisms were sampled from one living *Acropora* colony and one dead *Acropora* colony with intense overgrowth by turf algae. These turf algae consisted of a consortium, dominated by green algae of the genus *Cladophora*, red algae of the genus *Gelidium* and associated cyanobacteria assemblages.

Fragments (size: 7–13 cm, *Acropora* $n = 36$, turf algae $n = 18$) were generated from both colonies in-situ using side cutting pliers. All fragments were immediately transported into 1000 l flow-through tanks with in-situ seawater supply (exchange rate: 600–800 l h^{-1}), where they were separately kept for four days at in-situ temperature and light intensity in order to allow healing and regeneration.

Experimental set up

The thirty six living *Acropora* fragments were attached onto ceramic tiles (each 4 × 4 cm) using small amounts of coral glue (Reef Construct, Aqua Medic®). Thereafter, dead coral fragments with the turf algae were attached onto the tiles of 18 randomly selected corals in order to generate close contact (0.7–1.2 cm distance) between living corals and turf algae. Special care was taken to place the turf algae as close to the coral as possible without actual physical contact in order to avoid lesions by tangency, abrasion, or direct shading. All organisms were fixed on the site of fracture in order to avoid mechanical disturbance. Three of these combined coral–algae assemblages, together with three stand-alone coral fragments, were then transferred to each of six experimental tanks (volume: 500 l, length: 100 cm, width: 70 cm, water height: 70 cm). The tanks situated in an atrium shaded by skylights, were

provided with fresh untreated in-situ seawater flow-through (exchange rate: 70 l h^{-1}) and maintained at simulated in-situ conditions. Seawater inflow was 0.5 cm above the tank bottom and outflow on the opposite surface to provide for a consistent water exchange. Light intensity (lx) and temperature (°C) were recorded every 5 s by ONSET HOBO® Temperature/Light Data Loggers at the in-situ habitat (13,600–14,400 lx; 21.5–23.2 °C) of the collected organisms and during the mesocosm experiments. Differences to in-situ light conditions were compensated by the installation of halogen rays. Maximal deviations, relative to in-situ conditions, were 4% for temperature and 15% for light intensity.
All specimens were kept in the tanks for 90 days with experimental addition of nutrients to the tanks as described in Table 1. Nutrients were added twice a day (08:00h and 20:00h), maintaining a calculated average of 3-fold higher concentrations compared to the annual average concentrations in the study area as derived from Rasheed et al. (2002). Because nutrient concentrations could not be measured during the experiment, concentrations were calculated with a simple exponential formula for dilution in open systems (Fig. 1) with the assumption that nutrient uptake of the specimens was negligible when compared to the volume of the tank. As other factors, potentially leading to a faster depletion of nutrients (e.g. nutrient uptake by phytoplankton), could not be excluded, the average 3-fold higher concentration is a conservative calculation of the actual nutrient concentration. Consistent water exchange and the semi-daily addition of nutrients should however ensure a conservative but reliable approximation to the actual nutrient concentration.

Table 1 *Bleached coral tissue area and algae growth, listed for each treatment over total experimental period of 90 days. Values represent averages ± SD.*

Treatment	**Added nutrients**	**Sample**	**Area of bleached coral tissue (cm^2)**	**Algae growth (% $month^{-1}$)**
Seawater	-	Stand-alone coral	0.5 ± 0.9	
		Coral-algae assemblage	2.0 ± 1.0	2 ± 3
NH_4^+	340 ml NH_4Cl (1M)	Stand-alone coral	0.0 ± 0.0	
		Coral-algae assemblage	2.3 ± 2.3	33 ± 16
NO_3^-	420 ml $NaNO_3$ (1M)	Stand-alone coral	0.0 ± 0.0	
		Coral-algae assemblage	3.7 ± 1.2	-11 ± 9
PO_4^{3-}	90 ml Na_3PO_4 (1M)	Stand-alone coral	0.0 ± 0.0	
		Coral-algae assemblage	2.7 ± 1.2	-7 ± 10
Nutrient mix	All of the above	Stand-alone coral	0.0 ± 0.0	
		Coral-algae assemblage	1.7 ± 0.6	1 ± 6
Glucose	55g glucose monohydrate (s)	Stand-alone coral	0.0 ± 0.0	
		Coral-algae assemblage	5.3 ± 2.9	27 ± 19

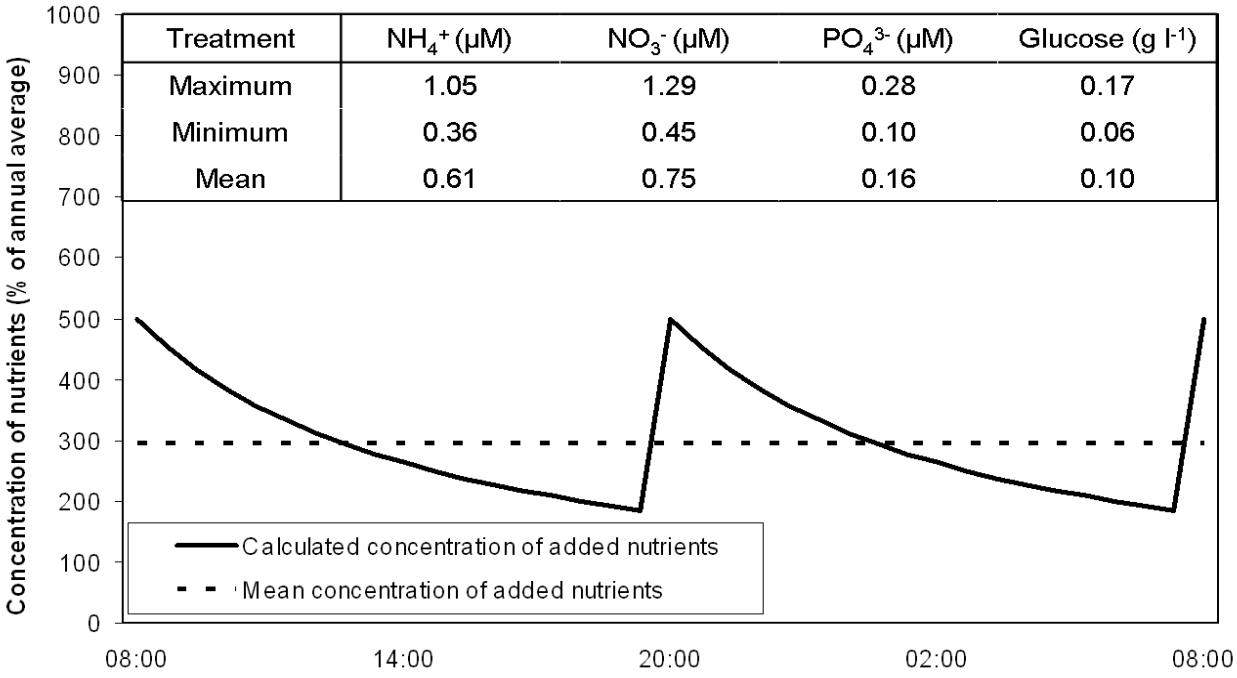

Fig. 1 *Calculated percentage of nutrient concentrations compared to the annual average in the Gulf of Aqaba over a time period of 24 h (start 8 a.m.) and corresponding minimum and maximum values of the single respective nutrients.*

Measured parameters

Photographs were taken from each of the 36 coral–algae assemblages once a week over the total duration of the mesocosm experiment in order to monitor changes in organism sizes as well as pigmentation. For this purpose, a *Sony Cybershot* digital camera (resolution: 5.1 megapixels) with underwater housing was used. Photographs were taken from directly above and sideward, while a ruler with millimetre scale was used as reference for the image plane. Algae growth rates were determined by measuring the height of the algal turf filaments growing on the coral skeleton using a calliper with millimetre scale. To determine the extent of visually detectable bleached areas on corals, one or more simple geometric forms, e.g. square, circle, triangle or cylinder, were assigned to the respective area and measured with a calliper.

In addition, the daily ranges of O_2 water concentrations for each of the mesocosm were measured prior to termination of the experiment (days 83–85) by deploying *EUREKA Midge*^{TM} *Temperature and Dissolved Oxygen Loggers*. These O_2 loggers were installed in the tanks for 24 h in a way that the sensor was located in the centre of the tank, within a distance of 15–25 cm towards all specimens at 3 cm above the tank bottom.

At the end of the experimental period, the combined coral–algae assemblages and stand-alone corals were transferred into a 30 l aquarium, placed in front of a homogenous white background and photographed with a *Panasonic DMC-TZ5* digital camera (resolution: 9.1 megapixels). In order to detect differences in coral tissue colour, the same shutter speed (200^{-1} s), aperture (F 3.3) and ISO sensitivity (ISO-200) was used for every picture. Differences in tissue colour were identified using the *Coral Colour Reference Card* developed by Siebeck et al. (2006) for standardizing changes in coral colour.

Thereupon, a defined area of coral tissue (5 to 9 cm^2) was removed from each coral colony for chlorophyll a analysis using a jet of high-pressure air from an artist's airbrush as described in Szmant and Gassman (1990). These tissue samples were immediately filtered onto GF/F filters (Whatman; 0.7 µm nominal pore size, 25 mm in diameter) and kept frozen at – 20 °C

and lightproof until further analysis. Chlorophyll a was extracted from the filters by immersion in 90% acetone for 24 h in the dark at 4 °C and measured by fluorometric analysis as described in Rathbun et al. (1997) using a *TD-700 Laboratory Fluorometer*.

Statistic analyses

Analyses of variance (ANOVA) were used for independent and normally distributed data that showed the same variance (Dytham 1999). Homogeneity of variance was tested with a Levene test for every ANOVA. The data were assigned to groups with one or more factors. For comparison of groups with more than one factor having potential influence on the measured parameters, a least significant difference (LSD) Post hoc test was conducted. For the analysis of the colour chart, a Mann–Whitney-*U* test was conducted as the data had less than 30 possible values, and the assumptions made for ANOVA were thus not valid. To test differences in the dissolved O_2 concentration, a Friedman test was conducted as non-parametric analogue to ANOVA (Dytham 1999).

Results

Over the duration of the experiment, in the tanks with addition of ammonium and glucose, growth rates of turf algae assembled to corals were 33% and 27% $month^{-1}$, respectively. These growth rates were significantly higher (one-way ANOVA, LSD Post hoc test; NH_4^+: $p = 0.009$, glucose: $p = 0.025$) compared to the seawater control (2% $month^{-1}$). A red algae of the genus *Peyssonnelia* additionally developed a phylloid in the organic nutrient mesocosm, but this exceptional occurrence was not included in the calculation of algae growth rates. No significantly different turf algae growth rates were found for the nitrate, phosphate and mixed nutrient treatments relative to the seawater control (Table 1).

At the end of the experiment, all corals assembled with turf algae had developed a significant area of visibly bleached tissue towards the algae (Fig. 2), when compared to the beginning of the experiment (one-way ANOVA; $p < 0.001$). This bleached area was only observed for one stand-alone coral in the seawater control. Amongst the coral–algae assemblages, only corals subjected to increased organic nutrient concentrations showed significantly larger bleached areas when compared to the sea water control (one-way ANOVA, LSD Post hoc test; $p = 0.014$). No significant effects of inorganic nutrient addition on the extent of bleached areas in coral–algae assemblages could be detected.

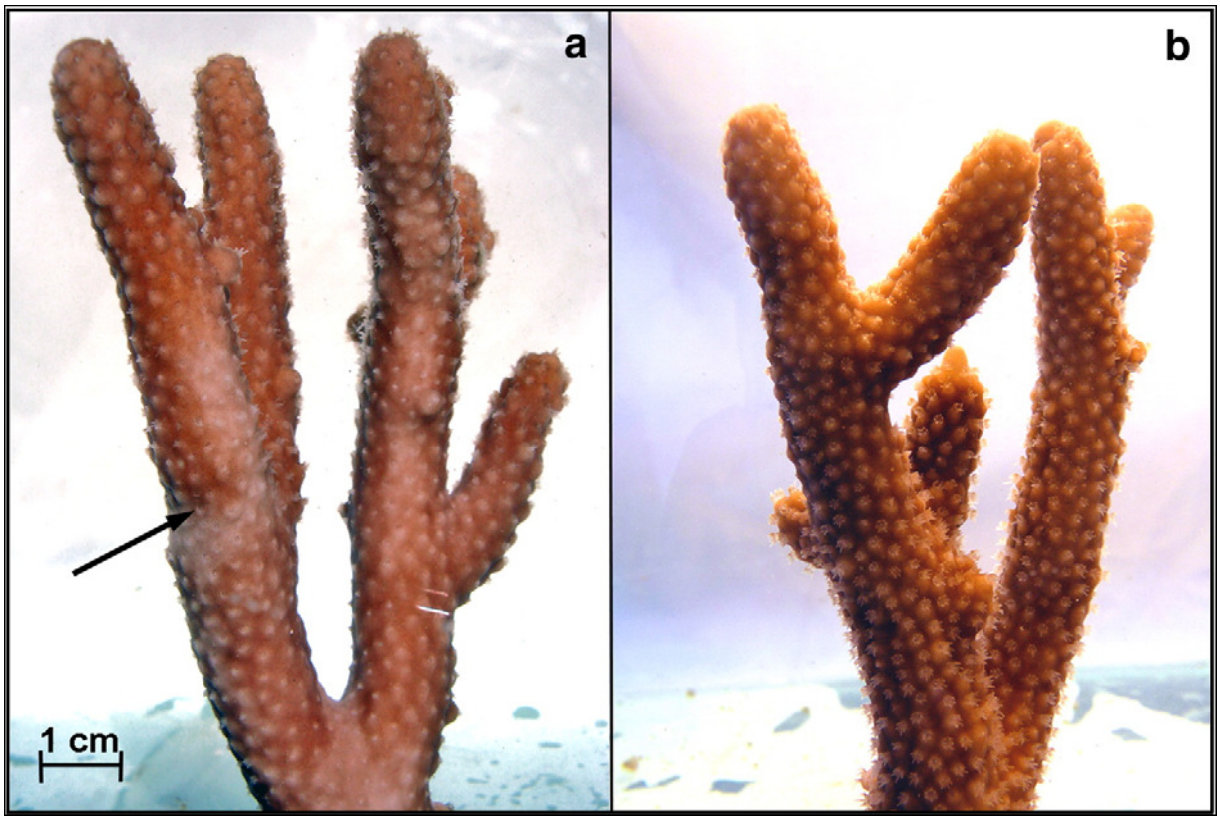

Fig. 2 *Exemplary Acropora fragments after three months exposed to elevated glucose concentration. Visible bleached areas (arrow) at the zones aligned with the turf algae consortium (a) are not apparent at the stand-alone coral colony (b).*

The addition of each single nutrient, but not the inorganic nutrient mix, led to significantly decreased chlorophyll a concentrations in the tissue of the stand-alone corals compared to the stand-alone corals in the control seawater tank (one-way ANOVA; $p < 0.001$, Fig. 3a). Coral–algae assemblages generally displayed significantly lower chlorophyll a tissue concentrations when compared to stand-alone corals in all treatments (two-way ANOVA; $p = 0.003$), whereby these differences were significantly more pronounced in the organic nutrient treatment compared to all other treatments (one-way ANOVA; $p = 0.007$).

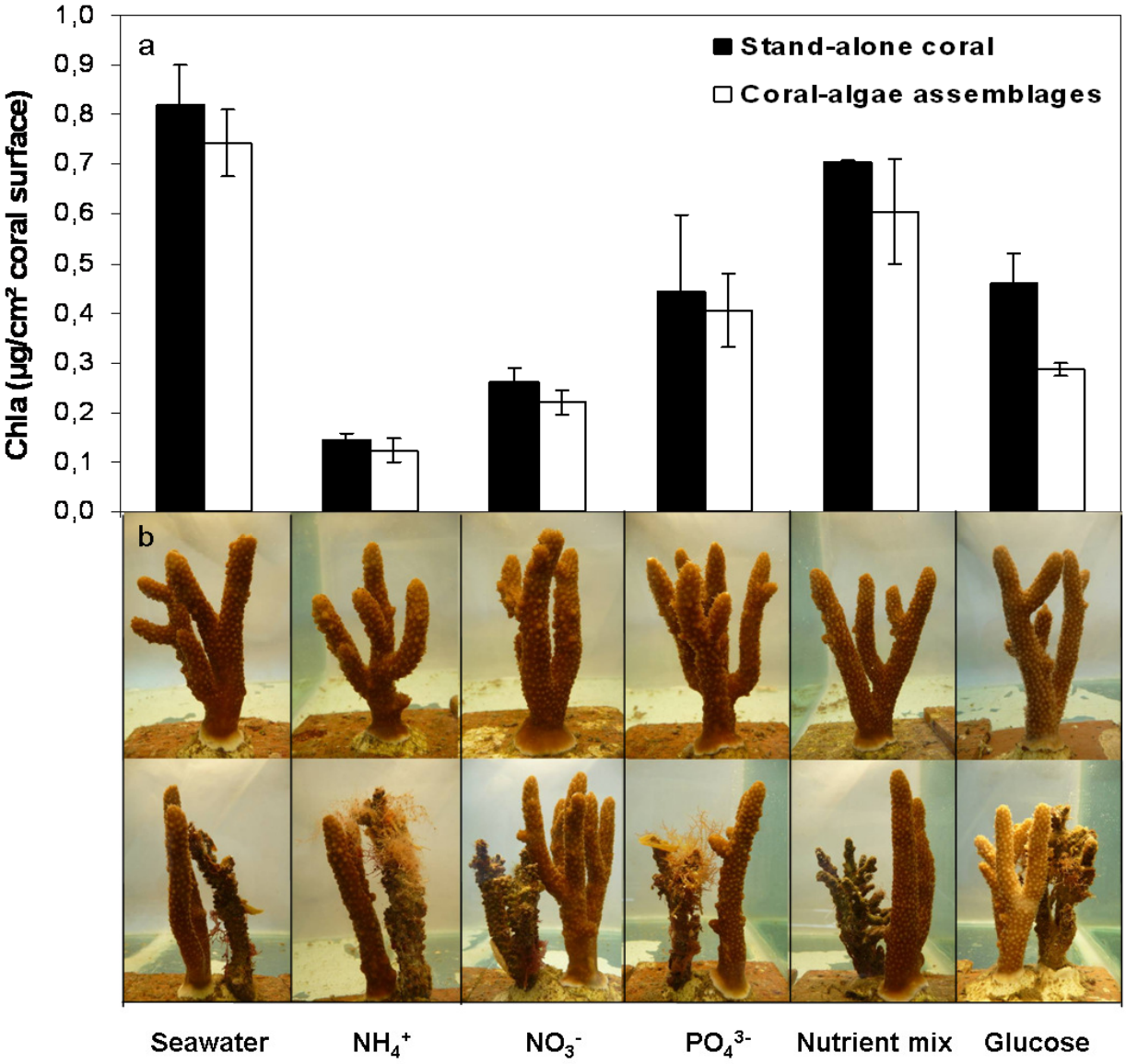

Fig. 3a *Chlorophyll a concentration of coral tissue samples collected at the end of the experiment.* ***b*** *Standardized photographs of stand-alone corals (first row) and coral–algae assemblages (second row) used in the long term-monitoring experiment, involving branching corals of the genus Acropora and typical consortia of benthic turf algae (photographs by M. Naumann).*

Standardized photographs revealed an overall significant stand-alone coral tissue colour decrease over the entire surface when exposed to enhanced concentrations of organic nutrients (Mann–Whitney-*U* $p = 0.002$) and ammonium (Mann–Whitney-*U* $p = 0.015$). But only in the organic nutrient treatment, this effect was significantly more pronounced for the coral–algae assemblages compared to the stand-alone corals (Mann–Whitney-*U* $p = 0.016$) (Fig. 3b).
Daily O_2 concentration ranges were not different in the water of all experimental treatments (6.7–7.8 mg l^{-1}), except for organic nutrient addition, which exhibited significantly (Friedman Test; $p < 0.001$) lower O_2 concentrations (5.7–6.8 mg l^{-1}) compared to all other treatments (Fig. 4).

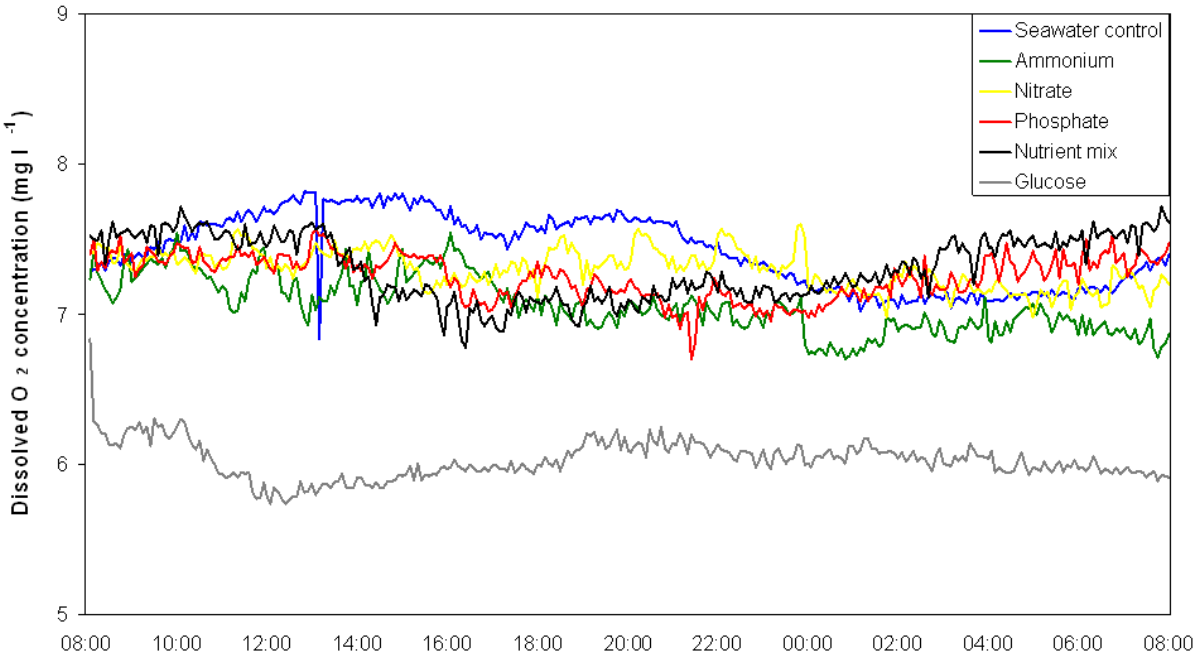

Fig. 4 *Dissolved O_2 concentrations measured with O_2 loggers (Midges) in the respective experimental tanks over a time period of 24 h (start 08:00 a.m.).*

Discussion

Effects of nutrient addition on stand-alone corals

The coral tissue analysis conducted in this study showed very specific chlorophyll a stand-alone coral tissue concentration dependent on the inorganic nutrient. Lowest chlorophyll a concentrations were recorded for the ammonium treatment, whereas the mixed nutrient treatment showed no significant difference compared to the seawater control. Moreover, the ammonium addition was the only inorganic treatment resulting in visibly decreased tissue colour of the entire coral surface.

This finding is consistent with the results presented by Kline et al. (2006), showing that ammonium addition had the most damaging influence on corals amongst inorganic nutrients. It further has been demonstrated that chlorophyll contents are highly responsive to changes in the concentration of inorganic nutrients (Hoegh-Guldberg; Smith, 1989). Inorganic nutrients influence abundance of symbiotic dinoflagellates in corals, and consequently coral pigmentation (Muller-Parker et al. 1994). In contrast to the present findings most studies concluded in a higher density of chlorophyll in coral tissues when exposed to elevated ammonium concentrations (Muscatine et al. 1989; Dubinsky et al. 1990; Ferrier-Pagés et al. 2001). Possible explanations for a decrease in chlorophyll concentration following nitrogen enrichment are however already mentioned in literature. Nordemar et al. (2003) concluded in enhanced growth rates of zooxanthellae populations, leading to space limitation (Fitt et al. 2001) and subsequent expulsion of surplus algae cells (Stimson and Kinzie 1991; Muller-Parker and D'Elia 1997). Smaller algal cells, as a result of higher cell division rate and increased cell density (Ambariyanto and Hoegh-Guldberg 1996; Koop et al. 2001), could be responsible for decreased chlorophyll concentrations in the coral tissue.

Another explanation for reduced chlorophyll content in coral tissue after the exposure to elevated ammonium concentrations could be a suggested toxic effect of free ammonia on the coral associated algae (Hoegh-Guldberg 1994; Ben-Haim et al. 1999). Free ammonia may

lead to a disturbance in the electron transport in photosystem II (Azov and Goldman 1982) and thus to the inhibition of photosynthesis with subsequent bleaching and lysis of zooxanthellae (Ben-Haim et al. 1999). The varying effects of inorganic nutrient addition on coral pigmentation support the assumption of species specific responses to changes in ambient inorganic nutrient concentrations (Hoegh-Guldberg and Smith 1989).
The finding that the mixed nutrient treatment showed no detectable effect on all measured parameters is congruent with other studies. Snidvongs and Kinzie (1994), investigating nitrogen and phosphorus addition effects on the hermatypic coral *Pocillopora damicornis*, suggested negative feed-back mechanisms, which enhance the effects of single-nutrient enrichments, but do not influence chlorophyll a tissue concentrations when both kinds of nutrients are available. Higher mortality in *Acropora longicyathus* and reduced growth rates for *Stylophora pistillata* were described by Koop et al. (2001) for single added nutrients, but not for the combined treatment with dissolved inorganic nitrogen and phosphorus. A possible explanation may be the generation of an imbalance in the exchange of nutrients between the zooxanthellae and the host coral (Dubinsky and Stambler 1996) by single-nutrient additions. Destabilizing effects on the functioning of the finely attuned symbiotic algae-host association due to a single-nutrient enrichment may be better compensated with the simultaneous addition of a range of nutrients. Also, the abovementioned toxic effect of ammonium ions may be suppressed by excessive anions of the nitrate and phosphate addition.
The present study demonstrated that the exposure to 3-fold higher organic nutrient concentrations in the form of glucose resulted in reduced chlorophyll a tissue concentrations and in a visible colour decrease of all corals exposed to this treatment. Recent studies observing corals exposed to 5-fold higher concentrations of nutrients have already revealed that elevated organic nutrient concentrations in reef waters can be more deleterious for hermatypic corals than addition of inorganic nutrients (Kuntz et al. 2005; Kline et al. 2006). These studies concluded in a higher mortality of coral polyps due to increased organic nutrient concentrations, but did not show influence on coral pigmentation as revealed by the present study.
The underlying mechanisms of organic nutrients effects on hermatypic corals are still poorly understood (River and Edmunds 2001; Vu et al. 2009). Elevated organic nutrient concentrations can stimulate microbial growth and activity (Cole et al. 1982), but it is discussed whether the resulting low O_2 concentrations (Kline et al. 2006; Smith et al. 2006) or the increased release of bacteria derived coral damaging secondary metabolites (Littler and Littler 1997, Jompa and McCook 2003; Nugues et al. 2004) are mainly responsible for the negative impact on hermatypic corals. The present study confirmed that O_2 deficiency was correlated to coral tissue pigmentation change in the organic nutrient treatment tank.

Supplementary effects of algal presence

As the abovementioned studies had their main focus on the effects of nutrients on the individual corals or algae, the principal intention of the present study was to evaluate the surplus effects of elevated nutrient concentrations on hermatypic corals in direct competition with benthic algae.
This long term-monitoring study confirmed that the occurrence of benthic reef algae in direct vicinity to hermatypic corals does affect entire coral colony colour and tissue pigmentation, regardless of nutrient addition. As changes in coral colour reflect change in symbiont abundance and chlorophyll a content, and therefore the bleaching state of the coral, they are a useful indicator for stress in hermatypic corals (McClanaham 2004; Siebeck et al. 2006). Differences, between the coral tissue colour observations and the chlorophyll a analysis, found in this study may, however, be attributed to the fact that the observed colour does not necessarily reflect the chlorophyll a concentrations found in the respective coral tissue sample (Fitt et al. 2000).

The only significant difference of inorganic nutrient addition relevant for the competitive process in coral–algae assemblages comprised the ammonium treatment via significantly promoting unilateral benthic algae growth. In contrast to the inorganic nutrient addition treatments, the added organic nutrient had significant influence on both organisms of the coral–algae assemblages, when compared to the seawater controls. This was reflected by the decrease of colour and chlorophyll a concentrations of coral colonies assembled with turf algae as well as by an intensified coral bleaching and algal growth. The observed increased algae growth rates could either have resulted from positive effects of the additional organic nutrients and ammonium on the algae competitor or diminished competitive ability of the coral competitor. The latter however seems more presumable as organic carbon in the form of exogenous acetate or saturated fatty acids can be used by algae (Neilson and Lewin 1974). Glucose though, as a photosynthetate (Norris et al. 1954; Cole 1982), is hardly a factor influencing algal growth in warm water coral reefs. Contrary to glucose, ammonium is known to influence algae growth rates (Fong et al. 1993), but is hardly a limiting factor for the associated cyanobacteria due to their ability of nitrogen fixation (Kratz and Myers 1955; Williams and Carpenter 1997). Thus the addition of glucose could potentially affect the growth rates of the turf algae associated cyanobacteria and addition of ammonium those of the associated benthic algae.

Decrease of colour and chlorophyll a concentrations in the tissue of corals assembled with algae and subjected to increased organic nutrient concentrations may potentially have resulted from photo- and heterotrophic plasticity due to increased organic nutrient availability. Anthony and Fabricius (2000) showed however, that carbon loss due to decreased photosynthesis cannot be entirely compensated by heterotrophic feeding and results in a decline of tissue mass and lipid content. The intensified bleaching of corals assembled with algae in the organic nutrient treatment further indicates the negative effects on corals, as bleaching is known to be a general response to stress (Glynn 1996).

This study suggests that an addition of glucose may have exceeded the threshold of tolerable organic nutrient concentration for the corals (Kline et al. 2006) already associated with benthic algal, which release dissolved organic matter (Hackney and Sze 1988). Damage inflicted on corals by the presence of benthic algae could thus be based on similar mechanisms as the damage associated with enhanced organic nutrient concentrations. Both can potentially be responsible for a change in the carbon limited microbial community (Segel and Ducklow 1982; Rohwer and Kelley 2004) associated with the coral surface mucopolysaccharide layer (Kline et al. 2006) and, thus for coral bleaching and other coral diseases (Rosenberg et al. 2007). However, lower O_2 concentration in the glucose treatment compared to all other treatments strengthens the hypothesis that elevated organic nutrient concentrations are reducing the competitive ability of corals assembled with benthic algae via stimulated biological O_2 demand, leading to O_2 depletion and subsequent damage of hermatypic corals in the direct vicinity (Smith et al. 2006).

Ecological implications

In the context of the ongoing discussion on the influence of nutrients for coral reef ecosystem functioning (McCook 1999; Szmant 2002; Vu et al. 2009), the present study supports the hypothesis that dissolved organic nutrients affect the outcome of direct competition processes between corals and benthic algae (Smith et al. 2006). It further suggests that the exclusive addition of only one kind of inorganic nutrient, e.g. spillage of industrial phosphate (Walker and Ormond 1982) or agricultural fertilization (Smith et al. 1999), predominantly containing nitrogen (Cerrato and Blackmer 1990), likely has stronger deleterious effects on corals than broad eutrophication of several inorganic nutrients, potentially owing to a disturbed nutrient flux balance in the reef ecosystem. This could provide an additional aspect to evaluate

contradictory findings of various studies on the influence of eutrophication in coral reef ecosystems (McCook et al. 2001; Smith et al. 2001; Szmant 2002).
The elevated organic nutrient concentrations not only had an already demonstrated direct negative influence on the coral specimen (Kuntz et al. 2005; Kline et al. 2006), the effect on coral tissue pigmentation was additionally enhanced when the coral was assembled with benthic algae. Extent of bleaching, entailed by the assemblage to benthic algae and growth of the algal competitor, were also accelerated by elevated organic nutrient concentrations. These findings indicate that organic nutrient input into coral reefs either from the algae themselves or from anthropogenic sources like waste water (Smith et al. 2006) can affect physiology and metabolism of both corals and benthic turf algae. Reinforcing interaction between the two investigated dominant groups of hermatypic corals and benthic reef algae along with involvement of microbes may facilitate phase shifts in coral reefs.

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3

Composition analysis of organic matter released by cosmopolitan coral reef associated green algae

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Abstract

Coral reef associated benthic algae can control important metabolic processes in reef ecosystems via organic matter release. Recent studies provided first information about quantity and microbial degradability of these algae-derived exudates. However, information about the chemical composition of macroalgae exudates is very limited. This study therefore presents total and dissolved organic carbon (TOC, DOC) and total and dissolved bound nitrogen (TON, DON) quantification of exudates released by two cosmopolitan coral reef-associated green algae species, *Halimeda opuntia* and *Caulerpa serrulata*. Additionally, first glycosyl and lipid composition and content analyses, along with protein and chlorophyll a (chl a) quantifications of algae exudates were conducted. Findings revealed that organic matter released from both algae predominately consisted of carbohydrates (56 ± 7 %) and proteins (35 ± 9 %) which dissolve in surrounding waters. Traces of fatty acids (C16:0; C18:0) and chl a were also found in algae incubation waters, but in a quantitatively negligible amount. Carbohydrate analysis further showed that glucose was the dominant glycosyl released by algae, accounting for 77 ± 8 % of the carbohydrate fraction and 42 ± 8 % of TOC. Galactose (9 ± 4 % of carbohydrate fraction), mannose (6 ± 3 %), xylose (4 ± 1 %), rhamnose (3 ± 1 %) and fucose (2 ± 1 %) were also detected in all incubation water samples. High glucose and protein contents of algae exudates found in the present study confirm assumptions on its fast

microbial degradability with ensuing potential effects on O_2 availability and coral-algae interactions in coral reef ecosystems.

Introduction

Coral reef ecosystems worldwide are facing degradation in reef biota from coral to macroalgae dominance (McCook et al. 1997, Bellwood et al 2004, Work et al. 2008). These "phase shifts" (Done 1992) may lead to alterations in quantity and quality of organic matter released by the different primary producing organisms into the ecosystem (Wild et al. 2009). Previous studies showed that organic matter derived from hermatypic corals (Wild et al. 2004) and benthic algae (Hedges 2002) are key components in reef biogeochemical cycles. Comparison of organic matter derived from various reef algae, hermatypic corals and seagrasses has however revealed significant differences in quantity, quality and microbial degradability (Naumann et al. submitted, Wild et al. submitted, Haas et al. submitted). The described differences may lead to alterations in nutrient cycles and O_2 availability in the reef ecosystem (Wild et al. 2009 ICRS, Niggl et al. submitted).
Bio-labile organic matter derived from benthic algae has a highly stimulating effect on the activity of bacterioplankton in the ambient water column (Jonas 1997, Wild et al. 2009). Algae-derived organic matter may therefore negatively affect coral health, thus facilitating a negative feedback loop in reef degradation (Smith et al. 2006). This may potentially be explained by a high content of rapidly degradable organic carbon (Kline et al 2006). In this context, the glycosyl composition is a key factor for microbial degradation (Arnosti 2000).

While organic matter derived from various hermatypic corals (Richards et al. 1983, Coffroth 1990, Wild et al 2005) and phytoplankton (Hellebust 1965, Hellebust 1974, Fogg, 1966, Abdullah & Fredriksen 2004, Nguyen et al. 2005) has already been subject to analyses, there is only little data available on the chemical composition of macroalgae released organic matter (Wada 2007) and none that specifically addresses released matter of coral reef-associated algae.
This study therefore aims to provide first data on the composition of organic matter derived from coral reef associated green algae. For this purpose, total organic carbon (TOC) and nitrogen (TON) as well as dissolved organic carbon (DOC) and nitrogen (DON) released by two cosmopolitan coral reef-associated green algae species, *Halimeda opuntia* and *Caulerpa serrulata*, were quantified. Additionally, glycosyl and lipid composition and content analyses, along with protein and chlorophyll a (chl a) quantifications of algae exudates were conducted.

Material and methods

Experimental set up

This study was conducted in the aquarium facilities of Coral Reef Ecology Group (CORE), at Geo-Bio Center^{LMU}. Aquarium water was adjusted to salinity (40 ± 1 ‰) and temperature (27 ± 1 °C) conditions, which imitated natural conditions in the Northern Red Sea during summer. Comparable light conditions were provided by artificial illumination (AQUAMEDIC® Aqua-star-light) 50 cm above water level (resulting light intensity: 70720 lx) for a daily illumination period of 12 h. Assessed water parameters were: pH: 8.4; inorganic nutrient concentrations: PO_4 < 0.1 mg l^{-1}; NO_2: not detectable; NO_3 < 1 mg l^{-1}; NH_3- / NH_4 < 0.01 mg l^{-1}; measured weekly using Tropic Marin® Expert Test kits.
Two green algae of the order *Bryopsidales* (*Chlorophyta*, *Bryopsidophyceae*), namely *Halimeda opuntia* and *Caulerpa serrulata* were used for this experiment. Three different algae specimens of each genus (8 – 15 cm height) were carefully checked to exclude organisms infested by epibionts that could have affected experimental results by release or uptake of organic matter. For each of the 6 specimens, an independent beaker incubation experiment was conducted after the method described in Herndl & Velimirov (1986) with some modifications. Autoclaved 5 l glass beakers were filled with 2000 ml sterile sea water each. Sterile sea water was generated by dissolving a pure marine salt mixture (Tropic Marin® Sea Salt) in nanopure water. The beaker was provided with the respective algae and coated with transparent plastic foil in order to avoid external contamination, leaving small channels for air circulation. The beaker was left for 24 h in a water bath under identical conditions as described above. At the end of each incubation experiment, algae specimens were removed from the beakers using sterilised forceps to prevent contamination of the incubation water, and the remaining incubation water was sampled and analyzed as described below.
Subsequently, algae surface area was determined by spreading each of the incubated algae specimen 2-dimensionally on a scaled paper. Photographs were then taken directly from above with a *Sony Cybershot* digital camera (resolution: 5.1 megapixels), and surface area was calculated from the image plain with a digital image processing software (*ImageJ*, V. 1.37m, National Institutes of Health, USA).

Organic carbon and nitrogen quantification

Subsamples of incubation water volumes were taken from each of the 6 treatments with a sterile syringe for subsequent organic carbon and bound nitrogen quantification. Samples for total organic carbon (TOC) and total bound nitrogen (TN) analysis (10 ml) were immediately transferred into precombusted (450 °C; 4 h) glass vials. Samples for dissolved organic carbon (DOC) and dissolved bound nitrogen (DN) analysis (10 ml) were filtered through 0.2 µm

sterile polyethersulfone membrane filters before transferring them into the glass vials. Samples were then kept frozen at –20 °C until analysis by high-temperature catalytic oxidation with a Dimatec DIMA-TOC 100 TOC analyser. Non-purgable organic carbon was measured after sample acidification with hydrochloric acid to pH < 3, while sparging with O_2. For organic carbon quantification, potassium hydrogen phthalate, and for bound nitrogen, potassium nitrate and ammonium sulfate were used as elemental standards (standard deviation < 3%). POC and PN concentrations were calculated by subtracting the dissolved fraction from the respective total content. Organic carbon release rates were then normalized to algae surface and duration of release.

Glycosyl content and composition

Algae incubation water subsamples (6 x 1000 ml) were desalted prior to glycosyl composition analysis by dialysis. Dialysis was performed using Spectra/Por dialysis membranes with a molecular weight cut off of 1000 Daltons. Samples were placed in 3 l deionized water and stirred for 8 h while the water was continuously refreshed. Samples were then transferred to 3 l of nanopure water and kept at 4° for 48 h. Dialysis water was refreshed midway through the 48 h interval. After dialysis, each sample was lyophilized and glycosyl composition was performed on the resultant material.

Glycosyl composition analysis was performed by combined gas chromatography/mass spectrometry (GC/MS) of the per-O-trimethysilyl (TMS) derivates of the monosaccharide methyl glycosides produced from the sample by acidic methanolysis.

A 200µl aliquot was used from each sample and added to separate tubes with 20µg of Inositol as internal standard. Methyl glycosides were then prepared from the dry sample following the mild acid treatment by methanolysis in 1 M HCL in methanol at 80° C for 16 h, followed by re-N-acetylation with pyridine and acetic anhydride in methanol for the detection of amino sugars. Samples were then per-O-trimethylsilylated by treatment with Tri-Sil (Pierce) at 80° C for 20 minutes. This analysis was done according to Merkle & Poppe (1994). GC/MS analysis of the TMS methyl glycosides were performed on an AT 6890N GC interfaced to a 5975B MSD, using a Supelco EC-1 fused silica capillary column (30m x 0.25mm ID).

Lipid content and composition

For this analysis, a 100 ml subsample of each incubation water volume was used. Extraction of lipophilic components was achieved with a polystyrol-divinylbenzol-copolymer-phase. Elution was conducted with methanol, and samples were then dried by sparging. The residue was dissolved in 990 µl methanol and 10 µl trimethylsulfoniumhydroxide, and lipid identification was carried out by GC/MS (Varian 3800 GC with Split-/Splittless-Injektor 1177 interfaced to a 1200 MS with CTC CompiPAL for Liquid-Injektion) with helium as carrier gas.

Protein content

Protein content was determined by a protein assay described by Lowry et al. (1951) with bovine serum albumin as protein standard. One ml subsamples of undiluted incubation water were used for the procedure, and sterile sea water was used as blank. Protein assay was conducted in replicates of 3 for each of the 6 treatments and the standard error was < 5 % for each single treatment.

Chlorophyll a content

Chl a content was determined from a 30 ml incubation water subsample for each of the 6 incubations. Chl a concentrations were analyzed with a GAT TD-700 Fluorometer after the method described by Holm-Hansen et al. (1965).

Results

Organic carbon and nitrogen quantification

Organic carbon quantification of algae incubation waters showed that *H. opuntia* released 2.26 ± 0.18 mg TOC m^{-2} surface area h^{-1} (all values are given in mean ± SE) during the incubation period. DOC was released in quantities of 2.09 ± 0.14 mg m^{-2} h^{-1} (93 % of TOC), whereas POC release accounted for only 0.17 ± 0.17 mg m^{-2} h^{-1} (7 % of TOC). Similar results were obtained for *C. serrulata* incubations with 3.43 ± 1.22 mg TOC m^{-2} h^{-1} released by this alga species, composed of 3.06 ± 1.05 mg DOC m^{-2} h^{-1} (89 % of TOC) and 0.37 ± 0.17 mg POC m^{-2} h^{-1} (11 % of TOC). Incubation waters of both algae therefore showed a high DOC:POC ratio of 13 ± 5.

Total bound nitrogen was below the detection limit of 0.5 mg l^{-1} in all incubation water samples except one *C. serrulata* incubation sample. This single incubation indicated that nitrogen containing compounds were also released primarily in dissolved form (DN:PN ratio = 44) and that algae exudates exhibit high C:N ratios of 16. Organic carbon and nitrogen contents of all incubation water samples are given in detail in Table 1.

Table 1 *Analysed contents of algae incubation waters. (DOC = dissolved organic carbon, POC = particulate organic carbon, TOC = total organic carbon, DN = dissolved bound nitrogen, PN = particulate nitrogen, TN = total bound nitrogen, Chl a = Chlorophyll a, n.a. = not assessed, n.d. = not detectable)*

Species	***Halimeda opuntia***			***Caulerpa serrulata***			Mean ± SE
Replicate	1	2	3	1	2	3	
DOC (mg l^{-1})	1.71	1.60	3.30	2.46	3.36	8.94	3.56 ± 1.12
POC (mg l^{-1})	0.45	n.a.	0.10	0.24	0.34	1.25	0.39 ± 0.19
TOC (mg l^{-1})	2.16	1.53	3.40	2.70	3.70	10.19	3.95 ± 1.29
$C_{Carbohydrates}$ (mg l^{-1})	1.24	0.38	2.14	1.48	2.22	8.01	2.58 ± 1.12
$C_{Proteins}$ (mg l^{-1})	0.55	1.16	0.91	1.14	1.16	1.06	0.99 ± 0.10
C_{Lipids} (mg l^{-1})	>0.01	n.d.	>0.01	n.d.	>0.01	>0.01	n.a.
DN (mg l^{-1})	>0.50	>0.50	>0.50	>0.50	>0.50	0.56	n.a.
PN (mg l^{-1})	>0.50	>0.50	>0.50	>0.50	>0.50	0.02	n.a.
TN (mg l^{-1})	>0.50	>0.50	>0.50	>0.50	>0.50	0.58	n.a.
Chl a (µg l^{-1})	1.29	0.12	1.49	1.23	1.27	1.87	1.21 ± 0.23

Composition analyses

Composition analysis of algae released organic matter revealed that the main component of algae exudates were carbohydrates (56 ± 7 % of TOC) followed by proteins (35 ± 9 %). Also traces of chl a (> 0.1 %) and fatty acids (< 1 %) were found (Table 1).

Glycosyl composition analysis showed that the dominant carbohydrate component in all algae exudates was glucose (77 ± 8 % of all carbohydrates) followed by galactose (9 ± 4 %), mannose (6 ± 3 %), xylose (4 ± 1 %), rhamnose (3 ± 1 %) and fucose (2 ± 1 %). Traces (> 1 %) of arabinose and N-acetyl glucosamine were detected in *H. opuntia*, but not for *C. serrulata* exudates (Fig. 1). Palmitic acid (C16:0) and stearic acid (C18:0) were found in 2 of 3 incubation water samples of each algae genus, but not in a quantitatively determinable concentration (< 10 µg l^{-1}).

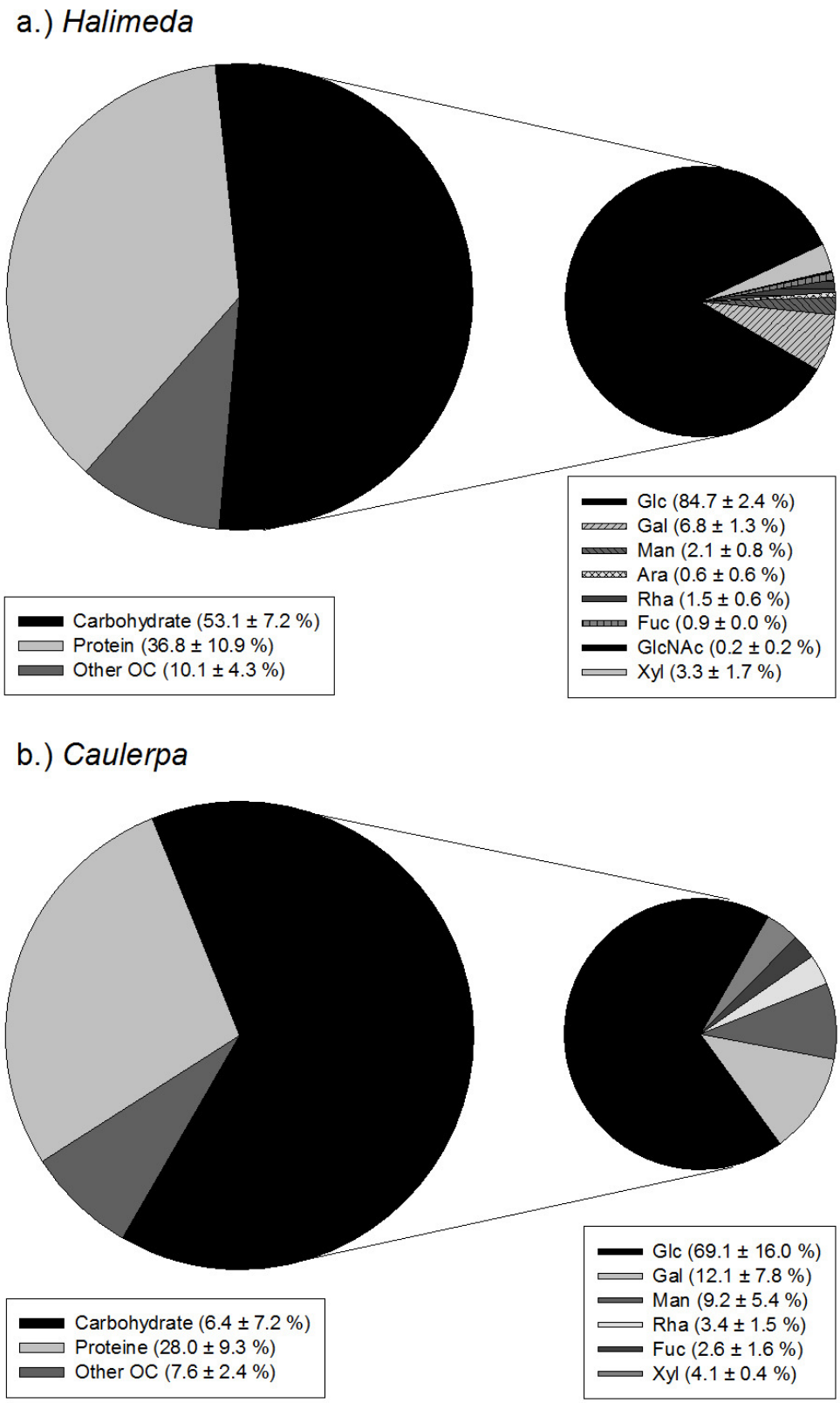

Fig. 1 *Structural composition of a.) H. opuntia and b.) C. serrulata exudates (% of total organic carbon) with detailed depiction of glycosyl composition (mol % of all detected carbohydrates). (OC = organic carbon, Glc = glucose, Gal = galactose, Man = mannose, Ara = arabinose, Rha = rhamnose, Fuc = fucose, Xyl = xylose, GlcNAc = N-acetyl glucosamine).*

Discussion

Organic matter quantification conducted in the present study revealed that both investigated coral reef-associated green algae, *H. opuntia* and *C. serrulata*, released organic matter primarily in dissolved form into their surrounding. This is in line with previous studies (Khailov & Burlakova 1969; Sieburth 1969; Abdullah & Fredriksen 2004; Haas et al. submitted) which congruently suggested that algae-derived organic matter may primarily dissolve into the ambient waters. High C:N ratios found in the present study are confirmed by previous studies (Biddanda & Benner 1997, Haas et al. submitted) and can be explained by a high fraction of carbohydrates in algae derived organic matter.

Total carbohydrate fractions of the analysed algae incubation water samples were 56 ± 7 % of TOC, which is similar to carbohydrate contents described for exudates of various phytoplankton cultures (52 ± 2 % of TOC) by Biersmith & Benner (1998). Glucose was found to be the main component of the bulk carbohydrate fraction, accounting for 42 ± 8 % of the total organic carbon released by algae. Next to glucose, other C6 sugars (mannose, galactose) followed by smaller amounts of 6-deoxysugars (rhamnose, fucose) represented principal carbohydrate components of algae derived organic matter. The only C5 sugar found in noticeable amounts in incubation waters was xylose. In a number of marine algae of the order *Bryopsidales*, including *Halimeda* and *Caulerpa*, cellulose, consisting of β(1→4) linked D-glucose units, was completely absent (Lewin 1974). Instead, cell walls contained β(1→4) linked xylan as structural polysaccharide (Frei & Preston 1964). Xylose glycosides did therefore likely derive from structural cell components, which have been shed during the incubation period.

Glycosyl composition analysis, together with DOC and TOC quantification, revealed that the main part of algae derived organic matter was released as dissolved C6 sugars and primarily as glucose. Glucose is used as universal energy source in most organisms (Vollhardt & Schore 2000) and, followed by mannose, the most substantial component for heterotrophic bacterial production in marine environments (Rich et al. 1996). Glucose and, to a lesser extent, mannose, have together been shown to fuel more than 40 % of the total bacterial respiration in aquatic ecosystems (Rich et al. 1996). Other dissolved monosaccharides, especially the more refractory deoxysugars (Amon et al. 2001; Ogawa et al. 2001), play a rather subordinate role in heterotrophic bacterial production (Rich et al. 1996).

Proteins, the second largest fraction in algal exudates next to carbohydrates, can significantly contribute to the dissolved combined amino acid (DCAA) pool in marine ecosystems (Billen 1991). DCAAs also serve as rapidly degradable substrate for bacterial growth (Hagstrom et al. 1984, Keil & Kirchman 1993) and contribute 12 – 50 % to heterotrophic bacterial N requirements (Tupas & Koike 1990) in the generally nutrient poor coral reef ecosystem (Lapointe 1997). Other components of algae exudates (lipids, chl a, N-Acetyl Glucosamine) were only found in negligible amounts (> 1 %) during the present study. This confirms the rather homogeneous structure of algae exudates, with two substance groups (carbohydrates and proteins) accounting for 92 ± 3 % of the total released organic carbon.

Ecological implications

Comparison with glycosyl composition analysis of mucus released by various hermatypic corals (Wild et al. submitted) showed that corals release a more heterogeneous carbohydrate mix (glucose 19 ± 14 % of all carbohydrates, galactose 1 ± 1 %, mannose 20 ± 6 %, fucose 39 ± 14 %, arabinose 11 ± 11 %, N-acetyl glucosamine 10 ± 8 %). Contrary to the two tested algae genera, coral mucus carbohydrate content and composition proofed to be highly genus-specific (Wild et al. 2005/submitted) and contained significantly lower proportions of glucose (one way analysis of variance, df = 12, p = 0.006). This coral mucus generally provided a

good substratum for microbes in the pelagic and benthic environment of coral reef habitats with ensuing positive effects on microbial activity (Wild et al. 2004, 2005).
However, coral reef associated algae exudates may provide an even more rapidly degradable substratum for the ambient heterotrophic microbial community (Wild et al. 2009/submitted, Haas et al submitted). This is supported by the present study as high concentrations of glucose and DCAAs released by algae represent a matrix readily available for heterotrophic microbial utilisation.
This study therefore strengthens the hypothesis of alterations in cycles of matter and O_2 availability following changes in benthic community structure from coral to algae dominance (Wild et al. submitted, Niggl et al. submitted). It also provides essential chemical composition information to link findings documenting deleterious effects of dissolved glucose on corals (Kuntz et al 2005, Kline et al. 2006) and corals in interaction with algae (Haas et al. 2009), with those recent findings hypothetically assigning algae exudates a key role in microbe-induced coral mortality (Smith et al. 2006).

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Organic matter release by coral reef associated benthic algae in the Northern Red Sea

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Abstract

Recent research indicates that coral reef associated benthic algae may control important metabolic processes in reef ecosystems via organic matter release. Yet little information is available about quantity and chemical composition of these algae-derived exudates. Therefore first comprehensive studies on algal organic matter release were conducted at a fringing reef ecosystem in the Northern Red Sea. Dissolved organic carbon (DOC), particulate organic carbon (POC) and nitrogen (PN) release by dominant reef associated benthic algae (*Caulerpa serrulata*, *Peyssonnelia capensis*, turf algae assemblages) were quantified during 4 seasonally resolved expeditions. Additionally, 4 seasonal blooming (*Ulva lactuca*, *Enteromorpha flexuosa*, *Liagora turneri*, *Hydroclathrus clathratus*) and 2 patchy growing algae species (*Lobophora variegata*, *Saragassum dentifolium*) were included in these investigations. To complement the data set organic matter release by *Caulerpa* was studied under different light conditions, simulating water depths of 1, 5, 10 and 20m. Environmental parameters (temperature, light availability, inorganic nutrient concentrations) were monitored simultaneously to assess potential effects on algal organic matter release. All 9 investigated genera of benthic algae exuded DOC and POC in amounts of 12.2 ± 2.1 and 4.2 ± 0.3 mg organic C m^{-2} algae surface area h^{-1}, respectively. Resident algae, primarily turf algae assemblages, displayed highest and seasonal blooming algae lowest organic matter release rates. Results therefore indicate that organic matter release rates are rather influenced by functional properties (growth form, life strategy) of algae than by taxonomic affiliation. Quantities of organic matter release showed seasonal and depth-mediated variations and were positively correlated with temperature and light availability within photosynthetically active radiation intensities of 0 to 300 µmol quanta m^{-2} s^{-1}, suggesting photoinhibition as limiting factor for productivity and subsequent organic matter release. Stable isotope signatures of algae-derived organic carbon were within a common range and likewise subjected to seasonal variations ($\delta^{13}C$ summer: -11.2 ‰ ± 0.2 ‰; δ^{13} winter: -16.7‰ ± 0.4‰). These data provide first comprehensive information about a) the potential contribution of different benthic reef algae to cycles of matter and b) environmental key factors influencing organic matter release by benthic algae in the investigated fringing reef ecosystem.

Introduction

In the last decades, coral reefs have undergone a worldwide decline in coral cover. This decline prevalently results in an ecosystematic "phase shift" (Done 1992), describing a degradation in reef biota from coral to macroalgae dominance (Work et al. 2008). Although potential causes for such phase shifts have been identified (Pandolfi et al. 2003, Hoegh-Guldberg et al. 2007), there is little information available on how population, community and ecosystem structure and function differ in degraded from un-degraded reefs (Done 1992).
Wild et al. (2004) showed that hermatypic corals, being most adversely affected by this phase shift, act as fundamental engineers of their entire ecosystem. They modulate their environment by constructing three dimensional frameworks (Scheffers et al. 2003), by generating biocatalytically active reef sands (Wild et al. 2005a) and particularly, by the release of organic matter, that functions as particle trap in the reef ecosystem (Wild et al. 2004). It is also known that macroalgae influence their environment, not only in their role as primary producers, but also through the release of a considerable part of their photosynthetic products (23 to 62 %) as organic matter (Khailov and Burlakova 1969, Sieburth 1969). Recent studies indicate that organic matter derived by benthic algae is a key organic group in marine biogeochemical cycles (Hedges 2002) and plays an essential role as a carbon source to heterotrophic bacteria in microbial loops (Azam et al. 1993). But the stimulating effect of bio-labile DOC derived from benthic algae on the activity of either the coral surface mucopolysaccharide layer associated microbial community (Kline et al. 2006) or bacterioplankton in the ambient water column (Jonas 1997), is assumed to entail negative effects on coral health. Kline et al. (2006) experimentally showed that increased concentrations of dissolved organic carbon (DOC) were more deleterious for reef corals than increased concentrations of inorganic nutrients such as nitrate, phosphate or ammonium. Studies conducted by Smith et al. (2006) suggested algae-derived dissolved organic matter to be indirectly responsible for death of corals by stimulating microbial oxygen consumption that subsequently results in oxygen depletion.
While algae-derived organic matter may influence competition between benthic algae and hermatypic corals in coral reef ecosystems, only a small number of quantitative studies have been conducted regarding organic matter derived from macroalgae with various outcomes (reviewed in Wada et al. 2007). Quantitative studies using ^{14}C as tracer (Brylinsky 1977, Pregnall 1983) revealed that only small proportions (0 – 6 %) of the photosynthetic products are released as organic matter. DOM release by macrophytes in temperate environments however may account for 2 – 62 % of photosynthetic production (Khailov and Burlakova 1969, Abdullah and Fredriksen 2004). These high variations in the proportion of photosynthetic products released as organic matter can potentially be ascribed to methodical differences and pronounced seasonal and environmental variations (Wada et al. 2007), but there is no experimental evidence for such hypothesized interrelationship.
The present study therefore investigates quantity of organic matter, released as DOC, particulate organic carbon (POC) and particulate organic nitrogen (PN) by the dominating representatives of benthic macroalgae and turf algae assemblages in a high latitude reef of the Northern Red Sea. Experiments were conducted in a seasonal resolution in order to study natural variations in environmental factors which influence productivity of macroalgae (Hatcher et al. 1977, Wada et al. 2007).

Material and methods

Study site

This study was conducted at the Marine Science Station (MSS) in Aqaba, Jordan (29°27´ N, 34°58´ E). The MSS is located at a marine reserve in a fringing reef in the northern Gulf of Aqaba, a segment of the Red Sea, east of the Sinai-peninsula and west of the Arabian mainland. The high latitude reefs in the Northern Red Sea are subject to considerable seasonal variations in temperature, light and inorganic nutrient availability (Rasheed et al. 2002). To account for this variability, this study was conducted during four seasonal expeditions (Fall: 7 November to 12 December 2006; Summer: 9 August to 13 September 2007; Winter: 11 February to 19 March 2008; Spring: 6 May to 28 May 2008). During each of these expeditions, identical experiments were carried out in order to allow comparability between datasets.

Environmental parameters

To investigate the influence of environmental parameters on organic matter release rates, temperature, light availability and inorganic nutrient concentrations were measured during the entire study period from November 2006 until May 2008. In-situ temperature was measured by Onset HOBO® Water Temp Pro v2 temperature loggers at a water depth of 10 m every 30 min. Onset HOBO® Pendant UA-002-64 light and temperature loggers were used to measure in-situ light availability at 1, 5, 10 and 20 m water depth. Inorganic nutrient concentrations (NH_4^+, NO_3^-, NO_2^-, PO_4^{3-}) in samples collected from 1 - 2 m water depth above the MSS reef were measured monthly as described by Grasshoff et al. (1999).

Investigated reef algae

Results of seasonally conducted benthic community assessments (Haas et al. in press) were used in order to identify the dominant benthic macroalgae and turf algae communities which covered between 19 and 22 % of the available substratum at the study site. These dominants included the green algae *Caulerpa serrulata*, the brown algae *Peyssonnelia capensis* and various consortia of turf algae predominately composed of green algae of the genus *Cladophora*, red algae of the genus *Gelidium* and associated cyanobacteria assemblages. Organic matter release by *Caulerpa*, *Peyssonnelia* and turf algal assemblages was determined in seasonal resolved incubation experiments. *Caulerpa serrulata* was additionally incubated at different light levels simulating 1, 5, 10 and 20 m water depth and in complete darkness (night conditions). Benthic macroalgae species occurring only in patches (*Lobophora variegata*, *Saragassum dentifolium*) or in seasonal blooms (*Ulva lactuca*, *Enteromorpha flexuosa*, *Liagora turneri*, *Hydroclathrus clathratus*) were also used for incubation experiments to establish a comprehensive overview of algae-derived organic matter release. The algae species, used in this study, accounted for almost 100% of the benthic algae coverage between 1 and 20 m water depth in the study area. A summary of all conducted incubation experiments, including information on species identity, simulated water depth, and season is given in Table 1.

Experimental setup

Specimens of each algae species (6 – 11 cm height) were collected using SCUBA in replicates of 5 from different colonies. Turf algae samples were generated by using side cutting pliers to detach dead *Acropora* fragments to which the algae consortium was attached. All algae were sampled at least 24 h before the respective incubation experiment in order to avoid leakage of intracellular organic matter due to potential injuries from removing the algal holdfast from its original substrate. Algae samples were transferred without air-exposure and kept at in-situ temperature and light conditions in maintenance tanks (volume 1000 l) with fresh seawater flow-through (exchange rate: 600 - 800 l h^{-1}) until the start of the subsequent incubation experiments. For each species of the investigated benthic algae, an independent beaker incubation experiment was conducted after the method described in Herndl and Velimirov (1986) with some modifications. For each of these experiments, ten 1 l glass beakers were used and filled with 1000 ml freshly collected local sea water to mimic in-situ conditions. Five beakers were provided with aliquots of the respective algae, whereas the remaining served as control beakers. All beakers were then coated with transparent plastic foil in order to avoid external contamination, leaving small channels for air circulation. All 10 beakers were left for 6 h in a water bath at in-situ temperature as recorded every 60 s by ONSET HOBO® Pro v2 Water Temperature Data Loggers in natural daylight. To further simulate in-situ conditions, light intensity (lx) was recorded every 5 s by ONSET HOBO® Pendant UA-002-64 Temperature/Light data loggers at the natural habitat of the collected algae and during incubation experiments. Layers of plastic gauze were used in order to generate similar light intensities as at the site of collection. Regarding the low water current velocity generally found in the studied reef (Manasrah et al. 2006), beaker contents were not stirred during incubations to avoid water current influences on organic matter release rates. This also allows for comparability with previous beaker incubation studies on other reef organisms (Herndl and Velimirov 1986; Wild et al. 2005b, Naumann et al. in press). At the end of each incubation experiment, algae specimens were removed from the beakers using sterilised forceps to prevent contamination of the incubation water and subsequently prepared for surface area determination. The remaining incubation water was sampled and analyzed as described below.

Quantification of algae-derived organic matter

Ten ml of the incubation water from each beaker were immediately collected for subsequent DOC measurements using sterile syringes. A sterile syringe filter (0.2 µm particle retention by a polyethersulfone membrane) was then mounted, the initial 4 ml of the filtered water discarded and the remaining 6 ml were sealed in 10 ml pre-combusted (450 °C; 4 h) amber ampoules. Samples were immediately frozen at -20 °C until analysis by high-temperature catalytic oxidation (HTCO) using a Rosemount Dohrmann DC-190 TOC analyzer. Non-purgable organic carbon (actual DOC) was measured by sample acidification with orthophosphoric acid to pH < 2 and sparging with oxygen. Specific concentrations of potassium hydrogen phthalate were measured as elemental standards (standard deviation < 3%).

Samples for particulate organic carbon (POC) and nitrogen (PN) were obtained by filtering 900 – 960 ml of the remaining incubation water onto precombusted GF/F filters (Whatman; diameter: 25 mm, nominal particle retention: 0.7 µm). The filters were stored in *Eppendorf* cups and dried for at least 48 h at 40 °C and kept dry until further analysis as described below. POC and PN contents on the filters were measured using a THERMO™ 1112 Flash EA elemental analyser. A THERMO/Finnigan MAT Delta V isotope ratio mass spectrometer, coupled to the elemental analyser, simultaneously measured stable C and N isotope ratios of the POM samples. Peptone, Atropine and cyclohexanone-2,4-dinitrophenylhydrazone were used as standards, and standard deviations of replicate measurements were < 3%.

Surface area determination

The reference parameter for the quantitative organic matter release data was the surface area, for it is shown to be a significant ecological interface because of its functional importance in the system (Dahl 1973). This parameter was measured for each of the incubated benthic macroalgae by spreading them 2-dimensionally on a scaled paper and taking photographs directly from above with a *Sony Cybershot* digital camera (resolution: 5.1 megapixels). Surface areas were then determined with the digital image processing software *Image J* (*ImageJ*, V. 1.37m, National Institutes of Health, USA) by calculating the surface from the image plane. For turf algae surface area determination, the coral skeleton serving as substratum was referred to and surface calculated using the *Advanced Geometry* protocol and the respective *Approximation Factors* for branching coral growth forms established by Naumann et al. (2009).

Data and statistical analysis

To calculate organic matter release rates for algae, mean values of the measured parameters found in the control beakers were subtracted from those found in the single algae treatments. To generate release rates, control corrected organic matter contents were then normalized to algae surface area and incubation time.

For statistical analysis of organic matter release by algae compared to their controls, a paired t-test was conducted as the data were normally distributed and variances were the same in both groups. For independent samples with the same variance that were normally distributed, analysis of variance (ANOVA) was used as statistical test. Homogeneity of variances was tested with a Levene test for every analysis (Dytham 1999).

Results

Environmental parameters

Long term monitoring of seawater temperature revealed temperature changes from a minimum of 21 °C during February 2008 to a maximum of 29 °C during July 2007. Typically for the Northern Red Sea, the low water temperatures of winter months were accompanied by high concentrations of inorganic nutrients in surface waters (NH_4^+: 0.31 µmol L^{-1}; NO_3^-: 0.83 µmol L^{-1} ; NO_2^-: 0.18 µmol L^{-1}; PO_4^{3-}: 0.07 µmol L^{-1}) which showed minimum values during summer (NH_4^+: 0.21 µmol L^{-1}; NO_3^-: 0.14 µmol L^{-1} ; NO_2^-: 0.02 µmol L^{-1}; PO_4^{3-}: 0.03 µmol L^{-1}).

Data obtained from the light logger revealed a maximum average daytime (10:00–16:00 h) photosynthetically active radiation (PAR) at 5 m water depth of 400 µmol quanta m^{-2} s^{-1} during August 2007 (summer) and a minimum PAR of 216 µmol quanta m^{-2} s^{-1} in February 2008 (winter). Dependent upon water depth, PAR availability in the studied reef varied from 946 (1 m depth) to 144 (20 m depth) µmol quanta m^{-2} s^{-1} during August 2007 and 527 (1 m depth) to 78 (20 m depth) µmol quanta m^{-2} s^{-1} during February 2008.

DOC release by reef algae

DOC concentrations were always higher (paired t-test, $p < 0.001$) in the incubation water from all beakers containing algae ($n = 117$) when compared to the control beakers. The amount of DOC released by algae varied significantly between different species (one way ANOVA, $p = 0.019$) under the same environmental parameters (Table 1). Turf algae showed the highest rates of DOC release (winter 2008: 10.5 ± 6.8 mg m^{-2} h^{-1}; spring 2008: 39.8 ± 2.2 mg m^{-2} h^{-1}), which were 6-fold higher than those of the seasonally blooming algae *Enteromorpha* during winter (1.7 ± 0.3 mg m^{-2} h^{-1}) and 8-fold higher than for *Hydroclathrus* during spring (4.8 ±

1.4 mg m^{-2} h^{-1}). Permanently resident algae (*Caulerpa*, *Peyssonnelia*, turf algae, *Lobophora*, *Saragassum*, *Liagora*) generally displayed significantly (one way ANOVA, p = 0.001) higher release rates than seasonally blooming species (*Ulva*, *Enteromorpha*, *Hydroclathrus*). No significant difference in DOC release rates the 3 broader taxonomic groups (green algae: *Caulerpa*, *Ulva*, *Enteromorpha*; red algae: *Peyssonnelia*, *Liagora*; brown algae: *Lobophora*, *Saragassum*, *Hydroclathrus*) could be detected. Among the three most abundant algae in the study area, a significant seasonal variability in DOC release (one way ANOVA, $p < 0.001$), was found for *Peyssonnelia* (fall: 35.5 ± 7.6; winter: 2.9 ± 1.4) and turf algae (fall: 66.4 ± 15.6; winter: 6.3 ± 1.9), but not for *Caulerpa* (Fall: 13.3 ± 12.3; Summer 6.7 ± 1.5). However, the green algae *Caulerpa* showed significant differences in DOC release rates when tested in a vertical resolution (one way ANOVA, p = 0.023) with highest release rates at a simulated water depth of 5 m (Table 1).

POC and PN release by reef algae

POC release was less variable between species and seasons and significantly lower (paired t-test, $p < 0.001$) than DOC release with DOC/POC ratios of 5.4 ± 0.9 (mean ± SE). All investigated algae released significant amounts of POC compared to the controls during every season (paired t-test, $p < 0.001$). Significant species specific differences in the amount of released POC were found between the tested benthic algae (one way ANOVA, $p < 0.001$). As with DOC release rates, permanently resident algae released significantly more POC than seasonally blooming algae (one way ANOVA, p = 0.017), but no significant difference was detectable among the broader taxonomic groups. Again, as was true for DOC release, significant seasonal variations were found for POC release of algae tested in a seasonal resolution (one way ANOVA; *Caulerpa*: p = 0.009; *Peyssonnelia*: p = 0.002; turf algae: p = 0.002), with all algae displaying the highest release rates in fall.

PN release rates were variable between different species (one way ANOVA, $p < 0.001$) and seasons (one way ANOVA, $p < 0.001$). PN release rates of seasonally tested algae at 5 m water depth were highest in fall (*Caulerpa*: 0.39 ± 0.06; *Peyssonnelia*: 0.42 ± 0.01; turf algae: 0.84 ± 0.09) and showed a minimum in summer (*Caulerpa*: 0.12 ± 0.02; *Peyssonnelia*: 0.17 ± 0.02; turf algae: 0.33 ± 0.11). Subjected to the same environmental parameters during winter 2008, turf algae exhibited the highest PN release rates of 0.4 ± 0.2 mg m^{-2} h^{-1}, whereas the seasonally blooming algae *Ulva* and *Enteromorpha* displayed no (0.00 ± 0.02) or very low release (0.05 ± 0.02 mg m^{-2} h^{-1}), respectively. Again, the resident algae showed significantly higher release rates of PN (one way ANOVA, $p < 0.005$) than the seasonally blooming species with no significant differences among the broader taxonomic groups.

Significantly elevated POC/PN ratios were found for all algae treatments compared to the seawater controls (paired t-test, $p < 0.001$) during all seasons except for winter (Table 1). Significant differences in POC/PN ratios of algae derived POM were detectable between the tested algae species (one way ANOVA, $p > 0.001$). Among those algae for which seasonal data was available, only *Caulerpa* exhibited significant seasonal differences in POC/PN ratios (one way ANOVA, $p < 0.009$) with the highest proportion of carbon in spring (13.9 ± 1.2) and lowest in winter (5.9 ± 1.3).

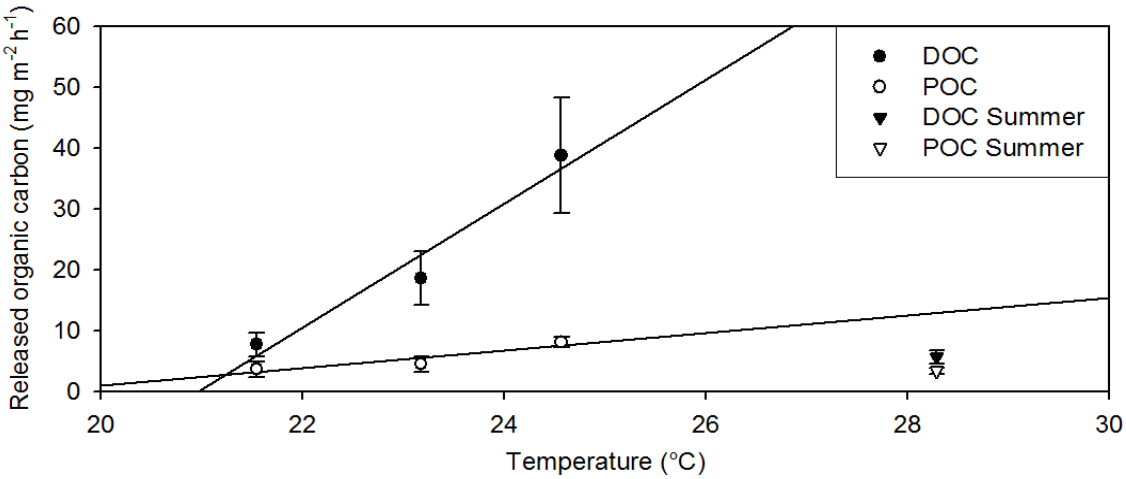
Table 1 *Organic matter release rates and ratios (mean ± SE) for 9 benthic algae species from the northern Red Sea sampled from 5 m water depth (exceptions are noted). The 3 dominant algae species were tested in a seasonal resolution. Values are given as ratios of released organic material per square meter algae surface area and hour. Abbreviations: POC = particulate organic carbon, DOC = dissolved organic carbon, PN = particulate nitrogen, n.a. = not analyzed.*

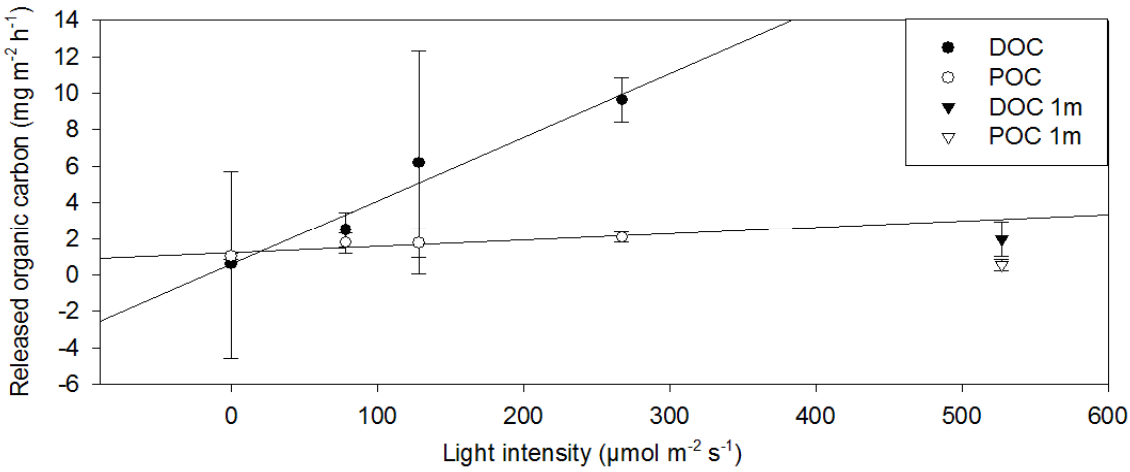
Algae	Seasons	POC release (mg m^{-2} h^{-1})	DOC release (mg m^{-2} h^{-1})	PN release (mg m^{-2} h^{-1})	DOC : POC	POC : PN	N
Caulerpa	Fall	4.6 ± 0.5	13.3 ± 12.3	0.39 ± 0.06	2.7 ± 2.6	13.8 ± 1.4	10
	Summer	1.7 ± 0.3	6.7 ± 1.5	0.12 ± 0.02	5.4 ± 1.1	11.4 ± 1.1	5
	Winter (1m)	0.5 ± 0.3	2.0 ± 1.0	-0.10 ± 0.07	2.1 ± 2.0	n.a.	5
	Winter	2.1 ± 0.9	9.6 ± 1.2	0.26 ± 0.12	11.6 ± 2.0	5.9 ± 1.3	5
	Winter (10m)	1.8 ± 0.7	6.2 ± 6.1	0.15 ± 0.02	6.2 ± 4.2	13.0 ± 0.6	5
	Winter (20m)	1.8 ± 0.5	2.5 ± 1.0	0.10 ± 0.04	1.6 ± 0.5	14.1 ± 0.4	5
	Winter (dark)	1.0 ± 0.2	0.6 ± 5.1	0.07 ± 0.01	1.3 ± 0.5	9.2 ± 0.4	5
	Spring	2.9 ± 2.4	10.1 ± 2.6	0.10 ± 0.03	14.7 ± 10.2	13.9. ± 1.2	5
Peyssonnelia	Fall	9.2 ± 0.7	35.5 ± 7.6	0.42 ± 0.01	4.0 ± 1.2	16.7 ± 1.5	5
	Summer	3.4 ± 0.3	4.0 ± 2.0	0.17 ± 0.02	1.7 ± 0.5	13.9 ± 1.1	5
	Winter	3.5 ± 0.5	2.9 ± 1.4	0.29 ± 0.05	1.3 ± 1.2	12.0 ± 0.5	5
	Spring	7.9 ± 2.6	5.6 ± 1.7	0.43 ± 0.14	1.1 ± 0.6	15.0 ± 3.5	5
Turf algae	Fall	10.8 ± 1.2	66.4 ± 15.6	0.84 ± 0.09	8.3 ± 2.0	13.2 ± 1.8	10
	Summer	4.9 ± 0.7	6,3 ± 1,9	0.33 ± 0.11	1.6 ± 0.6	13.6 ± 0.9	10
	Winter	5.3 ± 2.7	10.5 ± 6.8	0.41 ± 0.23	2.2 ± 1.0	8.9 ± 2.0	5
	Spring	6.4 ± 1.4	39.8 ± 2.2	0.36 ± 0.07	9.1 ± 2.6	16.0 ± 0.9	5
Ulva	Winter	0.3 ± 0.1	3.4 ± 1.5	0.00 ± 0.02	12.1 ± 7.8	n.a.	10
Enteromorpha	Winter	0.4 ± 0.2	1.7 ± 0.3	0.05 ± 0.02	10.8 ± 6.5	9.1 ± 2.7	5
Lobophora	Winter	1.7 ± 0.4	4.8 ± 1.0	0.16 ± 0.05	3.3 ± 1.0	13.8 ± 1.2	5
Saragassum	Winter	4.7 ± 0.3	5.6 ± 1.4	0.42 ± 0.04	1.6 ± 0.4	13.1 ± 0.3	5
Liagora	Spring	n.a.	4.9 ± 1.1	n.a.	n.a.	n.a.	5
Hydroclathrus	Spring	n.a.	4.9 ± 1.4	n.a.	n.a.	n.a.	5
Mean		4.2 ± 0.3	12.2 ± 2.1	0.29 ± 0.03	5.4 ± 0.9	12.4 ± 0.7	∑ = 130

Correlation with environmental parameters

The seasonal (*Caulerpa*, *Peyssonnelia*, turf algae) and vertical (*Caulerpa*) differentiation in organic matter release rates of the respective algae species showed a positive correlation to both light availability (DOC: $r^2 = 0.9573$, ANOVA, $p > 0.05$; POC: $r^2 = 0.7869$, ANOVA, $p = 0.041$) and temperature (DOC: $r^2 = 0.9529$, ANOVA, $p = 0.006$; POC: $r^2 = 0.8599$, ANOVA, $p = 0.019$), up to a PAR availability threshold of 300 - 400 µmol quanta m^{-2} s^{-1} (Fig. 1a + b). However, the applied linear regression of DOC release and light intensity was statistically not significant.

Caulerpa PN release was positively correlated to depth-mediated light intensity ($r^2 = 0.9754$, ANOVA, $p = 0.024$) when the 1 m release rate, with a PAR intensity of 527 µmol quanta m^{-2} s^{-1}, was excluded (Fig. 1c). POC/PN ratio showed no correlation with light intensity and showed mean negative values for the 1 m incubation, due to the PN uptake found for *Caulerpa* in the respective incubation experiment. No correlation was found between the amount of algae-derived organic matter and inorganic nutrient availability on a seasonal basis.





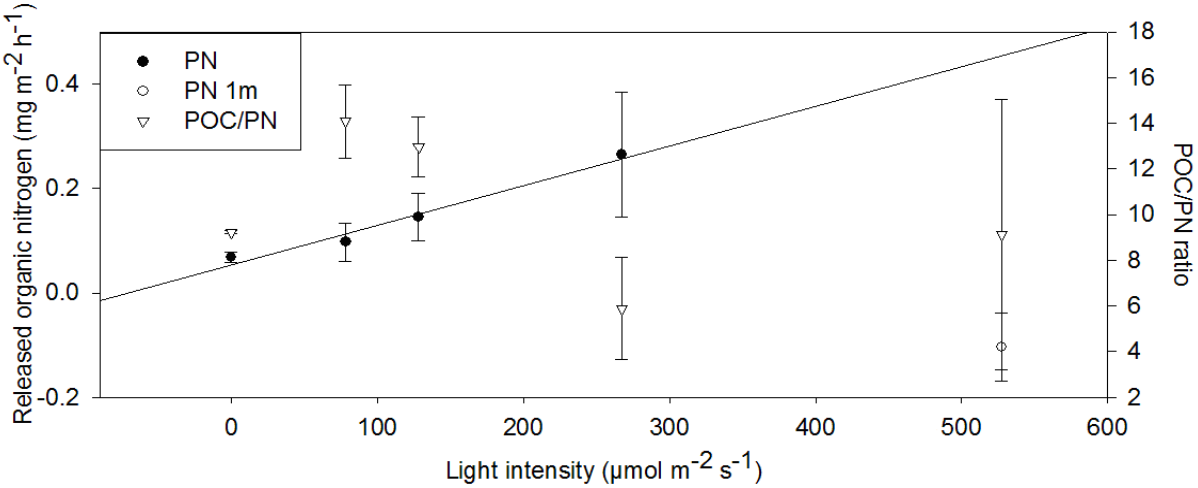


Fig. 1a *Seasonal resolution of mean organic carbon release (mean ± SE) for the three most abundant algae in the study area (Caulerpa, Peyssonnelia, turf algae), compared to the respective temperature regime (summer data excluded from the applied linear regression).* ***b*** *Vertical resolution of organic carbon release (mean ± SE) for Caulerpa (1m, 5m, 10m, 20m), compared to the respective light intensity (1m data excluded from the applied linear regression).* ***c*** *Vertical resolution of PN release and POC/PN ratio of released organic matter (mean ± SE) for Caulerpa (1m, 5m, 10m, 20m), compared to the respective light intensity (1m data excluded from the applied linear regression)*

POM stable isotope signatures

Stable carbon isotope signatures ($\delta^{13}C$) of algae derived POC (average ± SE: -14.4 ‰ ± 0.4 ‰) were significantly (paired t-test $p < 0.001$) higher than those of the seawater controls (-20.6 ‰ ± 0.3 ‰) on annual average. In contrast to $\delta^{13}C$ values of seawater controls, which showed no substantial seasonal variation, $\delta^{13}C$ of algae-derived POC showed significant fluctuations (ANOVA, $p < 0.001$), with highest values during summer (-11.2 ‰ ± 0.2 ‰) and lowest values during winter (-16.9 ‰ ± 0.4 ‰) (Fig. 2). Stable nitrogen isotope signature ($\delta^{15}N$) of algae-derived PN (average ± SE: 1.6 ‰ ± 0.3 ‰) was significantly higher (ANOVA, $p < 0.001$) when compared to the seawater controls (-0.4 ‰ ± 0.5 ‰). There was no significant difference in $\delta^{13}C$ between algae species during each season. $\delta^{15}N$ of algae-derived POM showed no significant difference between the single species except for turf algae which displayed significantly lower $\delta^{15}N$ values (-0.7 ‰ ± 0.4 ‰) than all other analyzed algae (2.3 ‰ ± 0.3 ‰) (ANOVA, $p < 0.001$) during each season (Fig. 2).

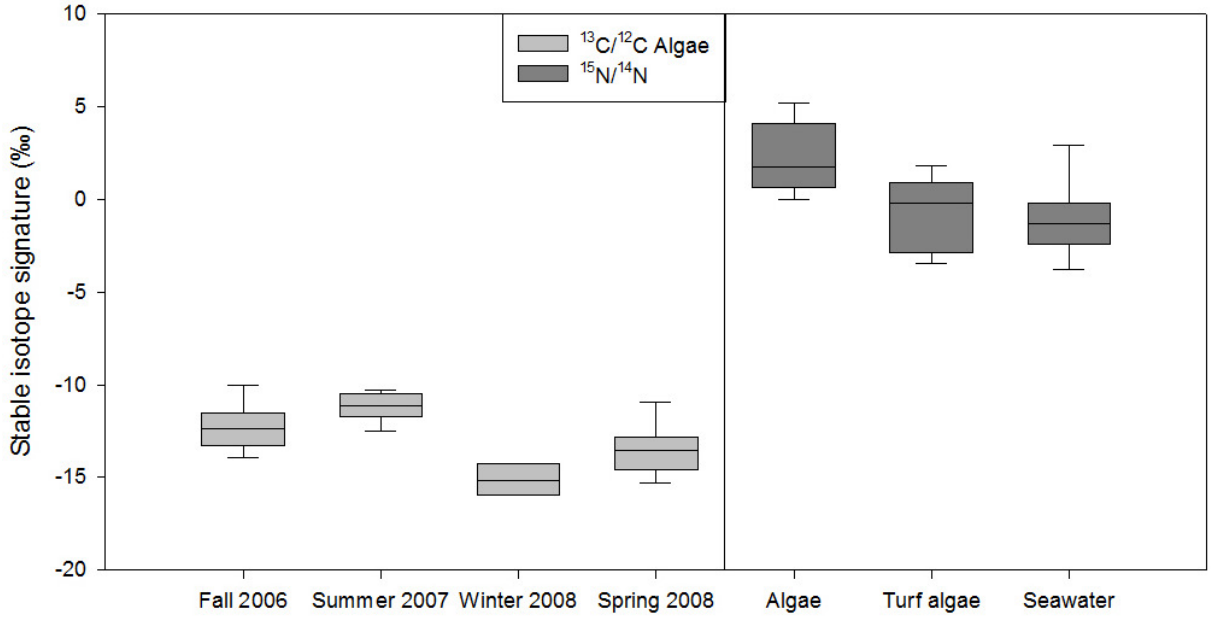


Fig. 2 *Carbon stable isotope signature ($\delta^{13}C$ values) from algae-derived organic matter in a seasonal resolution and overall nitrogen stable isotope signature ($\delta^{15}N$ values) of algae, turf algae with associated cyanobacteria, and seawater controls*

Discussion

Algae-derived organic matter

This study presents the first overview of organic matter release by benthic algae in a fringing reef of the Northern Gulf of Aqaba. All of the 9 investigated benthic algae species always showed an organic matter release regardless of season and water depth. DOC/POC ratios of 5.4 ± 0.9 indicate that the majority of the algae-derived organic matter immediately dissolved in the ambient reef waters, thus contributing to the bio-available DOC pool of the reef ecosystem (Hedges 2002). However, congruent with the findings of Wada et al. (2007), DOC release rates of all investigated benthic algae were highly variable throughout all seasons. These variations were also reflected in DOC/POC ratios of the algae-derived organic matter, as POC release rates were subjected to the same seasonal variations, but in a minor degree.

Release of organic matter has been considered an important pathway of the photosynthetic products of algae (Wada et al. 2007). Although organic matter release by phytoplankton has been studied (Zlotnik and Dubinsky 1989, Baines and Pace 1991), not much is known about organic matter released by macroalgae, which often exceed the biomass related productivity of phytoplankton in coastal regions (Mann 1973). Compared to a maximum release of less than 2 mg DOC m^{-3} h^{-1} from phytoplankton (Thomas 1971), macroalgae release rates of up to 66 mg DOC m^{-2} h^{-1} were found in the present study. C/N ratios of 4.1 to 14.1 described for phytoplankton-derived organic matter by Biddanda and Benner (1997) were in the same range as those found in the present study for macroalgae (5.9 to 16.0). This confirms the importance of benthic algae derived organic matter in coastal regions as source for organic carbon and nitrogen. Yet, species specific variations and adaptations to the respective environmental parameters, primarily to light and temperature, were found for algal organic matter release rates as discussed in the following section.

Species specific organic matter release by reef algae

The release of all investigated organic matter sources (DOC, POC, PN) varied between species regardless of environmental conditions. Although there were no differences found among the broader taxonomic groups of algae, the varying life strategies were reflected by the respective organic matter release rates. Organic matter release by turf algae exceeded those of all other specimens, while the lowest release rates were found for seasonal blooming algae such as *Enteromorpha, Ulva* and *Hydroclathrus*. These differences may potentially be attributed to a higher demand of photosynthates by the algae itself, to obtain high adult growth rates required by the short life cycles of seasonally blooming algae species (Lotze et al. 1999). The release of exudates causes energy and resources to be unavailable for use in other algae functions and structures. This is supported by studies on the brown algae *Ecklonia radiate* by Steinberg (1995), showing a negative relationship between seasonal changes in algal growth rates and the release of organic matter in form of phlorotannins. Low nitrogen contents of POM released by seasonally blooming algae species also indicate that metabolic resources are mainly used for growth relevant, nitrogen enriched substances, such as amino acids and cytokinin (De-Lin et al. 1996).

The lack of differences in primary production and thus organic matter release rates between taxonomic groups of algae has already been described in studies by Littler and Arnold (1982). In their overview on the productivity of macroalgal groups, similarities were found within morphological groups rather than within systematic groups. The findings of Littler and Arnold (1982) that the filamentous algae group displayed the highest productivity, are also reflected by highest organic matter release rates measured for turf algae assemblages in the present study. Lesser proportions of structural tissue in filamentous compared to more complex morphologies (Littler and Littler 1984) and a higher ratio of surface to volume, allowing for increased exchange rates with the ambient surroundings, may enable higher organic matter release rates. The present study therefore suggests that organic matter release

rates of different algae species are determined by functional properties (morphology and, primarily, life strategy) rather than by systematic properties (e.g. pigmentation).

Influence of environmental parameters

All seasonally incubated algae displayed a positive correlation in release rates of POC, PN and, most pronounced, DOC, to temperature and light availability up to a specific threshold. Temperature is one of the most important environmental factors controlling growth and distribution of marine plants and algae (Lüning 1990). Metabolic rates, and thus extracellular organic matter release are known to increase exponentially with temperature across a limited temperature range (Gillooly et al. 2001). Kübler and Davison (1993) suggested a high-temperature inhibition (30 °C), in high and low temperature adapted algae, for the synthesis of components in the photosynthetic apparatus. This inhibition was attributed to a breakdown in the ability to transfer energy of the light harvesting pigments to photosystem II. Incubation experiments conducted in the present study during summer 2007 came close (29 °C), but never exceeded this suggested temperature threshold, indicating that a light intensity threshold was the limiting factor for organic matter release by the investigated benthic reef algae rather than elevated temperatures.

Incubation experiments conducted with the green algae *Caulerpa* in a vertical resolution (1, 5, 10 and 20 m water depth) and under no-light conditions revealed a pronounced correlation of DOC, POC and PN release rates to light availability under identical temperature conditions. This correlation however, was only found for a limited PAR intensity range (0 – 300 µmol quanta m^{-2} s^{-1}), with organic matter release rates above 400 µmol quanta m^{-2} s^{-1} being comparable to those during no-light conditions. The dominant impact of light intensity on DOC release of phytoplankton has already been shown by Zlotnik and Dubinsky (1989), who isolated the effects of light from other environmental factors in their experiments. Congruent to the present study, Zlotnik and Dubinsky (1989) discovered a nearly linear increase of DOC release with light availability until a limiting light intensity threshold. As organic matter release rates of algae are believed to be connected to photosynthetic production (Verity 1981), the reduction of organic matter release rates above a PAR intensity threshold of 300 – 400 µmol quanta m^{-2} s^{-1} can be explained by a possible onset of photoinhibition. The primary mechanism of photoinhibition is the blocking of electron transport through photosystem II (Critchley 1981). The resulting reduced photochemical efficiency of photosystem II leads to non-photochemical quenching and a conversion of incoming excitation energy to heat (Kok et al. 1965). Photoinhibition, commonly known within aquatic plants (Henley et al. 1992), is documented congruent to the present study, to start at PAR intensities above 300 µmol quanta m^{-2} s^{-1} (Belay 1981). This is supported by observations of Häder et al. (1997), suggesting a water depth of approximately 5 m as a photosynthetic optimum for various benthic and pelagic algae. Furthermore, Mergner and Svoboda (1977), examining multiple photosynthetic active organisms in the study area, discovered a maximum O_2 productivity per unit dry weight at 5 m water depth, with a decrease of 25 to 50% at 1 m water depth.

Due to its high latitude, the investigated coral reef is subjected to pronounced seasonal variations, not only in temperature and light availability, but also to seasonally determined changes in nutrient concentrations (Rasheed et al. 2002). Although Littler and Littler (1992) showed that photosynthetic rates of benthic algae increased with elevated nutrient concentrations, no correlation with the quantity of algae-derived organic matter and nutrient availability was found in the present study.

Thus, temperature regime and foremost light availability are suggested as the dominant factors influencing and limiting organic matter release by benthic algae in the investigated reef of the Northern Gulf of Aqaba.

Stable isotope signatures of algae-derived POM

Carbon content of algae-derived organic matter was enriched in $\delta^{13}C$ values compared to the seawater controls, which were within the expected range of about -20 ‰ (Fry 2006, Swart et al. 2005). The $\delta^{13}C$ values of organic matter sampled from benthic algae incubations were assumed to be a composite of seawater contained POC (-20 ‰) and POC released by the respective algae. As fractionation during benthic algal photosynthetic carbon fixation usually results in characteristic $\delta^{13}C$ values of about -10 ‰ to -15 ‰ (France 1995, Schouten et al. 1998), the $\delta^{13}C$ values found in the algae incubation samples of -11 ‰ to -17 ‰ indicate that benthic algal-derived carbon was the dominating source in those samples. This is further supported by findings of France (1995) who showed that $\delta^{13}C$ values of organic matter derived from benthic algae were generally about 5 ‰ higher than those from marine phytoplankton derived organic matter.
The seasonal differences in $\delta^{13}C$ contents of algae-derived organic matter found in the present study further reflect environmental influences (e.g. elevated temperature and/or light availability) on isotope fractionation and thus on metabolic processes (Keeling 1958, Pataki et al. 2003) of benthic algae. Products of metabolic processes are usually lighter than inputs, because the heavier ^{13}C fraction is more inactive than the lighter ^{12}C fraction (DeNiro and Epstein 1976). Environmental conditions accelerating metabolic processes, though, lead to conditions where most of the carbon is utilized for product formation reactions, with an accompanying decrease in fractionation, as all incoming carbon is processed regardless of isotopic composition (Goericke et al. 1994).
$\delta^{15}N$ values of seawater PN (-0.4 ‰) and algae released PN (2.3 ‰) found in this study were in a common range (Muscatine and Kaplan 1994, Wild et al. 2008). That turf algae displayed significantly lower $\delta^{15}N$ values than all other algae can be ascribed to different nitrogen assimilation strategies. As algae have to rely on NH_4^+, NO_2^- and NO_3^- as sources of inorganic nitrogen, they have naturally higher $\delta^{15}N$ values than associations of turf algae, which include cyanobacteria that fix N_2 as main source of nitrogen (Goericke et al. 1994).

Ecological implications

The present study demonstrates that coral reef associated benthic algae exhibit high organic matter release rates, particularly of DOC. These rates were generally higher than those found for the majority of hermatypic coral species in the study area (Naumann et al. in press). Wild et al. (2004) described how corals, as sessile organisms, strongly influence the cycles of matter in their ecosystem via the release of organic matter as mucus, thereby initiating element cycles, which help to conserve essential nutrients in the reef ecosystem. A phase shift in the ecosystem coral reef will, thus, likely alter those cycles of matter with possible consequences on ecosystem functioning (Wild et al. in press).
Because it predominately dissolves immediately in the ambient water, algae derived organic matter can hardly substitute the particle trapping function of coral mucus (Wild et al. 2004). This may lead to a reduction of essential nutrients in the reef ecosystem with a concomitant increase of biolabile DOC. High C/N ratios of 12.4 ± 0.7, found for algae-derived organic matter in the present study, point to carbohydrates as dominant compound (Biersmith and Benner 1998) of algae-derived organic matter. Brylinsky (1977) showed that 20 to 30 % of the algae-derived organic carbon was metabolized by heterotrophic organisms within a 2 h period. Supplementary studies of Wild et al. (in press) revealed that algae released organic matter stimulated microbial oxygen consumption rates significantly more than organic matter released by other coral reef associated benthic primary producers. The negative implications on oxygen availability in the ecosystem could also be verified for in-situ conditions by oxygen logger measurements in the investigated reef (Wild et al. submitted; Niggl et al. submitted). In the light of investigations conducted on the influence of DOC on hermatypic corals (Kuntz et al. 2005, Kline et al. 2006) and the suggested influence of algae released DOC on oxygen availability for proximate hermatypic corals (Smith et al. 2006), an increased

macroalgal benthic cover may be liable to further favour organisms competing with hermatypic corals for the limited substratum in the coral reef ecosystem.

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Salinity and temperature effects on release of sexual products and other organic matter by coral reef-associated green algae of the genus *Caulerpa*

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Abstract

Mass spawning of coral reef-associated benthic green algae potentially results in extensive release of labile organic material, as previously documented for coral spawning. This could imply negative effects onto oxygen availability in coral reefs, because of intense microbial degradation of the algae-derived organic material, with indirect consequences for coral health. Yet, organic matter released during green algae mass spawning has never been quantified nor chemically characterized, and potentially relevant environmental factors have not been identified. This study therefore presents first quantification of release rates of different kinds of dissolved and particulate organic matter (POM) by two cosmopolitan green reef algae species of the genus *Caulerpa* in response to reduced and increased salinity and temperature conditions. Microbial degradability of the released organic matter was subsequently studied in incubation experiments. Results show that decreased, but not increased, salinity served to induce gametogenesis in both *Caulerpa* species, whilst the factor temperature showed species-specific effects and was generally less influential. Findings also indicate a strong relationship between gametogenesis and increases in POM release. C:N ratios of released POM were always low (4.4 to 7.7), and microbial activity positively correlated to the quantity of released algae-derived POM. This confirms that spawning of green reef algae may indeed stimulate microbial activity through the release of labile organic matter with concomitant effects on oxygen availability likely resulting in coral damage. Environmental effects such as strong rainfalls may trigger such events.

Introduction

"Phase-shifts" occurring on many coral reefs today are resulting in significant increases in the abundance of coral reef-associated macro algae (Done 1992). From 1977 to 1993, for example, there was an increase in cover of fleshy macro algae from 4 to 92 % along more than 300 km of Jamaica's coastline (Hughes 1994). Mass spawning, i.e. highly organised patterns of reproductive synchrony, with tens to thousands of con-specific algae thalli becoming simultaneously fertile overnight (Clifton 1997), is an important reproductive mechanism of benthic coral reef-associated green algae influencing patterns of their distribution and abundance (Clifton and Clifton 1999). Given the holocarpic nature of tropical algae (Hillis-Colinvaux 1984, Clifton 1997), whereby the entire thallus is converted into spores or gametes and released into the surrounding waters (Clerck et al. 2008), organic matter release during algae spawning events may be extensive. Mass spawning of corals, for example, has been shown to result in high concentrations of particulate organic matter (POM) in reef waters and sediments, with subsequent strong stimulation of microbial activity (Wild et al. 2008). This may lead to severe oxygen deficiency in coral reefs (Simpson et al. 1993).
Smith et al. (2006) have recently suggested that dissolved compounds released by reef algae can mediate coral mortality via stimulation of microbial activity. Although these compounds could not be identified nor quantified, labile dissolved organic carbon (DOC) was hypothesised, in line with findings of other investigations (Kuntz 2005, Kline et al. 2006). Elevated concentrations of POM can also induce bleaching of corals (Kline et al. 2006), leaving them more vulnerable to disease and mortality. If organic matter is extensively released by coral reef-associated benthic green algae, it may therefore have implications on oxygen availability in coral reefs, particularly those with high algae abundance.
However, few studies have quantified organic matter release by coral reef-associated benthic green algae (Hass et al. submitted) and none have quantified organic matter release during spawning events. In addition, relatively little is known of the phenology of benthic algae mass spawning (Tussenbrook et al. 2006), i.e. potentially relevant environmental factors (reviewed in Brawley and Johnson 1992). Some factors, e.g. temperature, appear to have been confounded by interacting variables during previous investigations, preventing isolation of those factors for analysis (Brawley and Johnson 1992). Others, e.g. salinity, have received little attention in the literature (see Shultz and Trainor 1968, and Brawley 1992), whereas preliminary findings of Haas et al. (unpublished data) demonstrate that salinity, as singular factor, may serve to induce coral reef-associated benthic green algae spawning.
The present study will therefore investigate the influences of decreased and increased salinity and temperature on spawning inducement in two coral reef-associated benthic green algae (*Caulerpa taxifolia* and *Caulerpa serrulata*). Further, concomitant organic matter release and its influence on microbial oxygen consumption rates will be determined.

Material & Methods

Experimental conditions and study objects

The present study was conducted in the aquarium facilities of the Coral Reef Ecology Group (CORE), at Geo-Bio Centre ^{LMU}, between December 2008 and March 2009. The aquarium water exhibited a salinity and temperature of 40 ± 1 ‰ and 27 ± 1 °C respectively, as measured daily using a refractometer and an AQUAMEDIC T2001HC thermometer. Illumination was achieved via AQUAMEDIC Aqua-star-light lighting, installed 50 cm above water level, providing a light intensity of 70720 lx, on a 12:12 (Light:Dark) cycle. Assessed water parameters were: pH: 8.4; PO_4 concentration: < 0.1 mg l^{-1}; NO_2 concentration: 0 mg l^{-1}; NO_3 concentration: < 1 mg l^{-1}; NH_3-NH_4 concentrations: < 0.01 mg l^{-1}, as measured weekly using TROPIC MARIN® EXPERT TEST kits. The above noted aquarium parameters are hereafter referred to as 'normal aquarium' conditions. Algae investigated in this study were

coral reef-associated benthic green algae of the order Bryopsidales (Chlorophyta, Bryopsidophyceae), namely *Caulerpa taxifolia* and *Caulerpa serrulata*.

Macro- and microscopic observations

To investigate influences of the selected environmental parameters on spawning inducement and organic matter release of the coral reef-associated benthic green algae, manipulative beaker incubation experiments were conducted. During all incubation experiments, macroscopic observations were performed in order to identify spawning events and to correlate any changes with organic matter release data. Descriptions of gametangia formation and associated changes by Goldstein and Morral (1970), Clifton and Clifton (1999) and Panayotidis and Zuljevic (2000) were used as the basis for recognition of fertility and observations made thereafter. These observations were recorded daily during the whole incubation time, with particular emphasis on the commencement of light phases, described to be the prevalent period for gamete release of coral reef-associated benthic green algae species (Clifton and Clifton 1999). Aliquots of incubation water (1 - 2 ml) were taken from the water column and areas directly surrounding gametangial structures (using sterile syringes) and were subsequently inspected with a LEICA LB30T BZ:00 microscope equipped with a LEICA DFC 400 digital camera.

Experimental setup

Fragments of *Caulerpa taxifolia* and *Caulerpa serrulata* approximately 10 cm in rhizomal length were cut from the main stock of algae in the aquarium and incubated after the method described by Herndl & Velimirov (1986), with the following modifications: fragments (n = 5) of both species were incubated in decreased salinity (15 ‰), increased salinity (50 ‰), decreased temperature (16 ± 1 °C) and increased temperature (35 ± 1 °C) conditions. In addition, control incubations with normal aquarium water (n = 10) for each species were conducted parallel to the manipulated treatments. For this purpose, glass beakers (1 l volume) were filled with 1000 ml of untreated aquarium water. Algae fragments of both species were then separately transferred into the beakers without air exposure. All control beakers were placed on a platform 2/3 their height within the main aquarium in order to maintain normal aquarium temperature and light conditions. For the salinity manipulation treatment, the salinity of the water was gradually reduced or increased through slow addition of freshwater or pre-prepared hyper-saline water (normal aquarium water + TROPIC MARIN® reef mix salt) to the normal aquarium water in the beakers using a 100 ml sterile syringe over a total duration of 30 min in order to avoid osmotic stress for the incubated algae. Once a salinity of 15 ‰ for decreased salinity incubations or 50 ‰ for increased salinity incubations was achieved, the beakers were placed next to the control beakers. Salinity was monitored every 2 h during light periods of the total incubation period. For the temperature manipulation treatments, replicate beakers (n = 5 each) were placed within 25 l water baths and placed next to the other beakers in the main aquarium. For the decreased temperature treatment, a TITAN 1500 AQUA MEDIC cooler was connected to one of the 25 l water baths via an EHEIM Compact $^{+}$3000 pump and set to a temperature of 16 °C. For the increased temperature treatment, four AQUAEL® EASYHEATER (239V~50Hz 50W) aquarium water heaters were set to a temperature of 35 °C and attached to the inside faces of the other 25 l water bath. Temperatures in both water baths were monitored using an AQUAMEDIC T2001HC thermometer or ONSET HOBO® Pro v2 water temperature data loggers until temperatures were stable at 16 ± 1 °C or 35 ± 1 °C respectively, thus marking the beginning of the incubation period.

All incubations were started at approximately 12:00 noon and lasted 47 - 50 h, thereby including two dark phases within the incubation period to allow sufficient time for possible gametangia formation as suggested by Clifton and Clifton (1999). At the end of all

incubations, algae fragments were removed from beakers using sterilised forceps to prevent contamination of incubation waters and subsequently prepared for surface area determination, while the remaining incubation water was sampled and analyzed as described below.

Quantification of organic matter release by reef algae

For dissolved organic carbon (DOC) measurement, the incubation water of each beaker was homogenised through gentle stirring for at least 30 seconds, and 10 ml aliquots collected using a sterile syringe. Following attachment of a VWR polyethersulfone membrane filter (0.2 µm pore size), 5 ml was purged in order to clean the respective filter and the remaining 5 ml filtered into and sealed within a pre-combusted (4 h at 450 °C) 10 ml brown glass vial. Samples were immediately frozen at -20 °C and kept frozen until analysis by high-temperature catalytic oxidation (HTCO) using a Maihak TOC analyzer. Sample acidification with orthophosphoric acid to pH < 2 and sparging with oxygen was performed to remove inorganic carbon and thus non-purgable organic carbon (actual DOC) was measured. Specific concentrations of potassium hydrogen phthalate were measured as elemental standards (standard deviation < 3 %).

For particulate organic carbon (POC) and particulate organic nitrogen (PON) concentration measurements, 400 ml aliquots of each homogenised incubation water were filtered onto pre-combusted (4-6 h at 500 °C) GF/F filters (Whatman; diameter: 25 mm, nominal particle retention: 0.7 µm). Filters were dried for at least 48 h at 40 °C and kept dry until later analysis by a THERMO^{TM} NC 2500 elemental analyser.

For chlorophyll *a* and pheophytin concentration measurements, a further 400 ml of each homogenised incubation water was filtered onto GF/F filters. These filters were frozen at -20 °C in the dark until later fluorometric analysis, as described in Rathbun et al. (1997), using a TD-700 laboratory fluorometer.

Organic matter release rates were calculated by subtraction of mean control incubation concentrations from treatment incubation concentrations and related to incubation time and surface area of the incubated algae.

Quantification of planktonic microbial O_2 consumption in incubation water

For these measurements, ca. 40 ml aliquots of each incubation water volume were separately filled into 40 ml Winkler glass bottles. Initial O_2 concentration of each aliquot was determined using a luminescent dissolved oxygen sensor (HACH LANGE HQ10, accuracy ± 0.05 % of the effective range). Bottles were sealed and incubated in the dark for a period of 24 h at aquarium temperature. Following this period, O_2 concentrations were measured as described above and subtracted from the initial O_2 concentrations. O_2 consumption was then related to incubation time and normalized to the surface area of the incubated algae.

Algae surface area quantification

The surface area of each algae fragment was determined to serve as reference for the other measured parameters. Fragments were spread 2-dimensionally in very shallow water (10 mm depth) on scaled paper and photographed from directly above with a *Casio Exilim* (resolution: 7.2 megapixels). Surface areas were then determined using the digital image processing software *Image J* (*ImageJ*, V. 1.37m, National Institutes of Health, USA), by calculating the surface area from the image plane.

Statistical analysis of data

Statistical analysis was performed using SPSS © software packages. All data were normally distributed (Kolmogorov-Smirnov test, $p > 0.05$), and therefore independent samples t-tests

were used for comparison of data with homogenous variances, and Mann-Whitney U tests for data without. Results were regarded as statistically significant at $p < 0.05$.

Results

Observations

Observation results of all salinity and temperature incubations are presented in Table 1. Among the 20 control algae fragments, only 2 demonstrated any notable pigmentation decrease during incubations.

In decreased salinity treatments, changes indicating gametogenesis were observed for the majority of algae fragments of *Caulerpa taxifolia* (80 %, Fig. 1, B) and *Caulerpa serrulata* (60 %, Fig. 1, E). Microscopy of incubation water samples taken directly adjacent to gametangial structures of both *Caulerpa* species revealed the presence of gamete-like cells with a length of 5-10 µm. Microscopy of cellular extrusions developed on *C. serrulata* fragments (60%) revealed the presence of an irregular cytoplasmic mass, containing chloroplasts, and amyloplasts (Fig. 1, G). Neither motile gametes, nor active gamete release, were observed.

During increased salinity incubations of both *Caulerpa* species, changes observed in all fragments (n = 10) did not denote development of structures associated with gametogenesis (Fig. 1, C and F). Microscopy of incubation waters did not reveal differences to controls and when returned to normal aquarium conditions, fragments regained normal colouration after 24 h.

Decreased temperature had no macroscopic effect on *Caulerpa taxifolia* at any stage of incubation in any fragment (n = 5). Changes consistent with the development of gametangia did occur for *Caulerpa serrulata* fragments incubated in decreased temperature conditions (60%, Fig. 1, I), suggesting that gametogenesis in this species was induced by such conditions. However, in comparison to changes observed during decreased salinity incubation of *C. serrulata*, fragments incubated in decreased temperature conditions appeared to have fewer discolourations with a smaller part of the whole algae displaying macroscopic changes.

During increased temperature incubations, gametangia formation occurred for the majority of fragments of *Caulerpa taxifolia* (60%, Fig. 1, H). However, changes occurred slower than during other incubations, and only approximately 50 % of the thalli of the affected fragments underwent visible changes. Increased temperature did apparently not induce gametogenesis in *Caulerpa serrulata* fragments. No microscopic differences were evident between incubation waters of any temperature incubation of either *Caulerpa* species as compared to controls.

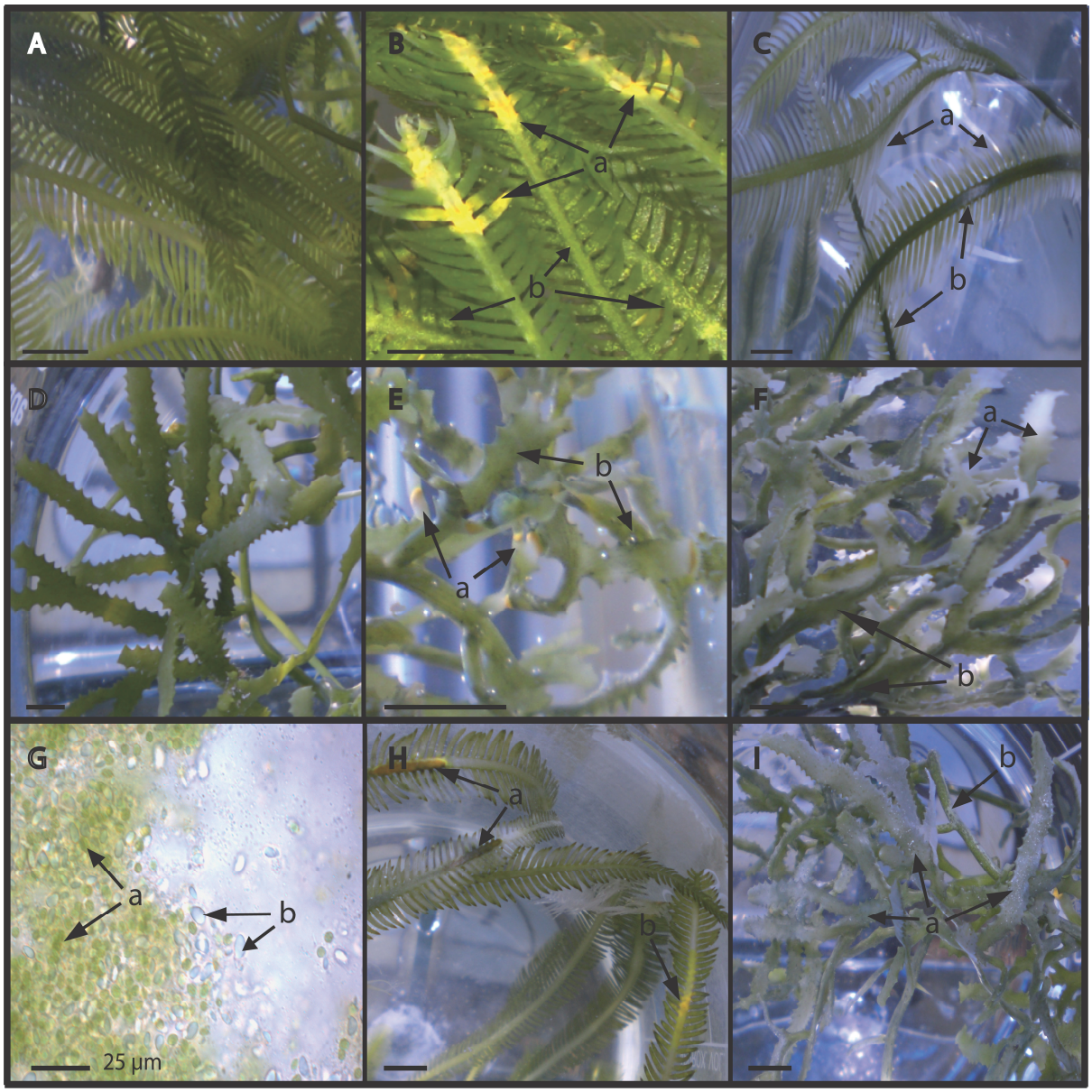

Fig. 1 *Caulerpa taxifolia (CT), Caulerpa serrulata (CS). Scale bars = 1 cm unless otherwise stated.* ***A)*** *CT non-fertile control fragment (+47 h).* ***B)*** *CT fertile fragment in decreased salinity incubation (+ 44 h) showing (a) distinct yellow discolouration of apical thalli and (b) reticulate cytoplasm of thalli.* ***C)*** *CT non-fertile fragment in increased salinity incubation (+47 h) showing migration of cytoplasm from (a) pinnules of thalli into (b) the thalli stem.* ***D)*** *CS non-fertile control fragment (+47 h).* ***E)*** *CS fertile fragment in decreased salinity incubation (+44 h) showing (a) apical yellow discolouration of cellular extrusions and (b) whitish reticulate cytoplasm of blades.* ***F)*** *CS non-fertile fragment in increased salinity incubation (+47 h) showing migration of cytoplasm resulting in (a) whitened apical blades and (b) deeper coloured distal blades.* ***G)*** *Microscopic observation (1000x) of an irregular cytoplasmic mass of a cellular extrusion of CS, showing (a) chloroplasts and (b) amyloplasts.* ***H)*** *CT fertile fragment in increased temperature incubation (+47 h) showing (a) yellow-brown discolouration of distal thalli and (b) yellow discolouration of apical thalli.* ***I)*** *CS fertile fragment in decreased temperature incubation (+47 h) showing (a) whitish reticulate appearance of cytoplasm within blades and (b) migration of cytoplasm from rhizomes.*

Table 1 *Caulerpa taxifolia, Caulerpa serrulata. Summary of macroscopic changes observed during decreased and increased salinity and temperature incubations of algae fragments. 'No change' indicates that fragments remained the same as described in the previous time period.*

Incubation treatment	Species	No. of aliquots undergoing change	Observations following first dark phase (+20 h)	Observations following second dark phase (+44 h)	Observations at the end of incubation (+47-50 h)	+24 h after cessation of incubations
Decreased Salinity	*C. taxifolia*	4 of 5	Migration of cytoplasm from rhizomes into thalli; distinct yellow discolouration of apical thalli	Increase in number of discoloured areas of thalli (Fig. 1, B)	Bleaching of several thalli; whitish turbidity of incubation waters	Holocarpy
	C. serrulata	3 of 5	Migration of cytoplasm from rhizomes into blades; development of cellular extrusions from blades	Development of apical yellow colouration of extrusions; whitish reticulate cytoplasm of blades (Fig. 1, E)	Whitened blades with reticulate cytoplasm	Holocarpy
Increased Salinity	*C. taxifolia*	5 of 5	Migration of cytoplasm from pinnules of all thalli into thalli stem, with resultant 'bleached' appearance of pinnules	Increases in described changes	No change (Fig. 1, C)	Return to normal colouration
	C. serrulata	5 of 5	Migration of cytoplasm from apical, to distal, regions of blades, with resultant whitened apical, and stronger coloured distal, blades	Increases in described changes	No change (Fig. 1, F)	Return to normal colouration
Decreased Temperature	*C. taxifolia*	5 of 5	No change	No change	No change	No change
	C. serrulata	3 of 5	Migration of cytoplasm from rhizomes into blades; development of whitish reticulate appearance of ~20% of blades	Increased development of whitish reticulate cytoplasm to ~60% of blades	No change (Fig. 1, I)	Partial return to normal colouration
Increased Temperature	*C. taxifolia*	3 of 5	Some migration of cytoplasm from rhizomes into thalli	Yellow and yellow-brown discolouration of apical and distal thalli, respectively, of ~50% of thalli; bleaching of a small number of thalli	No change (Fig. 1, H)	Holocarpy
	C. serrulata	2 of 5	No Change	Partial migration of cytoplasm from rhizome into blades	Development of whitish reticulate cytoplasm of few blades	No change

Table 2 *Caulerpa taxifolia, Cauerpa serrulata. Organic matter release rates (mean treatments - mean controls ± SE) (POC = particulate organic carbon, PON = particulate organic nitrogen, Chl a = chlorophyll a, Pheo. = pheophytin) of algae during decreased and increased salinity and temperature incubations, and microbial oxygen consumption (Microbial O_2 Consumption) rates of incubation waters. Significance (Sig.) of rates in comparison to mean respective controls is denoted by conventional * systems representing p-values (* = $p < 0.05$, ** = $p < 0.005$, *** = $p < 0.001$).*

Treatment	Species	POC ($\mu g\ m^{-2}\ h^{-1}$)	Sig.	PN ($\mu g\ m^{-2}\ h^{-1}$)	Sig.	Chl *a* ($\mu g\ m^{-2}\ h^{-1}$)	Sig.	Pheo. ($\mu g\ m^{-2}\ h^{-1}$)	Sig.	Microbial O_2 Consumption ($\mu g\ cm^{-2}\ d^{-1}$)	Sig.
Decreased Salinity	*C. taxifolia*	55.14 ± 9.10	*	11.20 ± 1.95	***	6.60 ± 2.30	*	5.92 ± 3.13		-3.60 ± 3.67	
	C. serrulata	42.68 ± 13.61	*	8.11 ± 1.67	***	-0.59 ± 1.08		2.77 ± 2.44		23.77 ± 2.55	***
Increased salinity	*C. taxifolia*	-1.92 ± 1.30		-0.10 ± 0.02		0.28 ±0.30		-0.10 ± 0.42		-6.14 ± 0.28	***
	C. serrulata	7.31 ± 8.16		1.91 ± 1.58		5.37 ± 2.60	**	7.04 ± 4.16		-5.57 ± 2.03	*
Decreased Temperature	*C. taxifolia*	0.05 ±0.67		-0.04 ± 0.16		-2.14 ± 0.33		-4.38 ± 0.54		-1.23 ± 1.39	
	C. serrulata	15.10 ± 3.68	*	1.72 ± 0.76	*	-1.58 ± 0.17		1.36 ± 0.89		8.55 ± 2.68	
Increased Temperature	*C. taxifolia*	10.25 ± 3.44		2.03 ± 0.80		1.75 ± 1.24		0.91 ± 1.01		11.64 ± 3.89	*
	C. serrulata	-8.93 ± 6.29		-0.81 ± 0.78		6.21 ± 2.61	*	8.29 ± 3.13	*	-5.42 ± 2.57	

Organic matter release

DOC release was measurable for 60 % of decreased salinity incubations of *Caulerpa taxifolia*, at a rate of 118 ± 12 µg DOC m^{-2} h^{-1} (average ± SE). DOC concentrations of all other incubations were below the 0.3 mg C l^{-1} resolution of the Maihak TOC analyzer.

POM release was detectable for all incubated algae fragments (Table 2). Species-specific differences in POM release were recorded between the control treatments, whereby POC and PON concentrations were on average 4-fold higher in *Caulerpa serrulata* incubations than in *Caulerpa taxifolia* incubations (Mann-Whitney U test, $p < 0.05$). Highest POM release rates were observed for both *Caulerpa* species during decreased salinity incubations. *C. taxifolia* incubations exhibited ca. 20 fold higher POC and 24 fold higher PON concentration increases than the controls. *C. serrulata* demonstrated comparable rates of POC and PON release during decreased salinity incubations, however, this translated to a smaller increase in POC(ca. 4 fold) and PON (ca. 5 fold) release, in comparison to controls. Significantly elevated POC release was also observed during decreased temperature incubations for *C. serrulata* (Mann-Whitney U-test, $p < 0.05$), although to a lesser extent than during decreased salinity incubations. Molar POC:PON ratios ranged from 4.4 to 7.7 across all incubations and controls. Salinity and temperature incubations had no significant effect on POC:PON ratios of released organic matter.

There was no significant difference in chlorophyll *a* or pheophytin release rates of control incubations between species. Significantly higher chlorophyll *a* release rates than in the control treatments were observed for *Caulerpa taxifolia* during decreased salinity incubations only (Mann-Whitney U test, $p < 0.05$). There was no significant difference in pheophytin release rates during any salinity or temperature incubation of that species in comparison to controls. *Caulerpa serrulata*, in contrast, released significantly higher amounts of chlorophyll *a* during increased salinity incubations as compared to controls (Mann-Whitney U test, $p < 0.01$). In addition, chlorophyll *a* and pheophytin release of *C. serrulata* was significantly elevated during increased temperature incubations (Mann-Whitney U test, $p < 0.05$).

Microbial O_2 consumption

No species-specific difference in microbial O_2 consumption rates in the water of control incubations was evident. Microbial O_2 consumption rates (Table 2) were significantly elevated in decreased salinity (3.1 ± 0.2 fold) incubation waters of *Caulerpa serrulata* and increased temperature (2.2 ± 0.4 fold) incubation waters of *Caulerpa taxifolia* (Independent samples t-test, $p < 0.001$ and < 0.05, respectively), when compared to the control treatments. Significant reduction in microbial O_2 consumption was observed in increased salinity incubation waters of both species (Independent samples t-test, $p < 0.05$ and < 0.01, respectively).

Microbial O_2 consumption rates of incubation waters did not correlate with chlorophyll *a* or pheophytin release during any incubation of either *Caulerpa* species. Significant positive correlation to POC and PON release during temperature incubations of *Caulerpa taxifolia* (Spearman rank-order correlation, $p < 0.005$ and < 0.001 respectively), and both salinity and temperature incubations of *Caulerpa serrulata,* was identified (Spearman rank-order correlation, $p < 0.001$ in all cases).

Discussion

Spawning induction by environmental parameters

Results of the present study highlight that decreased salinity can serve to induce gametogenesis in two coral reef-associated benthic green algae. This was indicated by macroscopic changes in the majority of *Caulerpa taxifolia* and *Caulerpa serrulata* fragments consistent with descriptions of gametogenesis in the literature; including migration of cytoplasm from rhizomes into thalli, development of distinct yellow discolouration of apical thalli and/or cellular extrusions, and bleaching and whitening of thalli (see Goldstein and Morall 1970, Clifton and Clifon 1999, Panayotidis and Zuljevic 2000). Microscopic inspection further confirmed the presence of gamete-like cells within decreased salinity incubation waters of both *Caulerpa* species and identified irregular cytoplasmic masses within cellular extrusions of fertile *C. serrulata,* consistent with descriptions of cellular structures that cleave to give rise to gametes in *Caulerpa* species (Goldstein and Morall 1970). Lack of observation of active gamete release during the present study, nor motile gametes in incubation waters, suggest that whilst gametangia formation was induced by such conditions, incubation length was not sufficient to include a holistic gamete release. Clifton and Clifton (1999) showed that 48-60 h can pass before release of gametes from *Caulerpa* species following the development of gametangia. Observed holocarpy of fragments, within 24 h after cessation of incubations, apparently owed to release of gametes once fragments had been returned to normal aquarium conditions during the present study.

Reproductive seasonality in algae, coordinated by environmental cues, can cause reproduction to occur when deteriorating conditions require the production of resting stages, or when conditions favourable to growth are declining (Brawley and Johnson 1992). As hypo-saline conditions can negatively impact *Fucoid* algae (Brawley and Johnson 1992), centric diatoms (Schultz and Trainor 1968) and corals (Hoegh-Guldberg 1999), it could be supposed that responses of the two *Caulerpa* species during decreased salinity incubations were inline with such reproductive life history traits. This is supported by findings of Clifton and Clifton (1999), who described a delay in the recruitment of sexually derived algae into the population, probably allowing green algae to persist through unfavourable periods in a manner analogous to terrestrial seed banks.

When exposed to different temperature conditions, both *Caulerpa* species responded differently and less strongly than when exposed to salinity treatments. Low temperature inducement of gametogenesis in some *Caulerpa serrulata* fragments reflects findings of previous studies with kelp (Lüning 1980) and brown algae species (Muller 1981). Tussenbrook et al. (2006) more recently also attributed observed development of fertility in *all* dominant species of siphonous green algae on a Caribbean reef to a registered drop in seawater temperature, owing to the passing of hurricane Wilma. The absence of holocarpy following incubation of *C. serrulata* fragments in decreased temperature conditions suggests the occurrence of some form of fertility reversal. Possible failure of gametes to fully develop, internal release, or re-absorption of gametes were hypothesised by Vroom et al. (2003), when an individual (*Halimeda tuna*) that had displayed all the characteristics of a reproductive algae failed to release gametes, returned to pre-reproductive levels of pigmentation and remained healthy.

Caulerpa taxifolia, in contrast, behaved in a similar manner to observations of *Halimeda* species, whereby increases in temperature may have induced fertility in *H. cuneata* and *H. tuna* (Chihara 1956) with a minimum threshold temperature of 26 °C being required for Great Barrier Reef individuals (Drew and Abel 1988, Vroom et al. 2003). Significant decreases in *C. taxifolia* productivity above 30 °C (Gacia et al. 1996), compared with survival at temperatures down to 10 °C (Chisholm et al. 2000, Burfeind and Udy 2009), suggest that high

temperature inducement of gametogenesis in *C. taxifolia* in the present study is a response to deteriorating conditions, as with decreased salinity.
Results are consistent with the literature whereby species-specific differences in response to temperature have previously been described (Brawley et al. 1992). Temperature is fundamental to marine species growth and distribution (Lüning 1980), and is strongly associated with other variables, e.g. light intensity (Hadall and French 1958). Lüning (1980) reported that the amount of light required for gametogenesis in three *Laminaria* species increased exponentially with increasing temperature. Isolation of temperature during the present study suggests that, as a singular factor, it may not have such a strong effect as other factors, e.g. salinity, on inducing gametogenesis in two *Caulerpa* species.

Influence on organic matter release and microbial oxygen consumption

Inducement of gametogenesis in both *Caulerpa* species by decreased salinity conditions is accompanied by significant reductions in gross photosynthetic rates (data not shown) owing to conversion of cytoplasm to structures concerned with reproduction (Drew and Abel 1988), and concomitant holocarpy, a consequence of spawning of tropical benthic green algae (Hillis-Colinvaux 1984). As entire cell contents are often released during green algae spawning (Clifton 1997) significant increases in chlorophyll *a* and pheophytin release into incubation waters would be expected. This was evident for chlorophyll *a* release of *Caulerpa taxifolia* during decreased salinity incubations, likely reflecting the more pronounced gametogenesis in that species compared to *Caulerpa serrulata*. Absence of elevated chlorophyll *a* concentrations in other incubations where reproductive structures were detected is concurrent with apparent gamete release following cessation of incubations.
This study presents first evidence of a significant relationship between gametogenesis and increases in release of POM by coral reef-associated benthic green algae. Significant increases in POC and PON release accompanied gametogenesis during decreased salinity incubations (20- and 24- fold for *Caulerpa taxifolia* respectively). Results contrast findings of other investigations describing positive correlation between salinity and organic matter exudation by *Fucus vesiculosus* Sieburth (1969), therefore suggesting that elevated release during the present study most probably owed to the induction of gametogenesis and not simply a direct response to changes in environmental conditions. The absence of elevated POM release during gametogenesis of *C. taxifolia* fragments incubated in increased temperature may be an artefact of the conditions. Previous studies have suggested an inhibitory role of increased temperature on organic matter release of algae. Kübler and Davison (1993) demonstrated that temperatures above 30 °C disrupt energy transfer to photosystem II in algae chloroplasts, resulting in reduced metabolic rate and therefore organic matter release. Yet, microbial activity was elevated in incubation waters, as evidenced by increased oxygen consumption rates, which may therefore indicate microbial utilisation of an alternative organic matter source, e.g. DOC. Such elevated DOC concentrations were only found for reduced salinity treatments of *C. taxifolia*, while all other treatments displayed DOC concentrations beneath the detection limit. As DOC release by benthic macro algae is well documented (Khailov and Burlakova 1969, Brylinsky 1977, Andrews 1979, Cole et al. 1982, Wada et al. 2007), the absence of recordable DOC concentrations during incubations of the present study was likely owing to the length of incubation employed and not a lack of release. Cole et al. (1982) demonstrated that heterotrophic micro-organisms rapidly assimilate a substantial portion of released DOC during algal growth, therefore resulting in underestimation of released DOC by sampling methods. Khailov and Burlakova (1969) and Brylinsky (1977) conducted incubations for the measurement of DOC release by algae ranging from 0.5-6 h, whilst 47-50 h incubations were conducted during the present study to allow sufficient time for development of gametangia (Clifton and Clifton 1999). Algae uptake and/or microbial

utilisation of DOC most probably occurred during these relatively long incubations (Fries 1973, Moebus and Johnson 1974, Brylinsky 1977, Cole 1982).
C:N ratios ranging from 4.4 – 7.7 demonstrate that released POM was rich in nitrogen (around the Redfield C:N ratio of 6.6). These are comparable to C:N ratios of coral spawning products presented by Wild et al. (2008). As nitrogen is often a limiting element in oligotrophic reef systems (Hallock and Schlager 1986), results of the present study suggest that POM released during the processes of gametogenesis in two *Caulerpa* species may be attractive to planktonic microbial communities. Indeed, microbial activity was significantly increased in decreased salinity incubation waters of *Caulerpa serrulata*, correlating positively with POM release of that species. The absence of increased microbial activity in the presence of *Caulerpa taxifolia*-derived POM during decreased salinity incubations probably reflects the role of secondary metabolites, e.g. caulerpenyne. These can exist at greater concentrations in *C. taxifolia* than other *Caulerpa* species, particularly in areas involved with reproduction, and can serve to inhibit the activity of micro-organisms (Meyer and Paul 1992, Amade and Lemèe 1998, Dumay et al. 2004). The detectable DOC concentrations in decreased salinity incubation waters of *C. taxifolia,* support the hypothesis of microbial activity inhibition by release of secondary metabolites.

Ecological implications

The present study demonstrates for the first time that reduced salinity conditions can serve to induce spawning in two species of coral reef-associated benthic green algae of the genus *Caulerpa*. Owing to the brief, often inconspicuous nature of fertility of tropical green algae, such information will allow for more accurate assessment of reproductive activity in the future (Clifton and Clifton 1999). Hurricane-associated heavy rainfall can lead to extensive flooding that often results in large amounts of freshwater runoff onto reefs, exposing them to short-term salinity reductions (Jokiel et al. 1993, Rogers et al. 1982, Lugo et al 2000). For example, following severe storm related flooding on a Hawaiian reef, Jokiel et al. (1993) recorded reduced salinity conditions down to 15 ‰, which remained below 20 ‰ for ca. 4 d; comparable salinities to- and longer exposure times than- those adopted by this study. Such destructive rainfall has recently damaged areas of the Great Barrier Reef (Great Barrier Reef Marine Park Authority, in press), and has been shown to pose risk to annual reproductive cycles of corals (Veron et al. 2009). Given the effects of climate change, e.g. increasing sea surface temperatures, tropical hurricanes are predicted to increase in both frequency and intensity in the future (Rogers et al. 1982, Emanuel 2005, Webster et al. 2005). These expected changes in tropical weather patterns, coupled with the results of the present study, indicate that an increase in the frequency of mass spawning events of coral reef-associated benthic algae may be expected; particularly on reefs near elevated land masses or major rivers, owing to more severe runoff effects in such locations (Rogers et al. 1982).
In the short term, local decreases in algae abundance following spawning events can be expected owing to holocarpy (Clifton and Clifton 1999), with concomitant increases in organic matter release to reef systems, as demonstrated by this study. Investigations addressing the stimulation of microbial growth within the surface mucopolysaccharide layer (SML) of coral species have suggested benthic algae-derived DOC (Smith et al. 2006, Vermeij et al. 2009) and reef water POM (Kline et al. 2006) as the critical substrate, causing mortality and significant bleaching of coral fragments, respectively (Kline et al. 2006). Considering the magnitude of coral reef-associated benthic algae spawning reported in previous studies, i.e. Clifton and Clifton (1999), Tussenbrook et al. (2006), and the associated significant release of labile organic matter as shown here, benthic algae spawning may hold potential to indirectly negatively impact corals via stimulation of SML microbial communities. In the longer term, the ecological implications of such effects could see the establishment of a positive feedback loop between algae-derived organic matter, coral

mortality and increases in algae abundance on reefs, as proposed by Kline et al. (2006). Spawning inducement may further allow coral reef-associated benthic algae to persist through unfavourable conditions expected to increase in frequency on reefs (Clifton and Clifton 1999, Brawley and Johnson 1992, Emanuel 2005, Webster et al. 2005), stabilising their presence and further preventing reversal of phase shifts to coral-dominated systems.

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6

Carbohydrate composition of mucus released by scleractinian warm and cold water reef corals

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This chapter is in revision for Aquatic Biology

Abstract

Mucus, a primarily carbohydrate complex, released by scleractinian warm and cold water reef corals can function as an important carrier of organic material from corals to a range of consumers, in particular microbes. However, information about mucus chemical composition is rare for warm water corals and non-existent for cold water corals. This study therefore presents comparative carbohydrate composition analyses of mucus released by the dominant and cosmopolitan warm and cold water corals. Findings hint to a genus-specific mucus carbohydrate composition. Arabinose was the major mucus carbohydrate component for the genus *Acropora* and not found in cold water coral mucus. Mucus derived from corals of the genus *Fungia* contained significantly more fucose than the mucus of all other investigated corals. However, comparison of carbohydrate mucus composition for the investigated cold water corals with those described for warm water corals in the present study and the literature revealed no significant differences. This indicates use of similar carbohydrate components during mucus synthesis by corals largely irrespective of zooxanthellate or azooxanthellate carbon supply mechanisms.

Introduction

Both scleractinian warm and cold water corals continuously release mucus into their surrounding (Wild et al. 2005b; Wild et al. 2008) for various purposes (reviewed in (Brown, Bythell 2005). This mucus is released in such quantities that it can dominate the suspended matter around warm water coral reefs (Johannes 1967; Marshall 1968) and may also affect the carbon cycle in the water column above cold water coral reefs (Wild et al. 2008; Wild et al. 2009b).

Previous studies confirmed the important function of warm water coral-derived mucus as an energy carrier and particle trap in the reef ecosystem (Wild et al. 2004a). Coral mucus was rapidly degraded by microbes in the pelagic and benthic environment at reef locations in the Australian Great Barrier Reef (Wild et al. 2004b) and the Northern Red Sea (Wild et al. 2005a). For cold water coral-derived mucus, fauna-microbe interaction via this material and its fast recycling by planktonic microbes was observed (Wild et al. 2008). Supplementary research confirmed these findings and revealed similar planktonic microbial degradation of mucus released by the cold water coral *Lophelia pertusa* compared to the carbohydrates starch and glucose (Wild et al. 2009b).

However, information about the chemical composition of warm water coral-derived mucus is very limited and non-existent for cold water coral-derived mucus. Warm water coral-derived mucus has been described as a primarily carbohydrate complex (Coffroth 1990), but more detailed chemical analyses revealed that the main component of mucus released by the staghorn coral *Acropora formosa* consisted of a proteoglycan (Richards et al. 1983). (Wild et al. 2005b) further analysed the carbohydrate composition of mucus released by six different coral species within the genus *Acropora* and found arabinose, mannose, galactose, glucose, and N-acetyl glucosamine present in all samples, whereas rhamnose, fucose and xylose could only be detected in some samples. Such differences in mucus composition may control microbial community composition in warm (Allers et al. 2008) and cold water coral reef habitats (Schöttner et al. 2009) with ensuing effects on microbial activity.

In comparison with zooxanthellate warm water corals, azooxanthellate cold water corals in addition likely release mucus with a distinctly different carbohydrate composition as they do not receive any photosynthetically produced transfer metabolites from their endosymbionts. Up to half of the carbon assimilated by the endosymbiotic algae can be released as mucus by warm water corals (Crossland et al. 1980; Davies 1984), and chemical analyses showed that carbohydrate mucus components such as arabinose may be directly transferred from the algae to the coral host (Meikle et al. 1988).

Substrate specificity in marine polysaccharide complexes is however critical for microbial degradation and concomitant organic matter recycling (Arnosti 2000). This study therefore presents carbohydrate compositions of mucus released from some of the most dominant warm water coral genera (*Acropora*, *Stylophora*, *Pocillopora*, *Fungia*, *Ctenactis*) in comparison to the two cosmopolitan cold water coral genera *Lophelia* and *Madrepora*. In addition, all literature data available for carbohydrate composition of warm water coral-derived mucus are compared to cold water coral mucus carbohydrate composition data, which are presented in this study for the first time.

Material and Methods

Collection of mucus samples

Warm water scleractinian corals were collected by SCUBA from water depths of ca. 5 m within a fringing reef close to the Marine Science Station in Aqaba, Jordan (29° 27′ N, 34° 58′ E) during three seasonal expeditions (Aug/Sep 2007, Feb/Mar 2008, May 2008). Information about sampling time is given in Table 1. For each mucus sampling, 4 to 6 intact *Acropora*, *Stylophora* or *Pocillopora* colonies (diameter: 21 to 45 cm) or polyps of *Fungia* or *Ctenactis* (diameter: 21 to 43 cm) were used. All coral colonies or polyps were kept in flow-through aquaria at in-situ temperature and light availability for 24 to 48 h prior to mucus sampling in order to avoid mucus contamination because of lesion leakage. Mucus was then collected from each coral genus by using the methodology described in (Wild et al. 2005b). Briefly, corals were turned upside-down and exposed to air for 2 min. They immediately began to release fluid, transparent mucus in variable volumes. The dripping mucus was collected in a clean container after discarding the initial 30 s of dripping. Mucus collected from colonies or polyps of the same genus were then pooled and frozen at -20 °C in volumes of 8 to 12 ml until further analysis.

Cold water corals were collected either by the manned submersible JAGO (IFM-Geomar, Kiel, Germany) during 3 dives at Røst Reef (67° 31.11' N, 9° 28.43' E, water depths: 310 to 380 m), Norway, during RV Polarstern expedition ARK-XXII/1a or by a remotely operated vehicle (ROV) of type Sperre SUB-fighter 7500 DC from dives at Tisler Reef (58° 59.81' N, 10° 57.98' E, water depth: ca. 100 m), located in the Skagerrak at the border between Sweden and Norway. From both Røst and Tisler Reef, 4 to 8 fragments (length: 10 to 25 cm) from different colonies of *Lophelia pertusa* (both reefs) and *Madrepora oculata* (only Røst Reef) were collected and kept in local seawater for at least 5 d prior to mucus sampling in order to avoid mucus contamination because of lesion leakage. Mucus was then collected from both coral genera during the two expeditions as described above in volumes of 2 to 10 ml. Coral mucus samples were kept frozen at -20 °C until further analysis as described in the following.

Carbohydrate composition

Coral mucus samples were desalted prior to carbohydrate composition analysis using a Spectra/Por Biotech cellulose ester dialysis membrane with a molecular weight cutoff of 100 to 500 Daltons. A length of membrane sufficient to hold 2 ml of liquid was cut off from the 10 m strip and washed using deionized, sterile water. The membrane was then filled with approximately 2 ml of sample and placed in a 4 l bucket that was continuously filled with new deionized, sterile water from the bottom and emptied from the top. A stir bar was employed to aid mixing at 4 °C. After 3 d, the samples were removed, frozen and lyophilized. Glycosyl composition analysis was performed by combined gas chromatography/mass spectrometry (GC/MS) of the per-*O*-trimethylsilyl (TMS) derivatives of the monosaccharide methyl glycosides produced from the sample by acidic methanolysis. An aliquot was taken from each sample and added to separate tubes with 40 µg of inositol as the internal standard. Methyl glycosides were then prepared from the dry sample following a mild acid treatment by methanolysis in 1 M HCl in methanol at 80° C for 16 h, followed by re-*N*-acetylation with pyridine and acetic anhydride in methanol (for detection of amino sugars). The sample was then per-*O*-trimethylsilylated by treatment with Tri-Sil (Pierce) at 80 °C for 0.5 h. These procedures were carried out as previously described in (York et al. 1985) and (Merkle, Poppe 1994). GC/MS analysis of the TMS methyl glycosides was performed on an AT 6890N GC interfaced to a 5975B MSD, using a Supelco EC-1 fused silica capillary column (30m × 0.25 mm ID).

Results and Discussion

Carbohydrate composition analyses shown in Table 1 revealed that C6 sugars (glucose, mannose, and galactose) occurred most often followed by deoxysugars (fucose, rhamnose), amino sugars (N-acetyl glucosamine) and C5 sugars (arabinose, xylose). The monosaccharide arabinose, often detected as compound of biopolymers such as hemicellulose and pectin, was only found in mucus released by warm water corals of the genus *Acropora* (Table 1), where it was the major carbohydrate component. Analysis of all available similar data sets on the carbohydrate composition of warm water coral mucus from the literature (Richards et al. 1983; Meikle et al. 1988; Wild et al. 2005b) confirmed that *Acropora* mucus (n = 9 samples) contained significantly (one-way ANOVA, $p < 0.001$) more arabinose than all other investigated samples (n = 8) from five different coral genera. Similarly, mucus derived from corals of the genus *Fungia* (n = 4 samples) contained significantly (one-way ANOVA, $p < 0.001$) more fucose than the mucus of all other investigated corals. This indicates similar carbohydrate composition at the genus level. Similarities with studies carried out in the Australian Great Barrier Reef (Richards et al. 1983; Meikle et al. 1988; Wild et al. 2005b) suggest that warm water coral-derived mucus shows rather genus-specific than location-specific differences. Seasonal differences in carbohydrate composition were not pronounced as indicated by a comparison between the three mucus samples from *Fungia* collected during spring, summer and winter (Table 1).

Table 1 *Carbohydrate composition (in mole percentage of all detected carbohydrates) of mucus released from scleractinian warm and cold water corals (Ara = arabinose, Rha = rhamnose, Fuc = fucose, Xyl = xylose, Man = mannose, Gal = galactose, Glc = glucose, GlcNAc = N-acetyl glucosamine, nd = not detected). Glucuronic acid, galacturonic acid, N-acetyl galactosamine and N-acetyl Mannosamine could not be detected in any of the samples.*

Genus	Season	Origin	Ara	Rha	Fuc	Xyl	Man	Gal	Glc	GlcNAc
Acropora	summer 2007	Aqaba, Jordan	76.4	nd	6.5	nd	5.7	3.7	1.2	6.6
Ctenactis	winter 2008	Aqaba, Jordan	nd	nd	5.2	nd	22.1	6.0	5.9	60.8
Fungia	spring 2008	Aqaba, Jordan	nd	nd	68.4	nd	31.6	nd	nd	nd
Fungia	summer 2007	Aqaba, Jordan	nd	nd	78.7	nd	15.0	0.7	0.9	4.7
Fungia	winter 2008	Aqaba, Jordan	nd	nd	85.8	nd	14.2	nd	nd	nd
Pocillopora	winter 2008	Aqaba, Jordan	nd	nd	25.3	nd	49.5	nd	25.2	nd
Stylophora	winter 2008	Aqaba, Jordan	nd	nd	nd	nd	nd	nd	100	nd
Madrepora	spring 2007	Rost Reef, Norway	nd	31.4	nd	nd	42.6	nd	26.0	nd
Lophelia	spring 2007	Rost Reef, Norway	nd	nd	8.0	1.5	18.8	4.7	9.8	57.2
Lophelia	spring 2008	Tisler Reef, Sweden	nd	nd	nd	nd	40.4	nd	59.6	nd

In the present study, the only carbohydrate component found in the mucus of all investigated genera was glucose (Table 1), which is a universal energy source in most organisms. Thus, glucose contents in mucus from both warm and cold water corals may explain its excellent microbial degradability described by several previous studies (Ducklow 1990; Wild et al. 2004a; Wild et al. 2004b; Wild et al. 2005a). Besides glucose, the neutral monosaccharides arabinose, galactose, xylose and mannose, as well as the amino sugar N-acetyl-glucosamine,

have been identified as important substrates supporting bacterial growth and contributing to the flux of labile dissolved organic matter (DOM) in marine waters (Rich et al. 1996; Riemann, Azam 2002). The concentration of labile monosaccharides in marine waters is usually low (Benner et al. 1992), as hydrolysable neutral sugars are subjected to rapid microbial decomposition (Ogawa et al. 2001). Thus, the finding that the carbohydrate fraction of coral mucus includes a heterogeneous mixture of labile monosaccharides further elucidates the significant influence of warm and cold water coral mucus on planktonic and benthic bacterial metabolism observed in previous studies (Wild et al. 2005a; Wild et al. 2008). The remaining monosaccharide constituents of coral mucus found here, fucose and rhamnose, likely contribute to the large pool of refractory marine DOM, as previous studies attested a low bacterial degradability of these deoxysugars (Amon et al. 2001; Ogawa et al. 2001).
Arabinose could not be detected in any of the azooxanthellate cold water corals, likely because this monosaccharide is usually not a constituent of animal cells, but a characteristic monosaccharide for photosynthetic organisms (Meikle et al. 1988). Rhamnose and xylose were only found in the present study for the cold water coral genera *Madrepora* and *Lophelia*, respectively (Table 1). Both sugars are predominately known as plant cell wall compounds such as pectin and hemicellulose, but they could also be released by bacteria (Cowie, Hedges 1984) potentially associated with the coral surface or mucus. Fucose, Mannose, Galactose, Glucose and N-Acetyl Glucosamine were found in both the mucus of zooxanthellate warm and azooxanthellate cold water corals without any significant differences between the two groups of corals (U-Test after Wilcoxon, Mann and Whitney, $p > 0.05$), therefore likely representing principal carbohydrate components of the matrix of scleractinian coral mucus.
Comparison of results of the present study with all available similar data sets from the literature (Richards et al. 1983; Meikle et al. 1988; Wild et al. 2005b) revealed no significant carbohydrate composition differences between warm ($n = 17$ samples) and cold-water corals ($n = 3$ samples) (MANOVA, $p = 0.618$). This indicates use of similar carbohydrate components during mucus synthesis by corals largely irrespective of energy supply mechanisms in zooxanthellate or azooxanthellate corals. The carbohydrate composition similarity of mucus from both corals indicates that this material may function as a cosmopolitan trophic link for intense interactions between the different reef organisms.

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Organic matter release by dominant hermatypic corals of the Northern Red Sea

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Abstract

Particulate organic matter (POM) and dissolved organic carbon (DOC) release by 6 dominant hermatypic coral genera (*Acropora*, *Fungia*, *Goniastrea*, *Millepora*, *Pocillopora* and *Stylophora*) were measured under undisturbed conditions by laboratory incubations during 4 seasonal expeditions to the Northern Red Sea. In addition, the influence of environmental factors (water temperature, light availability and ambient inorganic nutrient concentrations) was evaluated. Particulate organic carbon (POC) and particulate nitrogen (PN) release were always detectable and genus-specific, with *Stylophora* releasing most POM (6.5 mg POC and 0.5 mg PN m^{-2} coral surface area h^{-1}) during all seasons. The fire coral *Millepora* released significantly less POM (0.3 mg POC and 0.04 mg PN m^{-2} coral surface area h^{-1}) than all investigated anthozoan genera. The average POC:PN ratio of POM released by all coral genera was 12 ± 1 indicating high carbon / low nitrogen content of coral-derived organic matter. POM release showed little seasonal variation, but average values of POC and PN release rates correlated with water temperature, light availability and ambient nitrate concentrations. DOC net release and elevated DOC:POC ratios were detectable for *Acropora*, *Goniastrea* and *Millepora*, revealing maximum values for *Acropora* (30.7 mg DOC m^{-2} coral surface area h^{-1}), whilst predominant DOC uptake was observed for *Pocillopora*, *Fungia* and *Stylophora*. Depth-mediated light availability influenced DOC fluxes of *Acropora* and *Fungia*, while fluctuations in water temperature and ambient inorganic nutrient concentrations showed no correlation. These comprehensive data provide an important basis for the understanding of coral reef organic matter dynamics and relevant environmental factors.

Introduction

Scleractinian corals of tropical and cold water reef ecosystems are known to release organic matter in particulate and dissolved forms into the surrounding seawater (Crossland 1987; Ferrier-Pages et al. 1998; Wild et al. 2008). The bulk of organic matter released by tropical reef corals originates from coral mucus (Crossland 1987), a transparent exopolymer principally composed of glycoproteins and lipids (Krupp 1985; Meikle et al. 1987). Coral mucus is continuously synthesised by mucus gland cells (mucocytes) located in the coral ectoderm (Marshall and Wright 1993), which subsequently exude the mucus onto the epidermal tissue surface. Synthesis of mucus by corals relies on energy-rich photosynthates, which are transferred by the phototrophic endosymbiotic zooxanthellae and stored in the lipid pools of the host (Brown and Bythell 2005). Mucus on the coral surface serves as a transport medium for mucociliary feeding and sediment shedding processes (Schuhmacher 1977), and as a surface protection layer against desiccation during air-exposure (Krupp 1984), harmful UV-radiation (Drollet et al. 1997) and invasive microbes (Ritchie 2006). Once exuded by the mucocytes, a substantial fraction of the mucus enters the dissolved organic matter (DOM) pool of reef waters (Wild et al. 2004a), while the remaining particulate fraction is exposed to physical environmental factors (e.g., water current) ultimately leading to detachment from coral surfaces followed by the formation of suspended mucus aggregates (Wild et al. 2004a; Wild et al. 2005a). This particulate organic matter (POM) released by corals can dominate the suspended matter pool of reef waters (Marshall 1968), thereby contributing to reef ecosystem functioning (Wild et al. 2005b). Coral mucus can act as important energy and nutrient carrier in benthic-pelagic coupling processes (Wild et al. 2004a; Naumann et al. 2009a), influences planktonic and benthic (in particular microbial) metabolism (Wild et al. 2005a; Huettel et al. 2006; Wild et al. 2009a), and facilitates the fast recycling of essential nutrients via the initiation of element cycles (Ferrier-Pages et al. 2000; Wild et al. 2004b; Wild et al. 2005a). Therefore, quantification of coral-derived organic matter release represents a fundamental basis for the understanding of coral reef element cycles and ecosystem functioning.

However, little information is available on species-specific POM and DOM release rates and relevant environmental factors. Although a number of previous studies have investigated coral-derived organic matter release, most of these studies focused on single coral species (Herndl and Velimirov 1986; Ferrier-Pages et al. 1998) or only reported total organic matter release (e.g., Johannes 1967; Richman et al. 1975), rather than the fractionation of mucus into POM and DOM. Only Crossland (1987) and Tanaka et al. (2008) have measured the fractionated release of coral-derived organic matter in terms of POM and DOM by two warm water coral species, respectively.

To date, two studies have quantified coral-derived organic matter release in relation to environmental factors. Wild et al. (2005b) showed that POM release rates by two species of *Acropora* from the Great Barrier Reef (Australia) increased during aerial exposure, while Crossland (1987) showed that in situ organic carbon release by two coral species (*Acropora variabilis* and *Stylophora pistillata*) was strongly dependent on the availability of light under submerged conditions.

The present study presents a comprehensive dataset on the release of POC, PN and DOC by dominant hermatypic corals on a Northern Red Sea fringing reef under conditions similar to those in situ. The release rates of coral-derived organic matter in incubation experiments was evaluated in the context of environmental factors (water temperature, light availability and ambient inorganic nutrient concentrations) measured during four independent seasonal periods.

Material and methods

Study site

These studies were carried out during four visits to the Marine Science Station (MSS) at Aqaba, Jordan (latitude: 29°27′N, longitude: 34°58′E). MSS is situated approximately 10 km south of the city of Aqaba with exclusive access to a marine reserve including a typical Red Sea fringing coral reef. These visits occurred during four seasons: November–December 2006 (fall), August–September 2007 (summer), February–March 2008 (winter) and May 2008 (spring).

Environmental monitoring

Environmental monitoring was carried out during the entire study period (November 2006 to May 2008). Temperature at 10 m water depth was measured in 30 min intervals by data loggers (Onset HOBO® Water Temp Pro v2; accuracy: ± 0.2°C). Monthly measurements of inorganic nutrient concentrations in surface seawater samples (water depth: 1 m) were conducted by the MSS staff according to Grasshoff et al. (1999). Light availability was assessed in triplicates in water depths of 1, 5, 10 and 20 m (spring, summer and winter), and at 5 m depth during fall season using data loggers (Onset HOBO® Pendant UA-002-64; spectral detection range: 150–1200 nm).

Identification of dominant hermatypic coral genera

The dominant hermatypic coral genera in the study area were identified using line-point intercept (LPI) transect approaches of Loya (1978) and Nadon and Stirling (2006). Duplicate transects (n = 44) of 50 m length with regular 0.5 m point intervals were conducted at 0.5, 1.0, 5.0, 10.0 and 20.0 m water depth parallel to the reef crest. Percentage coverage data revealed that the anthozoans *Acropora*, *Fungia*, *Goniastrea*, *Pocillopora* and *Stylophora*, and the hydrozoan *Millepora*, were among the top 10 dominant coral genera at all depths, accounting for 24–66% (mean ≈ 45%) of total live coral coverage depending on water depth.

Coral samples

During each of 4 seasonal sampling periods, fragments from 5 different coral colonies of each of the dominant hermatypic coral genera or 5 individual *Fungia* polyps were collected from 5 m water depth using pliers or hammer and chisel, if necessary. In addition, corals of the genera *Acropora* and *Fungia* were sampled from 1, 10 and 20 m water depth in replicates of 5 during spring and winter season, respectively. Colonies infested by endolithic bioeroders (e.g. boring bivalves; Lazar and Loya 1991) were excluded. Coral samples from 5 m water depth were transported to the laboratory avoiding aerial exposure and kept in a 1000 l maintenance tank supplied with freshly pumped seawater (flow-through rate ~ 20 l min^{-1}) at in situ seasonal temperature (annual range: 21 (winter) – 29 (summer) °C), salinity (43 ± 1) and irradiance (integrated daylight average: 216 (winter) – 400 (summer) µmol photons m^{-2} s^{-1}). Temperature in maintenance tanks was constantly monitored by data loggers and corresponded to in situ conditions measured in parallel. Light levels were adjusted to in situ conditions by using layers of black plastic gauze covering the cultivation tanks. Samples of branching (*Acropora*, *Millepora*, *Pocillopora* and *Stylophora*; length: 4–8 cm) and massive (*Goniastrea*; diameter: 5–8 cm) corals were affixed to ceramic tiles (4 x 4 cm) using small amounts of coral glue (Reef Construct, Aqua Medic®) to reduce mechanical stress during experimental handling. Corals collected from 5 m depth were subsequently allowed to heal and acclimatise in the maintenance tanks for 2 weeks. During this time, any epibionts on ceramic tiles and glue junction were removed using a soft tooth brush. Fragments of *Acropora* specimens (length: 4–8 cm) sampled from 1, 10 and 20 m depth were treated similarly, but were subsequently transported back to the reef at their original water depths to heal under in situ conditions. These were returned to the laboratory on the evening before the day of experiments. *Fungia* polyps (diameter: 6–9 cm) from 1, 10 and 20 m depth were collected from the reef during dusk of the day prior to incubation. The skeletal surface area of all corals was measured using geometric techniques (Naumann et al. 2009b); advanced geometry for *Acropora*, *Millepora*, *Pocillopora* and *Stylophora*, and simple geometry for *Fungia* and *Goniastrea*) using respective approximation factors. The overall mean surface area was 122 ± 63 cm^2 (average ± SD) for all incubated corals.

Quantification of organic matter release

The release of organic matter by the 6 coral genera was measured in the laboratory using the beaker incubation method (Herndl and Velimirov 1986; Wild et al. 2005b) during all 4 seasonal periods. Beaker incubations were carried out during daylight hours (10:00–16:00 hrs). In addition, 2 night incubations (20:00–02:00 hrs) were done using *Acropora* and *Fungia* corals from 5 m water depth. Only samples from a single coral genus were incubated on a given day. Ceramic tiles and glue junctions were cleaned using a soft tooth brush and coral colony surfaces were exposed to a smooth stream of seawater inside the cultivation tanks to remove attached organic and inorganic particles immediately before experiments. The corals were transferred without aerial exposure into acetone-cleaned and seawater-rinsed 1000 ml glass beakers filled with 830–987 ml seawater, fully submerging the corals. Beakers containing corals (n = 5) and control beakers containing only (1000 ml) of fresh seawater (n = 5) were placed in a water bath continuously flushed with seawater freshly pumped from the reef. Control beakers did not contain ceramic tiles or glue samples, as preliminary studies indicated that these materials did not affect particulate and dissolved organic matter concentrations. The water bath containing the incubation beakers was covered with transparent cellophane foil to avoid the input of airborne particles, leaving 2 small side openings for air exchange. Light conditions were adjusted to those at water depths of 1, 5, 10 and 20 m using layers of black plastic gauze. Temperature inside the water bath was continuously monitored using data loggers and corresponded to in situ conditions due to the continuous flow-through of freshly pumped in situ seawater. To allow comparisons with

previous studies (Herndl and Velimirov 1986; Wild et al. 2005b) and to rule out the influence of water currents on organic matter release beaker contents were not stirred during incubations. Powder-free gloves were used during all experimental procedures to prevent contamination of the incubation water.
After 6 h corals were removed from the incubation beakers and transferred back to the maintenance tank. DOC samples were immediately taken from the incubation water. The content of coral and control incubation beakers was thoroughly homogenise and sterile syringes were used to collect one 10 ml water sample from each beaker. These samples were subsequently filtered through 0.2-µm-pore-sized sterile polyethersulfone membrane filters. The first 5 ml of sample were used to wash the filter membrane, and the filtrate was discarded. The remaining sample (5 ml) was filtered dropwise into pre-combusted (450°C; 4 h) amber glass vials. These samples were kept frozen at –20°C until analysis by high-temperature catalytic oxidation (HTCO) using a Rosemount Dohrmann DC-190 TOC analyser. Non-purgable organic carbon was measured after acidification with orthophosphoric acid (20%) to pH<2 and sparging with oxygen. A certified TOC standard (potassium hydrogen phthalate, ULTRA Scientific, cat. no. IQC-106-5) was used for 10-point instrument calibration and as a regular quality control after every four samples. Analytical precision was <3% of the certified value Following DOC sample collection, dissolved O_2 concentration in all beakers was measured using an O_2 optode (Hach Lange, HQ 10) to ensure that oxic conditions (>92% O_2 saturation) existed in coral incubations and controls of all conducted experiments.
The remaining seawater in the incubations (770–900 ml for coral treatments and 900 ml for controls) was used for POM determination. No later than 3 h after incubation, these samples were collected on pre-combusted (450°C; 4 h) GF/F filters (Whatman™, 25 mm diameter), dried at 40°C for at least 48 h and subsequently analysed using a Thermo™ NA 2500 elemental analyzer. Contents of POC and PN were derived from calculation using certified elemental standards (atropine for N, cyclohexanone-2,4-dinitrophenylhydrazone for C; Thermo Quest; analytical precision <3% of the certified value). As the presence of particulate inorganic carbon could be ruled out by test measurements of the incubation water, samples were not treated with HCl prior to analysis.

Data analysis

For calculation of organic matter release rates (POC and PN as well as DOC) per coral surface area and incubation time, values of control beakers were subtracted from those measured in the incubation water of the beakers containing the corals. Resulting net concentrations of coral incubations were normalised to coral surface area and incubation time for each coral to generate organic matter release rates. POC:PN ratios for coral-derived organic matter were calculated from molar contents of POC and PN. Organic matter release rates were compared on inter-generic and intra-generic levels applying one-way ANOVA combined with Fisher LSD post hoc tests inside SPSS© software. Correlation to variable environmental factors was assessed by linear regression analysis (evaluated by ANOVA) and Spearman rank-order correlation. Statistical results were regarded as significant at $p<0.05$, unless stated differently.

Results

Environmental monitoring

Seawater temperature during the study period ranged from 21–29°C with highest values during summer (July–August) and minimum values during winter and early spring (January–March). Integrated average daytime (10:00–16:00 hrs) light availability at 5 m water depth

varied between 216–400 µmol photons m^{-2} s^{-1} from winter to summer season, respectively. Depth-mediated average light availability ranged from 527 (1 m depth) to 78 (20 m depth) µmol photons m^{-2} s^{-1} during winter, and 946 (1 m depth) to 144 (20 m depth) µmol photons m^{-2} s^{-1} during summer. Ambient inorganic nutrient concentrations reflected the hydrographic setting of the study area, where deep advection and convection of the water column regularly occur during winter season (Wolf-Vecht et al. 1992). As a result, concentrations of nitrate, nitrite, ammonium and phosphate in surface waters showed peak values during winter (mid-season average ± SD: 0.83 ± 0.10, 0.18 ± 0.06, 0.31 ± 0.01 and 0.07 ± 0.01 µmol l^{-1}, respectively) and reached a minimum during summer season (0.14 ± 0.04, 0.021 ± 0.001, 0.21 ± 0.02 and 0.0319 ± 0.0004 µmol l^{-1}, respectively).

POM release

POM concentrations measured in coral beakers were significantly increased in comparison to controls. Including all conducted experiments, POC and PN concentrations in coral incubated seawater (0.32 ± 0.04 and 0.035 ± 0.003 mg l^{-1}, average ± SE, n = 118, respectively) were more than 2-fold higher than in control beakers (0.13 ± 0.03 and 0.015 ± 0.003 mg l^{-1}, average ± SE, n = 120, respectively). Consequently, POM release was detectable for all investigated corals originating from 5 m water depth and during all seasons (Table 1). POC:PN ratios for coral-derived POM (5–20) were significantly elevated in comparison to seawater controls (4–12), indicating higher carbon / lower nitrogen contents. POC release rates, averaged over all investigated seasons, were significantly genus-specific, except for *Acropora*, *Goniastrea* and *Fungia* corals (Table 1). Branching scleractinians (*Acropora*, *Pocillopora* and *Stylophora*) were the dominant POC and PN exuding growth forms, followed by massive *Goniastrea*. Overall average POC release calculated from summer and fall season results representing full genera coverage accounted for 2.6 ± 0.6 mg POC m^{-2} coral surface area h^{-1} (average ± SE). *Stylophora* and *Millepora* corals showed the highest and lowest POC and PN release rates, respectively. On average, *Stylophora* POM release rates exceeded those of *Millepora* 19 and 11-fold, for POC and PN respectively. Except for the direct comparison of *Acropora, Goniastrea* and *Fungia*, annual-averaged PN release also showed significant differences in the comparisons of all investigated coral genera. Taking into account results from summer and fall experiments only, the overall average PN release rate amounted to 0.27 ± 0.05 mg PN m^{-2} coral surface area h^{-1} (average ± SE, Table 1).

Table 1 *Particulate organic matter release rates and ratios (average ± SE) of 6 dominant hermatypic coral genera from 5 m water depth for all investigated seasons (POC = particulate organic carbon, PN = particulate nitrogen)*

Coral	Seasons	POC release (mg m^{-2} h^{-1})	PN release (mg m^{-2} h^{-1})	POC:PN	n
Acropora	Spring	1.2 ± 0.3	0.11 ± 0.04	7 ± 2	4
	Summer	2.9 ± 0.4	0.42 ± 0.12	9 ± 1	5
	Fall	2.0 ± 0.3	0.20 ± 0.05	15 ± 1	4
	Winter	1.0 ± 0.1	0.06 ± 0.22	5 ± 2	5
	Mean	1.8 ± 0.4	0.20 ± 0.08	11 ± 1	
Fungia	Spring	1.2 ± 0.6	0.18 ± 0.17	9 ± 3	5
	Summer	1.6 ± 0.7	0.32 ± 0.07	12 ± 3	5
	Fall	1.4 ± 0.9	0.20 ± 0.10	12 ± 2	5
	Winter	0.9 ± 0.1	0.26 ± 0.13	10 ± 2	5
	Mean	1.3 ± 0.2	0.21 ± 0.04	11 ± 1	

Stylophora	Spring Summer Fall Winter	6.3 ± 0.8 7.0 ± 0.8 6.6 ± 1.6 6.0 ± 2.5	0.52 ± 0.03 0.55 ± 0.14 0.52 ± 0.10 0.47 ± 0.16	14 ± 3 12 ± 3 17 ± 1 15 ± 1	5 4 4 5
	Mean	6.5 ± 2.1	0.52 ± 0.02	15 ± 1	
Goniastrea	Summer Fall Winter	0.9 ± 0.1 1.8 ± 0.7 1.5 ± 1.2	0.07 ± 0.01 0.22 ± 0.11 0.08 ± 0.15	14 ± 1 11 ± 3 11 ± 1	5 5 5
	Mean	1.4 ± 0.3	0.12 ± 0.05	12 ± 1	
Pocillopora	Summer Fall	3.9 ± 0.3 2.8 ± 0.4	0.24 ± 0.01 0.32 ± 0.11	20 ± 1 14 ± 1	5 5
	Mean	3.3 ± 0.5	0.28 ± 0.04	17 ± 3	
Millepora	Summer Fall	0.3 ± 0.1 0.3 ± 0.3	0.03 ± 0.01 0.04 ± 0.03	10 ± 2 11 ± 2	5 5
	Mean	0.3 ± 0.1	0.04 ± 0.01	11 ± 1	
Mean Summer / Fall		**2.6 ± 0.6**	**0.27 ± 0.05**	**13 ± 1**	**∑ = 91**

Corals investigated during all seasons (*Acropora*, *Fungia* and *Stylophora*) showed no significant seasonal variation in POC and PN release (Fig. 1a–d), except for *Acropora*, which displayed lower POC release rates during winter (Fig. 1b). However, linear regression analysis of average POC and PN release rates and variable seasonal parameters (temperature and daytime average light availability at 5 m depth) revealed correlations for these genera (Fig. 2a, b). An exception in this regard was found for light-dependency of PN release by *Stylophora* corals (r^2 = 0.788; p = 0.112 by regression ANOVA), otherwise showing correlation to environmental factors (temperature: POC (r^2 = 0.959; p = 0.020), PN (r^2 = 0.988; p = 0.006); light availability: POC (r^2 = 0.982; p = 0.009)). Seasonal fluctuations in ambient nitrate concentrations correlated significantly to POC and PN release rates for *Acropora*, *Fungia* and *Stylophora* corals (Spearman rank-order correlation, 2-tailed, $p<0.001$). Increase in nitrate concentrations resulted in a decrease in POC and PN release (Fig. 2c); while seasonally variable concentrations of other inorganic nutrients (i.e., nitrite, ammonium and phosphate) showed no correlation (Spearman rank-order correlation, 2-tailed, $p>0.05$).

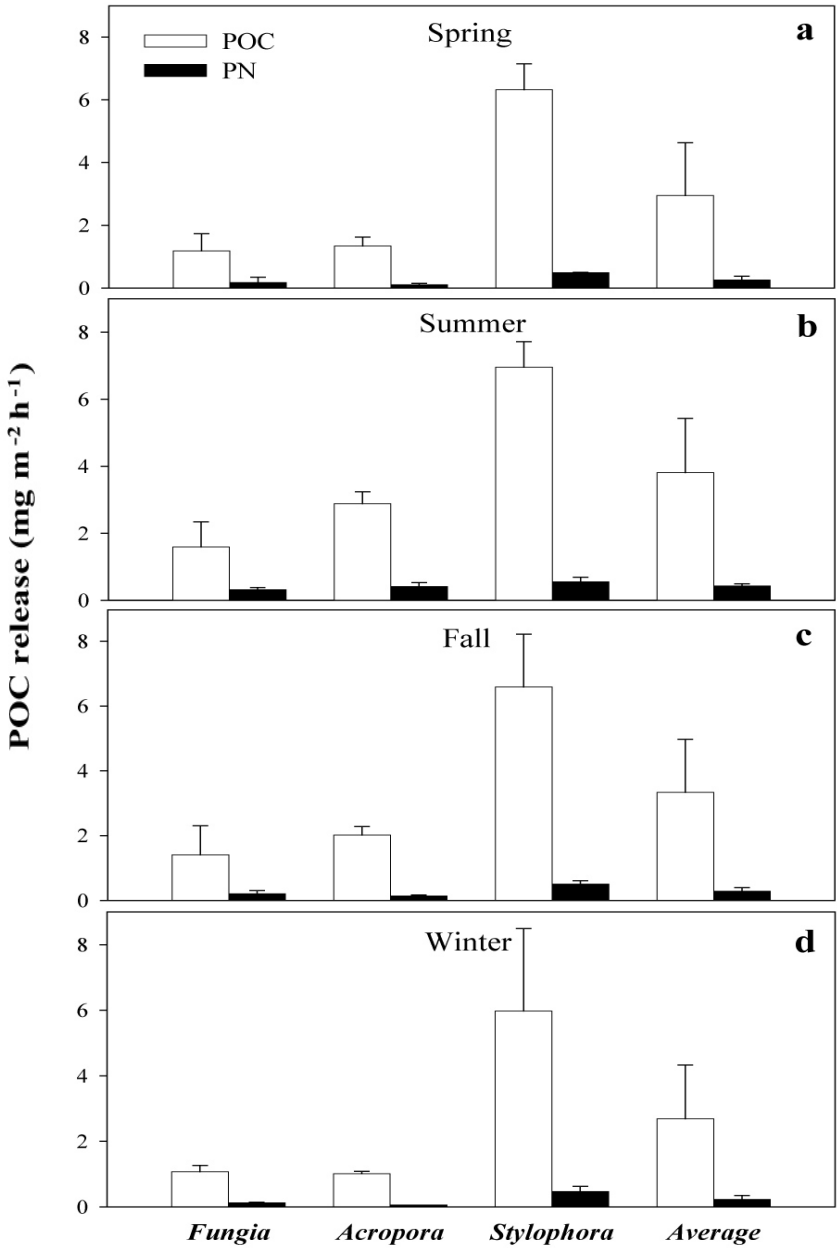

Figure 1 *Genus-specific average seasonal particulate organic matter release rates (POC and PN) for Acropora, Fungia and Stylophora corals from the investigated Red Sea fringing reef. a: spring; b: summer; c: fall; d: winter. Values are given as average ± SE for POC and PN release rates of n ≥ 4 coral specimens per genus and season.*

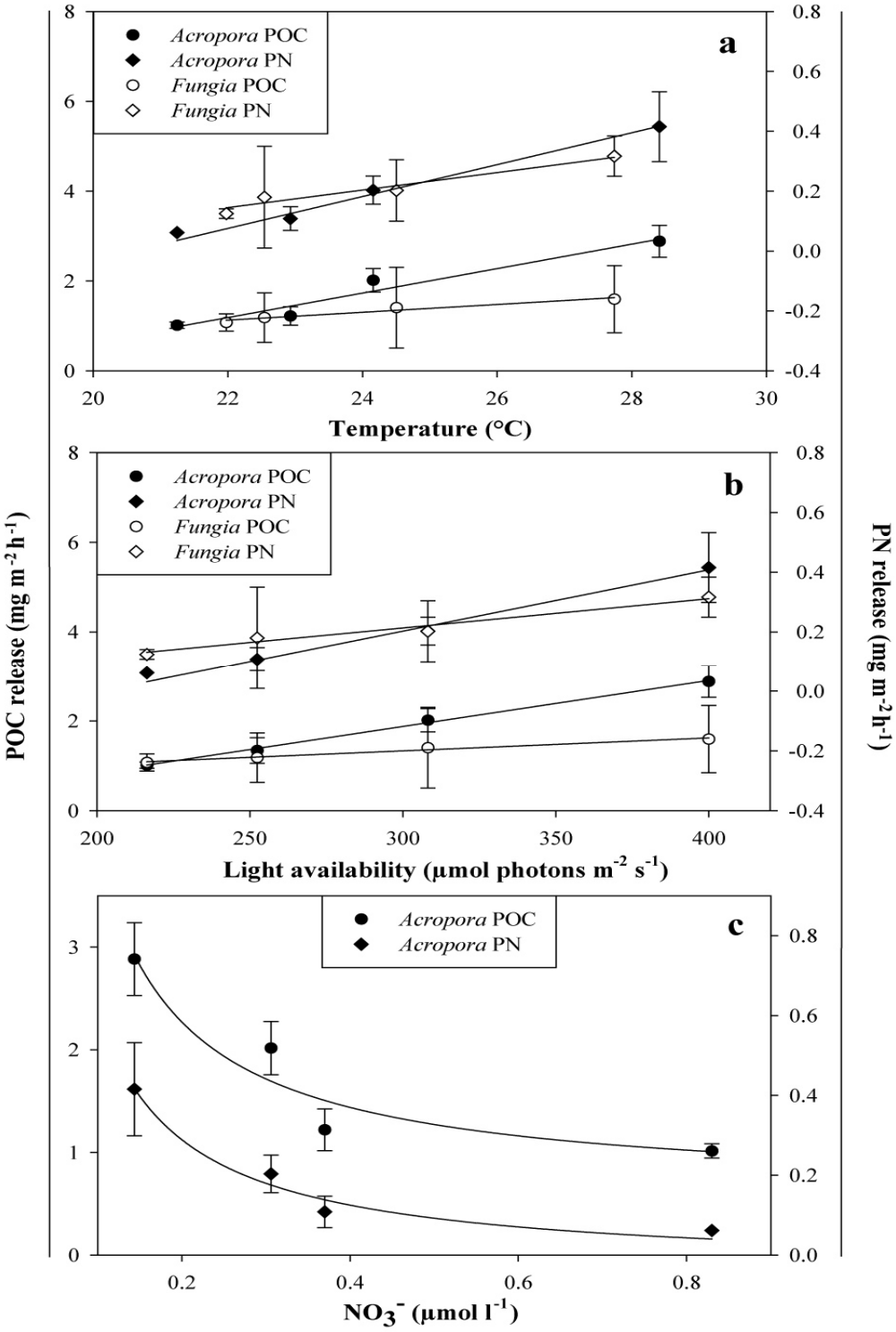

Fig. 2 *POM release rates by corals from 5 m water depth influenced by seasonal fluctuations in some environmental parameters. a: temperature; b: light availability; c: in situ seawater nitrate concentration. Regression analysis of average POC and PN release rates, a: Acropora (POC (r^2 = 0.953; p = 0.024), PN (r^2 = 0.988; p = 0.006)); Fungia (POC (r^2 = 0.923; p = 0.039), PN (r^2 = 0.967; p = 0.016)); b: Acropora (POC (r^2 = 0.989; p = 0.006), PN (r^2 = 0.987; p = 0.006)); Fungia (POC (r^2 = 0.918; p = 0.042), PN (r^2 = 0.969; p = 0.016)). Curve fitting in panel c conducted by general power function ($y = a * x^b$, where x is in situ seawater nitrate concentration and y is Acropora POC and PN release) inside SigmaPlot© SPSS Inc. software packages.*

Average POC and PN release rates of *Acropora* and *Fungia* corals originating from different water depth (1–20 m) were positively correlated (Spearman rank-order correlation, 2-tailed, $p<0.05$) to depth-mediated light availability (Fig. 3a). However, this was only statistically significant for *Acropora* corals originating from 1 m depth in comparison to other investigated depths (10 and 20 m) and during night time. POC content of released POM increased with increasing irradiance for both corals (Fig. 3a), as reflected by POC:PN ratios ranging from 17 to 8 and 11 to 8 for irradiance representative of 1 m water depth and night incubation for *Acropora* and *Fungia*, respectively (data not shown).

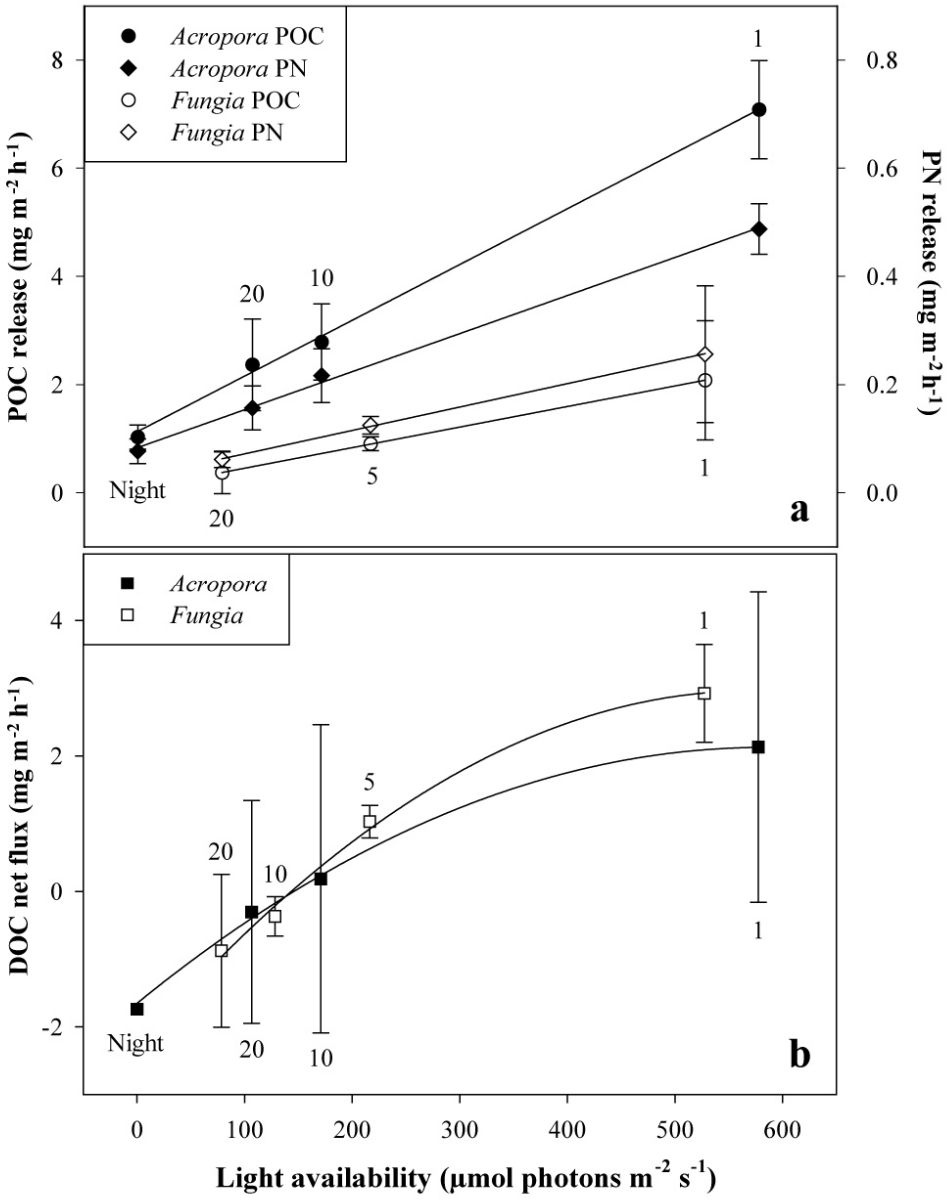

Figure 3. *POC, PN and dissolved organic carbon (DOC) release rates for Acropora and Fungia corals at different light regimes. a: POC and PN release; b: DOC net flux, curve fitting conducted by quadratic polynomial function ($y = y_0 + a * x + b * x^2$, where x is in situ light availability and y is coral DOC net flux) inside SigmaPlot© SPPS Inc. software packages. Numbers above and below data points represent the original water depth (m) of incubated corals and the corresponding light availability applied during incubations.*

DOC release

DOC concentrations measured from coral and control beakers ranged from 0.8–28.9 mg l^{-1} and were significantly higher than the respective POC concentrations (0.04–0.84 mg l^{-1}). DOC in control and coral beakers ranged from 1.0 to 2.6 mg l^{-1} and 0.8 to 5.2 mg l^{-1}, respectively, with the exception of fall season samplings, when DOC concentrations were significantly elevated in control (1.7–29.6 mg l^{-1}) and coral beakers (1.8–28.9 mg l^{-1}). Higher DOC concentrations in control compared to coral beakers were measured in 39% of all incubations, which consequently resulted in the calculation of negative DOC fluxes, i.e. DOC uptake, for 50% of the investigated genera (*Fungia*, *Pocillopora* and *Stylophora*). Average DOC net release was detectable and similar, i.e. not significantly different, for *Acropora* (30.7 ± 27.4 mg DOC m^{-2} coral surface area h^{-1}, average ± SE), *Goniastrea* (22.0 ± 67.9) and *Millepora* (9.2 ± 12.8). *Pocillopora* corals showed DOC uptake throughout all seasons (-263.4 ± 105.2 mg DOC m^{-2} coral surface area h^{-1}), which was significantly increased compared to all remaining genera ($p<0.001$). *Fungia* and *Stylophora* displayed nearly identical DOC uptake rates on annual average (-14.2 ± 5.5 and -14.1 ± 12.8 mg DOC m^{-2} coral surface area h^{-1}, respectively). Although DOC fluxes were highly variable for all genera, significant seasonal variation was only detectable for *Fungia*, which showed increased DOC uptake during fall (-38.5 ± 5.3 mg DOC m^{-2} coral surface area h^{-1}) in comparison to summer, winter and spring seasons (-5.4 ± 1.1, 1.0 ± 0.2 and -1.8 ± 0.6 mg DOC m^{-2} coral surface area h^{-1}, respectively). In the majority of cases where DOC net release was detected, the substantial part of organic carbon was released in form of DOC, as evident from high DOC:POC ratios (20–50). Analysis of DOC release rates revealed a positive trend with increasing light availability for *Acropora* and *Fungia* corals (Spearman rank-order correlation, 2-tailed, $p<0.001$). Low irradiance (at 10 and 20 m water depth and night time) resulted in DOC uptake, while corals from shallow waters (5 and 1 m water depth) released DOC with indication of a saturation level reached at around 1 m water depth (Fig. 3b). DOC fluxes of all genera showed neither correlation to variable seasonal inorganic nutrient concentrations nor to temperature fluctuations (Spearman rank-order correlation, 2-tailed, $p>0.05$).

Discussion

The results presented here provide the first comprehensive overview of organic matter release rates by hermatypic corals in warm water coral reef ecosystems. Net flux rates of organic matter (POC, PN and DOC) are presented for 6 dominant coral genera (*Acropora*, *Fungia*, *Goniastrea*, *Millepora*, *Pocillopora* and *Stylophora*), together representing approximately 45% of total live coral coverage between 0.5 and 20 m water depth in a Red Sea fringing reef. In addition, the influence of variable environmental factors (water temperature, light availability and ambient inorganic nutrient concentrations) on organic matter release was investigated by seasonal and manipulative incubation experiments.

Genus-specific POM release

All coral genera investigated by the present study released POM to the surrounding seawater, thereby providing a source of energy-rich carbon and nitrogen compounds (Benson and Muscatine 1974) to reef trophodynamics (Wild et al. 2004a, 2004b). The released POM was predominantly composed of POC, as shown by POC:PN ratios (5–20). The wide range of POC:PN ratios may be indicative for the heterogeneity of coral-derived POM, as reviewed in Coffroth (1990), and is in agreement with some results from previous studies. Wild et al. (2004a) found molar C:N ratios of 5–14 for the particulate fraction of *Acropora* coral mucus, while Tanaka et al. (2008) mentioned molar C:N ratios of 16 and 19 for POM released by *Acropora pulchra* and *Porites cylindrica*, respectively.

The finding that branching anthozoan coral genera (*Acropora*, *Pocillopora* and *Stylophora*) showed similar or higher POM release than the massive genus (*Goniastrea*) (Table 1), contradicts the results of Richman et al. (1975), who reported enhanced mucus release rates for several massive coral genera in comparison to *Acropora variabilis* and *Stylophora pistillata* in the study region. Unfortunately, mucus release rates by Richman et al. (1975) were not normalised to coral surface area, but to "coral head", thus these results cannot be directly compared to values obtained here. Nevertheless, the comparably invasive quantification technique of enclosing coral heads in situ using plastic bags (Richman et al. 1975) may have increased organic matter release of massive coral genera by exposure of larger tissue areas to mechanical stress through contact with the plastic cover.

POM release rates of all coral genera were in the range reported by Wild et al. (2005b), who also used the beaker incubation technique. In particular, POC and PN release of *Acropora* corals from shallow (water depth: 1 m) Red Sea waters (7.1 ± 0.9 mg POC and 0.5 ± 0.1 mg PN m^{-2} coral surface area h^{-1}; Fig. 3b) are nearly identical to values generated by Wild et al. (2005b) for *Acropora aspera* (7 ± 3 mg POC and 0.8 ± 0.4 mg PN m^{-2} coral surface area h^{-1}) from the reef flat of Heron Island (Australia), possibly indicating conformity within this genus. In contrast, *Acropora pulchra* originating from a similar water depth in Japan was found to release substantially less (3.5 ± 0.5 and 0.3 ± 0.1 mg m^{-2} coral surface area h^{-1}) POC and PN, respectively (Tanaka et al. 2008). However, this large variation may account for methodological differences in the applied techniques, as Tanaka et al. (2008), contrary to all studies compared here, applied direct stirring during incubations accompanied by a prolonged experimental duration of 4 d. These factors may be responsible for an altered partitioning of coral-derived organic matter into POM and DOM fractions. POC release rates of *Acropora* corals from 5 m depth were comparable to values reported by Crossland (1987) for *Acropora variabilis* from the same water depth in the study region. Although different quantification techniques were applied, in situ perspex chamber incubations, carried out by Crossland (1987) during late summer, resulted in POC release rates (3.6 ± 0.4 mg POC m^{-2} coral surface area h^{-1}), which are similar to the summer values obtained by the present study (2.9 ± 0.4 mg POC m^{-2} coral surface area h^{-1}; Table 1). Nevertheless, the latter does not hold for summer POC release rates of *Stylophora* corals from 5 m water depth, which show substantially higher values (7.0 ± 0.8 mg C m^{-2} coral surface area h^{-1}) in comparison to *Stylophora pistillata* (3.5 ± 0.2 mg C m^{-2} coral surface area h^{-1}), obtained by Crossland (1987). This difference may be explained by a general variability within *Stylophora* corals assessed by the present study, possibly affected by the abundance of energy-providing zooxanthellae (Falkowski and Dubinsky 1981), as some *Stylophora* specimens demonstrated lower POC release rates ranging from 2.9 to 3.3 mg C m^{-2} coral surface area h^{-1} (data not shown).

POM release by the hermatypic hydrozoan *Millepora* was investigated for the first time in the present study. Wild et al. (2005a) investigated 12 hermatypic coral genera and found the highest occurrence of particulate mucus strings attached to the surface of *Millepora* colonies (72% of inspected corals). Surprisingly, POC as well as PN release rates of *Millepora,* measured here, were low in comparison to all investigated anthozoan genera (Table 1). This could be explained by the overall highest contribution of DOC to total organic carbon released by *Millepora* (DOC:POC ratio: 50 ± 9).

As established for coral mucus, hermatypic corals constantly release endosymbiotic zooxanthellae to the surrounding seawater (Hoegh-Guldberg et al. 1987; Stimson and Kinzie 1991) which consequently add to the measured POM release. However, Hoegh-Guldberg et al. (1987) showed that carbon loss due to expulsion of zooxanthellae represents a minor part of the daily budget of photosynthetically-fixed carbon by *Stylophora* corals (0.01%). Further, calculations applying zooxanthellae release rates measured by Stimson and Kinzie (1991) for *Pocillopora* corals and zooxanthellae carbon contents (0.3 ng POC $cell^{-1}$; Niggl et al. 2009) attest only a minor contribution (<5%) by zooxanthellae expulsion to the overall *Pocillopora*

POC release presented here. This confirms coral mucus as the bulk component of organic matter released by tropical reef corals (Crossland 1987).

Relevant environmental factors for POM release

Constant POM release rates found for the 3 year-round investigated coral genera (*Acropora*, *Fungia* and *Stylophora*), together representing 30% of total live coral coverage in the MSS reef, confirm a steady input of coral-derived POC and PN into organic matter cycles of Red Sea reef environments. Although seasonal differences in POC and PN release rates were not pronounced (Fig. 1), correlation between average POC and PN release values and seasonally variable temperature and light availability suggests an influence by these parameters (Fig. 2a, b). Variation of temperature within the accustomed annual range influences specific components of coral metabolism (i.e., calcification and tissue carbon incorporation) (Crossland 1984), and may thus as well affect temperature-sensitive metabolic processes regulating extracellular organic matter release. The significant influence of light availability on POC release rates is confirmed by Crossland (1987), who studied organic carbon release under different light regimes and reported a substantial decrease in POC release with reduced irradiance. The effect of depth-mediated light availability is additionally highlighted by incubation experiments with *Acropora* from different water depths carried out here. Corals originating from 1 m depth released significantly more POC and PN than their counterparts in 10 and 20 m depth, and during the night (Fig. 3a). With the reduction of light availability between 1 and 20 m water depth, POC release also decreased by 66%. This is comparable to results obtained by Crossland (1987), who measured a loss of 40% POC release caused by a reduction of 69% irradiance between 5 and 23 m water depth. In addition, variable POC:PN ratios (8 – 17) for coral-derived POM at different water depths point to light availability as an environmental key factor influencing not only the quantity, but also the composition of coral-derived POM. Consequently, the results of the present study emphasise the important role of the light-dependent phototrophic zooxanthellae symbionts by supplying the coral host with photosynthates required for the synthesis and ensuing release of POM.

Significant seasonal variations in ambient inorganic nutrient concentrations have already been described by previous investigations in the study area (Rasheed et al. 2002). Corals are able to take up inorganic nutrients from ambient seawater to cover specific metabolic demands (Hoegh-Guldberg and Williamson 1999; Grover et al. 2002). Except for nitrate, seasonal concentration differences in inorganic nutrients showed no significant influence on POM release rates, which indicates that nitrate represents another key factor for coral-derived organic matter release. Increased nitrate concentrations during winter and spring season coincided with a decrease in coral-derived POM release, as shown here for *Acropora* corals (Fig. 2c). Nitrate represents an important source of nitrogen for hermatypic corals and is predominantly assimilated by the symbiotic zooxanthellae (Grover et al. 2003). In nutrient-poor reef waters, zooxanthellae growth is primarily limited by ambient nitrate concentrations, while low phosphate levels, as found for the study site, represent no significant limitation factor (Muscatine et al. 1989). As a consequence of nitrate limitation, zooxanthellae transfer most of the photosynthetically acquired carbon to the coral host, which can serve as an explanation for the observed increase in POM release during summer. Elevated nitrate concentrations during winter season may promote a nutrient-demanding zooxanthellae population growth, consequently resulting in a decreased transfer of photosynthates to the coral host, thereby impairing the release of POM, as shown for decreasing coral calcification rates (Marubini and Davies 1996). Reduced supply of photosynthates by the symbionts can be compensated by coral heterotrophy (Sebens et al. 1996). However, as seasonal variations in the abundance of prey organisms (i.e. zooplankton) are not pronounced in the study region (Yahel et al. 2005), the measured decrease in POM release during winter season seems, at least for *Acropora*, to be uncoupled from heterotrophic sources.

DOC flux

Only half of the investigated coral genera (*Acropora*, *Goniastrea* and *Millepora*) showed DOC net release. The remaining genera displayed a negative DOC balance after quantitative subtraction of seawater control contents, consequently concluding DOC uptake during incubation. Ferrier-Pages et al. (1998) demonstrate the role of coral-associated bacteria for substantial DOC uptake, measured for the coral *Galaxea fascicularis*. Similar processes likely explain the DOC uptake observed here for *Fungia*, *Pocillopora* and *Stylophora*. Differences in DOC uptake rates between coral genera may result from different bacterial community compositions (Rohwer et al. 2002) or by the dominance of a certain bacterial group, predominantly responsible for DOC uptake in certain genera (e.g. *Pocillopora*). In addition, variable DOC uptake capacities by different coral hosts (i.e. coral–zooxanthellae) may add to the observed genus-specific variability (Al-Moghrabi et al. 1993).

The majority of corals showing a positive DOC balance (DOC net release) released more DOC than POC to the surrounding water, indicating strong dissolution of organic matter (coral mucus) exuded by these corals (Wild et al. 2004a). This stands in contrast to Tanaka et al. (2008), who reported a higher contribution of POC to total organic carbon release, which may suggest species-specific characteristics of mucus synthesis and composition as a possible influence on dissolution ratios of coral-derived organic matter. Seasonal differences in DOC flux rates were only detectable for *Fungia*. This indicates that significant variability of ambient DOC concentrations, as measured here during fall season and concomitantly in situ by Wild et al. (2009b) may not significantly affect DOC flux rates for the majority of dominant hermatypic coral genera. DOC net release rates determined for *Acropora* corals during summer season are only half (4.0 ± 0.3 mg DOC m^{-2} coral surface area h^{-1}) of values reported by Crossland (1987) for *Acropora variabilis* during late summer (9.3 ± 1.4 mg DOC m^{-2} coral surface area h^{-1}), but twice as high as release rates found for *Acropora pulchra* (1.9 ± 0.2 mg DOC m^{-2} coral surface area h^{-1}) (Tanaka et al. 2008). This variability may result from *Acropora* species-specific associations to different clades of zooxanthellae symbionts, which have been shown to provide a clade-specific photosynthate composition transferred to the coral host (Loram et al. 2007). Clade-specific photosynthates may therefore affect the structural composition of coral mucus, its dissolution characteristics and the ensuing release of coral-derived DOC. However, as detailed in the following, variations in light availability provided during incubations, may as well be responsible for the observed differences in DOC release rates for *Acropora* species by the above compared studies.

Depth-mediated light availability influences DOC release rates of *Acropora* and *Fungia* corals, resulting in higher DOC release with increasing light availability and DOC uptake at low light conditions (10, 20 m water depth and during the night). In the present study, this correlation has only been investigated for *Acropora* and *Fungia* corals (Fig. 3b). However, decreased DOC release with increasing water depth is confirmed by Crossland (1987), who described a comparable relationship for *Acropora variabilis* and *Stylophora pistillata* in the study area, nevertheless without detection of DOC uptake. At greater depth, reduced photosynthesis, attenuated translocation of photosynthates and lowered exudation of coral mucus may initiate the enhanced uptake of DOC from ambient seawater by coral-associated bacteria (Ferrier-Pages et al. 1998). Further, corals exposed to lower light levels show decreased fatty acid contents (Treignier et al. 2008), suggesting a reduced generation and supply of these lipid compounds to coral mucus synthesis and ensuing DOC release with increasing depth. Additional studies are necessary to fully resolve DOC flux dynamics within the coral holobiont (animal – zooxanthellae – bacteria) and the exchange between corals and a variable environment.

In conclusion, genus-specific organic matter release rates generated here for 6 dominant hermatypic Red Sea coral genera provide fundamental information for continuing studies in coral reef biogeochemistry including quantitative calculations to assess the role of coral-

derived organic matter in reef energy budgets. Apart from the identification of environmental key factors for organic matter release by hermatypic corals presented here, the relative extend to which the individual identified variable physical (light availability and temperature) and chemical (nitrate) parameters influence coral-derived organic matter release remains to be resolved. Further discrimination of the relative importance of individual key factors and synergistic effects will provide essential information required for the understanding of element cycles and ecosystem functioning of warm water coral reefs in a changing environment.

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8

Comparative investigation of organic matter release by corals and benthic reef algae – implications for pelagic and benthic microbial metabolism

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Abstract

Global climate change and direct anthropogenic stress factors lead to gradual replacement of hermatypic corals by benthic algae at many reef locations, a process which is commonly referred to as phase shift. Recent research showed that corals via the release of organic matter and concomitant effects on cycles of matter can act as engineers of reef ecosystems. There are strong indications that reef associated benthic algae do also affect reef ecosystem functioning via organic matter release, but relevant information is lacking. To gain a better understanding of the biogeochemical consequences such phase shifts may entail, a series of comparative studies with corals and algae was conducted in reefs of the Northern Red Sea during four seasonal expeditions in 2006-2008. These investigations focused on the quantity and quality of the organic matter released by both groups of organisms involving dissolved organic carbon (DOC), particulate organic carbon (POC) and nitrogen (PN) along with the respective stable isotope signatures. Planktonic and benthic degradation of the released material were investigated using bottle incubation experiments and in-situ stirred benthic chambers. First outcomes show clear differences between organic matter release by corals and algae, thus suggest effects of phase shifts onto reef biogeochemical cycles.

Introduction

It is generally assumed, that the global climate change along with direct anthropogenic factors like eutrophication and overfishing lead to phase shifts in coral reefs, i.e. the gradual replacement of reef building corals by benthic algae (Hoegh-Guldberg 1999, Hughes et al. 2003, Pandolfi et al. 2005, Hoegh-Guldberg et al. 2007, Hughes et al. 2007). Recent studies also showed that hermatypic corals can act as engineers of the entire reef ecosystem, particularly by the release of organic matter and associated effects on biogeochemical key processes and element cycles (Wild et al. 2004a, Wild et al. 2005b, Wild et al. 2008). This is a newly discovered aspect of corals as ecosystem engineers besides their long known ability to generate structural frameworks. Moreover, the work of Smith et al. (2006) indicates that benthic reef algae can also affect processes such as microbial activity in their surroundings via a hypothetical release of organic matter. Reef algae may therefore act as (new) reef ecosystem engineers, but likely in a very different way. This pilot study presents first data based on comparative investigations with the dominant corals and benthic reef algae from four expeditions to the Northern Red Sea comprising the following three interrelated approaches: 1) Quantification of dissolved and particulate organic matter (DOM and POM) release, 2) Determination of POM stable isotope signatures, 3) Planktonic and benthic degradation of released exudates. These data will provide first comparative information on the quantity and quality of benthic algae-derived organic matter and its subsequent degradation in the different compartments of the ecosystem coral reef.

Material and Methods

The work for this study was conducted during four seasonal expeditions (Nov/Dec 2006, Aug/Sep 2007, Feb/Mar 2008, May 2008) to Marine Science Station (MSS), Aqaba, Jordan. Collection of all specimens took place in the MSS fringing reef in water depths of 5 to 7 m. During each of the field trips, 5 replicate fragments (coral branch length: 6 to 10 cm) were broken off in-situ from colonies of the dominant hard corals of the genera *Acropora*, *Pocillopora* and *Stylophora*, which were allowed to heal in a flowthrough aquarium for at least 7 d prior to the subsequent experiments. In addition, 5 replicate small pieces (lengths: 6 to 14 cm) of the 3 most dominant types of benthic algae were collected in-situ: the green algae *Caulerpa* spec., the red algae *Peyssonnelia* spec., and typical filamentous turf algae consortia growing on dead coral skeletons. All algae were left in a flow-through aquarium for at least 12 h prior to the subsequent experiments for cleaning and healing purposes. For the organic matter release quantification the beaker incubation technique described by Herndl and Velimirov (1986) was used. Corals and benthic algae were separately transferred into acetone- and seawater-rinsed 1000 ml glass beakers filled with 800 to l000 ml of untreated seawater freshly pumped from the field. Identical beakers, only filled with seawater, served as controls. Beakers were kept in a flow-through aquarium during day at in-situ temperature of 21 to 29 °C (caused by seasonal differences) as monitored by *Onset HOBO* temperature loggers. Nylon gauze was clamped above the beakers to simulate light intensities very similar to those at 5 m water depth as verified by *Onset Pendant* light loggers. After 6 h incubation duration, corals and algae were removed from the beakers and subsamples were taken from the incubation water for determination of the following parameters.

Dissolved Organic Carbon (DOC)

Circa 10 ml of the incubation water were filtered through 0.2 µm sterile syringe filters (polyethersulfone membrane). The first 4 ml of the filtrate were discarded, but the following 6 ml were collected in pre-combusted brown glass bottles or ampoules, which were instantly frozen at - 20 °C and kept frozen until analysis. DOC concentrations were determined by high temperature catalytic oxidation (HTCO) using a Rosemount Dohrmann DC-190 total organic carbon (TOC) analyser. After defrosting, each sample was treated by adding 100 µl of 20 % phosphoric acid and purging for 5 min in order to remove dissolved inorganic carbon. DOC concentration of each sample was measured five times. An outlier test was conducted and the DOC concentrations of the remaining samples were averaged. Potassium hydrogenphtalate was used as standard for calibrating the DC-190 TOC analyser.

Particulate Organic Carbon (POC) and Nitrogen (PN):

Between 400 and 940 ml of the incubation water were filtered onto pre-combusted GF/F filters (Whatman, 25 mm diameter), which were dried for at least 48 h at 40 °C and kept dry until analysis. POC and PN concentration measurements and respective stable isotope analyses were performed with a Carlo Erba NC 2500 elemental analyzer, coupled with a THERMO/Finnigan Conflo II- interface to a THERMO/Finnigan MAT Delta plus isotope ratio mass spectrometer. Elemental concentrations were calculated from certified elemental standards (Atropine, Cyclohexanone-2,4-dinitrophenylhydrazone; Thermo Quest, Italy) and typically showed standard deviations < 3 %. Stable isotope ratios are given in the conventional delta notation (δ 13C, δ 15N) relative to Vienna PeeDee Belemnite (VPDB) standard (Craig 1957, Coplen 1995) and atmospheric nitrogen (Mariotti 1984), respectively. Standard deviations for repeated stable isotope measurements of lab standard (Peptone) were better than 0.15 ‰ for nitrogen and carbon, respectively. Respective surface areas of all coral fragments and algae pieces were measured as reference parameter using geometric approximations (all corals and turf algae growing on dead coral fragments, see Naumann et al. (2009) or the image analysis software *Image J* to analyze digital photographs of the predominantly 2-dimensionally growing macro algae *Caulerpa* spec. and *Peyssonnelia* spec.

Planktonic microbial degradation:

Circa 140 ml of the incubation water from each beaker was used to fill two 60 ml gas-proof glass bottles. Oxygen concentration in one of the bottles was measured immediately and in the second bottle after incubation of the enclosed water for at least 16 h in the dark and at in-situ temperature using Winkler titration (Winkler 1888) or a *Hach HQ 10* optode. Microbial activity in the incubation water was determined by subtracting final from start oxygen concentration. Planktonic microbial degradation of the added TOC was calculated by using the respective POC + DOC amounts and the increase in O_2 consumption in the bottles relative to the controls assuming that 1 mol added organic material is oxidized by 1 mol O_2.

Benthic degradation:

Degradation of algae and coral exudates was studied in-situ by addition of algae- and coral derived organic material to stirred benthic chambers identical to those described by Huettel and Gust (1992). These in-situ experiments were conducted at a reef site with carbonate sands (2.5 m water depth) described in (Wild et al. 2005a). The duration of the individual chamber experiments ranged between 5 to 8 h. Prior to each experiment, chambers were gently inserted into the loose calcareous sands to a depth of about 12 cm, thus including a water column of approximately 20 cm height and 5.7 l volume. At the beginning of the first experiment, 81 µmol coral- and 310 µmol algaederived organic matters were added to two chambers each. In a second independent experiment, 91 µmol coral- and 186 µmol algae-derived organic matters were again added to two chambers each, but only one of these two replicate chambers was stirred (advection chamber) , whereas the other one was left without stirring (diffusive

chamber). All 8 chambers of both experiments were incubated for 8 h in the dark. Water samples were regularly (at least every 2 h) collected from all chambers through a sampling port using plastic syringes, whereby the water from the diffusive chambers was thoroughly mixed before sampling in order to avoid O_2 concentration gradients. Oxygen concentrations were measured in the chamber waters using Winkler titration and benthic TOC degradation of the added algae or coral exudates were calculated as described above.

Results

All investigated benthic reef algae released both DOM and POM in measurable quantities. Data from the first two seasonal expeditions showed that organic matter release by corals and benthic algae was very different. In particular, DOC fluxes were one order of magnitude higher during autumn 2006 compared to summer 2007 (Table 1). There was no correlation between organic matter release and water temperature. All investigated benthic reef algae during both seasons showed DOC release, whereas DOC release by the corals was highly variable (as indicated by the large error bars) with often negative values, i.e. DOC uptake (Table 1). POC release could be detected for all investigated specimens, but showed no seasonal differences with similar release rates in autumn and summer. However, corals generally released significantly more POC than algae (U-test after Wilcoxon, Mann and Whitney, $p < 0.05$). The C:N ratios and nitrogen stable isotope signatures of algae and coral-derived particulate organic matter (POM) were not significantly different, but carbon stable isotope signatures of algae-derived POM (δ 13C: -10.1 ± 1.4 ‰) were significantly more positive ($p < 0.05$) than those of coral-derived POM (δ 13C: -18.3 ± 0.3 ‰). POM C stable isotope signatures were very similar to that of sterile coral mucus (δ 13C: -18.2 ± 1.2 ‰; Naumann et al. unpublished data), thereby demonstrating the apparent dominance of this material in the coral beakers. The respirometric experiments from all 4 seasons revealed that microbial activity measured as O_2 consumption was only significantly higher in the algae incubation water compared to that of the corals in autumn, but not during the other three seasons. Resulting microbial Total Organic Carbon (TOC = POC + DOC) degradation rates in autumn were 0.57 ± 0.38 and 0.18 ± 0.02 % h-1 for the algae- and coral-derived exudates, respectively. Benthic degradation of both organic matter sources showed an opposite trend with twice as high TOC degradation rates for the added coral exudates (23.7 ± 4.8 % h-1) than those for the algae exudates (12.1 ± 3.9 % h-1) under advective conditions. Advective transport of matter induced by the stirred benthic chambers increased benthic C degradation by a factor of 8 for the coral exudates, but only doubled for the algae exudates.

Discussion

This study confirms that benthic reef algae similar to hermatypic corals release organic matter in dissolved and particulate form to their surrounding. The assumed differences in organic matter release between benthic reef algae and corals (please see introduction) are verified by the tendency that corals release more POC and algae more DOC as well as by the differences in carbon stable isotope signatures. The latter finding may be caused by a more pronounced photosynthetic C assimilation of the benthic reef algae (Fry 2006), but may also indicate different chemical composition of algae compared to coral exudates. This aspect needs further detailed chemical analyses, but the differences in natural C stable isotope signatures suggest the suitability of this material for natural tracer studies.

Table 1 *Organic matter release by the dominant benthic algae (Turf algae, green algae Caulerpa, red algae Peyssonnelia) and hermatypic corals (Acropora, Stylophora, Pocillopora) in the study area during the first two expeditions to the Northern Red Sea (means ± SE given as mg C m-2 coral or algae surface area h-1; n.m = not measured; data from other expeditions not measured yet).*

Autumn 2006		
	DOC net release	POC net release
Turf	66.0 ± 23.0	2.7 ± 1.3
Caulerpa	10.0 ± 8.0	0.8 ± 0.2
Peyssonnelia	22.0 ± 18.0	2.2 ± 0.3
Acropora	105.0 ± 193.0	2.5 ± 0.6
Stylophora	-75.0 ± 45.0	7.8 ± 1.5
Pocillopora	-435.0 ± 30.0	2.8 ± 0.8
Summer 2007		
	DOC net release	POC net release
Turf	1.46 ± 1.50	1.34 ± 0.34
Caulerpa	1.63 ± 0.81	0.48 ± 0.34
Peyssonnelia	1.57 ± 1.15	n.m.
Acropora	4.00 ± 0.70	2.24 ± 0.41
Stylophora	-3.81 ± 11.06	5.04 ± 1.77
Pocillopora	-6.75 ± 3.52	3.88 ± 0.58

The comparably high DOC release by benthic reef algae in combination with the observed stimulation of planktonic microbial activity supports previously postulated statements (Kline et al. 2006, Smith et al. 2006, Dinsdale et al. 2008), which suggested that DOM released by benthic algae could stimulate microbial O_2 consumption with subsequent damage of corals in direct vicinity via hypoxia or anoxia.

Generally, algae-derived organic matter is obviously rapidly degraded in the water column, whereas this applies for coral-derived organic matter in the reef sands. Reasons for that may be that a high proportion of the algae-derived organic matter enters the DOM pool and can be taken up by planktonic microbes via the microbial loop. Kuntz et al. (2005) could demonstrate that because of this interrelationship DOM is more deleterious for corals than inorganic nutrients in reef waters.

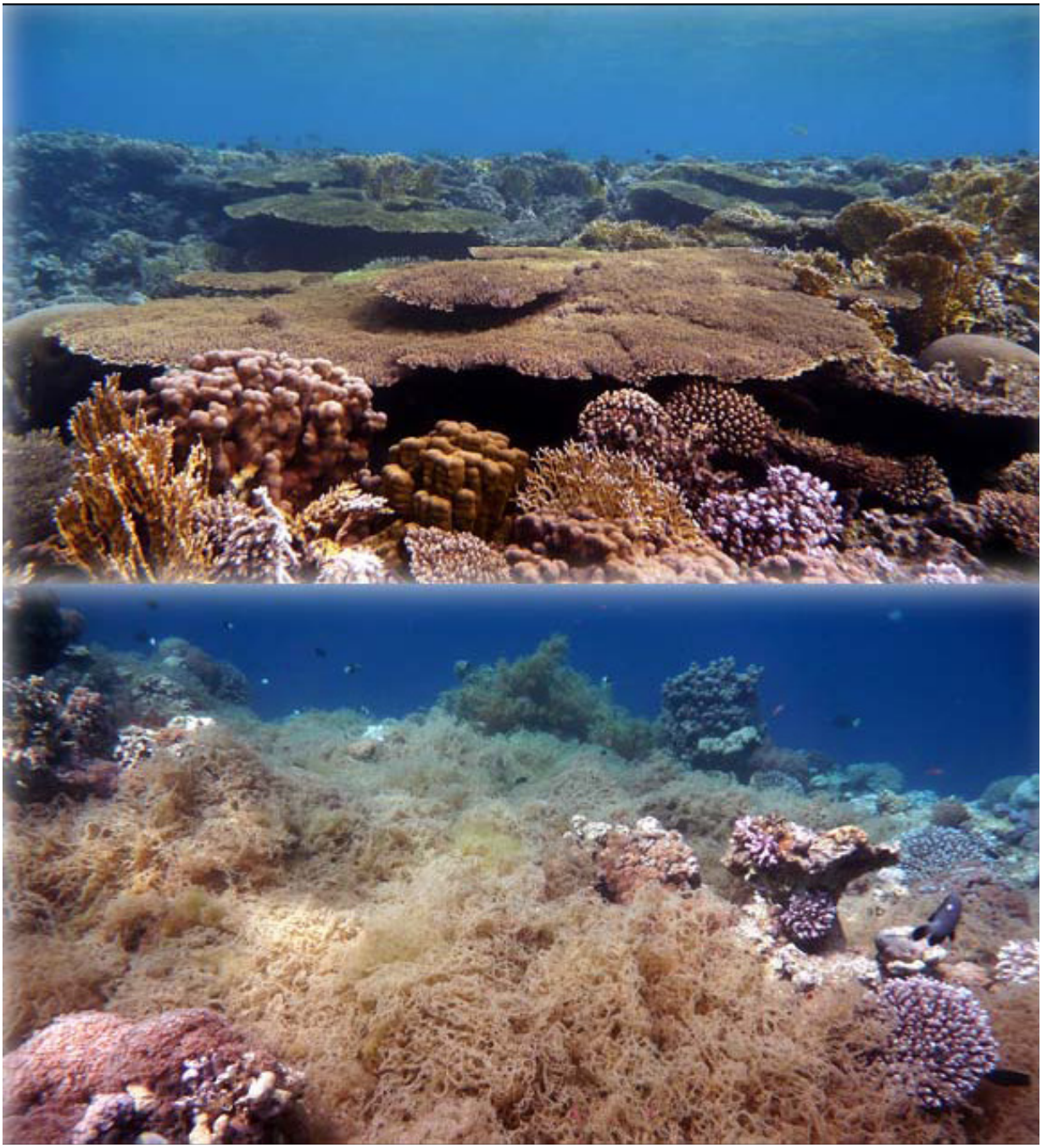

Fig. 1 *Coral (upper panel) versus benthic algae dominated (lower panel) fringing reef areas in front of MSS, Aqaba, Jordan, photographed during spring expedition 2008.*

Coral-derived organic matter in contrast contains more POM, which is often dominated by mucus. This material can be degraded by (specialized) microbes inhabiting the calcareous coral reef sands in high abundances (Wild et al. 2004b, Wild et al. 2005b, Wild et al. 2006), thus providing an explanation for the comparably high benthic degradation rates observed in the present study. Coral mucus in addition, because of its gel-like structure, can easily be transported via advection into the highly permeable reef sands, which act as biocatalytical particle filter systems. Such transport may not be possible to that extent for the particulate fraction of algae-derived organic matter, which can explain the pronounced advective stimulation of benthic coral-derived organic matter degradation. Algae-derived POM may in

addition have a distinctive refractory character (Buchsbaum et al. 1991, Kristensen 1994), which prevents rapid degradation and leads to deposition and ultimately blockage of the reef sands. This may compromise the important function of reef sands for the recycling of organic matter and thus has potential implications for reef management.

The observed strong seasonal differences concerning algae- and coral derived organic matter release in the study area between autumn and summer were probably caused by higher availabilities of inorganic nutrients in autumn due to colder temperatures and the beginning of deep water mixing typical for the Northern Red Sea (Rasheed et al. 2002). A higher availability of inorganic matter may have resulted in increased algae growth rates and associated high synthetisation of DOM. Monitoring of benthic reef algae coverage also showed strong seasonal differences (Haas et al. unpublished data) with temporal overgrowth of reef corals by algae during late winter and early spring (see Fig. 1). However, algae blooms collapsed soon after due to depletion of inorganic nutrients in late spring. Permanent phase shifts will thus likely not appear in the study area if inorganic nutrient input from land or mariculture facilities and direct reef damage are avoided.

In summary, both investigated groups of organisms can obviously act as reef ecosystem engineers via organic matter release. However, the hard corals as “old” engineers (i.e. before phase shift) contribute differently to reef processes than benthic algae as the “new” engineers after phase shift. Element cycles via coral-derived organic matter as described by Wild et al. (2004a) contributing to the conservation of essential nutrients in the reef ecosystem will likely not take place in an algae dominated post phase shift reef, as algae-derived organic matter can apparently not substitute the important particle trapping function of coral mucus. This pilot study therefore suggests that phase shifts from coral to benthic algae may have far reaching consequences for biogeochemical processes and general reef functioning.

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Organic matter release by benthic coral reef organisms in the Red Sea –its effect on planktonic microbial activity and potential implication for in-situ O_2 availability

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Abstract

Benthic coral reef organisms can control a range of processes important for reef ecosystem functioning via the release of organic matter, but related data are rare. This study therefore presents a comprehensive dataset (223 reef organisms separately incubated within 44 independent experiments during 4 seasonal expeditions) of dissolved and particulate organic matter (DOM and POM) release by the dominant benthic organisms from the Northern Red Sea, thereby including scleractinian and fire corals, upside-down jellyfish and reef-associated algae. Subsequently, the effect on microbial activity in the incubation water was determined. These studies were complemented by high resolution in-situ O_2 concentration measurements within reef environments dominated by corals or algae. Dissolved organic carbon (DOC) release was 14.5 ± 2.3 mg m^{-2} surface area h^{-1} for all nine investigated reef algae, and thereby significantly higher compared to scleractinian corals during all seasons except winter. POM release was observed for all investigated reef organisms, whereby the jellyfish released about one order of magnitude more POM compared to all other organisms. Benthic reef algae on average released 5.1 ± 0.5 mg POC m^{-2} h^{-1} and 0.35 ± 0.03 mg PON m^{-2} h^{-1}, thereby exhibiting significantly higher POC release rates than scleractinian corals in spring and autumn. Algae-derived organic matter, presumably its DOC fraction, stimulated microbial activity in adjacent water significantly more than that released by the investigated scleractinian and fire corals. Consequently, daily mean in-situ O_2 concentrations in the water directly above the reef (> 10 cm) were always significantly higher at coral-dominated compared to algae-dominated sites, thereby emphasizing in-situ relevance of previous laboratory findings. These findings suggest that benthic reef algae decrease O_2 availability in waters close to reef environments via the release of labile organic matter and its subsequent fast microbial degradation.

Introduction

Quantitative data on organic matter release by common benthic coral reef organisms other than corals are rare. Recent research showed that scleractinian corals, through the release of both dissolved and particulate organic matter (DOM and POM), can affect biogeochemical element cycles and establish fauna-microbe interactions in warm (Wild et al. 2005a) and cold (Wild et al. 2008; Wild et al. 2009b) water coral reef ecosystems. Ducklow & Mitchel (1979) previously demonstrated that other reef cnidarians, at least under manipulative stress conditions, were able to release organic matter into their surroundings. However, organic matter release rates of these organisms under undisturbed conditions are undetermined.

The study of (Smith et al. 2006) also indicates that benthic coral reef-associated algae may affect processes such as microbial activity in their surroundings via a hypothetical release of organic matter. This could reduce O_2 availability in coral reef environments and thus may have severe consequences for coral metabolism and ecosystem functioning. In coral reefs, hypoxia, i.e. dissolved O_2 water contents clearly below saturation, is a common phenomenon (Nilsson, Östlund-Nilsson 2004). Between the branches of coral colonies during night, when no O_2 is produced by the zooxanthellae, severe hypoxic conditions (down to 0.7 mg O_2 l^{-1}) can occur (Shashar et al. 1993; Kuehl et al. 1995). Although corals may overcome periods of low O_2 concentrations by extending their tentacles or decreasing their respiration (Shashar et al. 1993), severe hypoxia or anoxia can be responsible for wide coral mortality (Simpson et al. 1993a). A recent study also described the occurrence of hypoxia in interactions between corals and some turf or fleshy macroalgae in coral reef ecosystems (Barrot et al. 2009).

In this context, it is surprising that only few data are available on O_2 availability in coral reef ecosystems, in particular as most tropical organisms, including corals, are living near their critical tolerance levels for dissolved O_2 (Kinsey 1973). Low O_2 concentrations caused by the decomposition of organic materials may constitute a significant stress factor for corals (Johannes 1975), and the release of labile organic matter by reef organisms may stimulate microbial activity resulting in such O_2 deficiency (Simpson et al. 1993a).

This study therefore presents comparative quantitative data of organic matter release by the dominant benthic coral reef organisms investigated during four seasonal expeditions to a typical fringing reef in the Northern Red Sea. These studies were supplemented by

investigations on the effects of this release for 1) the planktonic microbial activity in the adjacent water measured as O_2 consumption, and the potential implication for 2) the in-situ O_2 availability within benthic communities dominated by different reef organisms.

Material and Methods

The work for this study was conducted during four seasonal expeditions (Nov/Dec 2006, Aug/Sep 2007, Feb/Mar 2008, May 2008) to a fringing reef close to the Marine Science Station (MSS), Aqaba, Jordan (29° 27′ N, 34° 58′ E). The dominant benthic target organisms were identified at the beginning of the first expedition using a Line Point Intercept (LPI) transect survey technique modified from (Loya 1978; Nadon, Stirling 2006). In water depths of 0.5, 1.0, 5.0, 10.0 and 20.0 m, duplicate 50 m transects with 0.5 m point intervals were carried out parallel to the reef crest at a Northern and Southern reef location using SCUBA. LPI transect data were analysed to derive the percentage coverage for the dominant benthic organisms in the study area. In total, 44 transects were carried out during the 4 seasonal expeditions.

Collection of specimens

Collection of all specimens took place in the MSS fringing reef in water depths of 5 to 10 m using SCUBA. All specimens were collected in replication of at least n = 5 for each subsequent incubation experiment.

During the field trips, replicate fragments (coral branch length: 6 to 10 cm) were broken off in-situ from colonies of the dominant hard coral genera *Acropora*, *Pocillopora*, *Stylophora* and the calcifying hydroid fire coral genus *Millepora*. In addition, similarly sized colonies and individual polyps of the scleractinian coral genera *Goniastrea* and *Fungia* respectively were collected. All corals except *Fungia* polyps were fixed onto ceramic tiles (4 x 4 cm) using small amounts of coral glue (Reef Construct, Aqua Medic®) on the site of fracture to reduce mechanical stress during experimental handling and allowed to heal in a flow-through aquarium with water directly pumped from the field at in-situ water temperatures (21 to 29 °C depending on season; monitored by *Onset HOBO* temperature loggers) and light intensity for 7 to 14 d prior to subsequent incubation experiments. This kind of maintenance therefore was very close to natural conditions, therefore minimizing any disturbance of the corals. Consequently, all corals looked healthy (no pigment change or tissue loss; polyps often expended) and no differences to corals in the field could be detected.

Benthic jellyfish of the genus *Cassiopea* (5 to 8 cm in diameter) were collected by carefully lifting them from the seafloor and transferring them into seawater filled polyethylene zip locked plastic bags (ca. 500 ml volume). Subsequently, *Cassiopea* specimens were transported to two 40 L flow-through tanks supplied with in-situ seawater at exchange rates of approximately 1.5 l min^{-1} and in-situ water temperatures for at least 2 d prior to the incubation experiments.

Additionally, small pieces (lengths: 6 to 14 cm) of the 3 most dominant types of benthic algae were collected in-situ: the green algae *Caulerpa* spec., the red algae *Peyssonnelia* spec., and typical filamentous turf algae consortia growing on dead coral skeletons were collected during each of the seasonal expeditions. The seasonally occurring algae genera *Ulva*, *Enteromorpha*, *Hydroclathrus* (all green), *Lobophora*, *Sargassum* (all brown) and *Liagora* (red) were only collected during winter or spring expeditions. All algae were left in a flow-through aquarium for at least 12 h prior to the subsequent experiments for cleaning and healing purposes.

Quantification of organic matter release

Organic matter release by all collected reef organisms was carried out as described in Herndl & Velimirov (1986). Animals and algae were separately transferred into 1000 ml glass beakers (acetone- and subsequently thoroughly seawater-rinsed) filled with 800 to l000 ml of untreated seawater freshly pumped from the field. Identical beakers filled with seawater served as controls. Beakers were kept in a flow-through aquarium during the day at in-situ temperature. Nylon gauze was clamped above the beakers to simulate light intensities very similar to those at 5 m water depth as verified by *Onset Pendant* light loggers. After 6 h incubation duration, organisms were removed from the beakers and sub-samples were taken from the incubation water for determination of the following parameters.

Dissolved Organic Carbon (DOC): Circa 10 ml of the incubation water were filtered through 0.2 µm sterile syringe filters (polyethersulfone membrane). The first 4 ml of the filtrate were discarded and the following 6 ml were collected in pre-combusted brown glass bottles or ampoules, which were instantly frozen at -20 °C and kept frozen until analysis. DOC concentrations were determined by high temperature catalytic oxidation (HTCO) using a Rosemount Dohrmann DC-190 total organic carbon (TOC) analyser. After defrosting, each sample was treated by adding 100 µl of 20 % phosphoric acid and purged for 5 min using pure O_2 in order to remove dissolved inorganic carbon. DOC concentration of each sample was measured five times. An outlier test was conducted, and the DOC concentrations of the remaining samples were averaged. Potassium hydrogenphtalate was used as standard for calibrating the DC-190 TOC analyser.

Particulate Organic Carbon (POC) and Nitrogen (PON): Between 400 and 800 ml of the incubation water were filtered on pre-combusted GF/F filters (Whatman, 25 mm diameter), which were dried for at least 48 h at 40 °C and kept dry until analysis. During this sampling, inclusion of larger, macroscopically visible particles such as faecal pellets (in the case of the jellyfish) or tissue fragments (in the case of algae) was avoided. POC and PON concentration measurements and respective stable isotope analyses were performed with a Carlo Erba NC 2500 elemental analyzer. Elemental concentrations were calculated from certified elemental standards (Atropine, Cyclohexanone-2,4-dinitrophenylhydrazone; Thermo Quest, Italy) and typically showed standard deviations < 3 %.

All organic matter release rates were related to the surface area of the incubated organism. Respective surface areas were measured as reference parameter using geometric approximations (all corals and turf algae growing on dead coral fragments, for detailed methodology please see (Naumann et al. 2009a) or the image analysis software *Image J* to analyze digital photographs of the other organisms.

Effects onto planktonic microbial O_2 consumption

Circa 140 ml of the incubation water from each beaker at the end of the incubation experiments was used to fill two 60 ml glass bottles. O_2 concentration in one of the bottles was measured immediately, and in the second bottle after incubation of the enclosed water for at least 16 h in the dark at in-situ temperature using the modified Winkler titration technique described by (Carpenter 1965) during autumn and summer expeditions or a *Hach HQ 10* optode during winter and spring expeditions. The optode was calibrated using Winkler titration. O_2 consumption by microbes (including bacteria, archaea, small protozoa) in the incubation water was determined by subtracting final from start O_2 concentration. Resulting values were then also related to the surface area of the incubated organism.

Supplementary in-situ studies

The effect of different benthic reef community composition on in-situ O_2 availability in the overlying water column was investigated during both spring and winter expeditions by deploying *Eureka* Midge Dissolved O_2 Loggers. During each of the two expeditions, two

loggers were simultaneously deployed in water depths of 4 to 7 m during 3 (spring) or 6 (winter) occasions for 24 h within different small (< 5 m^2) reef sections dominated by scleractinian corals (coral cover: 20 to 95 %, algae cover: 0 to 10 %) or benthic reef algae (algae cover: 35 to 100 %, coral cover: 0 to 15 %). O_2 concentration and water temperatures were measured and logged every 5 min over the entire deployment period (= 288 data points for each deployment). Water currents were measured in triplicates during each deployment occasion via tracking of natural suspended particles along known distances using ruler and watch.

Data analysis

Net organic matter release (DOC, POC, PON) by the investigated reef organisms and subsequent effects on microbial O_2 consumption rates were calculated by subtracting mean control values of the respective parameters from those of each treatment. For calculation of microbial organic carbon turnover rates, TOC concentrations (µM l^{-1}), released by the investigated reef organisms in the incubation water were related to microbial O_2 consumption (µM l^{-1}) in the respective treatment corrected for the control values. Statistical analyses were carried out using Mann-Whitney U-tests, as homogeneity of variances was given (Levene test), but the data were not normally distributed (Kolmogorov-Smirnov test). For comparison of data obtained from simultaneously deployed in-situ O_2 Loggers, paired t-tests were used. These data showed homogeneity of variances and were normally distributed.

Results

Fig. 1 gives an overview of the benthic coverage by the different groups of investigated organisms in the study area, the MSS fringing reef. Among the study organisms, hard corals exhibited highest benthic coverage followed by reef algae, fire corals and jellyfish. Seasonal algae only appeared during winter and spring expeditions and could account for up to 26 % of the seafloor area of the investigated coral reef. Water currents were low (3.2 – 7.1 cm s^{-1}) during all logger deployments.

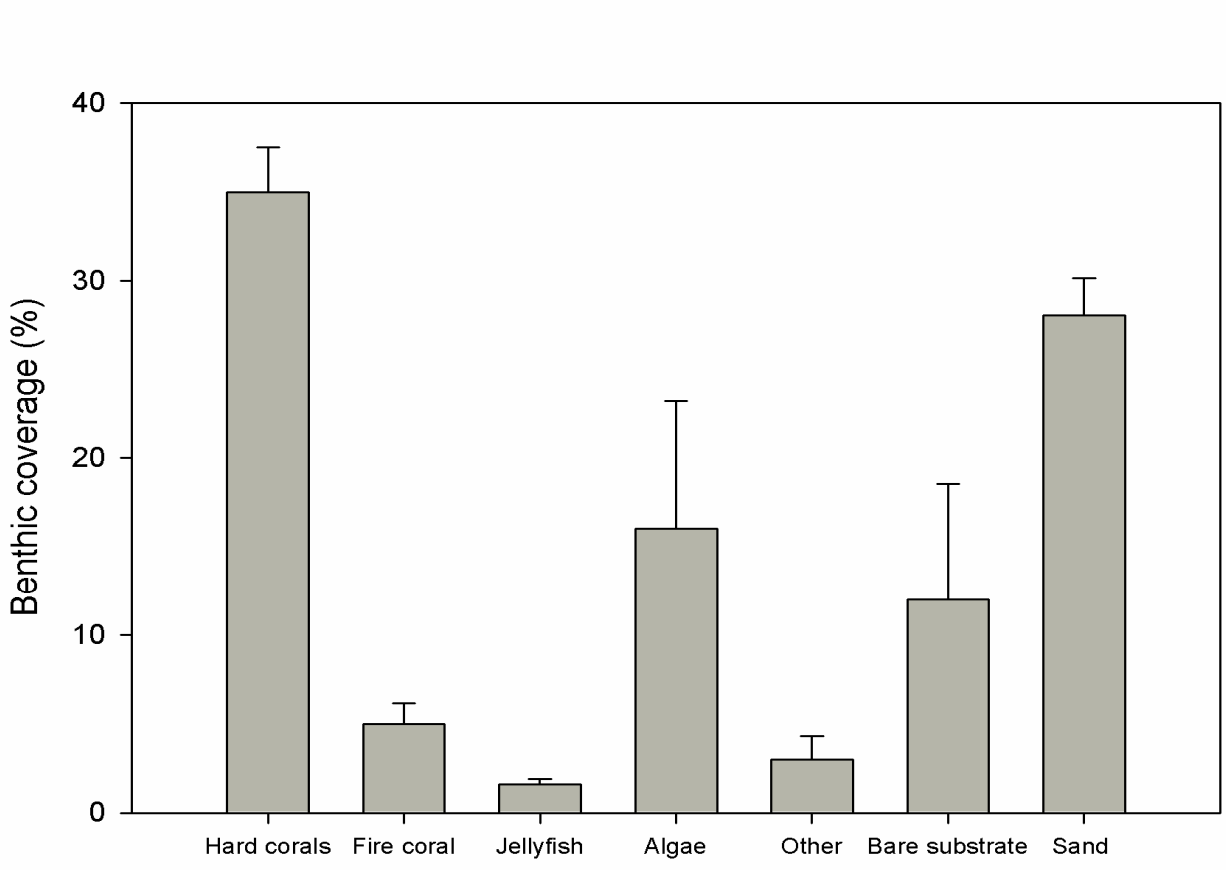

Fig. 1 *Benthic coverage by the different reef organisms at the MSS fringing reef as revealed by Line-Point-Intercept surveys. Values are displayed as means with SD indicated by the error bars.*

Comparative organic matter release

In total, 223 separate incubations of identical methodology with the different coral reef organisms were conducted in 44 independent experiments during the 4 different expeditions. Detailed information about temporal and spatial as well as species-specific resolution of these investigations are presented in the studies of (Naumann et al. in press) for scleractinian and fire corals, (Haas et al. submitted) for benthic reef algae, and (Niggl et al. 2010) for jellyfish. Fig. 2 summarizes mean organic matter release by the different groups of reef organisms investigated in the related studies and during the 4 different seasons.

Average net DOC release for all nine investigated different reef algae during all seasons was 14.5 ± 2.3 mg DOC m^{-2} h^{-1}, whereof turf algae released most (33.6 ± 6.9 mg DOC m^{-2} h^{-1}). While scleractinian corals (- 20.7 ± 21.2 mg DOC m^{-2} h^{-1}) and the jellyfish (- 1.2 ± 4.4 mg DOC m^{-2} h^{-1}) rather took up DOC, fire corals exhibited net DOC release of 9.2 ± 12.8 mg DOC m^{-2} h^{-1}. Benthic algae released significantly more DOC compared to the scleractinian corals during all investigated seasons except in winter and significantly more DOC than the jellyfish in spring (Fig. 2a).

POC and PON release was observed for all investigated reef organisms (Fig. 2b and c). The jellyfish *Cassiopea* released about one order of magnitude more POM compared to all other organisms. Benthic reef algae on average released 5.1 ± 0.5 mg POC m^{-2} h^{-1} and 0.35 ± 0.03 mg PON m^{-2} h^{-1}, thereby exhibiting significantly higher POC release rates than scleractinian corals in spring and autumn and significantly higher PON release rates in autumn. Fire coral POC (0.34 ± 0.14 mg POC m^{-2} h^{-1}) and PON (0.04 ± 0.01 mg PON m^{-2} h^{-1}) release was always

significantly lower when compared to benthic reef algae and scleractinian corals (POC: 2.8 ± 0.3 mg m^{-2} h^{-1}; PON: 0.29 ± 0.03 mg m^{-2} h^{-1}).

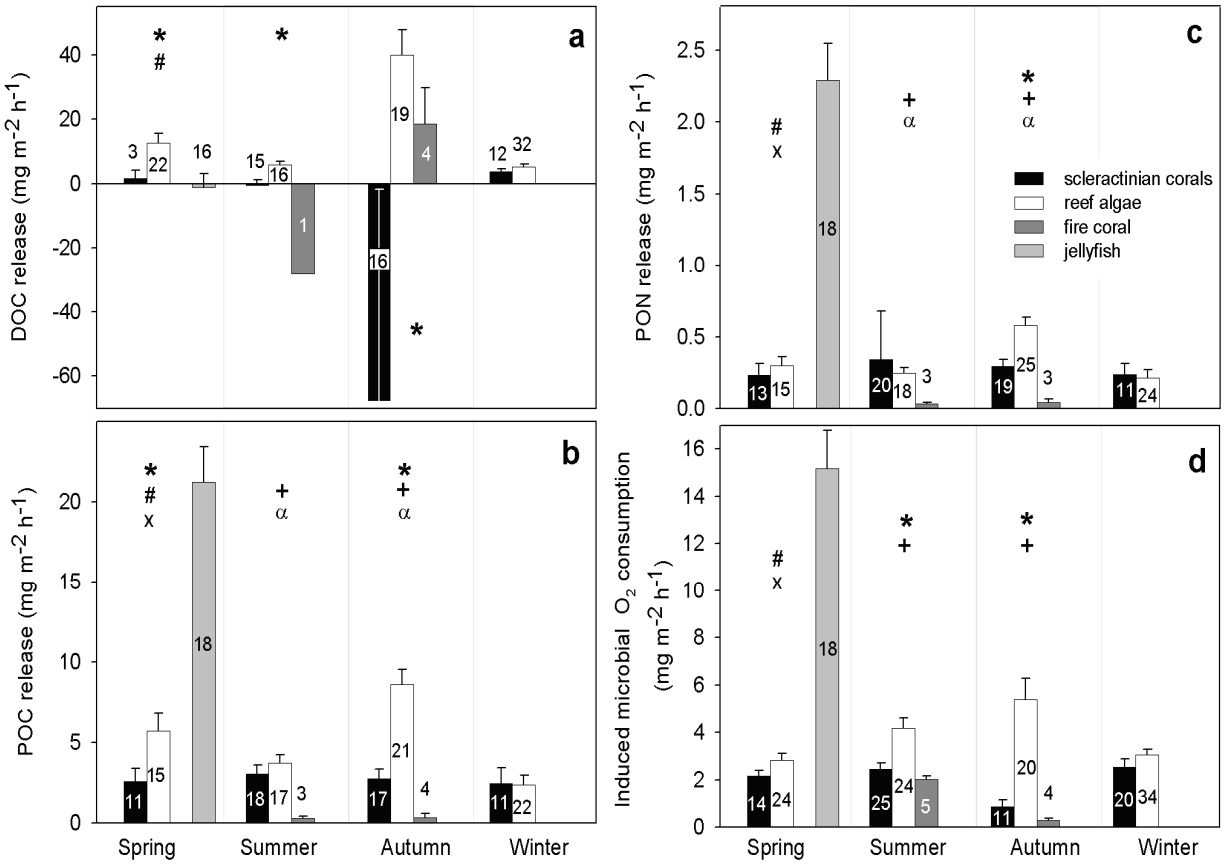

Fig. 2 *Comparative display of organic matter release by the investigated coral reef organisms ((a) DOC-, (b) POC-, and (c) PON- release) and its effects on microbial O_2 consumption (d) during the four seasonal expeditions. Values for microbial activity in (d) are normalized for 1 L of incubation water. Columns with error bars show means ± SE with number of replicate measurements (n) indicated by the numbers within/just above the columns. Symbols above columns give information about statistically significant differences ($p \leq 0.05$) between scleractinian corals and reef algae (*), fire coral (a), or jellyfish (x), as well as between reef algae and fire coral (+), or jellyfish (#).*

Effects on microbial activity and in-situ O_2 availability

Induction of microbial activity (Fig. 2d), measured as O_2 consumption, was highest for *Cassiopea*-derived organic matter (15.2 ± 1.6 mg O_2 l^{-1} h^{-1} normalized per m^2 surface area) thus significantly exceeded those of scleractinian corals and benthic algae. Corrected planktonic microbial O_2 consumption was 3.7 ± 0.2 mg O_2 l^{-1} h^{-1} for algae incubations, and 2.2 ± 0.2 mg O_2 l^{-1} h^{-1} or 1.2 ± 0.3 mg O_2 l^{-1} h^{-1} for scleractinian and fire coral incubations, respectively. Stimulation of microbial activity by reef algae-derived organic matter thus significantly exceeded those by scleractinian and fire coral-derived organic matter in summer and autumn.

During all nine parallel deployments of O_2 loggers at coral- or algae-dominated reef sites, daily mean O_2 concentrations in the water directly above the reef were significantly higher (two-sided, paired t-tests; $p < 0.05$) at the scleractinian coral-dominated compared to the benthic reef algae-dominated sites (Fig. 3).

Algae dominated sites showed a strong diurnal variation of O_2 concentrations ranging from 5.1 to 9.3 mg l^{-1} in spring and 5.1 to 10.3 mg l^{-1} in winter. In contrast, O_2 concentrations at coral dominated sites ranged from 6.6 to 8.5 mg l^{-1} in spring and 7.0 to 9.5 mg l^{-1} in winter.

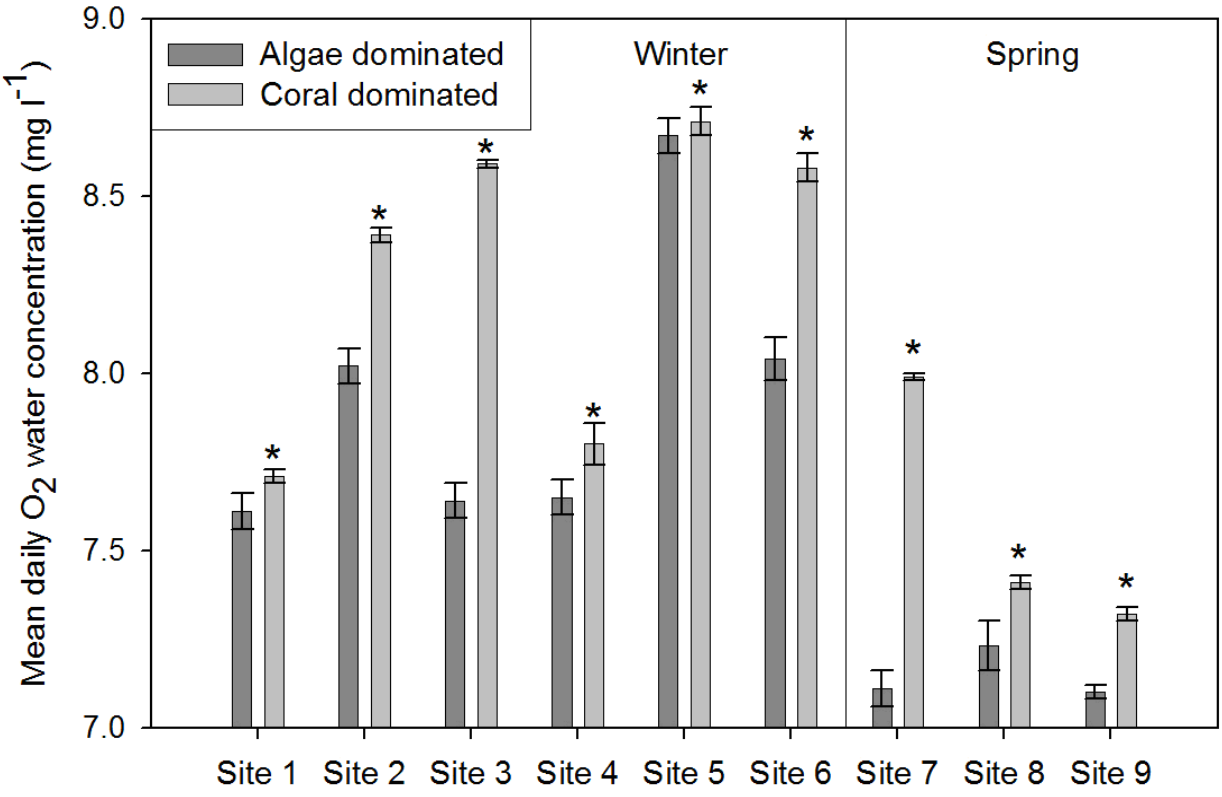

Fig. 3 *O_2 concentrations in the water column close (< 5 cm) above reef sites with benthic communities dominated by scleractinian corals or reef algae in-situ measured with Midge O_2 loggers. Water temperatures were 21.5 to 21.8 °C during winter and 22.3 to 22.7 °C during spring measurements. Columns display daily means of 288 data points (every 5 min over 24 h) for each deployment with respective SE as error bars. Statistically significant ($p \leq 0.05$) higher concentrations are indicated by asterisks.*

Discussion

Organic matter release by different groups of reef organisms

Organic matter net release by coral reef organisms has been investigated already in the 1970ies (Richman et al. 1975; Ducklow, Mitchell 1979). In contrast to those earlier studies, the present study delivers a comprehensive data set about organic matter net release by the dominant resident and seasonal benthic reef organisms in the Northern Red Sea under undisturbed conditions.

Results of the present study showed that all investigated benthic reef organisms released POM (POC and PON) in significant quantities into their surroundings. For corals, this can account for up to half of the carbon assimilated by their zooxanthellae (Crossland et al. 1980; Davies 1984; Muscatine et al. 1984). Coral-derived POM release rates were similar to those described

elsewhere (Crossland 1987; Wild et al. 2005b), while hydrozoan, scyphozoan and macroalgal POM release rates were quantified here for the first time. As release of organic matter has been attributed to surplus carbon fixation during intense photosynthesis (Fogg 1983; Davies 1984), similarities in POM release between algae and corals may be explained by similar photosynthetic overproduction and concurrent limited nutrient availability with very low nitrate (0.12 – 0.90 µM) and phosphate (0.03 – 0.07 µM) concentrations at the study site during the 4 expeditions (Wild et al. 2009a).
DOM net release by corals has been already demonstrated by Ferrier-Pages et al. (1998). In the present study however, net release of DOC was only observed for three of the investigated coral genera. Main reason for that finding was likely the variety of feeding mechanisms of zooxanthellate corals and jellyfish. Besides photosynthetic products from zooxanthellae (Muscatine 1990), corals are able to change to other feeding models, like the capture of zooplankton by polyps and uptake of dissolved organic compounds from the surrounding seawater (Sorokin 1973; Ferrier 1991; Muller-Parker, D'Elia 1996). This DOM uptake may have exceeded release for most of the investigated corals. The same likely applies for the jellyfish *Cassiopea sp*.. In contrast, all algal species, particularly turf algae, released DOC, which is in agreement with previous studies (Khailov, Burlakova 1969; Brylinsky 1977). In contrast to zooxanthellate corals and jellyfish, benthic algae are strictly photoautotrophic in terms of their energy and carbon requirement (Tuchman 1996), thus re-absorption of DOM is unlikely, but may still occur.
The exceedingly high organic matter release rates found for turf algae can potentially be attributed to an underestimation of surface areas, by not including their fine filaments. As larger proportions of structural tissue are needed for more complex morphologies, Littler & Littler (1984) suggested a higher performance of primary production of algae with filamentous morphology. This also creates an increased surface area, thereby providing large interface to the surrounding that may lead to a faster exchange of metabolic products with the ambient environment. Another reason may be the N fixation ability of turf algae associated cyanobacteria (Williams, Carpenter 1998), which can affect net organic matter production via overcoming of N limitation (Smith 1982).

Effects on microbial activity and potential implication for in-situ O_2 availability

The comparably high DOC release by benthic reef algae in combination with the observed high stimulation of planktonic microbial activity confirms previously postulated statements (Kline et al. 2006a; Smith et al. 2006; Dinsdale et al. 2008), suggesting that DOM released by benthic algae may stimulate planktonic microbial O_2 consumption. High microbial respiration of the biologically labile algae-derived organic matter may reduce O_2 availability in the surrounding seawater (Nguyen et al. 2005b). The results of several laboratory studies suggest that this can lead to the death of scleractinian corals (Mitchell, Chet 1975; Kuntz et al. 2005; Kline et al. 2006a; Smith et al. 2006). The present study supplements these laboratory studies by demonstrating that there may be similar in-situ effects via strong influence on O_2 availability in the reef by benthic algae, likely via the release of labile DOM. The study of (Haas et al. 2009b) in this context also indicated that DOC addition may negatively influence corals in interaction with algae via decreased O_2 water concentrations.
Various factors can influence the occurrence of hypoxia around corals in-situ. The morphology of coral colonies can promote hypoxic conditions by creating a region of weak water exchange between the inner branches (Chamberlain, Graus 1975). Hypoxia on corals has also been found along natural interactions between corals and algae (Barrot et al. 2009). In contrast, the mutualistic relationship of branching corals to sleep-swimming fish helps to aerate the colony, thus preventing hypoxia (Goldshmid et al. 2004). O_2 supply to corals is influenced by diffusion limitations around the organism surface, which may induce the development of diffusive boundary layers (DBL) (Shashar et al. 1993). Intense metabolic

activity (e.g. coral holobiont respiration during night) can result in niches of sharp O_2 gradients in the DBLs leading to anoxic conditions (Shashar et al. 1993). Low O_2 concentrations in the surrounding water may therefore decrease flux rates of O_2 towards the O_2 depleted corals. There are few studies investigating O_2 water concentrations in coral reefs using O_2 electrodes (Barnes 1983; Barnes, Devereux 1984a). The comparison of adjacent sites with different benthic communities to our knowledge has been conducted in the present study for the first time and revealed significantly lower O_2 water concentrations at algae dominated sites compared to adjacent coral dominated sites. Physical factors, such as water flow or topographic characteristics as well as biological factors such as respiration and photosynthetic activity may influence water O_2 concentrations (Kraines et al. 1996). The deployed O_2 loggers in the present study were placed at low water current sites where differences in topography and flow speeds between the respective algae and coral locations did not occur. In addition, higher respiration by benthic algae can not explain the findings of the present study, because coral respiration is usually higher than that of benthic reef algae (C. Jantzen, unpublished data). Lower O_2 concentrations at algae dominated sites may therefore very likely be attributed to strong stimulation of microbial activity by algae-derived organic matter release.
The supply of rapidly degradable organic matter may further not only lead to an increased growth and activity of the established microbial community, but also to a shift in microbial community structure. Recent research has demonstrated that corals contain large, diverse and specific populations of microorganisms (Rohwer et al. 2002; Kellogg 2004) that have co-evolved with them (Rohwer, Kelly 2004; Ritchie 2006). Changes in coral associated microbial communities can cause coral bleaching and other diseases in conditions of environmental stress (Rosenberg et al. 2007). Kuntz et al. (2005) also demonstrated that high DOM water concentrations are more deleterious for scleractinian corals than inorganic nutrient concentrations. By producing biologically labile DOC (Nguyen et al. 2005b), benthic reef algae may stimulate microbial activity stronger than corals (Cole et al. 1982; Aluwihare et al. 1997; Aluwihare, Repeta 1999) with ensuing potential alterations in microbial community structure.

Ecological implications

All investigated groups of organisms can obviously control reef processes, in particular interaction with microbes via organic matter release. However, corals likely contribute differently to reef functioning than benthic algae. The organic matter released by corals does stimulate microbial activity less than algae-derived organic matter. Further, corals mainly release POM in form of coral mucus (Crossland 1987), a transparent exopolymer (Krupp 1985; Meikle et al. 1987), which is able to trap particles and thereby fulfils an important role as an energy carrier and nutrient trap in coral reef ecosystem functioning (Wild et al. 2004a; Huettel et al. 2006; Naumann et al. 2009b). In contrast, algae release organic matter predominately in dissolved form, and the particulate fraction of algae-derived organic material mainly consists out of detritus and dead algae cells (Duarte, Cebrian 1996; Mannino, Harvey 2000). This does likely not substitute the important role of coral mucus as particle trap. Apart from lacking the function as particle trap, algae-derived organic matter potentially supports a different microbial community. Whereas coral-derived organic matter is mainly (> 90 %) degraded by the microbial community in the reef sands (Wild et al. 2004b), stimulating effects of algae-derived organic matter indicates that it is utilized predominately by the planktonic microbial community in the surrounding water column. This assumption is further supported by the conducted in-situ O_2 logger measurements, showing significantly lower water O_2 concentrations at algae- compared to coral-dominated reef areas.
Thus, this study supports assumptions about negative consequences on O_2 availability in reefs subjected to a phase shift from coral to algae dominated ecosystems, owing to organic matter released by benthic algae (Kuntz et al. 2005; Kline et al. 2006a; Smith et al. 2006). It further

indicates that these two key groups of primary producers may contribute differently to reef ecosystem functioning, owing to the rate and location of the microbial utilization of their released organic matter substrates.

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10

Benthic community composition affects O_2 availability and variability in a Northern Red Sea fringing reef

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Abstract

Many coral reef ecosystems experience shifts in benthic community composition from scleractinian corals to algae. However, consequences of such phase shifts on O_2 availability, important for many reef organisms, are unresolved. The present study therefore comparatively investigated potential in-situ effects of different benthic cover by reef macroalgae and scleractinian corals on water column O_2 concentrations in a Northern Red Sea fringing reef. Findings revealed that mean daily O_2 concentrations at algae-dominated sites were significantly lower compared to coral-dominated sites. Minimum O_2 concentrations and variability were significantly negatively correlated with increasing benthic cover by algae,

while no correlation with coral cover was found. These results indicate that shifts from corals to benthic algae may likely affect both in-situ O_2 availability and variability. This may be particularly pronounced in reef systems with low water exchange (e.g. closed lagoons) or under calm weather conditions and suggests potential O_2-mediated effects on reef organisms.

Main Text

While “pristine” coral reefs usually exhibit low fleshy macroalgal standing crop (Smith et al. 2001), degrading reefs often undergo a phase shift in which fleshy macroalgae become dominant over scleractinian corals (Done 1992; Hughes 1994; McCook 1999). In this context, many studies investigated phase shift promoting factors such as overfishing of herbivores and nutrient enrichment (e.g. McManus et al. 2000; Smith et al. 2001; Belliveau, Paul 2002; Ledlie et al. 2007) or focused directly on coral-algae interactions (McCook et al. 2001; Jompa, McCook 2003; Nugues, Roberts 2003; Haas et al. 2009a; Haas et al. 2010). Recent laboratory studies demonstrated the deleterious effect of coral reef macroalgae on scleractinian corals via the hypothetical release of biologically labile dissolved organic

compounds (Nguyen et al. 2005a) and a subsequent decrease in O_2 availability via stimulation of microbial activity (Kline et al. 2006b; Smith et al. 2006). However, in-situ relevance of these observations could not be demonstrated.

The present study aims to contribute in this context by the comparative investigation of in-situ O_2 availability and variability at reef sites dominated by algae or corals in the Northern Red Sea. Coral reefs in this area usually display high benthic coverage by living scleractinian corals (Wilkinson 2008). However, we observed extensive overgrowth of corals by benthic macroalgae during two field expeditions to the Gulf of Aqaba in winter and spring 2008 although the living hermatypic coral cover in investigated area was 36 – 41 % (Haas et al. 2010) thus exceeded typical living hard coral cover (30 %) of the Jordan coral reefs (Wilkinson, 2008). The high macroalgal abundance was likely caused by high availability of inorganic nutrients owing to seasonal water mixing in winter (Wolf-Vecht et al. 1992; Badran et al. 2005) and subsequent stimulation of algal growth, presumably at first on bare substrate. The resulting simultaneous occurrence of adjacent algae- and coral-dominated reef sites offered a good opportunity to study potential effects of benthic community composition on O_2 concentrations in the overlying water column in situ. Therefore, during the two expeditions, two *Eureka* Midge Dissolved O_2 Loggers were simultaneously deployed for 24 h within adjacent algae- (35 to 100 % benthic cover by genera *Hydroclathrus*, *Enteromorpha* or turf algae) and coral- (20 to 95 % benthic cover; ca. 60 % of total coral cover by genera *Acropora*, *Lobophyllia*, *Porites*) dominated reef sites at 9 different deployment occasions (6 in winter, 3 in spring) in water depths of 4 to 7 m (Fig. 1). Coral benthic coverage was less than 10 % on algae-dominated sites and vice versa. The remaining seafloor was covered with sand or dead coral rock. During each of these deployment occasions, the two O_2 loggers were centrally placed, one on the algae- and the other one on the respective adjacent coral-dominated site resulting in a distance between the loggers of 3 to 10 m. All 18 different sites were spread over a total distance of approximately 400 m. O_2 concentrations and water temperatures were measured and logged every 5 min over the entire deployment period of 24 h (= 288 data points for each deployment), whereby the sensors of the loggers were installed ca. 10 cm above the seafloor. Before deployment, the O_2 loggers were calibrated at in-situ temperature and salinity using O_2 concentrations obtained from the dissolved O_2 determination method described by Winkler (1888) as reference. All deployment sites were exposed to similar water current velocities of 3.2 to 7.1 cm s^{-1} (measured via in-situ tracking of suspended particles along known distances using ruler and watch). The orientation of algal- to coral-dominated sites relative to the main current direction varied between the 9 deployment occasions. Temperature ranged from 20.7 to 21.4° C during winter and from 22.0 to 22.7° C during spring expedition.

Analysis of data revealed that mean daily O_2 water concentrations were significantly lower at algae-dominated (7.69 ± 0.91 mg O_2 l^{-1}; mean ± SD of all 9 deployments, n = 2512) compared to adjacent coral-dominated (8.07 ± 0.61 mg O_2 l^{-1}; mean ± SD of all 9 deployments, n = 2512) sites (two-sided, paired t-tests; $p < 0.05$) (Fig. 2).

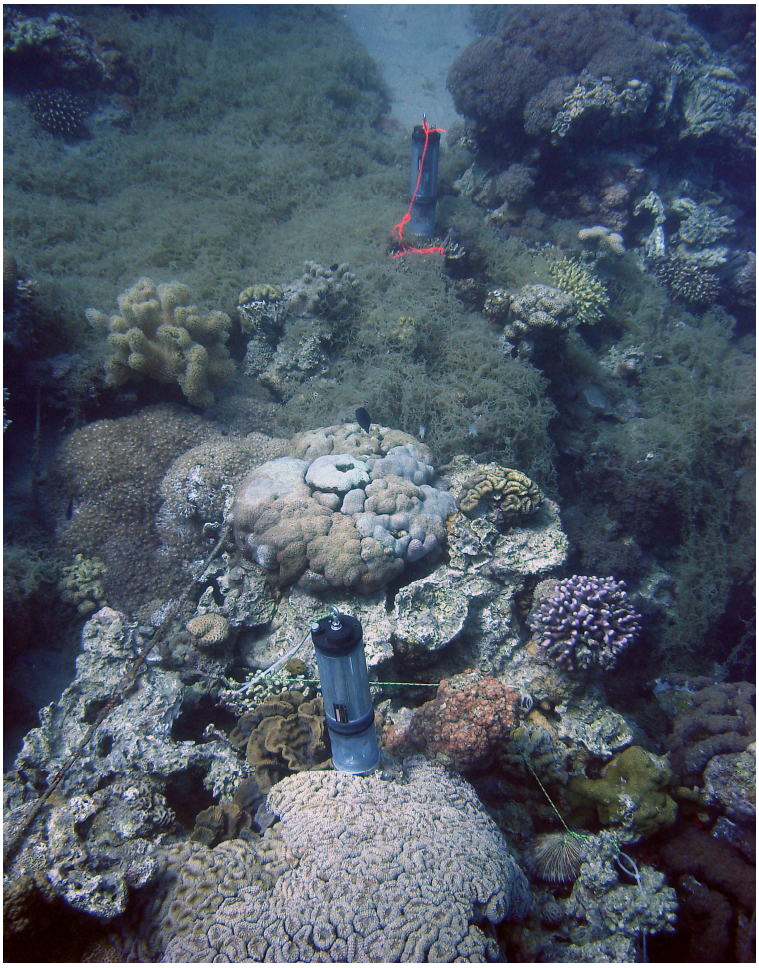

Fig. 1 *In-situ O_2 loggers deployed at adjacent reef sites dominated by scleractinian corals or benthic macroalgae.*

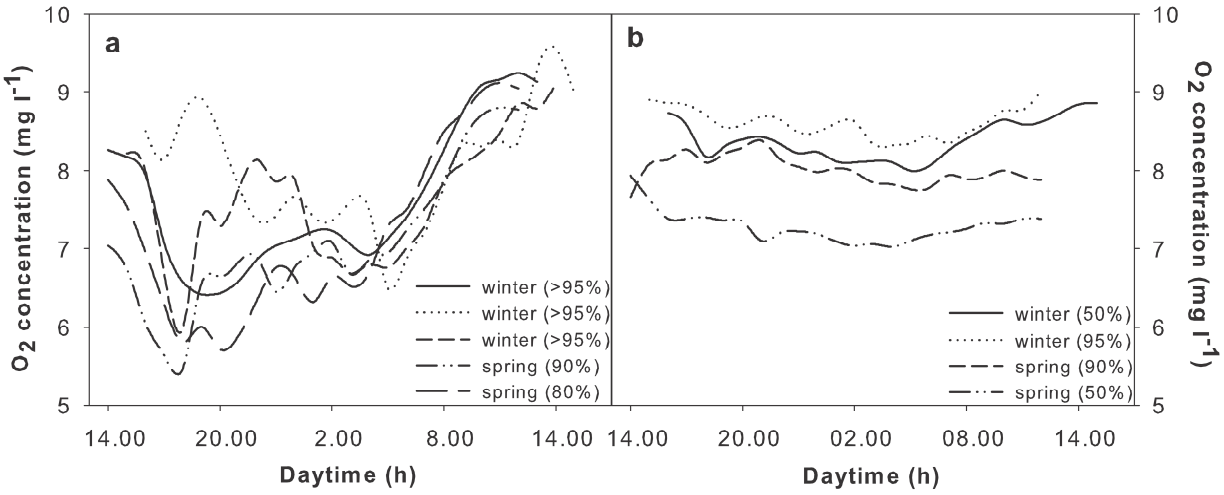

Fig. 2 *Hourly averaged O_2 concentrations above a) algae-dominated sites (algae-cover ≥ 80%) and b) coral dominated sites (coral cover ≥ 50%).*

The minimum daily O_2 concentrations exhibited a strong negative correlation with benthic algae cover (Pearson product-moment correlation, r = -0.842, p = 0.004) (Fig. 3a). In addition, diurnal variations in O_2 concentrations at algae-dominated sites were significantly higher than at coral-dominated sites and displayed strong positive correlation with benthic algae cover (Pearson product-moment correlation, r = 0.90, p = 0.001) (Fig. 3b). Coral cover in contrast did neither correlate to diurnal variation nor to minimum daily O_2 concentrations.

Several previous studies also investigated in-situ O_2 concentrations in coral reefs, but did not link these results to benthic community composition (Sournia 1976; Barnes 1983; Barnes, Devereux 1984b; Routley et al. 2002). Kinsey & Kinsey (1967) reported strong diurnal variation and minimum O_2 concentrations of 2.1 mg O_2 l^{-1} during low tide in the residual 1 ft water column over the coral reef platform of Heron Island, Australia, where O_2 production and consumption were primarily associated with areas of high coral abundance. In the present study however, only algae benthic coverage correlated with minimum O_2 concentrations and diurnal variations. Results therefore indicate that shifts to algae-dominated reef sites may likely lead to local in-situ decreases of O_2 availability, particularly critical during nights and early mornings or at low tides.

During the measurements, water from coral-dominates sites likely exchanged with water from the algae-dominated sites and vice versa because of alternating tidal currents. In addition, the pronounced reef topography and high sedimentary permeability at the study site (Wild et al. 2005a; Wild et al. 2009a) likely facilitated advective water exchange (Huettel, Gust 1992; Ziebis et al. 1996a; Ziebis et al. 1996b) that counteracted establishment of O_2 gradients. Supplementary measurements (Haas & Wild, unpublished) revealed no gradients in O_2 concentrations in the water column beyond 1 m above the seafloor up to the surface. However, the significant O_2 concentration and variability differences between neighbouring sites observed during all comparative logger deployments in the present study indicate in-situ establishment of spatially limited O_2 gradients despite such counteracting factors.

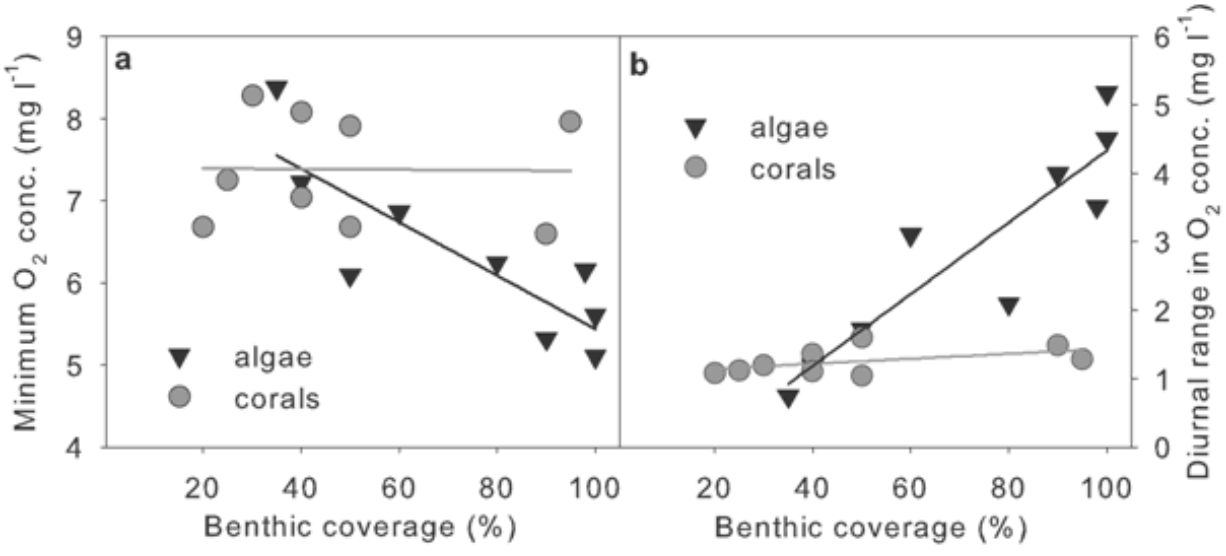

Fig. 3 *Minimum O_2 concentrations (a) and diurnal variation in O_2 concentrations (b) plotted against percent benthic coverage of corals or algae in the surveyed locations.*

The results of the present study also suggest more pronounced, longer-lasting hypoxia events under calm-weather or other advection-inhibiting environmental conditions (e.g. small sediment grain size and resulting low permeability, low surface topography, weak bottom currents). This may particularly apply for closed reef lagoons with low water exchange and high cover by benthic macroalgae.
Occurring severe hypoxia could cause altered distribution and behaviour of fishes (Breitburg 2002; Östlund-Nilsson, Nilsson 2004) or even lead to mass mortality of reef organism, particularly if microbial activity is stimulated by natural (Simpson et al. 1993b) or anthropogenic organic matter and nutrient input. But already low O_2 concentrations in the range of 4.5 to 6.0 mg O_2 l^{-1} which are similar to those of the present study (Fig. 3a), may negatively affect animal growth, behaviour and metabolism (Harris et al. 1999; Gray et al. 2002). While physiological processes may only be marginally affected by a transitory O_2 decrease, benthic fauna composition may change under these conditions (Montagne, Ritter 2006). Mobile dwellers can avoid low O_2 availability by migrating to sites with higher O_2 concentrations (Wu et al. 2002). But very active specimens which are unable to tolerate low oxygen levels (Nilsson et al. 2007) and some of the sessile benthic specimens could be particularly affected. Hypoxia-tolerant reef species (e.g. inactive organisms) could be favoured against those hypoxia-sensitive organisms, which may lead to changes in community composition in a particular habitat. Such changes in the benthic community composition due to periodically low dissolved O_2 concentrations have already been observed (Platon et al. 2005; Lim et al. 2006; Montagne, Ritter 2006).
Recent studies indicated negative effects of benthic algae on scleractinian corals by reducing O_2 availability likely via the release of DOC (Kline et al., 2006; Smith et al., 2006), as algae-derived DOC mostly consists of glucose and positively correlates with microbial O_2 consumption (Haas & Wild, unpublished). In contrast, reef algae-derived POM does not correlate with microbial O_2 consumption, thus exhibits much lower microbial degradability (Haas et al., unpublished). The findings of the present study therefore supplement previous laboratory studies (Kline et al., 2006; Smith et al., 2006) by demonstrating that there may be similar in situ effects of algae-derived DOC. Besides released organic matter, intense coral or algae respiration could also lead to decreased local O_2 concentrations. The observations of the present study do therefore likely reflect a combination of high DOC release and respiration by algae. The study of Haas et al. (2009a) in this context indicated that DOC addition may negatively influence corals in interaction with algae via decreased O_2 water concentrations. This could also imply positive feedback loops promoting reef algae in the competition with scleractinian corals. In contrast to the investigated seasonal phase shifts, which likely provide sufficient time for the reestablishment of original benthic community composition, permanent phase shifts may cause non-reversible subsequent changes.

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11

Coral sand O_2 uptake and pelagic–benthic coupling in a subtropical fringing reef, Aqaba, Red Sea

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Abstract

Calcareous sands are major sites for recycling of organic matter in coral reef ecosystems. O_2 uptake and pelagic–benthic coupling were studied in coral sands using benthic chambers and sediment traps during several seasonal expeditions between May 2004 and May 2008 along a fringing reef on the Jordanian Red Sea coast. A total of 12 independent dark chamber experiments were conducted at 2.5 to 16.5 m water depth on the highly permeable calcareous reef sands covering the seafloor at the reef and back-reef lagoon. Sedimentary O_2 uptake ranged from 20 to 39 mmol m^{-2} d^{-1} and was positively correlated with water depth in the lagoon, but not in the reef, where O_2 uptake was significantly lower. Comparison of sedimentary O_2 uptake rates recorded at the same locations revealed little temporal and seasonal variation, and no significant responses to changes in environmental factors in the water column, such as temperature and concentrations of organic or inorganic nutrients. These results suggest that efficient recycling in the pelagic food web of the nutrientdeprived coral reef limits the supply of degradable organic matter to the reef sediments. Increase of sedimentary O_2 uptake with water depth in the lagoon sands may therefore be a function of lateral transport of labile organic particles produced by reef organisms (e.g. benthic algae and corals) rather than sedimentation of water column production.

Introduction

Coral reefs are characterized by high turnover rates and efficient recycling of energy and essential nutrients (Crossland & Barnes 1983, Hatcher 1988, 1997, Richter & Wunsch 1999, Wild et al. 2004a). The reef sediments, in particular the permeable calcareous sands with their high abundances of phototrophic and heterotrophic microbes (Wild et al. 2006), contribute significantly to primary production, carbon mineralization and nutrient cycling of reef ecosystems (Johnstone et al. 1990, Clavier & Garrigue 1999, Wild et al. 2004b,c, 2005). Sedimentary production and decomposition processes control O_2 flux across the sediment-water interface, and investigations by Werner et al. (2006) suggest that O_2 is the dominant electron acceptor over sulphate in permeable coral reef sands. Sedimentary O_2 uptake not only reflects the aerobic degradation of organic matter, but also the microbial and chemical reoxidation of reduced electron acceptors derived from anaerobic organic matter decay (Canfield et al. 1993), which also plays an important role in coral reef sediments (Skyring & Chambers 1976, Skyring 1985, Werner et al. 2006). Thus, O_2 uptake is the parameter ultimately integrating sedimentary organic matter mineralization processes, and the spatial and temporal changes in sediment–water O_2 flux contain key information on the functioning of calcareous sands as sites for the recycling of carbon and nutrients in the coral reef ecosystem.

Despite increasing interest in carbon and nutrient cycling in coral reefs associated with the worldwide deterioration in reef ecosystems (Hoegh-Guldberg et al. 2007, Hughes et al. 2007), studies targeting the spatial and temporal variability of O_2 flux in reef sands are rare, and most measurements are from nonrecurring investigations exclusively focusing on one or a few shallow reef locations (Boucher et al. 1994, 1998, Rasheed et al. 2004, Reimers et al. 2004, Wild et al. 2005). To our knowledge, only Clavier & Garrigue (1999) have studied sedimentary O_2 uptake in the lagoon of a barrier reef in New Caledonia on both temporal and spatial scales; they found pronounced differences between seasons, locations and sediments with different mineralogy. All investigations of sedimentary O_2 uptake in coral fringing reefs, the world's most common tropical reef type, have been conducted at shallow locations (maximum water depth ca. 5 m), although large sections of reef lagoons are deeper (Riddle et al. 1990, Hansen et al. 1992, Kayanne et al. 1995, Chabanet et al. 1997) and may function as traps for organic material as reduced wave and current impact allows settlement of fine particles. Deeper sandy areas in the reef, therefore, may account for a large fraction of organic matter recycling. The relatively high permeability of coral sands permits water flow through the pore space of the upper sediment layers (Wild et al. 2004b,c), thereby waves and bottom flow can affect spatial and temporal O_2 distribution in the seabed (Booij et al. 1991, Ziebis et al. 1996, Falter & Sansone 2000). As the intensity of wavegenerated orbital water motion and wind-driven currents decreases with depth, it is expected that the hydrodynamic effects are less pronounced in the deeper areas of the reef lagoon, leading to reduced sediment flushing and higher concentrations of reduced compounds in the sediment. The deeper lagoon sites thus may accumulate more organic matter, but may also be characterized by a slower recycling rate due to reduced sediment–water solute exchange. Seasonal changes in waves, currents and organic matter deposition as well as benthos activities may cause spatial and temporal variability in sediment–water exchange processes affecting sedimentary organic carbon decomposition and thereby O_2 flux. The few nonrecurring measurements from shallow reef locations reported in the literature do not reveal the spatial and temporal dynamics of O_2 flux in coral reef sands or the contribution of deeper sand sites to the recycling in the reef. This lack of data impedes an assessment of the role of permeable sands for the cycling of matter in the reef and led to the initiation of the present study.

The main objectives were to: (1) assess magnitude and temporal variability of sedimentary O_2 flux in fringing reef sands in order to elucidate their function in the cycling of carbon in the reef, and (2) investigate the O_2 uptake of sandy sediments located at a deeper site in the lagoon. Working hypotheses were as follows: (1) sedimentary O_2 uptake shows temporal and spatial variation caused mainly by changes in organic matter supply and water column conditions (e.g. temperature, nutrient concentrations), and (2) sandy reef sediments in deeper waters can accumulate more organic matter and thus have a higher O_2 uptake. These working hypotheses were evaluated with a temporal series of *in situ* benthic chamber and sediment trap deployments in a Red Sea subtropical fringing reef over a total period of >3 yr.

Material and Methods

Study site

The present study was conducted at a fringing reef located near the Marine Science Station (MSS) in Aqaba, Jordan (29° 27' N, 34° 58' E). During 3 field expeditions (May–June 2004, Nov.–Dec. 2006, Aug. 2007), 12 independent *in situ* experiments with stirred benthic chambers were carried out at different water depths on calcareous sediment locations in the lagoon and reef as summarized in Table 1; 3 stations at 2.5, 5.5 and 9.5 m water depth were established in the lagoon sand area (lagoon sands) and 2 stations at 7.0 and 16.5 m depth in the reef area on small sand patches between the coral colonies (reef sands; Fig. 1). Mean grain

size at the long-term monitoring station at 2.5 m water depth was 559 µm, and organic content was 0.36% (Rasheed et al. 2003a). Sediment permeability at this station was 11.6 ± 1.1 × 10–11 m2 (Wild et al. 2005). Water temperatures at the study site ranged from 20°C in February to 28°C in August. Due to strong evaporation, salinity is relatively high (40.3 to 40.8) year-round (Manasrah et al. 2006). Water currents are relatively weak and typically do not exceed 25 cm s^{-1} at the surface and 5 cm s^{-1} at the bottom (Manasrah et al. 2006). Due to these calm conditions, suspended particles can settle out of the water column resulting in low turbidity and deep light penetration promoting coral growth.

Table 1 *Summary of all stirred benthic chamber deployments on calcareous sands in the lagoon (L) and coral reef (R) areas at the study site*

Expedition	Date (dd.mm)	Area	Depth (m)	No. of chambers
Spring 2004	27.05	L	2.5	4
	31.05	L	2.5	2
	04.06	L	2.5	1
	05.06	L	2.5	1
	14.06	L	2.5	2
Autumn 2006	22.11	L	9.5	2
	26.11	L	2.5	3
	29.11	L	5.5	4
	02.12	R	16.5	3
	04.12	R	7.0	2
	06.12	L	9.5	3
Summer 2007	21.08	L	2.5	2

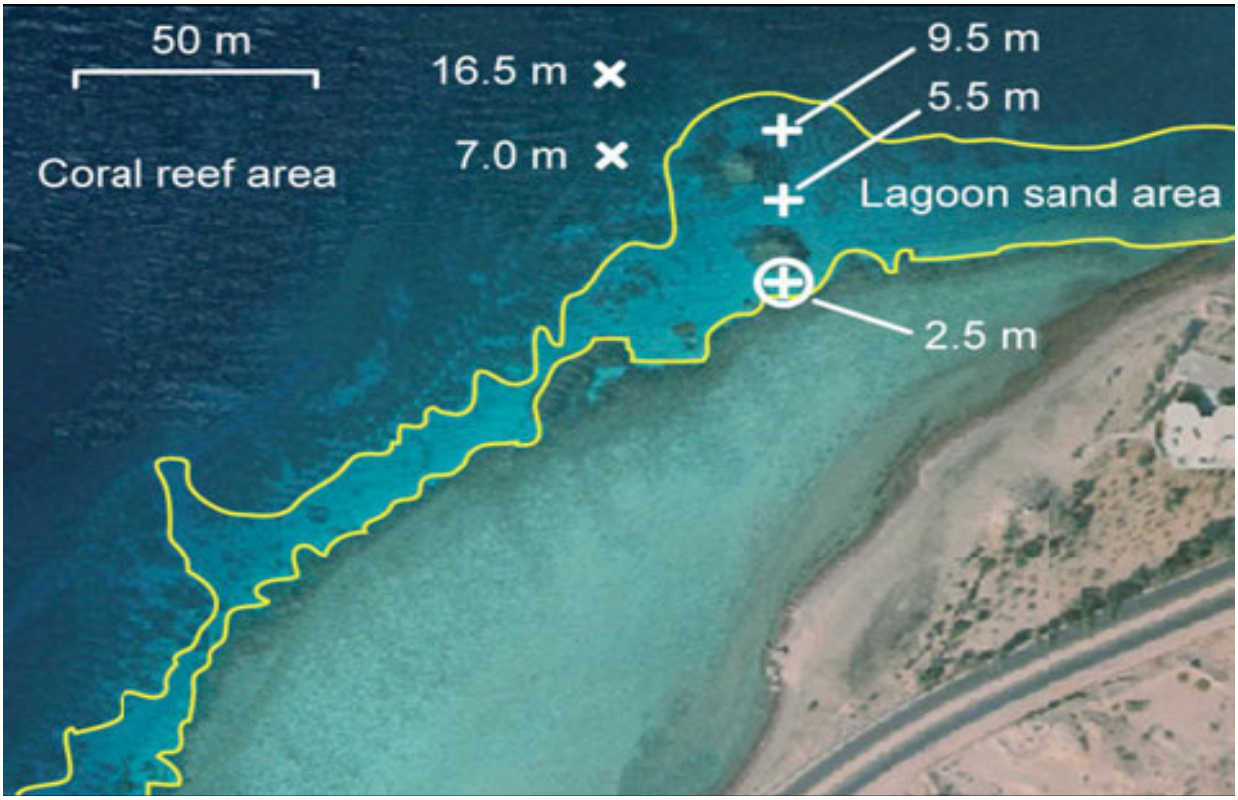

Fig. 1 *Aerial photograph of the study area off Aqaba with the locations of the chamber measurements on reef sands (x) and lagoon sands (+). The long-term monitoring station is indicated by the circle. The yellow line delineates the back-reef lagoon. The white building at right is the MSS (see 'Materials and methods—study site'). Photograph courtesy of R. Mobiedeen, ASEZA GIS Unit, Aqaba, Jordan*

In situ measurement of sedimentary O_2 uptake

For each experiment, between 1 and 4 stirred benthic chambers identical to those described by Huettel & Gust (1992) and Wild et al. (2004b,c, 2005) were used to measure sedimentary O_2 uptake. The opaque cylindrical chambers were 30 cm in height with an inner diameter of 19 cm and excluded all light from the enclosed water and sediment (benthic primary production rate was not addressed in the present study). A plastic lid containing a sampling port for water extraction and a second port permitting replacement of the sampled water volume covered each chamber. The water inside the chambers was circulated by a horizontally rotating disk 17 cm in diameter. The disk, driven by a 12 V DC motor, rotated about 8 cm above the sediment at a computer-controlled speed. For the deeper deployments, chambers were used with motors in pressure-proof titanium housings as described in Cook et al. (2007). In order to reproduce the advective pore water exchange that affects interfacial solute exchange in permeable sediments (Huettel et al. 1996, 2003), the flux chamber mimicked the lateral pressure gradients generated by the interaction of boundary layer currents with sea bed topography. The stirring in the chambers was set to produce a radial pressure gradient that corresponds in magnitude to the pressure gradients produced by the interaction of boundary currents and sediment topography at the study sites. Water currents ranged from 5 to 15 cm s^{-1} at ~10 cm above the bottom, as inferred from the movement of buoyant particles carried by the bottom flows. Topographical structures of the sandy bottom did not exceed 5 cm in height. For such settings, flume measurements have shown that lateral pressure gradients at the sediment–water interface range from 0.01 to 0.1 Pa cm^{-1} (Huettel & Gust 1992). For our flux measurements, the chamber stirring was adjusted to 20 rpm producing a radial pressure

gradient of 0.07 Pa cm^{-1} at the sediment-water interface, which can be considered a conservative setting for the study site. Details of the functioning of these chambers are given in Huettel & Gust (1992).
The duration of the individual chamber experiments ranged between 5 and 8 h. Prior to each experiment, chambers were gently inserted into the loose calcareous sands to a depth of about 12 cm marked by a ring of tape on the chamber wall, and thus included a water column of approximately 18 cm height and 5.7 l volume. Chambers were generally operated using SCUBA. Special care was taken to remove any air bubbles enclosed in the chambers. Chambers for parallel measurements and the assessment of spatial variability of flux were placed within an area of approximately 3 × 3 m. Water samples (60 ml) were extracted from the chambers using plastic syringes at preset time intervals (30 to 120 min) for later analyses of O_2 concentrations. Samples were fixed within 15 min after collection and measured using Winkler titration within 1 h after fixation. Sedimentary O_2 uptake was evaluated by linear regression of O_2 concentrations over time (at least 4 data points for each chamber) and related to the enclosed water volume and sediment surface.

Water column parameters

Water temperature was measured in direct vicinity to the chambers during the experiments using HOBO temperature loggers. Water samples were collected parallel to the benthic chamber experiments (see below) at 9 m water depth (1 m above the reef) in replicates of n = 4 in clean 5 l plastic containers using SCUBA. Water samples were then processed within 30 min or kept at 4°C for <12 h before processing. Subsamples were taken from the containers after homogenization through agitation. Salinity as measured with a hand refractometer was always
between 41 and 42.
For measurement of dissolved organic carbon (DOC) concentrations, ca. 10 ml of the sample solutions were filtered through 0.2 µm sterile syringe filters (polyethersulfone membrane, VWR International). The first 4 ml of the filtrate were discarded and the following 6 ml were collected in new, precombusted glass ampoules, which were instantly frozen at –20°C and kept frozen until analysis by high-temperature catalytic oxidation (HTCO) using a Rosemount Dohrmann DC-190 total organic carbon (TOC) analyzer. Non-purgable organic carbon (actual DOC) was measured by sample acidification with orthophosphoric acid to pH <2 and sparging with oxygen. Specific concentrations of potassium hydrogen phthalate were measured as elemental standards (SD < 3%).
Subsamples from each container were used in order to determine the microbial oxygen consumption rate. For this purpose the initial dissolved oxygen concentration of each subsample was measured using an optical dissolved oxygen sensor (HACH LANGE HQ10, accuracy ±0.05% of the effective range). The subsamples were then incubated in 60 ml Winkler glass bottles in the dark at *in situ* temperatures for 16 to 24 h. Oxygen concentrations at the end of the period were measured again as described and the difference was used to calculate microbial oxygen consumption rates.
Subsamples for particulate organic carbon (POC) and particulate nitrogen (PN) were obtained by filtering 500 ml (fall 2006) or 1000 ml (summer 2007, winter 2008, spring 2008) seawater from each container onto precombusted GF/F filters (Whatman, diameter: 25 mm, nominal particle retention: 0.7 µm). The filters were stored in Eppendorf cups and dried for at least 48 h at 40°C and kept dry until further analysis. POC and PN contents on the filters were measured using a THERMO™ NC 2500 elemental analyser. Peptone, atropine and cyclohexanone-2,4-dinitrophenylhydrazone were used as standards, and SD of replicate measurements were <3%.
Subsamples for chlorophyll *a* (chl *a*) analysis were obtained identical to POC and PN subsamples but stored frozen at –20°C and lightproof until further analysis. Chl *a* was

extracted from the filters by immersion in 90% acetone for 24 h in the dark at 4°C and measured by fluorometric analysis as described in Rathbun et al. (1997) using a TD-700 laboratory fluorometer.
Inorganic nutrient concentrations (nitrate and phosphate) were measured monthly and provided by Drs. M. Al-Zibdah and M. Y. Rasheed, MSS Aqaba.

Particulate organic matter (POM) reaching the seafloor

In order to determine amount and composition of POM reaching the seafloor, custom-made sediment traps were used. Traps were deployed in triplicate, spaced approximately 10 m apart from each other, at 1, 5, 10 and 20 m depth during each of the field expeditions in December 2006 (autumn), August 2007 (summer), February 2008 (winter) and May 2008 (spring). The 5 m depth traps were deployed on the lagoon sands using SCUBA, while at the remaining depths, all traps were placed on the reef sands. Each trap consisted of a plastic funnel (12 cm diameter)
attached to a 600 ml plastic sampling container weighted with a 1 kg piece of lead mounted underneath. Each sampling container was partly buried in the loose sand, and the funnel was fixed at a height of 7.5 cm above the seafloor. Traps were deployed for 48 h. After the collection period, all material that settled onto the funnel was carefully washed *in situ* using SCUBA into the container, using a 60 ml syringe. Subsequently, the funnel was detached from the sampling container, which was simultaneously closed with a lid and transported to the laboratory. The water with suspended material contained in the trap was decanted into a clean 1000 ml container. Organic particles remaining in the trapped sediment were extracted by resuspending the sediment 3 times with seawater and decanting the water with suspended organic matter after the heavier carbonate grains had settled. In total, 48 trap contents were sampled and analyzed. The collected contents of the traps were either processed immediately or kept at 4°C for <12 h before processing. Aliquots of the trapped material were prepared for the analysis of POM (POC + PN) content by filtering the particulate material onto precombusted GF/F filters (Whatman, 0.7 μm nominal pore size), which were subsequently dried for 48 h at 40°C. Filter samples for POC analysis were exposed to a fuming HCl atmosphere for 24 h before measuring, to remove remaining small carbonate grains. All data were related to the trapping area as determined by the funnel diameter.
Subsamples for chl *a* analysis in the trapped material were obtained identically to POC and PN subsamples, but stored frozen at –20°C and lightproof until further analysis. Chl *a* was extracted from the filters by immersion in 90% acetone for 24 h in the dark at 4°C and measured by fluorometric analysis as described above.

Results

Fig. 2 shows the O_2 concentration over the time course of 4 independent benthic chamber experiments on lagoon and reef sands at different water depths. O_2 concentration decreased linearly ($r2 > 0.95$) in all 29 chamber deployments during the 12 independent chamber experiments.

Temporal variability of sedimentary O_2 uptake

Over the observation period of >3 yr, O_2 uptake of the calcareous sands at the reference station at 2.5 m water depth ranged from 19 mmol m^{-2} d^{-1} in June 2004 to 27 mmol m^{-2} d^{-1} in November 2006 (Fig. 3). The temporal variability of O_2 uptake ranged from 15 to 25 mmol m^{-2} d^{-1} ($n = 10$) in spring 2004, and 26 to 28 mmol m^{-2} d^{-1} ($n = 3$) in autumn 2006. These temporal differences in sedimentary O_2 uptake were not statistically significant (Mann-Whitney-Wilcoxon *U*-test, $p > 0.05$). Sedimentary O_2 uptake showed no correlation with

water temperature, which ranged from 23 to 27°C (Fig. 3). Inorganic nutrient and chl *a* concentrations (Tables 2 & 3) were low from May to October, and high from November to April. The differences in chl *a* concentration in the water column in winter and spring, compared to summer and autumn, were significant (1-way ANOVA, $p < 0.001$).

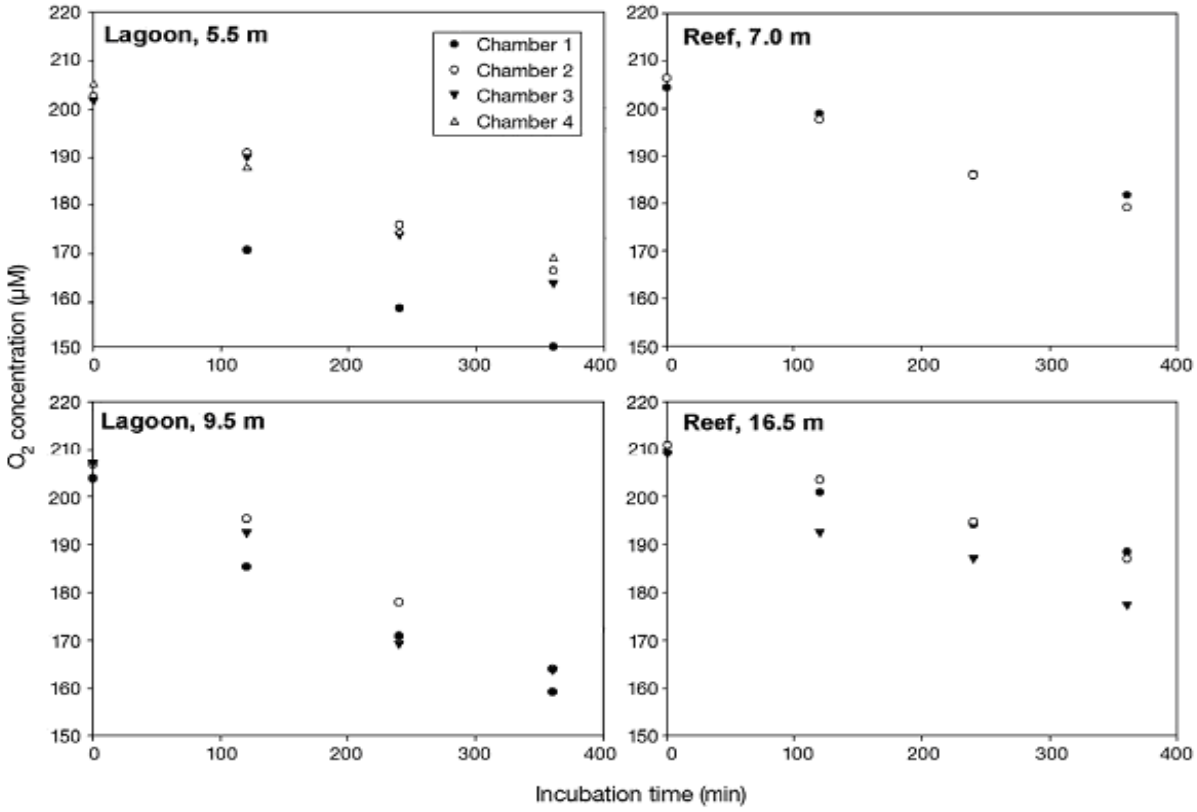

Fig. 2 *O_2 concentration development during 4 independent benthic chamber experiments on lagoon and reef sands at different water depths*

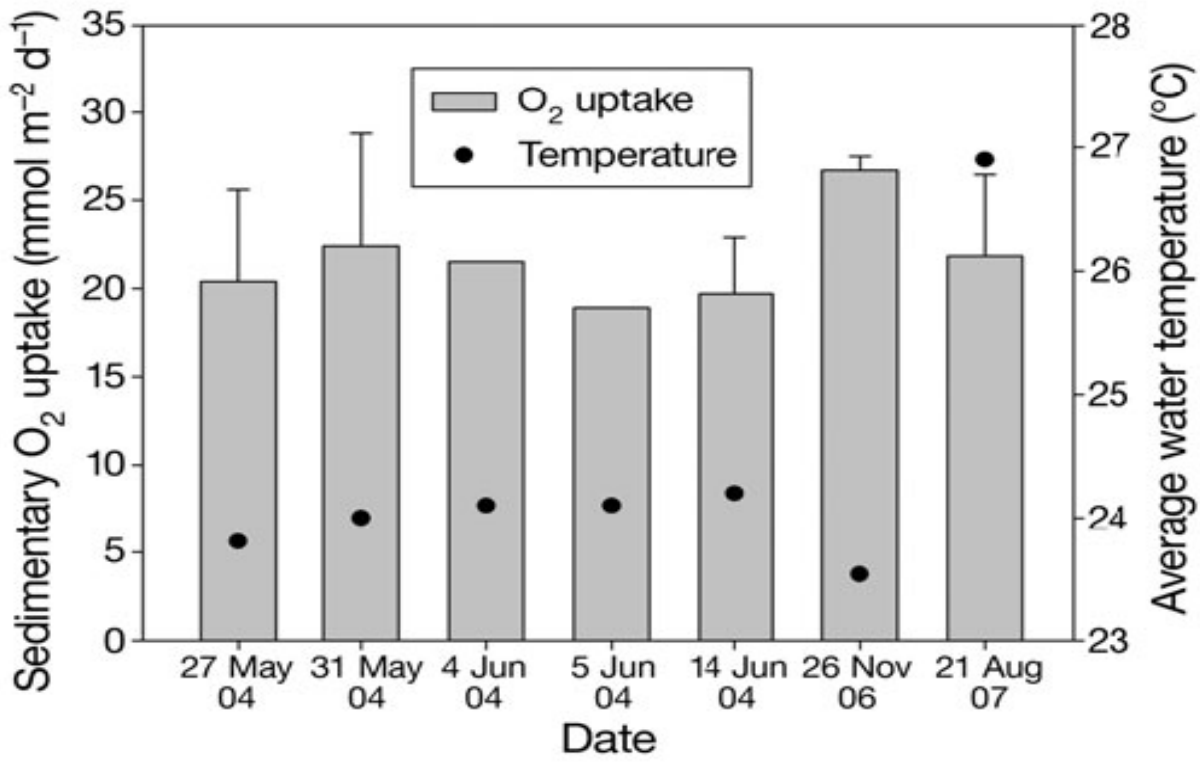

Fig. 3 *Temporal changes in sedimentary O_2 uptake measured with benthic chambers (mean + SD) and mean water temperatures at the long-term monitoring station at a water depth of 2.5 m. These chamber measurements were carried out from spring 2004 to summer 2007*

Depth variability of reef sand O_2 uptake

In the lagoon, sedimentary O_2 uptake of the calcareous sands was positively correlated with water depth ($R^2 = 0.85$, ANOVA of linear regression $p < 0.001$), with maximum values of almost 40 mmol m^{-2} d^{-1} at 9.0 m water depth (Fig. 4). In contrast, O_2 uptake at the reef sand patches located between coral colonies down to 16.5 m did not reveal such a trend with water depth (Fig. 4). In these reef sand patches, O_2 uptake was similar (around 20 mmol m^{-2} d^{-1}) to those rates measured at the reference station at 2.5 m water depth (Fig. 3).

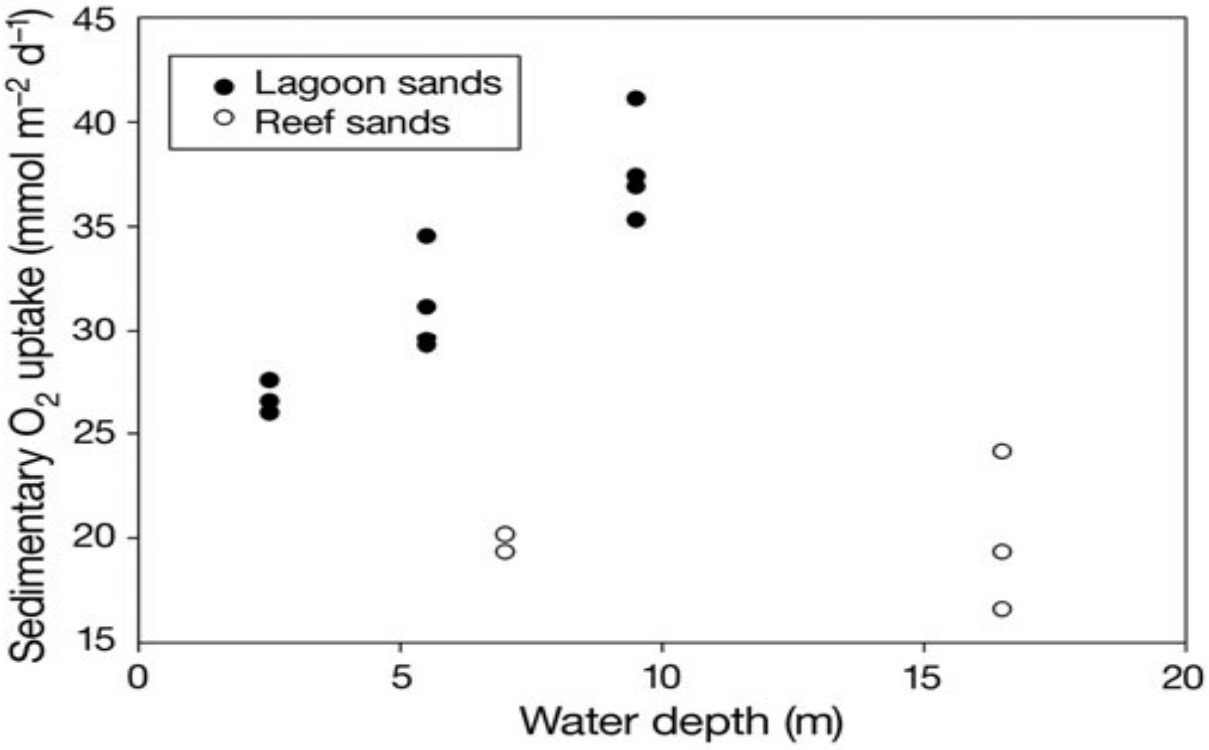

Fig. 4 *Sedimentary O_2 uptake at lagoon and reef sands versus water depth as measured with benthic chambers. All chamber measurements for these investigations were carried out during the autumn expedition in November–December 2006*

Organic matter sedimentation

POC and PN supply to the reef sediments, as measured seasonally by the sediment traps, did not show a positive correlation with water depth (Fig. 5). Statistical analysis revealed significantly higher annual POC supply to the reef sediments only at 10 m water depth compared to 5 m (paired *t*-test, $p = 0.029$), but the amount of sedimented material decreased again at 20 m. According to our trap samples, there were no significant temporal differences in POC and PN supply to the reef sediments, but the highest POC sedimentation rates were observed in summer (86 ± 40 mg POC m^{-2} d^{-1}) and the lowest in winter (39 ± 13 mg POC m^{-2} d^{-1}), whereas average PN sedimentation was highest in spring (7.3 ± 3.3 mg PN m^{-2} d^{-1}) and

lowest in winter (4.5 ± 1.5 mg PN m^{-2} d^{-1}). Likewise, chl a contents in sediment traps were highly variable and thus displayed no significant temporal differences (Fig. 6).

Table 2 *Water column temperature and inorganic nutrient concentrations measured parallel to the benthic chamber deployments*

	Temperature (°C)	Nitrate (µM)	Phosphate (µM)
Autumn 2006	22.8–24.6	0.27–0.34	0.04–0.05
Summer 2007	26.4–28.7	0.12–0.17	03–0.03
Winter 2008	20.6–21.	0.76–0.90	0.06–0.07
Spring 2008	22.0–25.7	0.37	0.04

Discussion

Temporal changes in sedimentary O_2 uptake and organic matter supply

Wild et al. (2005), by using transparent and opaque benthic chambers, revealed that gross primary production in the calcareous sands at the study site ranged between 15 and 23 mmol O_2 released m^{-2} d^{-1} and sedimentary O_2 uptake accounted for 13 to 25 mmol O_2 consumed m^{-2} d^{-1}, which characterizes these sands as largely independent of allochthonous carbon input. Sedimentary O_2 uptake at the long-term monitoring station at 2.5 m water depth (19 to 27 mmol m^{-2} d^{-1}) was lower than rates described for other coral reef sand areas in similar water depths and investigated with identical methodology, e.g. Heron Island, Australia, with O_2 uptake ranging from 49 to 93 mmol m^{-2} d^{-1} (Rasheed et al. 2004, Wild et al. 2004a,b, Glud et al. 2008). This was very likely caused by higher nutrient availability due to abundant vegetation and a dense bird colony at Heron Island, whereas no such landderived influence occurred at the present study site in the northern Red Sea. Sedimentary O_2 uptake at the shallow long-term monitoring site changed less than 10 mmol m^{-2} d^{-1} between our different measurements over the 3 yr study period. This was unexpected as inorganic nutrient and chl *a* concentrations in the water column site showed temporal variations. These observations agree with those of Rasheed et al. (2002, 2003b). Measurements conducted during the present study also revealed higher DOC water concentrations during the chamber deployments in autumn 2006, compared to those in summer 2007 (Table 3). Higher planktonic microbial activity measured as O_2 consumption in autumn and spring, compared to winter and summer (Table 3), indicate temporal differences between degradability of suspended organic matter.

Table 3 *Water column organic matter concentrations and microbial O_2 consumption measured parallel to the benthic chamber deployments. POC: particulate organic carbon; PN: particulate nitrogen; DOC: dissolved organic carbon; nm: no measurement*

	POC (mg l^{-1})	PN (mg l^{-1})	DOC (mg l^{-1})	Chl *a* (µg l^{-1})	O_2 consumption (µM d^{-1})

Autumn 2006	0.08–0.17	0.01–0.02	5.6–11.2	0.15–0.47	4.7–24.1
Summer 2007	nm	nm	0.8–1.1	0.15–0.28	3.7–9.5
Winter 2008	0.05–0.15	0.01–0.01	0.7–2.9	0.29–1.01	2.5–6.1
Spring 2008	0.08–0.31	0.01–0.04	1.0–1.6	0.27–0.40	8.0–21.6

While the water column processes thus went through an annual cycle with low nutrient and organic carbon availability in summer and higher availability in winter (Table 3), the benthic processes did not follow this trend. This decoupling of sedimentary from water column processes may be explained by a relatively constant supply of organic matter to the sediments at the long-term monitoring station, as demonstrated by the sediment trap data, despite the production changes in the water column. The relatively large fluctuations of the C:N ratios in the trap material reflect the contribution of pieces of refractory detritus material (e.g. pieces of macrophytes and seagrass) settling into the traps. The highest POM concentrations in the water column in autumn and spring (Table 3) did not result in higher amounts of POC and PN in the sediment traps. A pulse of organic matter to the sediment results in a response in sedimentary O_2 uptake that may last for a relatively long period; for example Wild et al. (2004c) and Glud et al. (2008) observed that the sudden organic matter supply to coral reef sands in the Great Barrier Reef caused by a coral mass spawning event increased O_2 uptake over several weeks. In the present study, such an increase was not detectable, neither in autumn nor in spring after the usual phytoplankton blooms. Another reason for the observed low variability in O_2 flux may be that the reef sediments have some buffer capacity for organic matter (i.e. sediments can rapidly pick up organic matter that is then degraded over a longer time period), despite their function as biocatalytical filter systems (Wild et al. 2004a,c, 2008). This is supported by Eyre et al. (2008), who demonstrated the buffer function of reef sediments for phosphorus. After a sedimentation event, organic matter may be adsorbed by the relatively large surface area of the porous carbonate grains (Wild et al. 2006), loading the porous matrix with degradable material like a sponge soaking up water. Although the degradation rates may be high due to the advective flushing of the permeable bed (Precht & Huettel 2004, Cook et al. 2007), this loading process can dampen oscillations in the O_2 consumption rates between periods of increased organic matter input to the sediment.

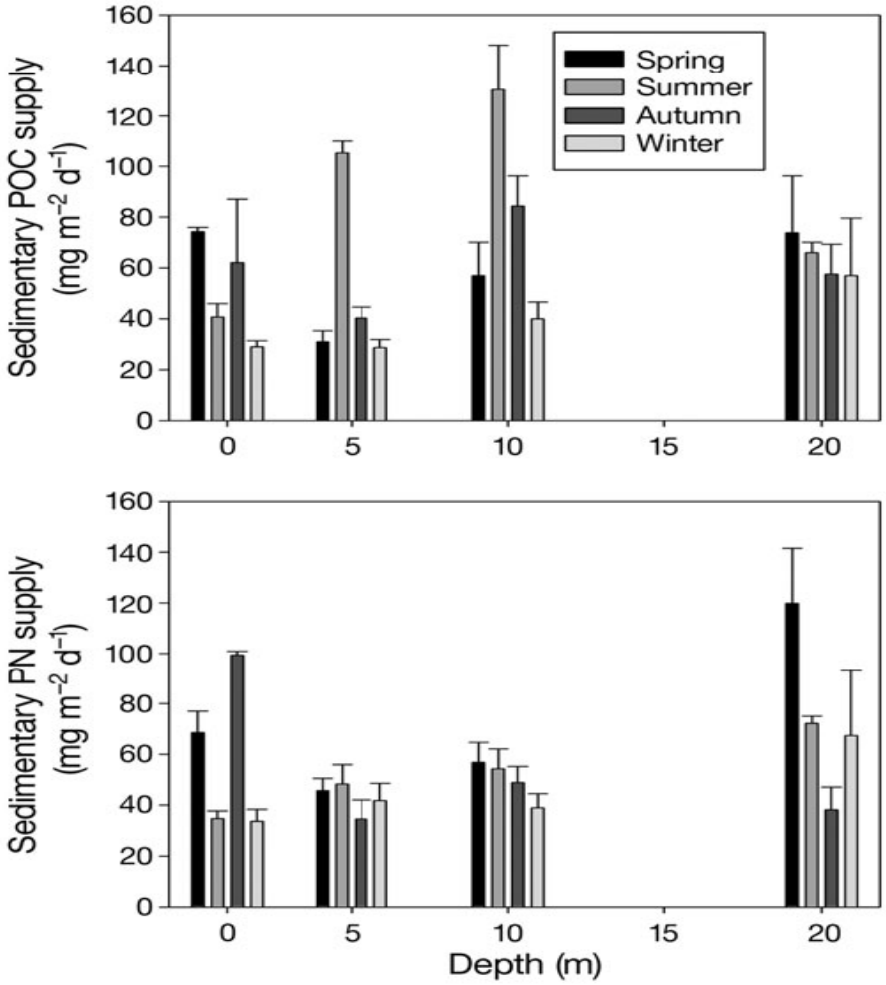

Fig. 5 *Particulate organic carbon (POC) and particulate nitrogen (PN) supply to reef sediments over time measured with sediment traps (mean + SD). All traps were deployed in the reef, except traps at 5 m water depth, which were deployed in the lagoon*

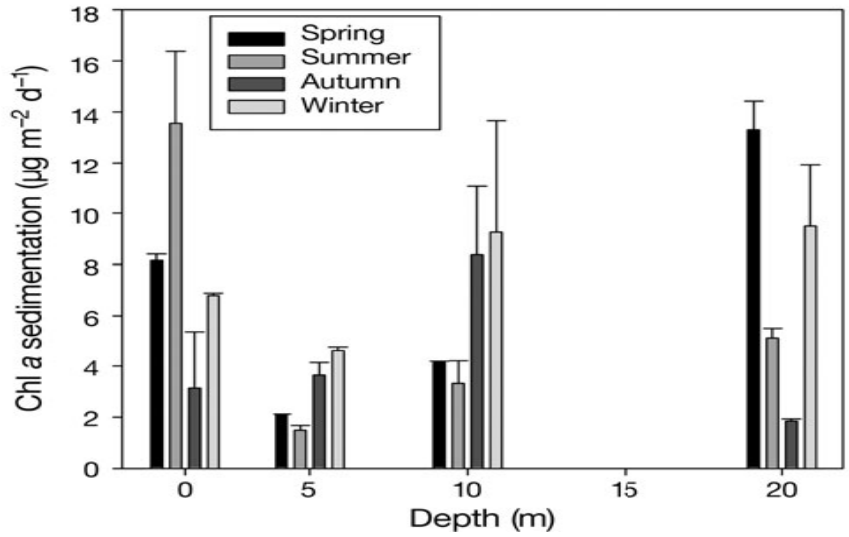

Fig. 6 *Chlorophyll a sedimentation over time measured with sediment traps (mean + SD). All traps were deployed in the reef, except traps at 5 m water depth, which were deployed in the lagoon*

Sedimentary O_2 uptake and organic matter supply at different depths

While temporal changes in O_2 flux were relatively small, larger increases in O_2 flux were observed with increasing water depths in the lagoon sands. This positive correlation may have been caused by: (1) the higher water column at the deeper sites, producing more particulate organic matter that could settle to the bottom than the shorter water column at the shallow site; (2) lateral transport processes providing more organic matter to the deeper sites; and (3) reduced sediment resuspension at the deeper sites, permitting accumulation of low-density organic particles and reduced substances (e.g. sulphide) in the sediment. In the clear oligotrophic waters where coral reefs grow, high light intensities can penetrate deeply, permitting photosynthesis at water depths of 60 m or more (Vooren 1981, Jarrett et al. 2005). Pelagic primary production here takes place throughout the water column above the reef. In the clear tropical waters, phytoplankton accumulations can form in deep water layers, where light levels are still sufficient for photosynthesis and nutrients are more concentrated than in the water near the surface (McManus & Dawson 1994, Gattuso et al. 2006). In such environments, the amount of POC integrated over the entire water column above the reef can thus increase with water depth. Where reef-forming corals grow, steep slopes can form as the reef framework, cemented by coralline algae and sponges, has more structural strength than, for example, sandy or muddy deposits. Consequently, particles that settle on the steep surfaces of the reef are easily entrained and then accumulate in the troughs and crevices of the reef framework or rush down the slopes of the reef. Our sediment traps accumulated materials settling from the water column and also materials that had been resuspended. A clear distinction of the 2 sources is difficult in the waveswept reef environment, because a large fraction of the suspended particle load in the water originates from resuspension. The important observation here is that the trapped material did not reflect the temporal production changes in the water column. As the intensity of the hydrodynamic forces caused by surface waves and wind-driven currents decrease with depth, the deeper zones of the reef are calmer, permitting deposition of low-density materials including organic particles. Accumulation of fine particles and organic detritus in the sand decreases the permeability of the sediments in the deeper sections of the reef. Higher organic content and less hydrodynamic flushing of the sediments can lead to oxygen depletion, which leads to the build-up of sulphides resulting from microbial sulphate reduction activities.

Nevertheless, the trend of increasing sedimentary O_2 uptake with increasing depth was not observed in the reef sand patches embedded between living coral colonies. One reason may be that around these small sand patches, in contrast to the large lagoon sand areas, benthic suspension feeders, in particular hermatypic corals, occur at high abundances, covering 29 to 67% of the seafloor (M. Naumann et al. unpubl. data). The intense feeding on POM by corals has been demonstrated by Anthony (1999), but other coral reef organisms such as gastropods (Kappner et al. 2000), bivalves (Monismith et al. 1990), sponges (Richter & Wunsch 1999), ascidians (Petersen 2007), and polychaetes (Jordana et al. 2000) also filter particles from the water column, thereby reducing organic matter flux to the reef sand patches. As the digestion of the trapped materials by these animals is often incomplete (Coffroth 1984, Kappner et al. 2000, Ribak et al. 2005), the sedimentary microbial community can benefit from the nearby high macrofauna abundance through a continuous lateral supply of organic matter (e.g. in the form of fecal pellets and coral mucus), which may support the low but relatively constant sedimentary O_2 uptake rates measured at these sites.

In conclusion, the observed lack of temporal benthic O_2 uptake changes in the Aqaba reef sands reflects efficient functioning and recycling in the oligotrophic reef ecosystem. Large variations in sedimentary O_2 uptake would require large variations in the organic matter input. This could only be caused by production of organic matter that is not consumed in the water column or by benthic reef organisms, and therefore settles to the sediments. In an organic matter-limited ecosystem such as this reef, any primary production likely is effectively

recycled in the food web of the water column and reef framework (Richter et al. 2001), with the detrital food web being of lower importance, as labile detrital matter cannot accumulate due to the efficient recycling. The observed sedimentary O_2 uptake increase with depth at the lagoon sands therefore is a function of the lateral supply of fresh organic particles produced by the adjacent reef organisms that actively filter particles from the water column. Coral mucus may play a major role in this process, because it often dominates suspended organic matter in coral reefs (Johannes 1967, Marshall 1968), and at the study location a majority of this material reaches the lagoon sands in very close vicinity to the reef (Wild et al. 2005, F. W. Mayer et al. unpubl. data).

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Organic matter release by the dominant primary producers in the Puerto Morelos reef lagoon, Mexican Caribbean - implication for in-situ O_2 availability

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Abstract

Coral reef lagoon associated benthic primary producers may control various processes important for ecosystem functioning, predominately via the release of organic matter, but related data are rare. The present study therefore comparatively investigated quantity and chemical composition of particulate and dissolved organic matter released by different benthic primary producers (seagrasses, macroalgae and scleractinian corals) from the coral reef lagoon of Puerto Morelos, Mexican Caribbean. Microbial degradability of the released organic matter was determined along with diurnal in-situ measurements of O_2 concentrations at lagoon sites dominated by different primary producers. Particulate organic carbon (POC) release was highest for corals (8.2 ± 4.2 mg m^{-2} h^{-1}) followed by benthic algae (3.9 ± 0.7 mg m^{-2} h^{-1}) and seagrasses (3.1 ± 2.0 mg m^{-2} h^{-1}). Dissolved organic carbon (DOC) release rates were highest for seagrasses (15.8 ± 6.0 mg m^{-2} h^{-1}) followed by algae (1.9 ± 2.0 mg m^{-2} h^{-1}), whereas corals displayed net DOC uptake. Benthic algae-derived organic matter stimulated planktonic microbial O_2 consumption significantly more, when compared to seagrass- or coral-derived organic matter. In-situ O_2 loggers revealed significantly lower average O_2 concentrations, particularly during the night, at algae-dominated sites compared to other benthic lagoon environments. This indicates negative effects of algae-derived organic matter on in-situ O_2 availability. The present study therefore suggests that shifts in benthic primary producer dominance affect ecosystem functioning owing to differences in quantity, composition and microbial degradability of the released organic matter.

Introduction

Caribbean coral reef lagoons typically accommodate different species of seagrasses, macroalgae and scleractinian corals (Williams 1987; Nagelkerken et al. 2000). These lagoon ecosystems are highly productive, with rates of primary production similar to those of coral reef ecosystems (Odum et al. 1959, Odum 1971). High associated biomass, productivity and turnover of organic material can serve as a reservoir for complex food chains (Ogden & Zieman 1977, Ziegler 1999). Caribbean coral reef lagoons thereby provide organic compounds derived from the occurring primary producers as resources for fish and invertebrates. Contrary to coral reef ecosystems, which usually display close linkage between trophic levels relevant for element recycling processes (Lesser 2004), reef lagoons display more open nutrient cycles (Ogden & Zieman 1977). Therefore, primary producer-derived organic compounds may even be exported to adjoining ecosystems (Jackson et al 2001), where they serve as substantial supply in extremely food-limited environments (Suchanek et al. 1985).

Tropical reef lagoon associated benthic communities are structurally and functionally complex habitats (Biber et al. 2004) and thus particularly susceptible to environmental changes (Eyre & Ferguson 2002; Fonseca et al. 2000; Kendrick et al. 2000). The typical lagoon community response to environmental disturbances (e.g. nutrient enrichment, terrestrial run offs, changes in herbivore abundance) is a sequential change in the dominance of primary producers (Nienhuis 1992, Castel et al. 1996, Raffaelli et al. 1998). Those primary producers, partly via their ability to influence the environment by the release of organic matter (Costanza et al. 1997; Hemminga & Duarte 2000; Wild et al. 2004a), display the basis of the food chain of reef lagoons and adjoining ecosystems.

Organic matter released by different marine primary producers may thereby affect activity and growth of microbial communities, which in turn play an important role in the transfer of energy to higher trophic levels (Tenore 1977, Azam et al 1993; Ferrier-Pages et al. 2000; Eyre & Ferguson 2002 Wild et al. 2004a; Wild et al. 2005). The effects of the released organic matter on the microbial community can significantly differ between primary producing organisms (Wild et al. 2009). For example, coral-derived organic matter in form of mucus can function as important energy and nutrient carrier in benthic-pelagic coupling processes (Wild et al. 2004b; Naumann et al. submitted). Thereby, it may influence planktonic as well as benthic microbial metabolism (Wild et al. 2005; Huettel et al. 2006). Such complex functions are likely not substituted by reef-associated benthic algae derived organic matter (Wild et al. 2009). Laboratory studies (Kline et al 2006; Smith et al 2006) further indicated that benthic reef algae may affect processes, such as microbial activity, by the release of more labile dissolved organic matter (DOM) compared to corals. The faster heterotrophic utilization of algae-derived organic matter may then influence planktonic O_2 dynamics (Eyre & Ferguson 2002) potentially leading to O_2 deficiency critical for some ecosystem inhabitants (Kline et al. 2006; Smith et al. 2006).

Phase shifts in the primary producer community of reef lagoons could therefore cause changes in cycles of matter (Eyre & Ferguson 2002), benthic and planktonic degradation and therefore O_2 availability. The basis to understand these changes is to characterize the organic matter (e.g. quantity, chemical composition and degradability) released by the different primary producers and its influence on the microbial community. A most recent study by Wild et al. (submitted) comparatively investigated those interactions for a fringing coral reef ecosystem in the Northern Red Sea. However, not much is known about the organic matter released by primary producers in coral reef lagoon systems (reviewed in Kaldy et al. 2002) and their contribution to ecosystem functioning (Ziegler & Benner 1999). Only few studies have investigated the production of organic matter relative to benthic community composition in tropic lagoon systems (Brylinsky 1977; Kirkman & Reid 1979; Ziegler & Benner 1999), and no study has carried out a comparative investigation on the related effects on microbial activity. The present study therefore aims to give a comprehensive overview of organic matter release by the dominant primary producers in a typical Caribbean reef lagoon and its subsequent influence on the activity of the planktonic microbial community as well as in-situ O_2 availability.

Material and Methods

Study site

The study was conducted at the Institute of Marine Sciences and Limnology (ICML, 20° 52´ N, 86° 52´ E) of the National Autonomous University of Mexico (UNAM) in Puerto Morelos, Mexico, from 15.07.2008 to 07.08.2008. An extensive coral reef lagoon is located alongside the ICML and is bordered at the seaward site by the north-south orientated Mesoamerican barrier reef system. Jordan et al. (1981) divided this reef system in front and rear reef, reef crest and reef lagoon. The reef lagoon extends from the coastline to the barrier reef that runs diagonally at a distance of 1500 m in the north and 200 – 300 m close to Puerto Morelos village. Mean water currents inside the lagoon are 0.1 m s^{-1} directed to the north, as recorded by previous studies of Coronado et al. (2007) and Merino-Ibarra & Otero-Dávalos (1991). The maximum water depth was 4 m in the central lagoon. The seafloor of the lagoon consisted mostly of calcareous sand, which was covered by seagrass meadows (Collado-Vides 1998) with occasional algae, soft and hard coral patches.

Environmental parameter monitoring

As previous studies showed that environmental parameters have considerable influence on organic matter release by benthic organisms (Ziegler & Benner 1999; Naumann et al. submitted; Haas et al. submitted), temperature and light availability were recorded during the whole study period. Both parameters were measured in triplicates within a radius of 10 m, using light and temperature loggers (Onset HOBO® Pendant UA-002-64) with a 1-min temporal resolution and in water depths of 3 m. To allow for better comparability with previous studies, light intensities (lx), were converted to photosynthetically active radiation (PAR, µmol quanta m^{-2} s^{-1}, 400 to 700 nm). This was done by the following approximation: 1 µmol quanta m^{-2} s^{-1} = 51.2 lx (Valiela 1984).

Benthic community assessment

To identify the dominant benthic organisms and their relative seafloor cover, 12 line point intercept transects (LPI, Hodgson et al. 2004) were conducted. The related water depths, surveyed distances and distances to shore of each LPI are given in Table 1. Benthic cover, including all occurring genera of scleractinian corals (*Manicina*, *Porites*), seagrasses (*Thalassia*, *Syringodium*), dominant green algae (*Avrainvillea*, *Halimeda*, *Rhipocephalus*,

Penicillus), and other green-, red- and brown algae as well as sand as substrate, were recorded at 0.5 m intervals directly below the measurement point and marked on a dive slate. LPI data were then analysed to derive the percentage coverage for the respective benthic organisms or substrate.

Collection of specimens

Two seagrass, six benthic algae and two coral genera, together amounting to 56.9 ± 6.0 % of the total lagoon seafloor coverage and 92.6 ± 2.8 % of all benthic macroorganism coverage, were selected for incubation experiments. Coral specimens of the genus *Porites* were further investigated in bleached and unbleached conditions. A summary on all conducted incubation experiments is given in Table 2.

Specimens (n = 5 for each species or bleaching condition) were collected from individual colonies using SCUBA and transferred to cultivation tanks without air exposure. Cultivation tanks (200 l) were provided with fresh seawater flow-through (exchange rate: 150 - 200 l h^{-1}). In-situ temperature and light conditions were verified by light and temperature logger (Onset HOBO® Pendant UA-002-64) measurements.

To avoid leakage of intracellular organic matter due to potential injuries from the sampling procedure, seagrass (12 – 26 cm height) and algal specimens (5 -12 cm height) were sampled at least 48 h before the respective incubation experiment and left for healing in the cultivation tank.

Porites colonies and individual *Manicina* polyps (diameter: 3 – 9 cm) were sampled one week prior to incubation experiments and fixed onto ceramic tiles (4 x 4 cm) using small amounts of coral glue (Reef Construct, Aqua Medic®) to avoid direct contact to the sensitive tissue during experimental handling. Corals were then left for healing and acclimatisation in the cultivation tanks whereby algal overgrowth on ceramic tiles and glue junction was removed regularly. For all collected organisms, special care was taken to exclude specimens infested by epibionts or endolithic boring organisms that could have possibly affected experimental results by release or uptake of organic matter.

Setup of experiments

Each investigated benthic species was incubated in an independent beaker experiment after the method of Herndl & Velimirov (1986) with some modifications as described in Naumann et al. (submitted). Briefly, 10 glass beakers were filled with exactly 1000 ml freshly collected sea water, whereby five beakers contained the selected specimens and the remaining 5 served as control. During incubation of seagrasses, roots were tightly sealed off with a light proof plastic cover to avoid rhizomal organic matter release. To assure in-situ conditions, light intensity (lx) and water temperature (°C) were recorded at the natural habitat of the collected specimens and during the incubation experiments every 5 s using light and temperature loggers (see above). After 6 h of incubation, the specimens were removed from the beakers using sterilised forceps and the remaining incubation water was sampled and analyzed as described below.

Quantification of released organic matter

Subsamples for DOC, POC and PON measurements were collected and processed as described in Naumann et al. (submitted). For DOC analysis, aliquots of the incubation water (5 ml) were filtered (0.2 µm nominal particle retention via polyethersulfone membrane) and immediately frozen at -20 °C until analysis. DOC was measured by high-temperature catalytic oxidation (HTCO) using a Rosemount Dohrmann DC-190 TOC analyzer. Potassium hydrogen phthalate was repeatedly measured as elemental standard (standard deviation < 3%). Samples for POC and PON measurements (680 - 940 ml) were filtered on precombusted GF/F filters (nominal particle retention of 0.7 µm). Filters were then dried for at least 48 h at 40 °C

and kept dry until further analysis. POC and PON contents on the filters were measured using an elemental analyser (THERMO^{TM} 1112 Flash EA). Peptone, Atropine and cyclohexanone-2,4-dinitrophenylhydrazone were used as standards, and standard deviations of replicate measurements were < 3%.

Quantification of microbial O_2 consumption rates

To determine the influence of the released organic matter onto microbial O_2 consumption rates, sub-samples of each incubation water volume were used at the end of each incubation experiment. The initial O_2 concentration of each sub-sample was determined using an Optode (luminescent dissolved O_2 sensor, HACH LANGE HQ10, accuracy ± 0.05 %), and the sub-samples were then kept at in-situ temperature in airtight 60 ml Winkler glass bottles in the dark. After 19 - 25 h, O_2 concentrations were measured again as described above. To calculate planktonic microbial O_2 consumption rates, end value of O_2 concentration was subtracted from start value and normalized by incubation duration.

Surface area determination

The surface area of all specimens was used as the reference parameter for quantification of organic matter release rates, because of its functional importance as ecological interface to the surrounding environment (Dahl 1973). Surface area was measured for each of the incubated seagrasses and macroalgae by spreading them 2-dimensionally on a scaled paper and taking photographs (*Sony Cybershot*, resolution: 5.1 megapixels) directly from above. Digital image processing software (*ImageJ*, V. 1.37m, National Institutes of Health, USA) was then used to calculate surface areas. Coral surface areas were calculated according to the *Advanced Geometry* protocol, in combination with the respective approximation factors, introduced by Naumann et al. (2009).

Measurement of in-situ O_2 concentration

The effect of different benthic community composition on in-situ O_2 availability in the water aloft was assessed by deploying dissolved O_2 Loggers (*Eureka* Midge). On 6 different occasions between July 19 and August 2, two loggers were simultaneously deployed in locations almost exclusively (> 90 %) dominated by one type of benthic organisms or substrates (seagrass, algae, bare calcareous sand) within a radius of 1 m around the sensor. In-situ O_2 concentrations at coral dominated locations could not be assessed in the study area, owing to their scattered abundance. Logger deployments took place in water depths of 3.0 – 3.5 m. O_2 concentrations and water temperatures were measured and logged every 5 min for at least 24 h during each deployment. Measurement conducted over a sand flat from July 29 to 30 was excluded from analysis because of presumable air entrapment under the membrane of the dissolved oxygen sensor, as O_2 concentrations exceeded all other measured values with maximum O_2 concentrations above 12 mg l^{-1}.

Analysis of data and statistics

Net organic matter release (DOC, POM) by investigated reef organisms was calculated by subtracting mean control values of the measured parameters from those of each treatment. Released amount of organic matter contents was then normalized to surface area of the incubated organisms and incubation time. To calculate the percentage organic carbon contribution of each primary producer to the lagoon organic matter pool, the released total organic carbon (POC + DOC) was related to the percentage benthic cover of the respective organism. For a comparable calculation of specimen-derived organic carbon turnover rates, the total organic carbon concentration (µM l^{-1}), released by the organisms was then related to the elevated O_2 consumption (µM l^{-1}) in the respective treatment. Statistical evaluation of release rates by the respective organisms was carried out by analysis of variance (ANOVA), and homogeneity of variances was tested with a Levene test (Dytham 1999). Correlation of

organic matter release and subsequent changes in the microbial O_2 consumption rates were statistically analyzed with a model I regression showing the coefficient of determination (R^2) and the probability (p). All values are given as mean ± SE.

Results

Environmental parameters

Water temperature was 30.4 ± 0.1 °C during the study period with diurnal fluctuations of 2.0 ± 0.1 °C. Maximum water temperature of 33.1 °C was measured on July 17 and minimum temperature of 28.4 °C on July 22 following the tropical storm "Dolly" that reached the coast on June 21. Effects of the tropical storm were also visible in light availability measurements (Fig. 1). Data obtained from in-situ deployed light loggers revealed a mean average daytime = incubation time (10:00–16:00 h) PAR availability at 3 m water depth of 508 ± 4 (µmol quanta m^{-2} s^{-1}) during the study period. A maximum average daytime PAR availability of 625 ± 17 (µmol quanta m^{-2} s^{-1}) was recorded on July 28 and a minimum of 41 ± 2 (µmol quanta m^{-2} s^{-1}) on the July 21 during the passing by of the tropical storm (Fig. 1).

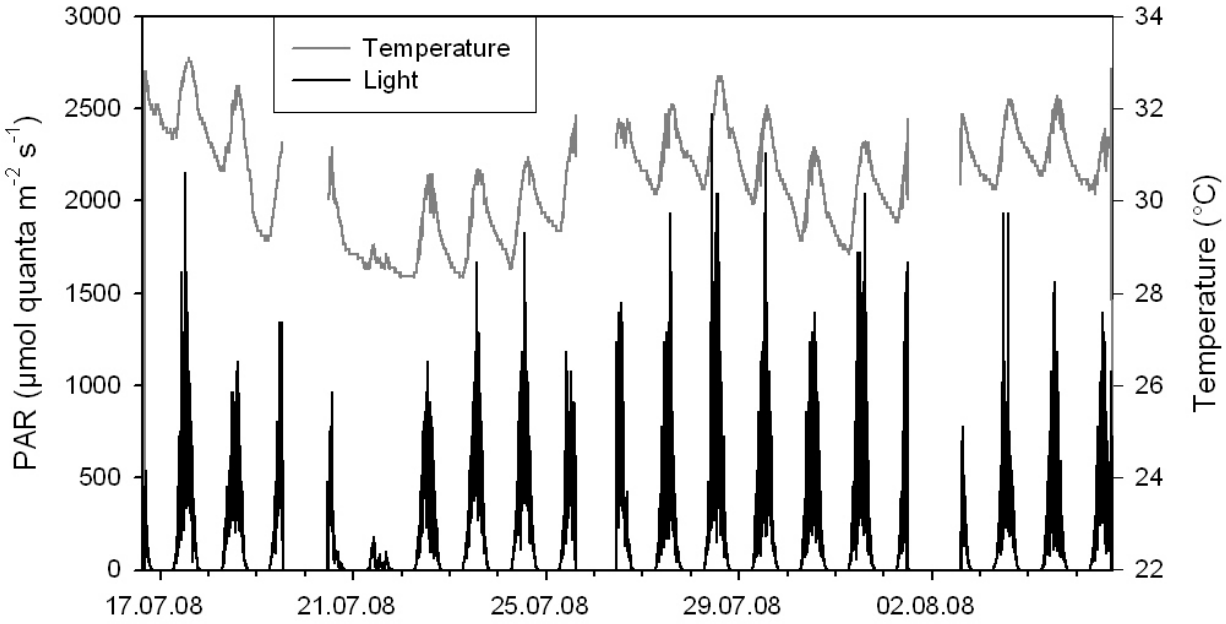

Fig. 1 *In-situ water temperatures (°C) and photosynthetic active radiation (PAR, µmol quanta m^{-2} s^{-1}) in the Puerto Morelos reef lagoon (water depth: 3 m) during the entire study period from July 16 to August 6.*

Benthic community assessment

The LPI transects revealed seagrasses as the most abundant benthic organisms in the study area (Table 1). The two dominant genera were *Thalassia* and *Syringodium* with 20.8 ± 1.6 % and 8.8 ± 1.4 % seafloor cover, respectively. Algae accounted for the second largest group with 25.3 ± 3.2 % seafloor cover. Dominant benthic algae were green algae of the genus

Halimeda (9.6 ± 1.6 %), *Avrainvillea* (7.9 ± 1.5 %), *Rhipocephalus* (1.3 ± 0.5 %) and *Penicillus* (1.0 ± 0.5 %), brown algae, mainly of the genus *Lobophora* (6.7 ± 3.7 %) and several unidentified red algae (6.1 ± 2.0 %). Scleractinian corals of the genus *Porites* (1.4 ± 0.6 %) and *Manicina* (0.6 ± 0.3) represented the third largest group of benthic primary producers. The largest proportion of seafloor in the Puerto Morelos reef lagoon was covered by bare calcareous sand (38.9 ± 4.0 %).

Table 1 *Seafloor coverage (%) by dominant benthic macro organisms in the Puerto Morelos reef lagoon obtained from LPI surveys. Values (mean ± SE) are given along with water depth, distance from the coast and length of transect.*

			Seafloor cover (%)					
Distance to coast (m)	Water depth (m)	Observed distance (m)	Scleractinian corals	Seagrass	Green algae	Red algae	Brown algae	Sand
100	2.0-2.3	2 x 50	1.0 ± 1.0	34.5 ± 2.5	15.5 ± 2.5	12.5 ± 4.5	13.0 ± 11.0	20.0 ± 3.0
200	2.5-2.8	4 x 50	4.3 ± 1.5	26.5 ± 1.6	29.5 ± 0.9	2.5 ±1.6	0.8 ± 0.8	31.3 ± 1.5
400	3.5	2 x 50	0.0 ± 0.0	24.5 ± 0.5	23.0 ± 6.0	4.0 ± 0.0	4.5 ± 4.5	43.5 ± 2.5
800	2.5	2 x 50	1.0 ± 0.5	35.0 ± 5.0	14.5 ± 1.5	0.0 ± 0.0	0.0 ± 0.0	49.0 ± 3.0
1000	2	2 x 50	1.5 ± 0.8	30.5 ± 0.5	8.0 ± 1.0	0.0 ± 0.0	1.0 ± 1.0	58.5 ± 3.5
Mean			**2.0 ± 0.7**	**29.6 ± 1.5**	**20.0 ± 2.6**	**3.4 ± 1.5**	**3.3 ± 2.0**	**38.9 ± 4.0**

Organic matter release quantification

All investigated benthic organisms showed a significant release of POC (one-way ANOVA, $p < 0.001$) and PON (one-way ANOVA, $p < 0.001$) over the incubation period. Highest POC release rates were found for corals (8.2 ± 4.2 mg m^{-2} h^{-1}), followed by benthic algae (3.9 ± 0.7 mg m^{-2} h^{-1}) and seagrasses (3.1 ± 2.0 mg m^{-2} h^{-1}), but differences between organism groups were not significant.

DOC release rates varied significantly between the different groups of organisms (one-way ANOVA $p < 0.001$). Highest release of the tested organism groups was accomplished by seagrass (15.8 ± 6.0 mg m^{-2} h^{-1}), followed by benthic algae (1.9 ± 2.0 mg m^{-2} h^{-1}). Corals displayed mean net DOC uptake of 28.6 ± 34.2 (mg m^{-2} h^{-1}) (Table 2).

Seagrass

All seagrass incubations showed significantly elevated concentrations of both, POC (one-way ANOVA $p < 0.001$) and DOC (one-way ANOVA $p < 0.001$) when compared to the seawater controls. Seagrass exuded the highest quantity of total organic carbon per surface area of all tested benthic organisms in the Puerto Morelos lagoon. However, release rates of *Syringodium* exceeded those of *Thalassia* by more than one order of magnitude. High DOC/POC ratios of 10.3 ± 4.4 revealed that most of the organic matter was released in dissolved form. C/N ratios of the released particulate organic matter (POM) of 9.1 ± 0.6 did not significantly differ from those found for POM suspended in the seawater controls.

Algae

POC (one-way ANOVA $p < 0.001$) and PON (one-way ANOVA $p < 0.001$) concentrations were significantly elevated in all algae incubations when compared to the seawater controls. DOC release rates were more heterogeneous and varied from 12.4 ± 10.2 (mg m^{-2} h^{-1}) for red algae to net DOC uptake of 6.0 ± 3.6 (mg m^{-2} h^{-1}) for the green algae *Avrainvillea*, when compared to the respective seawater controls. This was also reflected in low, but highly variable DOC/POC ratios of 0.53 ± 3.40. C/N ratios of algae released POM (15.3 ± 0.5) were higher than those found for the investigated seagrasses, corals and seawater controls, but not on a statistically significant level.

Corals

Organic matter release rates by scleractinian corals were highly species-specific. POC (3.5 ± 0.9 mg m^{-2} h^{-1}) and PON (0.53 ± 0.12 mg m^{-2} h^{-1}) release rates of both *Porites* samples, bleached and unbleached, were comparable to those of seagrasses and benthic algae. Release rates of POC (17.5 ± 12.1 mg m^{-2} h^{-1}) and PON (2.11 ± 1.01 mg m^{-2} h^{-1}) by *Manicina* significantly exceeded those of all other tested benthic organisms (one-way ANOVA $p < 0.001$). In contrast, *Porites* displayed the significantly highest DOC release rates of all organisms during the incubation period (one-way ANOVA $p = 0.011$), opposed to highest net DOC uptake rates by *Manicina* (one-way ANOVA $p < 0.001$). Particulate organic C/N ratios of all investigated corals (10.7 ± 0.3) were in the same range as those measured for seagrasses and algae and showed no significant difference to the respective seawater controls.

Taking into account the overall abundance of the investigated organism groups in the study area and the average combined total carbon release rates, this indicates that seagrasses were the main benthic contributors to the organic carbon pool in this lagoon ecosystem (64 %), followed by algae (48 %), whereas scleractinian corals (both species pooled) were found to be net consumers (-2 %) of organic carbon (Table 2).

Table 2 *Organic matter release rates and POC/PON ratio for dominant primary producers of the Puerto Morelos reef lagoon (mean ± SE). Organic carbon contribution to the ecosystem was calculated from OM release and benthic cover. Abbreviations: DOC = dissolved organic carbon, POC = particulate organic carbon, PON = particulate organic nitrogen, OC = organic carbon, N = number of replicate incubations, bl = bleached, n.d. = benthic cover not determined.*

Organism	Genus	DOC release (mg m^{-2} h^{-1})	POC release (mg m^{-2} h^{-1})	PON release (mg m^{-2} h^{-1})	POC : PON	OC contribution (%)	N
Seagrass	*Syringodium*	29.9 ± 10.3	5.6 ± 4.0	1.22 ± 1.03	7.9 ± 0.9	56	5
	Thalassia	2.2 ± 1.0	0.7 ± 0.1	0.08 ± 0.01	10.3 ± 0.5	8	5
	Mean	**15.8 ± 6.0**	**3.1 ± 2.0**	**0.65 ± 0.52**	**9.1 ± 0.6**	**64**	
Algae	*Halimeda*	-0.8 ± 3.4	1.9 ± 0.5	0.18 ± 0.07	16.2 ± 0.9	3	10
	Avrainvillea	-6.0 ± 3.6	2.7 ± 1.2	0.42 ± 0.23	13.4 ± 1.0	-4	10
	Rhipocephalus	12.1 ± 5.3	10.4 ± 2.7	0.64 ± 0.16	16.5 ± 1.4	4	5
	Penicillus	3.0 ± 0.8	1.4 ± 0.2	0.22 ± 0.15	15.3 ± 0.9	1	5
	Lobophora	10.2 ± 1.6	1.4 ± 0.6	0.09 ± 0.05	16.5 ± 2.2	11	5
	Red algae	12.4 ± 10.2	7.7 ± 0.4	0.56 ± 0.04	14.7 ± 0.3	23	5
	Mean	**1.9 ± 2.0**	**3.9 ± 0.7**	**0.34 ± 0.07**	**15.3 ± 0.5**	**48**	
Scleractinian coral	*Manicina*	-156.5 ± 43.0	17.5 ± 12.1	2.11 ± 1.01	11.1 ± 0.6	-14	5
	Porites	38.1 ± 19.9	4.5 ± 1.6	0.61 ± 0.20	9.3 ± 1.5	12	5
	Porites bl.	49.4 ± 9.9	2.5 ± 0.8	0.44 ± 0.14	6.2 ± 1.2	n.d.	5
	Mean	**-28.6 ± 34.2**	**8.2 ± 4.2**	**1.05 ± 0.38**	**10.7 ± 0.3**	**-2**	

Influence of released organic matter on microbial O_2 consumption

Microbial O_2 consumption in the incubation water of all different investigated specimens was significantly elevated when compared to the respective seawater controls (one-way ANOVA $p < 0.001$). However, benthic algae incubations led to a significantly higher stimulation (one-way ANOVA $p < 0.001$) of microbial O_2 consumption in the incubation water and higher total organic carbon turnover rates (one-way ANOVA $p = 0.001$) when compared to seagrass and coral incubations. Organic carbon quantities released by investigated benthic primary producers displayed significant, but group-specific correlations with subsequent changes in microbial O_2 consumption rates (Fig. 2) as summarized in the following:

Seagrasses and algae

A direct correlation was found for both seagrasses ($R^2 = 0.950$; ANOVA $p < 0.001$) (Fig. 2a) and algae ($R^2 = 0.647$; ANOVA $p < 0.001$) (Fig. 2b) between released DOC and stimulation of microbial O_2 consumption rates. Respective DOC turnover rates were 2.8 ± 1.0 % and 6.9 ± 1.0 % C h^{-1}, respectively. No correlation between the quantity of POC released by seagrasses and algae and the subsequently increased microbial O_2 consumption rates could be detected.

Corals

In contrast to seagrasses and algae, the quantity of POC released by corals showed a significant positive correlation to stimulation of microbial O_2 consumption rates ($R^2 = 0.561$; ANOVA $p = 0.029$) (Fig. 2c) with total organic carbon turnover rates of 3.0 ± 1.2 % C h^{-1}. While increased microbial O_2 consumption rates were found in all coral incubations compared to the seawater controls, the quantity of DOC in the respective coral treatments was negatively correlated to the stimulation of microbial O_2 consumption ($R^2 = 0.811$; ANOVA $p < 0.001$) (Fig. 2d).

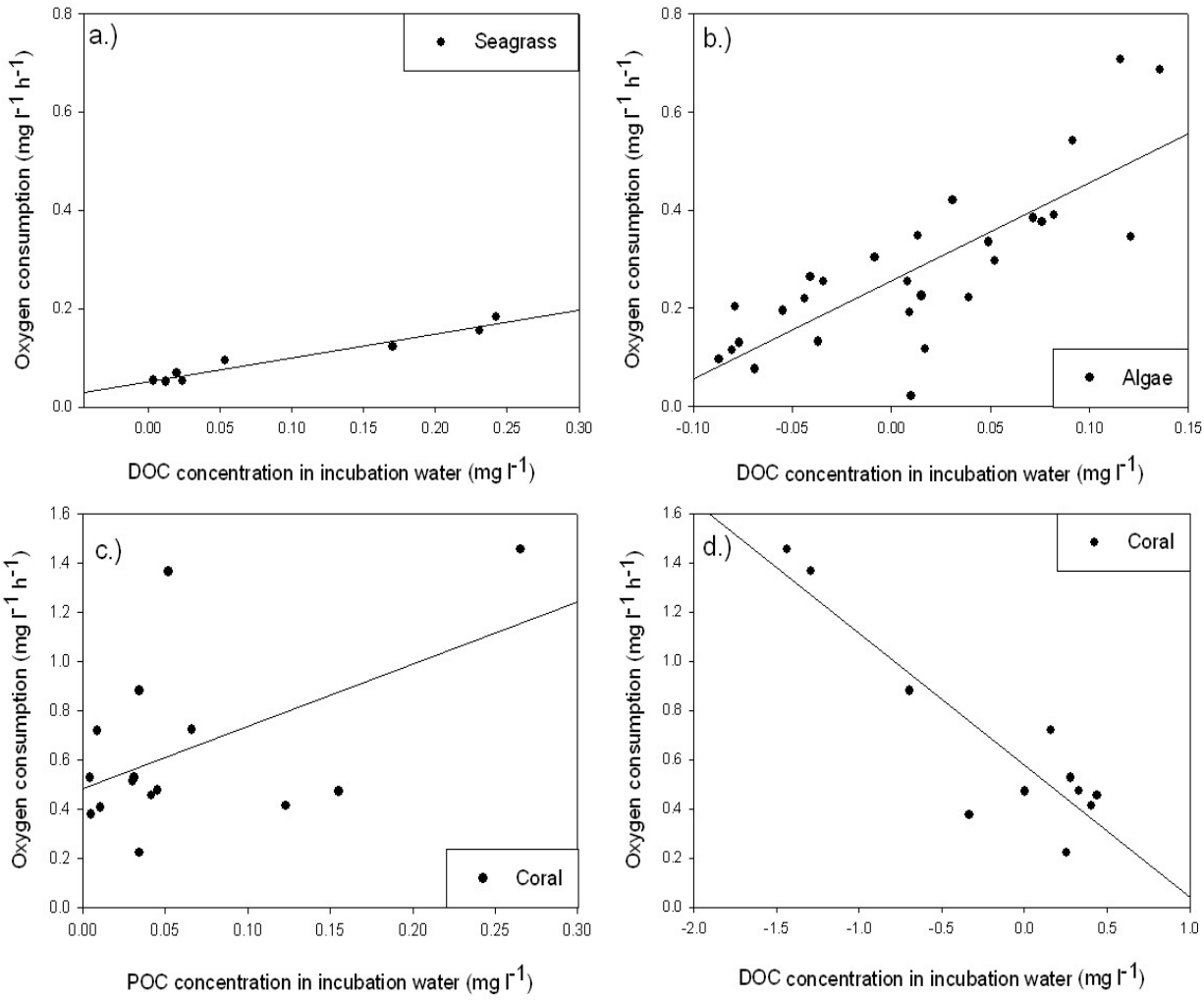

Fig. 2 *Correlation of organic carbon released by the investigated organisms and subsequent changes in microbial O_2 consumption rates in the incubation water. O_2 consumption rates are plotted against dissolved organic carbon (DOC) released by seagrasses (a), algae (b), particulate organic carbon (POC) released by corals (c) and DOC released by corals (d)*

In-situ O_2 measurements

Diurnal in-situ O_2 concentration records of all logger measurements are given in Fig. 3. Highest daily O_2 concentrations around 15:50h were measured directly above seagrass-dominated areas (9.1 ± 0.7 mg l^{-1}), followed by sand flats (8.3 ± 0.1 mg l^{-1}) and algae-dominated areas (7.9 ± 0.5 mg l^{-1}). Lowest O_2 concentrations, measured in the early morning (around 06:30 h) were found for algae-dominated areas (4.4 ± 0.1 mg l^{-1}), followed by sand flats (5.0 ± 0.1 mg l^{-1}) and seagrass dominated areas (5.2 ± 0.4 mg l^{-1}). Average O_2 concentrations in algae-dominated compared to sand flat- and seagrass-dominated locations were significantly lower (repeated measure ANOVA $p < 0.001$) and displayed the highest fluctuations.

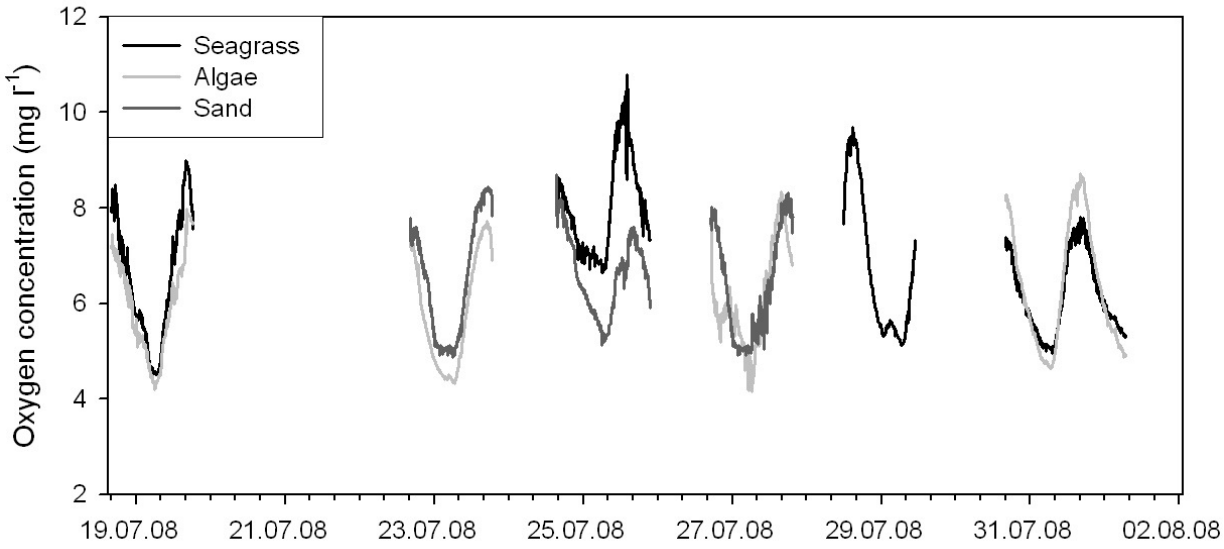

Fig. 3 *O_2 concentrations (mg l^{-1}) measured using in-situ dissolved O_2 loggers in the Puerto Morelos reef lagoon at seagrass- and algae-dominated sites as well as above sand flats.*

Discussion

Benthic coverage and organic matter release by different lagoon organisms

Seagrasses and algae

Results of the benthic community assessment in the Puerto Morelos reef lagoon identified seagrasses as the dominant benthic macro-organisms followed by algae and corals. This is in agreement with benthic community structure surveys of Ruiz-Rentería et al. (1998), conducted 10 years earlier. In addition to their predominant abundance, seagrasses also displayed the highest organic matter release rates measured for benthic organisms in the present study, mainly owing to the release of DOC. This supports their role as dominant primary producers (Ziegler & Benner 1999) and major contributors to the organic matter pool in shallow reef lagoon ecosystems (Ziegler & Benner 2000).

Even though the relatively tall seagrasses dominated the ecosystem visually, the mean contribution of the shorter grown macroalgae to benthic community structure was less only on a statistically insignificant level. Green algae, namely *Halimeda* and *Avrainvillea*, accounted for the majority of benthic algae cover in the study area. Because of their lower or negative net DOC release rates however, the overall contribution of green algae to the organic carbon pool was not significant. This is in contrast to preliminary studies from the Northern Red Sea (Wild et al. 2009; Haas et al. submitted), which showed a notable contribution of coral reef associated green macroalgae to the DOC pool. A possible explanation for these differences is the sensitivity of green macroalgae towards excessive irradiance (Hanelt et al. 1993; Larkum & Wood 1993). A connection between average daytime PAR levels above 300 - 500 (µmol quanta m^{-2} s^{-1}) and reduced DOC release rates, potentially owing to photoinhibition, was already suggested by a previous study (Haas et al. submitted). Furthermore, Hanelt (1992) showed that different algae species and seagrasses can display significant variations in susceptibility to high irradiance levels. Species-specific investigations on excessive irradiance

on photoinhibition showed a high irradiance compatibility of seagrasses and the brown algae *Lobophora*, whereas the onset of photoinhibition for green algae (e.g. *Ulva*, *Halimeda*) was at significantly lower irradiance levels (Hanelt 1992; Franklin et al 1996; Enríquez et al. 2002). This is in line with the present study, as particularly red algae and brown algae of the genus *Lobophora* showed high organic matter release rates and were next to seagrasses, substantial contributors to the organic carbon pool in the studied reef lagoon.

Corals

Overall, corals showed highest average release rates of POC and PON accompanied by the highest average net DOC uptake resulting in a negative total organic carbon balance. However, pronounced species-specific differences were visible. Scleractinian corals are commonly known to release organic compounds in particulate and dissolved forms into the surrounding seawater (Crossland 1987; Ferrier-Pages et al. 1998), thereby contributing to reef trophodynamics (Benson and Muscatine 1974; Wild et al. 2004a, b). However, they may also function as suspension feeders (Di Salvo 1971; Sorokin 1973; Porter 1974) and saprotrophs (Stephens 1962), via uptake of organic substances from seawater. Therefore, varying feeding modes may explain the pronounced differences between the incubated coral specimens in the present study.

POC and PON release of both *Porites* specimens, bleached and unbleached, was in the same range as previously described for various scleractinian corals in the Northern Red Sea (Naumann et al. submitted). *Porites* exudates also displayed high DOC/POC ratios, similar to those of the other major carbon contributors (seagrasses, brown- and red algae). In contrast to their relative small contribution to the overall benthic cover, *Porites* contribute notably to the total organic carbon pool in the investigated lagoon ecosystem, owing to their comparably high organic matter release rates.

Although *Manicina* displayed the highest POC and PON release rates of all incubated organisms, a significant uptake of total organic carbon was found as DOC uptake rates exceeded POC release rates by about one order of magnitude. A possible explanation for the striking differences in coral organic matter release rates can be found in the simultaneously measured background parameters. Incubation experiments conducted with *Manicina* coincided with the highest mean PAR availability (23 % above average) and temperature (1.9 °C above average) of all incubation days. High temperature (Glynn 1993; Brown 1997), excessive light availability (Lesser & Shick 1989) and foremost the combination of both (Hoegh-Guldberg & Smith 1989; Lesser et al 1990; Glynn et al. 1992) can result in the onset of bleaching as direct response of corals to these environmental stressors. Thereby, the release of zooxanthellae can be responsible for increased POM release rates by corals. Additionally, corals have been shown to increase the release rate of POM via mucoid exudates, as a general stress response (Niggl et al. 2009). Studies conducted on organic matter release by corals during the onset of bleaching (Niggl et al. 2009) showed increased release of mucoid exudates, presumably to decrease their vulnerability against pathogens (Brown & Bythell 2005).

During the onset of bleaching a decrease in photosynthetic activity and efficiency (Iglesias-Prieto et al. 1992; Fitt & Warner 1995, Warner et al. 1996) leads to a reduced net organic carbon production (Fujimura et al. 2001). To cover their metabolic needs when the symbiosis disrupts, corals may change to other feeding modes, like the capture of zooplankton by polyps and uptake of dissolved organic compounds from seawater (Muller-Parker & D'Elia 1997). Although *Manicina* revealed no visible changes in tissue color during the incubations, the significantly higher light intensities and temperatures may hint to an onset of bleaching. The consequently arising insufficient energy supply may then be satisfied by the uptake of DOC.

By contrast, bleached *Porite*s specimens showed no differences in DOC release compared to unbleached *Porites* specimens. This may be explained that in a later bleaching phase, nutrition has now switched to heterotrophy and no further DOC uptake is needed. Additionally, a

heterotrophic coral may release DOC via 'sloppy feeding' (Piniak & Lipschultz 2000). Furthermore, the equal quantity and quality of exudates by bleached and healthy *Porites* specimens (POM) may be explained by a study of Wild et al. (submitted), who assumed that mucus-POM release by corals may only be stimulated during the early phase of bleaching, but drops down to lower levels at longer bleaching periods.

Effects of released organic matter on microbial activity

Organic carbon released by the different benthic primary producers was group-specifically correlated to increases in microbial O_2 consumption rates. This indicates differences in the chemical composition of the released organic compounds with ensuing effects on microbial degradability as discussed in the following.

Seagrass and Algae

Seagrass and algae incubation samples showed a significant positive correlation of DOC release and elevated microbial O_2 consumption rates (Fig. 2a, b). This is in line with studies of Ziegler & Benner (1999), who demonstrated a positive correlation between O_2 consumption measured in the water column and fluctuations in DOC release by seagrasses and algae in-situ. Both groups of organisms have already been described to support their microbial environment by the release of organic carbon (Benner et al. 1986, Findlay et al. 1986). However, the present study indicates that algae-derived organic carbon provides a more attractive substratum for the ambient microbial community than organic carbon released by seagrasses. In general, algae incubations did not exhibit highest release rates for any of the measured parameters (DOC, POC, PON), but resulted in significantly highest microbial O_2 consumption rates, thus leading to the highest carbon turnover rates. The quantity of POM (POC and PON) released by both, seagrasses and algae, showed no direct effect on microbial O_2 consumption hinting to a more refractory nature of the particulate compared to the dissolved material. This is also congruent with studies of Ziegler & Benner (2000), who suggested that the benthic release of DOC is primarily responsible for the increases in bacterial production and that bacterioplankton production in seagrass ecosystems is not limited by bioavailable nitrogen.

Corals

In contrast to seagrass and algae treatments, increased POC concentrations in coral incubation waters were positively correlated with subsequent microbial O_2 consumption. Wild et al. (2004b) already summarized that the gel-like coral mucus carbohydrate complex (Coffroth 1990) contains energy-rich lipid compounds and proteins or peptides (Krupp 1985, Vacelet & Thomassin 1991), thereby providing an attractive energy source for the whole ecosystem. However, turnover rates of organic carbon released by corals were in the same range as for seagrass exudates, but significantly lower than those measured for exudates of benthic algae.

Even though microbial O_2 consumption rates were increased in all coral incubation samples, the amount of DOC in these samples was negatively correlated to elevations in microbial O_2 consumption. While the particulate fraction notably enhanced microbial O_2 consumption rates in coral incubation waters, this could indicate suppressive effects of the dissolved fraction on microbial activity (Fig. 2c, d). Coral mucus can act as a medium for secreted allelochemicals with antimicrobial properties (Brown & Bythell 2005). The findings of the present study therefore suggest that antimicrobial compounds potentially associated with coral mucus (Koh 1997; Geffen & Rosenberg 2005; Ritchie 2006) are mainly contained in the dissolved fraction of coral-derived organic matter.

In-situ O_2 availability

Diurnal O_2 records obtained from locations dominated by a specific type of benthic cover revealed significantly lower O_2 water concentrations at algae-dominated sites compared to adjacent sand flats and seagrass-dominated sites. Influences of topography and differences in

water currents are unlikely, owing to the regularity of the lagoon ground and homogeneous water currents within the lagoon (Merino-Ibarra & Otero-Dávalos 1991; Coronado et al. 2007). Overall, higher O_2 concentration in seagrass-dominated areas could be attributed to higher abundance and photosynthetic performance of seagrasses compared to algae at the given environmental conditions (Hanelt 1992; Franklin et al 1996). However, highest fluctuations between minimum and maximum O_2 concentrations at algae locations and more than two times higher turnover rates of organic carbon released by benthic algae compared to seagrasses indicate elevated microbial activity as prime reason for reduced O_2 concentrations in algae dominated areas. These assumptions are supported by previous studies, which showed that lagoons dominated by macroalgae often exhibit large diurnal changes in O_2 concentrations (Sand-Jensen & Borum 1991, Viaroli et al. 1995). The rapid changes in benthic metabolism may arise from a faster degradation of algae-derived organic matter, because of its high nutrient and low fiber content and its high C/N ratio (Enriquez et al. 1993). This confirms assumptions about the in-situ relevance of group-specific effects of released organic matter on their adjacent environment (Kline et al. 2006, Wild et al. 2009), whereby particularly algae-derived organic matter may lead to reductions in O_2 availability, potentially deleterious for other organisms in the ecosystem (Smith et al. 2006).

Ecological implications

Although the highest contribution to the organic matter pool was provided by seagrasses, algae had the strongest effect on planktonic microbial O_2 consumption rates, which could also be verified for in-situ conditions. Not only the turnover rate, but also the location of organic matter degradation may have implications for O_2 concentrations in the water column. Coral-derived organic matter is mainly (> 90 %) degraded in the sediment and not in the water column (Wild et al. 2004a). The primary location for seagrass-derived organic matter is also the sediment rather than the water column (Canuel & Martens 1996; Duarte & Cabrián 1996; Kaldy et al. 2006), as a main part of organic carbon is released through roots and rhizomes (Hansen et al. 2000) and it is generally more refractory, when compared to algae exudates. Algae exudates on the contrary are likely primarily degraded in the water column. The high planktonic turnover rates found in the incubation experiments of the present study are supported by investigations of Duarte & Cabrián (1996), who showed that the fraction of organic matter reaching the sediments was 4-fold higher for seagrass than for benthic macroalgae exudates.

This further strengthens the role of benthic algae exudates as main agent stimulating planktonic microbial O_2 consumption (Wild et al. 2009). This may also support the hypothesis of deleterious consequences on organisms in direct vicinity owing to microbial induced O_2 depletion (Kline et al. 2006, Smith et al. 2006, Dinsdale et al. 2008).

Overall, the present study shows that all investigated groups of organisms displayed the ability to alter the organic matter pool of the lagoon ecosystem. Organic matter release rates of Caribbean lagoon associated primary producers were thereby in the same range as those found for coral reef associated primary producers, scleractinian corals (Naumann et al submitted) and benthic macro- and turf- algae (Haas et al. submitted), in the Northern Red Sea. It simultaneously supports the assumption of organism-specific differences in ecological properties of the released organic matter (Wild et al. in 2009), because of variations in the rate and location of its (primarily microbial) utilization.

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