Biogeochemical consequences of organic matter release by reef-building scleractinian corals

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For my parents,
for introducing me to the wonders
of coral reefs from an early age
and for their everlasting support

"Coral reefs are threatened, not doomed – if we can avoid extreme climate change, runoff and over-fishing.

Prevention is better than cure, but recovery is possible.

The decisions we make now, or don't make, will have profound long-term consequences."

(Terry Hughes, 11th ICRS Fort Lauderdale 2008)

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Thesis abstract

This thesis is composed of 12 chapters presenting a series of biogeochemical and ecological investigations relevant to coral reef ecosystems. The major focus lies on the consequences of organic matter release by benthic reef organisms, in particular reef-building scleractinian corals, for biogeochemical element cycles of fringing coral reefs, globally the most common reef type. Warm water coral reefs, while thriving in oligotrophic marine environments, are nonetheless characterized by high primary production rates facilitated by efficient utilization, recycling and conservation of organic matter within reef biogeochemical processes. Scleractinian corals contribute substantially to biogeochemical processes and general reef ecosystem engineering by several synergistic features, which include (1) the construction of complex reef frameworks enhancing habitat and species diversity, (2) the erosion-induced supply of biogenic calcareous sands functioning as catalytic filter systems for organic matter recycling, and (3) the continuous release of organic matter, e.g. as mucus, initiating reef element cycles and conservation of essential nutrients via particle trapping.

Within this thesis, the ecosystem engineering feature of organic matter release by reef corals is examined by successive investigations on distinct pathways of this material within fringing reef ecosystems: ranging from its initial release and function as an energy and nutrient carrier, to its degradation and recycling by reef-associated organisms, in particular microbes. Quantitative investigations provide the first comprehensive overview of organic matter release rates by dominant scleractinian coral taxa and elucidate its substantial contribution to organic matter pools in reef-surrounding waters. In situ and laboratory studies exhibit the efficient microbial degradation of coral-derived organic matter in reef waters and sandy reef sediments, emphasizing its significant input to pelagic and benthic reef metabolism. Studies on the trapping efficiency of particulate coral mucus for picoplankton organisms indicate its potential role in benthic-pelagic coupling processes and biomass conservation within fringing reef ecosystems. Further, the function of coral-derived organic material in reef trophic interactions is demonstrated by tracer studies revealing its uptake by coral-associated organisms. These combined findings confirm organic matter release as a significant component of ecosystem engineering performed by corals in fringing reef systems.

In addition, comparative studies involving scleractinian corals and benthic reef algae are presented that evaluate the specific role of organic matter release by both, at times dominant, organism groups in biogeochemical processes and reef ecosystem functioning. In the light of global climate change, these studies suggest far reaching consequences for reef biogeochemical element cycles with concomitant implications for general ecosystem functioning, attributed to benthic community phase shifts towards reef algae dominance.

With reference to the findings for scleractinian corals, investigations on the release, biogeochemical cycling and ecological role of organic matter exuded by other dominant cnidarian reef taxa from warm and cold water coral reefs are included, which further underline the significance of organic matter release by reef cnidarians in biogeochemical element cycles of coral reef ecosystems.

Chapter 1 of this thesis, "A precise and non-destructive method to calculate the surface area in living scleractinian corals using X-ray computed tomography and 3D modelling", introduces a new technique, making use of high-accuracy medical computer tomography and digital 3D-modeling, enabling researchers to very precisely determine the surface area of scleractinian corals. As corals possess a complex physical architecture and intricate surface structure representing an important reference parameter for the standardization of growth rates, metabolic processes and matter fluxes (e.g. organic matter release), the development of this technique provides new possibilities for the precise and non-invasive surface area quantification of living coral specimens.

As most studies in coral science are conducted in locations without direct access to computer tomographic techniques, **Chapter 2**, "Coral surface area quantification – evaluation of established techniques by comparison with computer tomography", presents an evaluation of established methods for coral surface area quantification (e.g. geometric measurements) in comparison to the novel computer tomographic technique introduced in chapter 1. This comparative evaluation also provides conversion factors allowing for subsequent correction of surface area measurements produced by application of established methods during field studies. The new development and evaluation of coral surface area quantification techniques presented in chapters 1 and 2 provide a fundamental prerequisite for a series of studies presented here, including those investigations carried out in the principle study site of this thesis, a fringing reef system of the Northern Red Sea, which is introduced in detail starting with the following chapter 3.

Chapter 3, "Coral sand O_2 uptake and benthic-pelagic coupling in a subtropical fringing reef, Aqaba, Red Sea", opens the series of field investigations and introduces the principle study site of the following chapters, a fringing reef system of the Northern Red Sea. Further, this chapter presents results from 3 independent seasonal expeditions conducted to investigate the degradation of organic matter in calcareous reef sands by in situ measurements of oxygen flux rates. The results indicate a limited supply of degradable organic material to the reef sands by efficient recycling in the reef-overlying water column. In addition, a depth-mediated increase of oxygen flux into sands of the reef lagoon area points to an enhanced influence by lateral transport of organic matter released by benthic reef algae and scleractinian corals, which is further specified in the following chapter 4.

In **Chapter 4**, "Organic matter release by dominant hermatypic corals of the Northern Red Sea"**,** the release of organic matter is quantified for 6 dominant hermatypic (reef-building) coral genera in high spatio-temporal resolution. Genus-specific seasonal release rates for particulate organic carbon and nitrogen (POC and PN) are provided together with dissolved organic carbon (DOC) flux rates. The results indicate a genus-specific variation in particulate organic matter (POM) release without significant seasonal variations. However, fluctuations in POM release suggest a correlation to some seasonally variable environmental key factors in the Northern Red Sea (temperature, light availability and nitrate concentration). Genus-specific net DOC release and uptake concerns 50% of the investigated coral genera respectively, while those flux rates show no significant correlation to seasonality and variable

environmental key factors. This comprehensive data set provides essential information to our understanding regarding the role of coral-derived organic matter in fringing reef systems and supports several studies within this thesis, including the following chapter 5, where this information is applied to investigate the contribution of coral-derived organic C to ecosystem-wide organic C dynamics in the study area.

Chapter 5, "Contribution of coral-derived organic carbon to biogeochemical processes in a fringing reef of the Northern Red Sea"**,** combines information on coral-derived organic C release rates, its pelagic and benthic microbial degradation and seasonally recorded ecosystem background parameters of the study area (i.e. benthic reef coverage, pelagic and benthic primary production and microbial O_2 consumption, and POC sedimentation rates) to assess the contribution of coral-derived organic C to element cycles and recycling processes in a Red Sea fringing reef system. Findings reveal that coral-derived C contributes substantially (up to 8%) to the total organic carbon (TOC) pool in reef waters by a daily reef-wide release of 12.8 kmol C, representing 30 – 39% of total pelagic primary production. Rapid degradation of coral-derived TOC (8 – 10% h^{-1}) further provides a significant share of pelagic (up to 4.6%) and benthic (up to 41%) microbial O_2 consumption, thus confirming the important role of coral-derived organic matter in fringing reef element cycles, formerly only observed in platform reef systems. The significant contribution of coral-derived C to reef-wide organic C dynamics further provides evidence for an efficient recycling of the released organic material suggesting the ensuing nutrient conservation within fringing reef systems.

In **Chapter 6**, "Coral mucus as an efficient trap for picoplanktonic cyanobacteria – implications for pelagic-benthic coupling in the reef ecosystem", the topic of organic matter cycling in fringing reef ecosystems is continued by describing the function of coral-derived POM (i.e. particulate coral mucus) as an aggregation and transport medium for picoplankton biomass. The efficient trapping and enrichment of plankton dominating synechococcoid cyanobacteria (*Synechococcus* spp.) within the particulate fraction of coral mucus and the subsequent rapid vertical transport of this enriched material is demonstrated by laboratory and in situ experiments. The findings of this study connect to results of previous investigations describing the enrichment of larger particle classes in particulate coral mucus. Consequently, this study underlines previous results by providing new insights to the potential function of coral-derived POM in the pelagic-benthic transport of significant amounts of picoplankton biomass. Detection and tracing of picoplanktonic cyanobacteria is currently feasible by numerous techniques. However, for coral-derived organic matter its transparent mucoid properties accompanied by the absence of distinct markers hinder a direct visual tracing of this material in trophic processes.

This obstructive issue was approached in **Chapter 7**, "Stable isotope labeling of coral mucus reveals its uptake by epizoic *Waminoa* worms", by developing a technique allowing for the labeling of coral mucus using stable isotope markers. Incubation of individual coral polyps or entire coral colonies in stable isotope-enriched (^{13}C and ^{15}N) media produces strongly ^{13}C- and ^{15}N-labeled coral mucus subsequently applicable in the tracing of coral-derived organic material during laboratory and in situ investigations. The effective traceability

of stable isotope labeled coral mucus in food web processes is demonstrated here by proving its uptake by coral-associated epizoic *Waminoa* worms. These acoelomorphs, living closely attached to the surface mucus layer of numerous coral species show a constant enrichment of ^{15}N-labeled coral mucus, thus revealing a novel trophic relationship in coral reef ecosystems. Trophic relationships by close association of coral reef inhabitants are not solely restricted to anthozoan cnidarians. For example, a close association has been observed between the scyphozoan upside-down jellyfish *Cassiopea* sp. and zooplanktonic mysids (*Idiomysis tsurnamali*), leaving a possible trophic relationship in question.

The causality of this association is studied in **Chapter 8**, "Organic matter release by the benthic upside-down jellyfish *Cassiopea* sp. fuels pelagic food webs in coral reefs", hypothesising a possible trophic utilization of *Cassiopea*-derived organic matter by the associated mysids. Organic matter release rates by the scyphozoan *Cassiopea* exceed those measured for anthozoan corals, and results from tracer experiments reveal the continuous uptake of ^{15}N-labeled *Cassiopea*-derived organic matter by *Idiomysis tsurnamali*, thus providing evidence for a novel trophic linkage among reef-associated organisms. Supplementary incubation experiments further demonstrate the rapid mineralization of *Cassiopea*-derived organic matter by the mysids, as well as by the planktonic microbial reef community. These findings indicate that the release of organic material by *Cassiopea* into surrounding reef waters may represent a potential, so far overlooked, trophic pathway between the benthic and pelagic compartments of coral reefs and other tropical coastal ecosystems. The rapid microbial mineralization of organic matter found here for *Cassiopea*-derived organic material has also been observed in previous studies investigating the microbial turnover of organic matter released by anthozoan warm and cold water coral taxa. Coral-derived organic matter, or coral mucus, is predominantly a carbohydrate complex and its microbial degradability is distinctly founded by its chemical composition. Thus, the following chapter 9 continues this topic by presenting carbohydrate compositions of coral mucus released by a selection of dominant warm and cold water coral taxa.

Chapter 9, "Carbohydrate composition of mucus released by scleractinian warm and cold water reef corals", provides comparative information on the carbohydrate composition of mucus released by cosmopolitan warm and cold water corals. Analysis of mucus from 5 warm and 2 cold water coral taxa indicates a genus-specific carbohydrate composition. The plant-specific sugar arabinose is not included in the mucus of all cold water coral taxa, which is explained by the absence of photosynthetic endosymbionts (zooxanthellae) in cold water deep-sea taxa. However, the findings show no significant differences in the general carbohydrate composition of mucus released by warm and cold water corals, further indicating a utilization of similar carbohydrate components during the process of mucus synthesis. Consequently, the similarity of carbohydrate compositions in warm and cold water corals indicates an independence from direct photosynthetic sources and further provides an explanation for the similar rapid mineralization of mucus observed in both cold and warm water coral taxa. Scleractinian cold water corals of deep-sea reefs are relatively new to biogeochemical sciences. However, preliminary observational studies indicated that these

corals are able to release large quantities of mucoid organic substances into the surrounding environment, further suggesting a distinctive role of this material in reef ecosystem functioning.

Therefore, **Chapter 10**, "Organic matter release by cold water corals and its implication for fauna-microbe interaction", leads an excursion to the realm of the cold water corals dwelling in deep-sea reef ecosystems. The study provides quantitative data on the release of organic matter (POC, PN and DOC) by different cold water coral species in comparison to established release rates for warm water coral taxa. Further, the quality of the released organic material and the stimulation of microbial degradation processes are assessed during laboratory incubations and in situ samplings. The results indicate that POC and PN release rates are similar to those of warm water coral taxa, while DOC release is significantly increased. Qualitative analyses likewise attest similarities in dissolution characteristics (DOC:POC ratio) and nitrogen-richness (C:N ratio) in comparison to organic matter released by warm water coral taxa. Further, laboratory and in situ studies demonstrate a significant increase in planktonic microbial activity triggered by the presence of cold water coral-derived organic matter. This suggests that cold water corals, similar to their warm water counterparts, stimulate planktonic microbial processes in reef overlying waters by the release of labile and nutrient-rich organic substances, thereby initiating element cycles via the microbial loop and functioning as a carrier of energy and nutrients in coral reef ecosystems of the deep. In contrast to their deep-water counterparts, warm water corals of the photic zone thrive in direct competition to fast growing benthic reef algae from various groups, which likewise release organic matter into the surrounding seawater. However, our current understanding of the quantity and quality of organic matter released by benthic reef algae is still limited.

Thus, **Chapter 11**, "Organic matter release by coral reef associated benthic algae in the Northern Red Sea", presents investigations conducted to quantify and qualify the release of organic matter (POC, PN and DOC) by permanent and seasonally occurring benthic reef algae species of the Northern Red Sea in high spatio-temporal resolution. The findings reveal that all investigated algae species release POM and DOC in quantities, on average, exceeding those of scleractinian corals. Organic matter release by benthic reef algae shows no species-specific variability, but is rather influenced by functional properties (e.g. growth form) of the investigated taxa. Seasonal and depth-mediated differences in organic matter release rates are significant with positive correlation to variations in temperature and light availability. This comprehensive data set on organic matter release rates by benthic reef algae finally provides important insights to the contribution of this previously overlooked organism group to organic matter cycles of warm water coral reefs. Further, these findings allow for a direct comparison of benthic reef algae and scleractinian corals regarding their contribution to reef ecosystem functioning by the release of organic matter, which is continued within the following chapter.

In the final **Chapter 12**, "Comparative investigation of organic matter release by corals and benthic reef algae – implications for pelagic and benthic microbial metabolism", the wide-spread phenomenon of coral reef "phase shifts" (the shift from a coral to an algae dominated reef) is investigated by analysing the potential of both organism groups to act as

ecosystem engineers (essential contributors to reef ecosystem functioning) via the release of organic matter. Comparative analysis of organic matter release rates by scleractinian corals and benthic reef algae, accompanied by analyses of planktonic and benthic microbial degradation of the released material, indicate that both organism groups can support ecosystem functioning by the release of rapidly degradable organic material. However, the findings also suggest that corals ("pre-phase shift engineers") contribute differently to ecosystem processes than algae ("post-phase shift engineers"), ultimately resulting in significant alterations within biogeochemical element cycles after ecosystem phase shifts. As an example, the important function of coral-derived organic matter in reef element cycles by conservation of essential nutrients is diminished or lost after a shift to algae dominance, as algae-derived organic matter apparently cannot provide the significant particle trapping function of coral mucus. In addition, most of the algae-derived organic matter is released as dissolved organic matter (DOM) that predominantly stimulates planktonic microbial activity with no significant direct benefit to the benthic reef community. To the contrary, by stimulating microbial activity, labile algae-derived DOM shows potential to damage corals in direct vicinity via hypoxia or anoxia. In the light of global climate change and concomitant phase shifts, these findings suggest far reaching consequences for reef biogeochemical element cycles and general reef functioning caused by a shift towards benthic reef algae dominance.

Table of contents

1

A precise and non-destructive method to calculate the surface area in living scleractinian corals using X-ray computed tomography and 3D modeling

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Abstract

The surface area of corals represents a major reference parameter for the standardization of flux rates, for coral growth investigations and for investigations of coral metabolism. The methods currently used to determine the surface area of corals are rather approximate approaches lacking accuracy, or invasive and often destructive methods that are inapplicable for experiments involving living corals. This study introduces a novel precise and non-destructive technique to quantify surface area in living coral colonies by applying computed tomography (CT) and subsequent 3D reconstruction. Living coral colonies of different taxa were scanned by conventional medical CT either in air or in sea water. Resulting data volumes were processed by 3D modeling software providing realistic 3D coral skeleton surface reconstructions, thus enabling surface area measurements. Comparisons of CT data sets obtained from calibration bodies and coral colonies proved the accuracy of the surface area determination. Surface area quantifications derived from two different surface rendering techniques applied for scanning living coral colonies showed congruent results (mean deviation ranging from 1.32% to 2.03%). The validity of surface area measurement was verified by repeated measurements of the same coral colonies by three test persons. No significant differences between all test persons in all coral genera and in both surface rendering techniques were found (independent samples t-test: all n.s.). Data analysis of a single coral colony required approximately 15 to 30 minutes for a trained user using the isosurface technique regardless of the complexity and growth form of the latter, rendering the method presented in this study as a time-saving and accurate method to quantify surface areas in both living coral colonies and bare coral skeletons.

Introduction

Stony corals (Scleractinia) can be regarded as engineers of coral reef ecosystems. Large wave-resistant structures have accumulated by the precipitation of calcium carbonate forming a topographically complex habitat which is among the most diverse and productive ecosystems on Earth. Scleractinian corals occur in a variety of growth forms, and there is strong variation in coral shape even within a single species.

The question of how to determine the surface area in this phenotypically plastic organism has been of considerable interest in several studies in coral reef science. For instance, the rate of coral reef growth and related surface area is essential to assess population dynamics in reef ecosystems (Goffredo et al. 2004). Moreover, corals release dissolved and particulate organic matter and therefore, precise surface area estimation is indispensable to calculate the contribution of the latter to the nutrient and energy budgets of reef environments (e.g., Wild et al. 2004).

Hence, a variety of methodologies have been introduced and applied in coral reef science to determine the surface area of corals. Given that coral tissue is only a thin layer covering the coral skeleton in stony corals, the skeleton itself has been widely used to assess the surface area of coral colonies. A frequently used method is the foil wrap technique introduced by Marsh in 1970, which is based on a surface area to mass correlation (e.g., Hoegh-Guldberg and Smith 1989; Wegley et al. 2004). An alternative approach for estimating the surface area is to coat corals by dipping them into liquids such as vaseline (Odum and Odum 1955), latex (Meyer and Schultz 1985), dye (Hoegh-Guldberg 1988) or melted paraffin wax (e.g., Glynn and D'Croz 1990; Stimson and Kinzie 1991) and subsequently correlating the amount of the adhering liquid to the surface of the coral skeleton. Most coating techniques are harmful or completely destructive, thus inappropriate methods in studies requiring repeated measurements of living coral colonies, e.g., growth rate determination.

Hence, several non-destructive methods to assess the surface area of scleractinian corals have been introduced in coral reef science. Kanwisher and Wainwright (1967), for instance, used a 2-dimensional planar projection derived from photographs to assess the surface area of coral colonies. However, planar projections of 3-dimensional (3D) structures are unsuitable to determine the surface area accurately and likely underestimate the actual surface area of a coral colony.
Simplifying the complex 3D structure of a coral colony into geometric forms such as cylinders allows calculating the surface area of a single colony by the respective geometric formula. This method is effective in terms of time and therefore was used in numerous studies (Szmant-Froelich 1985; Roberts and Ormond 1987; Babcock 1991; Bak and Meesters 1998; Fisher et al. 2007). However, depending on the growth form of the coral species this may rather represent an inaccurate approximation of the actual surface area.

The implementation of computerized 3D reconstruction opened new avenues in surface area determination of living coral colonies. Both photogrammetry (Done 1981; Bythell et al. 2001) and X-ray computed tomography (CT) (Kaandorp et al. 2005) have been applied to achieve a suitable data set for image processing. Photographic and video-based techniques are applicable in field studies (Cocito et al. 2003; Courtney et al. 2007), but show their limitations when analyzing complex branching colonies due to “occlusion effects” of overlapping branches (Kruszynski et al. 2007). Since the introduction of X-ray computed tomography (Hounsfield 1973), applications of

this technique have been reported from various earth science disciplines such as sedimentology and paleontology (e.g., Kenter 1989; Ketcham and Carlson 2001). In studies on coral reefs X-ray CT has been frequently used to assess coral growth rates (e.g., Bosscher 1993; Goffredo et al. 2004). Recently, X-ray CT was applied to analyze the invasion of bioeroders (Beuck et al. 2007), morphogenesis (Vago et al. 1994; Kaandorp et al. 2005) and morphological variation (Kruszynski et al. 2006, 2007) in scleractinian corals. However, X-ray CT has not been applied to determine surface area in living coral colonies and image processing used to be sometimes a complicated and time-consuming procedure.

Computed tomography uses X-ray scans to produce serial cross-sectional images of a sample. The obtained volume of data is a stack of slices, each slice being a digital grey value image representing the density of an object corresponding to the average attenuation of the X-ray beam (Kak and Slaney 1988). A variety of software packages using sophisticated computations are available to subsequently reconstruct the scanned object in three dimensions and allow further data processing such as volume determination (Kruszynski et al. 2007). The high resolution and the ability to precisely reconstruct a virtual 3D model of the scanned object render this technique perfectly suitable for surface area determination of complex morphologies. Furthermore, X-ray CT is particularly appropriate to analyze calcified structures (Kruszynski et al. 2007). Given the fact that the attenuation of the X-ray beam in calcium carbonate differs extremely from the surrounding medium (e.g., salt water), the shape of the coral skeleton can be easily extracted during image processing. However, as in preoperative planning for bone surgery or for the evaluation of the accuracy of dental implants (Rodt et al. 2006; Kim et al. 2007) a precise adjustment of the grey scale threshold is indispensable to avoid a false estimation of the actual surface area from a virtual 3-dimensional model.

The purpose of this study was to present a novel non-destructive method to precisely calculate the actual surface area of coral colonies using X-ray CT-based computerized 3D modeling. This approach is especially useful in studies on living colonies used in time series analysis. Applying different kinds of calibration bodies aims to facilitate the accurate setting of the grey scale value during image processing. This in turn offers the opportunity to calculate the surface area from the isosurface of the volume data, an easy to use and time-saving procedure.

Material and methods

Data acquisition

Living zooxanthellate coral colonies of different genera (*Montipora* sp., *Acropora* sp., *Pocillopora* sp.) representing branching and plate-like growth forms were used for the surface area measurements. Coral samples fixed on unglazed ceramic tiles using coral glue (Aqua medic, Germany) were taken from the coral reef aquaria located at the Department of Biology II, LMU-Munich. All samples were placed in aquaria made of acrylic (12 l) to prevent artifacts caused by the container. Aquaria made of glass can cause “starburst” artifacts, which occur when scanning

materials of a high density e.g. crystals surrounded by materials of a much lower density (Ketcham and Carlson 2001).

Scans were performed either in “air” (for a maximum of 15 min exposure time) or in aquaria filled with artificial sea water (Aqua medic, Germany) at a temperature of 24 ± 1 °C.

Two types of calibration bodies were used in the study. A calibration cube (30.01 mm x 30.01 mm x 30.01 mm) made of polyvinyl chloride (PVC) was produced with an accuracy of 0.01 mm. The second calibration body was made of a special kind of marble originating from Laas, Italy. Laas marble is characterized by a high proportion of aragonite in its crystal structure, resulting from the metamorphism of limestone (Hacker and Kirby 1993). A micrometer-caliper was used to measure each side (a-d) and both diagonals (e and f) of all six faces of the cuboid made of marble (graining 800) with an accuracy of 0.01 mm. Subsequently the surface area of each quadrangle was calculated using

$$¼\sqrt{(4e^2f^2-(b^2+d^2-a^2-c^2)^2)}.$$

The sum of all six faces yielded the surface area of the cuboid.

X-ray computed tomography was performed on a medical scanner (Siemens Somatom Definition, Germany). The samples were scanned at a tube voltage of 140 kV (Care Dose 4D, Eff mAs 343) at virtually isotropic resolution of 0.400 mm x 0.400 mm x 0.4 mm (voxel size; voxel = volume pixel) by setting the field of view scan region to 205 mm in diameter. Scan time was 82.12 s resulting in a stack of 0.4 mm contiguous slices each having a size of 512 x 512 pixels. Hounsfield Units (HU; standard computed tomography units), which corresponds to the average X-ray attenuation values, ranged from - 1,024 to + 3,071 and were set at 0 for water and - 1,000 for air. Medical CT systems are generally calibrated using the latter HU values. Data acquisition was performed by the integrated Somaris software (Syngo CT 2007, Siemens, Germany) by using the U70 algorithm.

Data processing

The data sets (DICOM format files) were transferred to a personal computer (Fujitsu Siemens Celsius, 3GB memory, Germany) and further processed using the software package AMIRA 4.1 (Mercury Computer Systems, Inc., France). A variety of both commercial and non-commercial available software packages (e.g. digest listed at: http://biocomp.stanford.edu/3dreconstruction/software/index.html) can be applied to process DICOM data in a similar way, although some individual processing steps might differ between AMIRA 4.1 and other software packages. In the following the general procedure is described exemplarily using AMIRA 4.1.

The loaded data set was edited by the “Crop Editor” tool to reduce the entire data set to a volume containing the voxels of a single object, being either a coral colony or a calibration body. Reduction of the data set significantly increased processing time. No filters were used prior to image processing of the volume data. Surface area measurements of coral colonies were carried out by two different procedures.

A, "Isosurface"

Determination of the threshold that specifies the boundaries for the object of interest is a crucial part in surface rendering. In this study, the bright components of a single slice (higher Hounsfield unit values) represent the coral skeleton, whereas the dark areas (lower Hounsfield unit values) represent the lower density of the surrounding medium. Given that a coral colony has a high density near its surface (Kruszynski et al. 2006), and that the shade of grey of each voxel is corresponding to the density of the material, a distinct boundary between air or salt water and the calcium carbonate skeleton of the coral can be achieved by choosing the appropriate threshold for surface rendering. Among the techniques to extract the feature of interest from a set of data are volume rendering and isocontouring (Ketcham and Carlson 2001).

If internal structures of the object are not in focus of the study, isocontouring should be applied, since it can provide more detailed surface information compared to the volume rendering process (Ketcham and Carlson 2001). Isocountouring generates so called isosurfaces within a three-dimensional scalar field with regular Cartesian coordinates that define the boundaries of the coral colony or the calibration body in the scan. A lower threshold value for generating the isosurface adds voxels to the object. Setting the bounds too low might lead to an overestimation of the surface area caused by artifacts in the reconstruction process due to increased background noise. Raising the threshold value subtracts voxels from the material of interest leading to visible degradation of the reconstructed object of interest (Fig. 1).

Threshold setting in the module "Isosurface" corresponds to Hounsfield Units (-1,024 to +3,071) of the CT-scan. The threshold was set stepwise (50 units per step starting from 0) to identify the best fit to the known surface area of both calibration bodies. For each threshold a new triangulated surface was generated (vertex normal). If necessary, remaining artefacts originating from background noise were removed by applying the "Surface editor" tool followed by the creation of a new surface and the calculation of the surface area from the edited object.

Threshold values of both calibration bodies showing the best fit to the actual surface area were used to subsequently calculate the surface area of the coral colonies. To modify the isosurface of the latter, the same procedure was conducted as described above. In the process existing background noise and the ceramic tile as well as the coral glue were removed manually resulting in a virtual 3D model of a single coral colony. In order to ensure that the ceramic tile and the coral glue were removed equally at both threshold values, the obtained isosurfaces were congruently placed above each other to compare uniformity of the removal line. The results (output unit: cm^2 area) of both threshold values were stored in a spread sheet data object.

B, Segmentation

Image segmentation describes the process of dividing an object of interest in the entire 3D volume or in a single slice (2D) into different sub-regions. Boundaries or contours between two materials can manually or automatically be distinguished and extracted from the background. Thus the morphological structure or the region of interest can be viewed and analyzed individually. Depending on the data set and the aim of the study, segmentation can be a time-consuming process

compared to rendering an isosurface. However, segmentation is a suitable technique to e.g. remove background noise or to select single features (Ketcham and Carlson 2001).

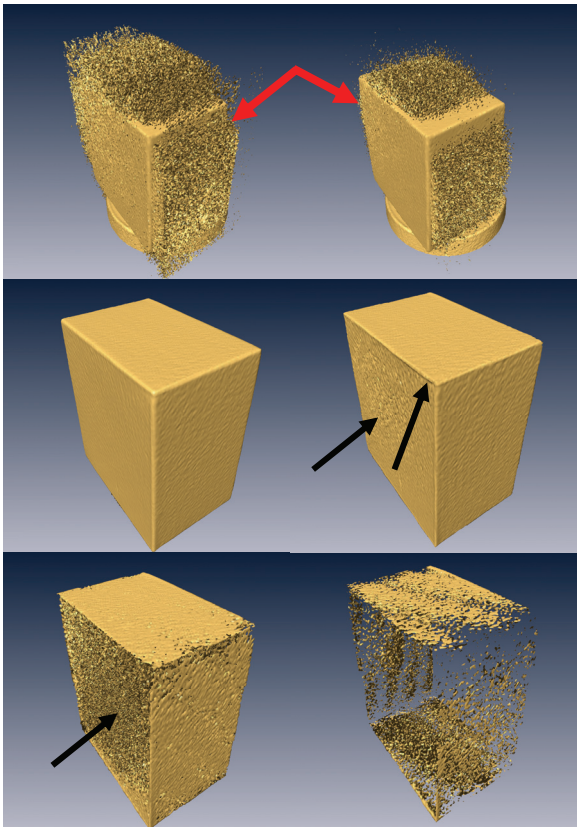

Fig. 1 *Isosurfaces of the calibration body made of Laas marble generated with six different threshold values corresponding to Hounsfield Units (a, - 800; b, - 900; c, + 350; d, + 2000; e, + 2500; f, + 2700). Setting a lower threshold value adds voxels to the object (red arrows). Raising the threshold value subtract voxels from the material (black arrows) leading to visible degradation of the reconstructed calibration body.*

Given that scanning coral colonies in sea water generates more background noise than scanning the samples in air (Fig. 2) segmentation is to be favored over computing an isosurface. In order to remove background noise which would cause artifacts in the rendering process, the structure of the coral colony needs to be delineated and separated out from the background (Fig. 3). Segmentation can be done either manually on each individual slice of a CT stack of 2D grey scale images or preferably processed automatically on the entire data volume (volume segmentation). Depending on the applied software a variety of algorithms are used for automatic or semi-automatic volume segmentation by detecting and selecting similar objects by their grey scale values representing the respective density of the material (aragonite vs. sea water). While scanning in sea-water threshold values needed for automatic segmentation will differ from the isosurface threshold because the HU for water itself is set at 0 and lower thresholds might therefore hamper the reconstruction algorithm. To remove background noise and to add or delete contours not belonging to the coral skeleton the application of filters and manual editing is needed. The surface mesh is then generated from the resulting contour data leading to a virtual 3D model of a coral colony for surface area calculation.

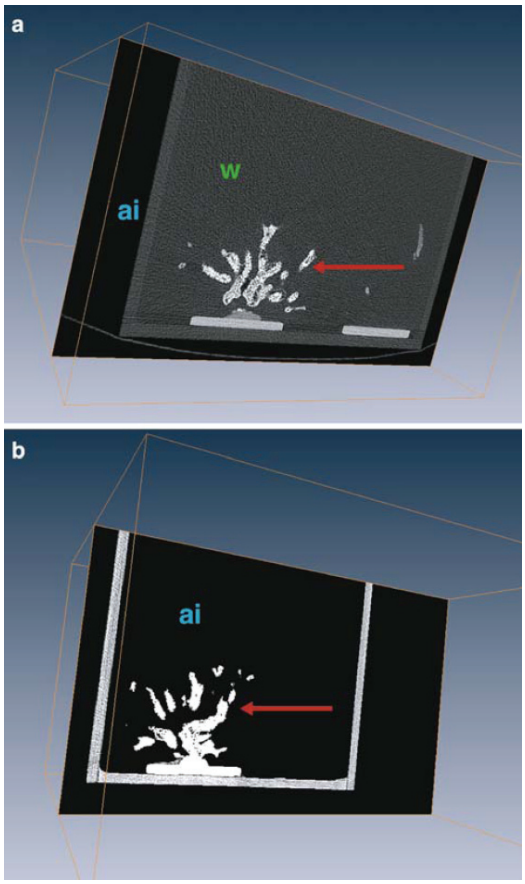

Fig. 2 *a, Orthoslice of a coral colony (red arrow) scanned in sea water (w). b, Orthoslice of a coral colony (red arrow) scanned in air (ai). The level of background noise is increased in the scan conducted in sea water.*

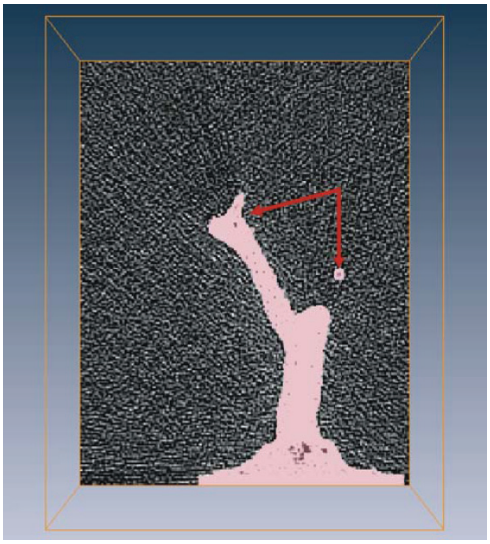

Fig. 3 *Image segmentation of a coral colony scanned in sea water. A single Orthoslice is devided into different sub-regions: coral colony (light red and red arrows) vs. background*

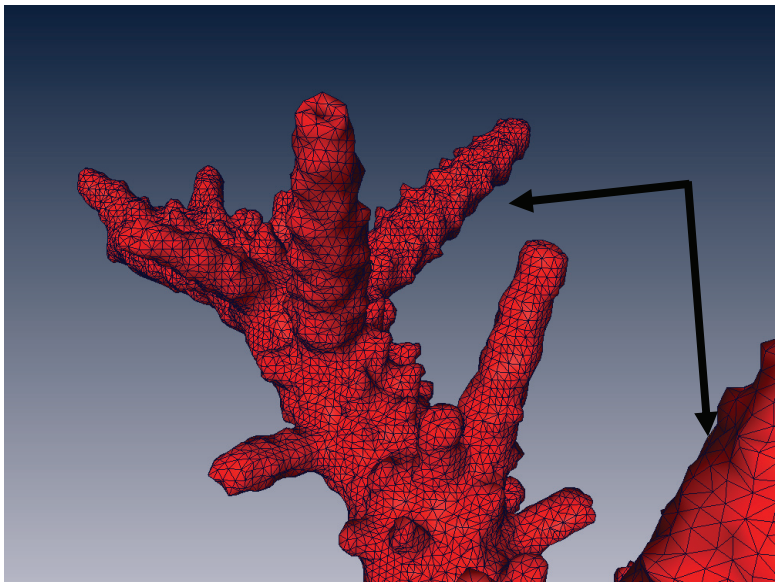

Fig. 4 *Triangulated surface (black arrows) of a coral colony created by the segmentation process.*

The "Segmentation editor" provided by Amira 4.1 was easily applicable to remove distracting artifacts. The first step was to apply a simple threshold segmentation algorithm called "Label voxel" to the volume data. In the process the exterior (sea water or air) and the interior region (coral skeleton) were subtracted (exterior-interior). In the "options field" of the "LabelVoxel" module, "subvoxel accuracy" was selected to create smooth boundaries. Threshold values were adjusted as described above. Given that partial volume effects (more than one scanned material type occurs in a voxel; Ketcham and Carlson 2001) occur stronger by scanning in sea-water the segmentation process was reworked manually to adjust the boundaries to the actual coral skeleton surface and to remove distracting features by using the "Segmentation Editor". The latter was used to remove background noise artefacts (islands) by simply applying the "Remove Islands" filter on the entire data volume. After removing all "islands" and manual adjustment the module "SurfaceGen" was applied, which computes a triangular approximation of the interface between different types of material (Fig. 4). The new surface model was subsequently processed as described in the Isosurface section.

Data analysis

Surface areas calculated with both applied thresholds (best-fit values of calibration bodies) were compared in three coral colonies of three different genera. Based on these results the best threshold value was chosen by visually comparing both virtual 3D models of each colony with the living colony and applied in the comparison of surface area determination in both the "Isosurface" and the "Segmentation" method. The validity of surface area measurement was verified by repeated measurements of the same colonies by three test persons (briefly introduced to AMIRA 4.1 and not familiar with 3D reconstruction software) and statistically analyzed using independent samples t-tests ($p < 0.05$; SPSS for Windows). The results for the threshold value determination and for the method comparison are presented using descriptive statistics.

Results

Threshold adjustment

Stepwise approximations to determine the best-fit threshold for three-dimensional surface area reconstruction of both calibration bodies showed remarkable differences between both materials. For instance, at a threshold value of + 1,000 HU the calibration body made of Laas marble and the coral skeleton were clearly visible, whereas surface area of the calibration body made of PVC showed a distinct degradation of the surface after the rendering process (Fig. 5 a). Setting the steps at 50 units matched the actual surface area of the calibration body made of Laas marble at a value of + 350 HU compared to the calibration body made of PVC for which an accurate concordance was achieved by setting the threshold value at - 350 HU (Table 1, Fig. 5 b). Visual verification of isosurfaces created with both threshold values (- 350 HU; + 350 HU) showed distinct artefacts at a threshold value of + 350 HU in each of the examined coral genera (Fig. 6). Variation of the greyscale threshold resulted in a difference in surface area measurements in *Acropora sp.* at 1,18 % by showing a lower surface area at the higher threshold value (+ 350 HU). In contrast, surface area measurements yielded an increased surface area in *Pocillopora sp.* and *Montipora sp.* at a threshold value of + 350 HU compared to the lower threshold value (Table 2). Additional visual comparison of the computed surface models of both surface rendering techniques with the shape of the respective living colony approved the setting of the threshold value to - 350 HU for all coral genera scanned in air (Fig. 7).

Table 1 *Surface areas of two types of calibration bodies (Actual surface area) made of different materials (densities). Surface areas were calculated from computed 3D - models using different thresholds (corresponds to Housfield Units) each showing a best-fit to the respective actual surface area.*

Surface area (cm^2)	Marble	PVC
Threshold + 350	99.157	53.513
Threshold - 350	111.076	54.433
Actual surface area	99.143	54.036

"Isosurface" vs. "Segmentation" and evaluation of the method

Surface area of the 3D models of the same coral colony computed with both methods (- 350) ranged from 1.32 % to 2.03 % difference depending on the coral genus (Table 2). Complex colony growth forms (*Pocillopora sp.*) showed the strongest deviation in the comparison of both methods. Applying the "segmentation" technique in *Acropora sp.* and *Montipora sp.* yielded a slightly higher surface area value than using the "Isosurface" module. Surface area determination in *Pocillopora* showed a converse result (Table 2).

A similar pattern was observed in the evaluation of both methods applied by three test persons (Fig. 8). Surface areas calculated from the same volume of data of a single coral colony showed no significant difference between all test persons in all coral genera and in both methods (independent samples t-tests: Person 1 vs. Person 2; Person 2 vs. Person 3; Person 1 vs. Person 3, all n.s.; df = 4; Fig. 8).

Table 2 *Surface areas of three different coral colonies of three genera computed with different thresholds (best-fit to calibration bodies) and surface rendering techniques "Isosurface" = "Iso"; "Segmentation" = "Seg"). Deviation of both thresholds and methods are shown in percent.*

	Surface area (cm^2)		
Threshold (HU) /method	*Acropora*	*Pocillopora*	*Montipora*
+ 350 / "Iso"	73.565	243.279	64.042
- 350 / "Iso"	74.444	223.241	57.176
- 350 / "Seg"	75.387	218.702	58.094
	Deviation (%)		
+ 350 / "Iso" vs. - 350 / "Iso"	1.18	8.24	10.72
- 350 / "Iso" vs. - 350 / "Seg"	1.32	2.03	1.58

Discussion

This study introduces an accurate and novel approach to quantify surface areas of coral colonies using X-ray computed tomography and subsequent 3D-modelling. An additional strength of this non-invasive and easy to learn method is its applicability in living colonies by scanning the latter in air or submerged in sea water. Moreover, data analysis of a single coral colony required approximately 15 to 30 minutes for a trained user applying the isosurface method, thus highlighting the rapid processing time as a further advantage of this method.

In studies primary aiming to quantify surface areas of coral colonies, surface rendering of volume data derived from X-ray CT is a sufficient technique to attain that goal. Although most coral species show different corallite assemblages, the robust coral skeleton allows equating the actual surface of the tissue of a living colony to the surface of the skeleton which is composed of calcium carbonate in the form of aragonite (Pingitore et al. 2002). The microstructure of the latter defines the density of the material, which is a crucial factor in X-ray CT and subsequent image processing. Setting the correct threshold for surface rendering is indispensable for topographical analysis in scleractinian corals. In this study, the use of calibration bodies with precisely known surface areas proved to be feasible to adjust the threshold for accurate image processing. Although the calibration body made of Laas marble is composed of almost the same material as the coral skeleton, the best-fit threshold value (+350 HU) was not applicable for isosurface reconstruction in corals (Fig. 6).

This fact resulted from the high density of the marble compared to the porous corallites. However, the best-fit threshold value of the calibration body made of PVC yielded an accurate result. Critical and accurate inspection of the visualized data is indispensable to achieve the optimal settings for image processing (Kruszynski et al. 2006, 2007). Thus, the computed 3D models of coral colonies were compared to the actual topographies of living coral colonies. The latter verification showed virtually identical colony morphologies demonstrating the accuracy of the applied threshold value for scanning coral colonies in air (Fig. 7).

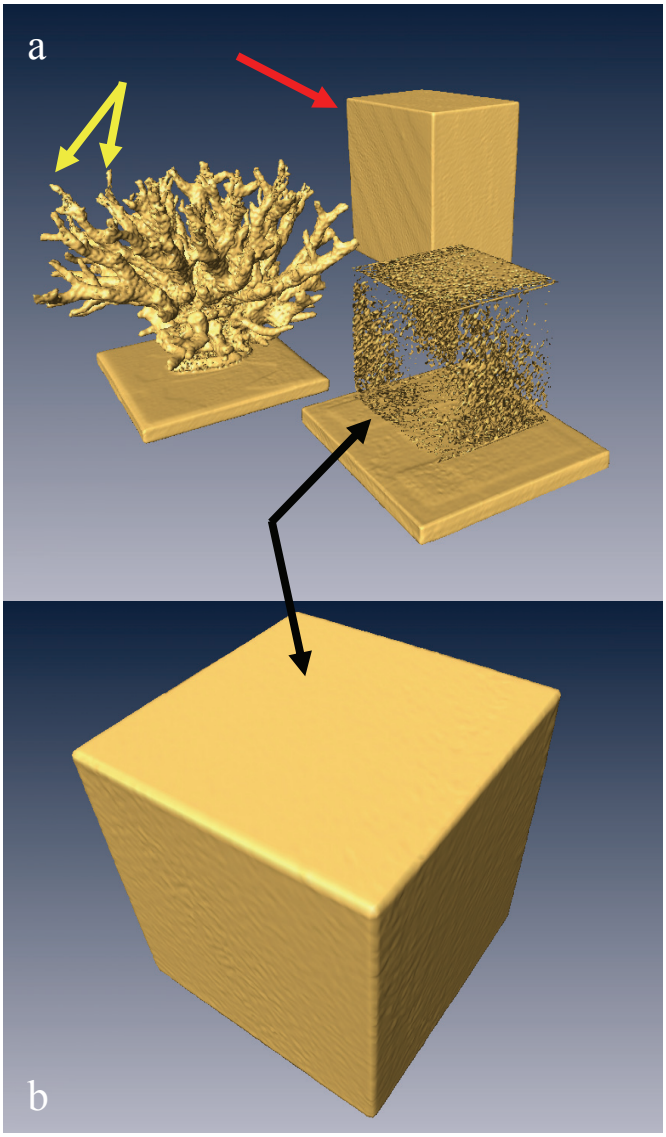

Fig. 5 *a, Isosurface of both calibration bodies created at a threshold value of + 1,000 HU. The silhouette of the calibration body made of Laas marble (red arrow) was distinctly visible, whereas surface area of the calibration body made of PVC (black arrow) showed a distinct degradation of the surface. Reconstruction of the coral colony showed artefacts (yellow arrows) using that threshold. b, Isosurface of the calibration body made of PVC (black arrow) reconstructed with the best-fit threshold of – 350 HU.*

The marginal difference of isosurfaces computed from both thresholds observed in the *Acropara sp*. colony (Table 2) may result from the more compact margins of the respective skeleton. Although 3D models of *Pocillopora sp.* and *Montipora sp.* showed degradation of the isosurface at a threshold value of + 350 HU, increased surface areas in comparison with the lower threshold were observed in both 3D models. This fact was likely caused by the formation of artificial islands in the rendering process. surface area determination are virtually identical to simple morphologies such as the *Montipora* colony (Fig. 6). At the optimum threshold level (-350 HU) both rendering techniques "Isosurface" and "Segmentation" showed almost identical results in each of the examined genera (Table 2).

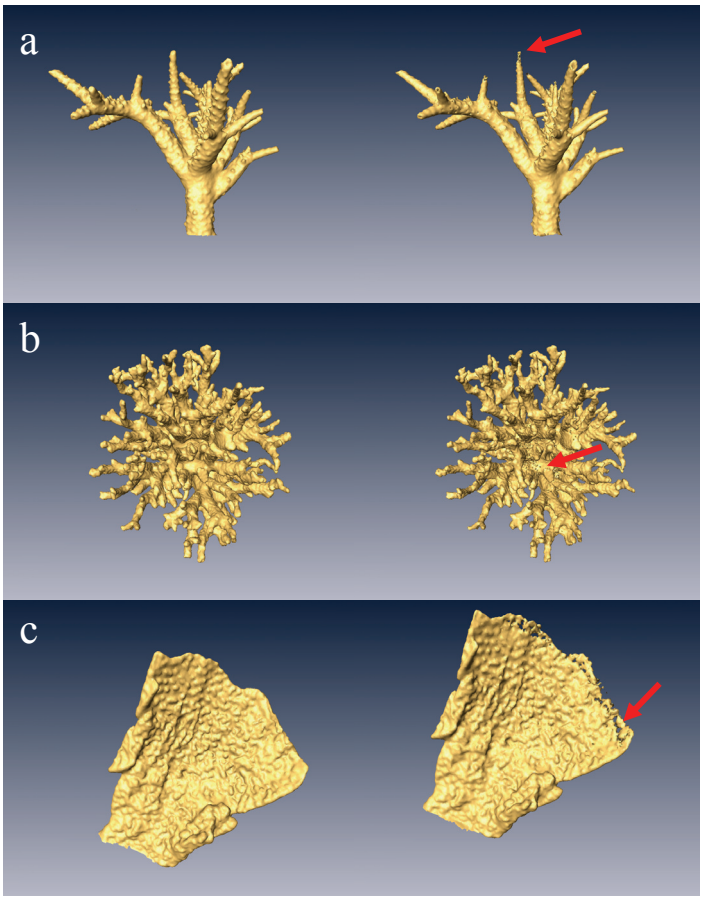

Fig. 6 *Comparison of isosurfaces of three coral genera (a, Acropora; b, Pocillopora, c, Montipora) created with both best-fit threshold values (left: - 350 HU; right: + 350 HU). Artefacts (red arrows) are clearly visible at a threshold value of + 350 HU.*

However, surface quantification by using the "Isosurface" module is less time-consuming than the segmentation process but only if background noise and the resulting artefacts are low. Moreover, partial volume effects might hamper the manual or automatic segmentation process in volume data gathered by scanning in sea-water, due to blurred material boundaries. Hence, scanning in air is to favor over scanning in water (Fig. 2). The high potential of surface rendering techniques in surface area quantification becomes obvious in the 3D model of the *Pocillopora* colony representing a highly complex morphology. Regardless of the complex assembly of overlapping branches processing time and accuracy of Short-time air exposure of corals regularly occurs in-situ, e.g. at extreme low tides (Romaine et al. 1997) without leaving damage and thus does not represent an artificial stress factor for corals.

If exposition to air of a living coral colony, even only for a couple of minutes during the scanning process, is not desirable in a projected study the specimens can also be scanned in sea water,followed by segmentation of the volume data to extract the surface topography of a coral colony. The segmentation editor provided by Amira 4.1 is a powerful tool to remove all artifacts caused by scanning in water. Even if more image processing steps are required in comparison to the "Isosurface" technique it is still a reliable and easily applicable method to quantify surface areas in

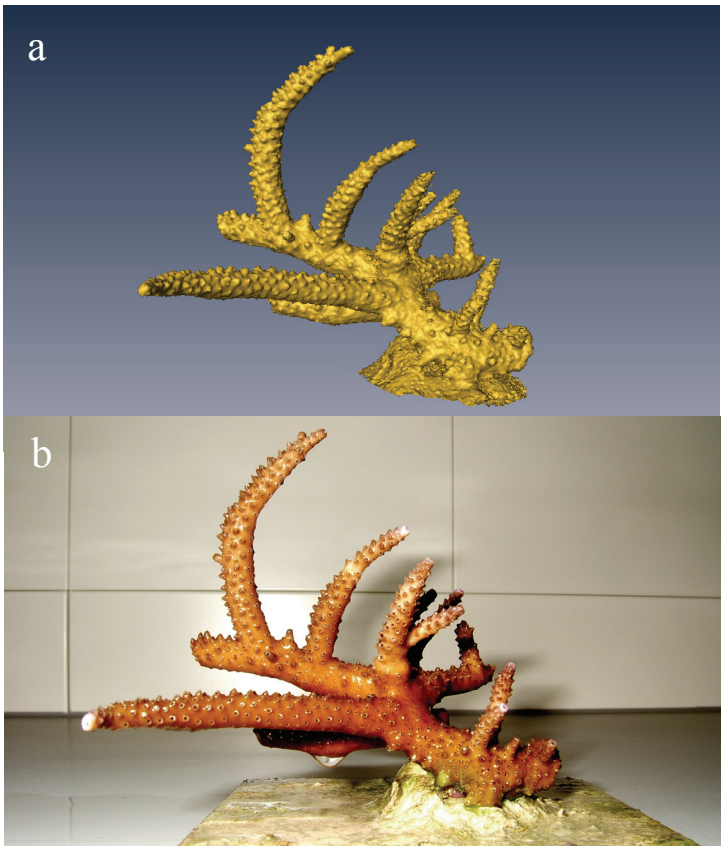

Fig. 7 *The visual comparison of the computed surface model using the segmentation method at a threshold value of - 350 HU with the shape of the respective living colony shows distinctly the accuracy of the reconstructed surface.*

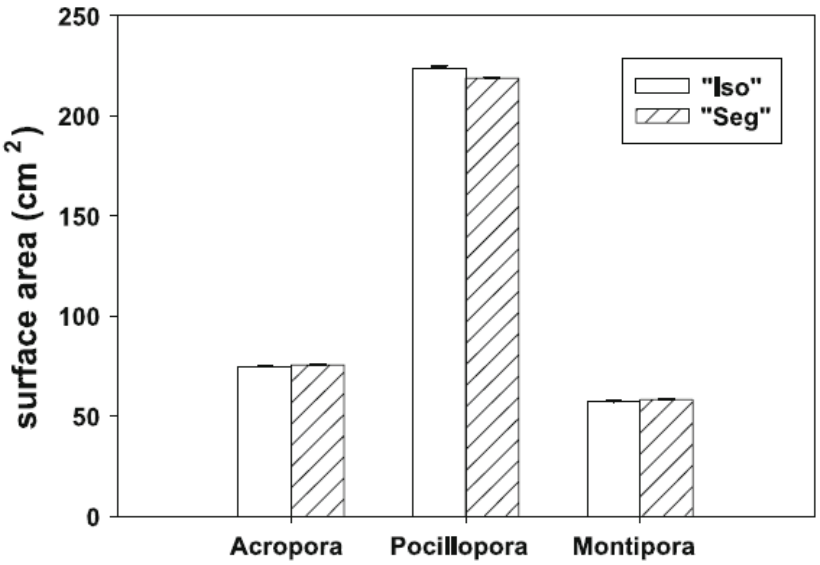

Fig. 8 *Bar chart of repeated surface area measurements conducted by three different persons. The bars indicate the mean surface area and the respective standard error of both surface rendering techniques ("Isosurface" white; "Segmentation" coarse lines) of the same coral colonies.*

coral colonies (Table 2). Both techniques (Isosurface and segmentation) are available in almost all software packages for processing DICOM data (e.g., Schicho et al. 2007).

Repeated measurements of the same coral colonies conducted by three different persons yielded mean deviations ranging from 0,13 % to 1,35 % (Fig. 8). This result shows the high reproducibility and accuracy of both surface rendering techniques and it is in concordance with the outcome of repeated surface area measurements in dental implants using Micro - CT (Schicho et al. 2007). The application of high resolution tomography such as Micro - CT is favourable in studies focusing on internal structures of coral skeletons as demonstrated for the impact of boring sponges on coral morphogenesis (Beuck et al. 2007). An image-processing algorithm labelled as "Skeletonization", which reduces the coral to a network of thin lines, has recently been introduced to analyze those morphometric and morphogenetic patterns in corals (Kruszynski et al. 2006, 2007). The high precision of Micro-CT reveals delicate structures of the coenosteum and the corallite of the scleractinian cold water coral *Lophelia pertusa* (Beuck et al. 2007). Depending on the species-specific coral morphology studied, detailed surface rendering of skeletal components such as septa, theca or columnella probably leads to an overestimation of the surface area of the coral tissue. Hence, to quantify surface areas in corals the use of a conventional medical CT scanner with the resolution set around 0.5 mm is to favour over the application of a Micro - CT scanner.

Surface models produced from a medical CT provide realistic surface views if compared to the respective tissue of a living colony (Fig. 4 and Fig. 7). However, even if a very high accuracy of surface area estimation could be achieved by this method the actual surface area of the polyps and the coenosarc could not be detected. Another limitation of X-ray computed tomography in surface area determination is that it is hardly applicable in the field although portable CT scanners are available (Mirvis et al. 1997).

Nevertheless, the precision and low processing-time highlight the potential of the novel approach presented in this study for surface area determination of living colonies and bare skeletons in laboratory and laboratory-based field studies. Moreover the volume of a virtual 3D model of a coral colony can be calculated accurately using the same set of data as used for the surface area determination without applying any further image processing steps. This fact renders this technique also perfectly suitable for accurate surface area to volume ratio calculations which are used in studies focusing on coral growth and metabolism such as nutrient uptake (Koop et al. 2001). Especially for complex branching taxa surface area and volume are often rough approximations. The latter are yielded for instance by geometric forms that best resemble the complex structure of the coral colony as applied in the analysis of whether growth forms are limited by coral physiology (Kizner et al. 2001).

Hence, the precision of the CT based method might be used to improve studies on several aspects in coral reef science. For instance time series analysis on a single coral colony can be conducted with a very high accuracy. In addition, measurements of a set of coral colonies by 3D modeling may be potentially utilised as a calibration factor for already established techniques (e.g. foil wrap, melted paraffin wax, photogrammetry or geometric techniques) to determine coral surface area. Such "standards" would offer the possibility to analyze surface areas of coral colonies

accurately independent from the scale of observation. The ubiquitous application of those "standards" might therefore improve the precision of surface area determination in studies where computed tomography is not affordable or impossible to use. This may also improve large scale surveys in the future, which are used to foster reef management strategies (e.g., Fisher et al. 2007).

Acknowledgments

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References

Babcock RC (1991) Comparative demography of three species of scleractinian corals using age- and size-dependent classifications. Ecol Monogr 61:225-244

Bak RPM, Meesters EH (1998) Coral population structure: the hidden information of colony size-frequency distributions. Mar Ecol Prog Ser 162:301-306

Beuck L, Vertino A, Stepina E, Karolczak M, Pfannkuche O (2007) Skeletal response of *Lophelia pertusa* (Scleractinia) to bioeroding sponge infestation visualised with micro-computed tomography. Facies 53:157-176

Bosscher H (1993) Computerized tomography and skeletal density of coral skeletons. Coral Reefs 12:97-103

Bythell JC, Pan P, Lee J (2001) Three-dimensional morphometric measurements of reef corals using underwater photogrammetry techniques. Coral Reefs 20:193-199

Cocito S, Sgorbini S, Peirano A, Valle M (2003) 3-D reconstruction of biological objects using underwater video technique and image processing. J Exp Mar Biol Ecol 297:57-70

Courtney LA, Fishera WS, Raimondoa S, Olivera LM, Davisa WP (2007) Estimating 3-dimensional colony surface area of field corals. J Exp Mar Biol Ecol 351:234-242

Done TJ (1981) Photogrammetry in coral ecology: a technique for the study of change in coral communities. Proc 4th Int Coral Reef Symp 2:315-320

Fisher WS, Davis WP, Quarles RL, Patrick J, Campbell JG, Harris PS, Hemmer BL, Parsons M (2007) Characterizing coral condition using estimates of three-dimensional colony surface area. Environ Monit Assess 125:347-360

Glynn PW, D'Croz L (1990) Experimental evidence for high temperature stress as the cause of El Nino-coincident coral mortality. Coral Reefs 8:181-191

Goffredo S, Mattioli G, Zaccanti F (2004) Growth and population dynamics model of the Mediterranean solitary coral *Balanophyllia europaea* (Scleractinia, Dendrophylliidae). Coral Reefs 23:433-443

Hacker BR, Kirby SH (1993) High-pressure deformation of calcite marble and its transformation to aragonite under non-hydrostatic conditions. J Struct Geol 15:1207-1222

Hoegh-Guldberg O (1988) A method for determining the surface area of corals. Coral Reefs 7:113-116

Hoegh-Guldberg O, Smith GJ (1989) The effect of sudden changes in temperature, light and salinity on the population density and export of zooxanthellae from the reef corals *Stylophora pistillata* Esper and *Seriatopora hystrix* Dana. J Exp Mar Biol Ecol 129:279-303

Hounsfield GN (1973) Computerized transverse axial scanning (tomography): Part I. Description of system. Br J Radiol 46:1016-1022

Kaandorp JA, Sloot PMA, Merks RMH, Bak RPM, Vermeij MJA, Maier C (2005) Morphogenesis of the branching reef coral *Madracis mirabilis*. Proc R Soc Lond B 272:127-133

Kak AC, Slaney M (1988) Principles of computerized tomographic imaging. Institute of Electrical and Electronics Engineers Press, New York

Kanwisher JW, Wainwright SA (1967) Oxygen balance in some reef corals. Biol Bull 133:378-390

Kenter JAM (1989) Applications of computerized tomography in sedimentology. Marine Geotechnology 8:201-211

Ketcham RA, Carlson WD (2001) Acquisition, optimization and interpretation of X-ray computed tomographic imagery: applications to the geosciences. Comput Geosci 27:381-400

Kim I, Paik KS, Lee SP (2007) Quantitative evaluation of the accuracy of micro-computed tomography in tooth measurement. Clin Anat 20:27-34

Kizner Z, Vago R, Vaky L (2001) Growth forms of hermatypic corals: stable states and noise-induced transitions. Ecol Model 141: 227-239

Koop K, Booth D, Broadbent A, Brodie J, Bucher D, Capone D, Coll J, Dennison W, Erdmann M, Harrison P, Hoegh-Guldberg O, Hutchings P, Jones GB, Larkum AWD, O`Neil J, Steven A, Tentori E, Ward S, Williamson J, Yellowlees D (2001) ENCORE: The effect of nutrient enrichment on coral reefs. Synthesis of results and conclusions. Mar Pollut Bull 42: 91-120

Kruszynski KJ, van Liere R, Kaandorp JA (2006) An interactive visualization system for quantifying coral structures. In: Ertl T, Joy K, Santos B (eds) Eurographics/ IEEE-VGTC Symposium on Visualization, pp 283-290. DOI: 10.2312/VisSym/EuroVis06/283-290

Kruszyjski KJ, Kaandorp JA, van Liere R (2007) A computational method for quantifying morphological variation in scleractinian corals. Coral Reefs 26:831-840

Meyer JL, Schultz ET (1985) Tissue condition and growth rate of corals associated with schooling fish. Limnol Oceanogr 30:157-166

Mirvis S, Shanmuganathan K, Donohue R, White W, Fritz S, Hartsock R (1997) Mobile computed tomography in the trauma/critical care environment: Preliminary clinical experience. Emerg Radiol 4:1435-1438

Odum HT, Odum EP (1955) Trophic structure and productivity of a windward coral reef community on Eniwetok Atoll. Ecol Monogr 25:291-320

Pingitore Jr. NE, Iglesias A, Bruce A, Lytle F, Wellington GM (2002) Valences of iron and copper in coral skeleton: X-ray absorption spectroscopy analysis. Microchem J 71:205-210

Roberts CM, Ormond RFG (1987) Habitat complexity and coral-reef fish diversity and abundance on Red-Sea fringing reefs. Mar Ecol Prog Ser 41:1-8

Rodt T, Bartling SO, Zajaczek JE, Vafa MA, Kapapa T, Majdani O, Krauss JK, Zumkeller M, Matthies H, Becker H, Kaminsky J (2006) Evaluation of surface and volume rendering in 3D-CT of facial fractures. Dentomaxillofacial Radiology 35:227-231

Romaine S, Tambutte E, Allemand D, Gattuso JP (1997) Photosynthesis, respiration and calcification of a zooxanthellate scleractinian coral under submerged and exposed conditions. Mar Biol 129:175-182

Schicho K, Kastner J, Klingesberger R, Seemann R, Enislidis G, Undt G, Wanschitz F, Figl M, Wagner A, Ewers R (2007) Surface area analysis of dental implants using micro-computed tomography. Clin Oral Implants Res 18:459-464

Stimson J, Kinzie RA (1991) The temporal pattern and rate of release of zooxanthellae from the reef coral *Pocillopora damicornis* (Linnaeus) under nitrogen-enrichment and control conditions. J Exp Mar Biol Ecol 153:63-74

Szmant-Froelich A (1985) The effect of colony size on the reproductive ability of the Caribbean coral *Montastraea annularis* (Ellis and Solander). Proc 5th Int Coral Reef Symp 4:295-300

Wegley L, Yu Y, Breitbart M, Casas V, Kline DI, Rohwer F (2004) Coral-associated Archaea. Mar Ecol Prog Ser 273:89-96

Wild C, Huettel M, Klueter A, Kremb SG, Rasheed M, Jørgensen BB (2004) Coral mucus functions as an energy carrier and particle trap in the reef ecosystem. Nature 428:66-70

Vago R, Shai Y, Ben-Zion M, Dubinsky Z, Achituv Y (1994) Computerized tomography and image analysis: a tool for examining the skeletal characteristics of reef-building organisms. Limnol Oceanogr 39:448-452

2

Coral surface area quantification – evaluation of established techniques by comparison with computer tomography

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Abstract

The surface area of scleractinian corals represents an important reference parameter required for various aspects of coral reef science. However, with advancements in detection accuracy and novel approaches for coral surface area quantification, evaluation of established techniques in comparison to state of the art technology gains importance to coral researchers. This study presents an evaluation of methodological accuracy for established techniques in comparison to a novel approach composed of computer tomography (CT) and 3-dimensional surface reconstruction. The skeleton surface area of reef corals from six genera representing the most common morphological growth forms was acquired by CT and subsequently measured by computer-aided 3-dimensional surface reconstruction. Surface area estimates for the same corals were also obtained by application of four established techniques: Simple and Advanced Geometry, Wax Coating and Planar Projection Photography. Comparison of the resulting area values revealed significant differences between the majority (82 %) of established techniques and the CT reference. Genus-specific analysis assigned the highest accuracy to geometric approximations (Simple or Advanced Geometry) for the majority of assessed coral genera (maximum accuracy: 104 %; Simple Geometry with *Montipora* sp.). The commonly used and invasive Wax Coating technique reached intermediate accuracy (47 – 74 %) for the majority of genera, but performed outstanding in the measurement of branching *Acropora* spp. corals (maximum accuracy: 101 %), while the Planar Projection Photography delivered low accuracy genera-wide (12 – 36 %). Comparison of area values derived from established techniques and CT additionally yielded approximation factors (AF) applicable as factors in the mathematical improvement of surface area estimates by established techniques in relation to CT reference accuracy.

Introduction

Scleractinian corals exhibit an array of different growth forms and intricate skeletal structures. In coral reef studies, the surface area of corals serves as an important reference parameter, for example, regarding the standardisation of metabolic processes such as photosynthesis, respiration and the release of coral-derived organic material to the environment (Wild et al. 2005). Measurement of the actual coral tissue surface area remains a difficult approach. The challenge to develop feasible methodologies, adequately determining the surface area of the complex and species specific skeletal structures of corals, has led to the publication of various methodological approaches over the past decades.

Geometric measurement techniques are probably the earliest approach used to assess the surface area of marine organisms (Odum et al. 1958). Geometric forms or shapes (e.g., cylinders, spheres, circles) that best resemble the complex structure and topography of the investigated organism are selected and basic dimensional parameters of the organism are measured; the surface area is calculated by the respective geometric formula. This approach offers important advantages as it can be rapidly carried out and is non-invasive; this has led to the frequent application of geometric approaches in ecological and physiological coral reef science (Szmant-Froelich 1985; Roberts and Ormond 1987; Babcock 1991; Bak and Meesters 1998) as well as in coral monitoring studies (Fisher et al. 2007).

Several coating techniques, involving the dipping of corals in liquids and the subsequent correlation of the amount of coating to the assessed surface area, have been described to date. These include coating of coral fragments with latex (Meyer and Schultz 1985), paraffin wax (Stimson and Kinzie 1991), or vaseline (Odum and Odum 1955) and the use of the dye Methylene Blue in a method designed for finely branched coral species (Hoegh-Guldberg 1988). In addition, coating with aluminium foil of known weight per unit area (Marsh 1970) has found application in several field studies (Fagoonee et al. 1999; Vollmer and Edmunds 2000; Wegley et al. 2004). Of these techniques, coating with paraffin wax appears most frequently in the literature (e.g., Glynn and D'Croz 1990; Chancerelle 2000; Vytopil and Willis 2001; Wild et al. 2005). All coating techniques require prior tissue removal or lead to mechanical damage of the tissue and are therefore unsuitable for experimental studies where continuous investigations on living corals are necessary.

The projected planar area of coral colonies has been used in numerous studies to estimate surface areas in combination with geometric assumptions (Falkowski and Dubinsky 1981; Muscatine et al. 1989) or by plain calculation of the covered benthic area (Jokiel and Morrissey 1986). In attempts to compare the projected planar area of corals with geometric approximations of substratum topography and coral morphology, surface indices (SI) were developed to find suitable means for the 3-D approximation of benthic reef coverage (Dahl 1973; Alcala and Vogt 1996) and coral colony surface area (Pichon 1978). Photography of the planar projection of corals, as a method to assess surface area (Kanwisher and Wainwright 1967), has found application in coral reef science on different scales of observation. Visual underwater surveys for benthic coverage make use of photographs taken from above the reef substratum to quantify and monitor reef community structures (Bohnsack 1979; Mergner and Schuhmacher 1979; Hughes and Jackson 1985). For studies on individual coral colonies, specific methods for surface area determination

were developed, involving computer-aided digitising of photographs (Benzion et al. 1991; Rahav et al. 1991; Tanner 1995). The shortcoming of the planar photographic approach is the 2-dimensional (2-D) projection of a 3-dimensional (3-D) form, which significantly underestimates the actual surface area. In order to reduce this limitation, photogrammetric methods have been described using object photographs from various perspectives, which are then combined by computer-aided design (CAD) to form a 3-D object surface reconstruction (Done 1981; Bythell et al. 2001; Cocito et al. 2003; Courtney et al. 2007; Jones et al. 2008). As the most recent advancement of methods applying optical surface detection and computer-aided object reconstruction, 3-D laser scanner systems have successfully been used for coral surface area quantification (Courtney et al. 2007; Holmes 2008).

Another computer-aided 3-D technique, computer tomography (CT), has found some application in coral reef sciences since its introduction (Hounsfield 1973). CT provides high resolution X-ray images, which have shown to be particularly useful in studies focussing on coral growth (Bosscher 1993; Bessat et al. 1997; Goffredo et al. 2004) and have additionally found broad applications in geosciences (Ketcham and Carlson 2001). In recent years, computer-aided methods using CT-derived data were applied to virtually reconstruct coral morphological structures (Kruszynski et al. 2006) and to simulate coral growth patterns (Kaandorp et al. 2005). With the help of software packages, 3-D surface reconstructions of coral colonies can be generated using CT-derived data from which the virtual surface area is subsequently calculated, thereby providing high accuracy area measurement of the actual skeleton surface area (Laforsch et al. 2008). This procedure can be applied to bare skeletons as well as on living coral specimens. A limitation of CT measurements of corals is the restriction to measurements of the skeleton topography only, while coral tissue components remain undetected.

The present study aimed to evaluate the accuracy of surface area estimates derived from four established techniques in coral reef science (Simple and Advanced Geometry, Wax Coating and Planar Projection Photography) in direct comparison to a high accuracy CT-based methodology, used as reference. Analysis of method accuracy was extended to generate approximation factors (AF) applicable in the mathematical improvement of surface area estimates by established techniques in relation to CT reference accuracy.

Material and Methods

A total of 72 coral skeletons from 6 genera and 4 growth forms (warm water corals: *Acropora* spp., *Fungia* spp., *Galaxea fascicularis*, *Montipora* sp., and *Pocillopora damicornis;* cold water coral: *Lophelia pertusa*) were used in this comparative investigation of surface area quantification (see Fig. 1, panels a-f). The skeleton surface area of each of these coral colonies was determined by the use of established techniques comprising geometric approximations, Wax Coating and Planar Projection Photography. In addition, skeleton surface area measurements for all coral colonies were carried out by conventional medical CT and subsequent 3-D surface reconstruction, in order to allow for a direct comparison to the results derived from established techniques. Coral skeletons

were obtained from collections of aquarists. For each genus, 11-13 colonies ranging from 1-17 cm maximum diameter were selected to account for differences between size classes. The bases of the colonies were ground to achieve an exact reference plane for all mentioned techniques and then glued onto ceramic tiles (4 * 4 cm). The skeleton surface area was quantified by the following procedures. An example for the genus *Acropora* (*Acropora* specimen # 10) illustrating the application of the different techniques is displayed in Figure 2.

Geometric approximations

Geometric measurements of coral colonies were divided into two approach categories, Simple Geometry and Advanced Geometry, defined as follows: Simple Geometry assesses the whole structure of a coral colony at once and assigns a geometric body that shows closest morphological similarity (e.g., cylinder, hemisphere or disc). Only few basic dimensional parameters (e.g., radius, height) need to be recorded once (Fig. 2, panel a). Advanced Geometry divides the coral colony into several sections and assigns an approximate geometric form or shape to each. Single measurements of dimensional parameters for each section are therefore necessary (Fig. 2, panel a). Measurements were carried out using conventional callipers (accuracy: ± 0.05 mm) and a flexible tape measure (accuracy: ± 1 mm). For both approaches, measured parameters of all geometric forms and shapes were put into the respective surface area equations (Table 1) to calculate the approximate area. Simple and Advanced Geometry were applied to all coral genera, with the exception of *Fungia* spp. and *G. fascicularis,* for which only Simple Geometry was used.

Simple Geometry

Branching growth form

Acropora spp. (n = 12), *L. pertusa* (n = 13), and *P. damicornis* colonies (n = 12) (small single branches and branched colonies) were interpreted as cylinders. The total height and the maximum and minimum horizontal diameters of the whole colony were measured in order to calculate the average horizontal diameter and radius. Height and average radius were used to determine the cylinder shell surface to which the cylinder cover, calculated as a circle using the average radius, was added.

Massive growth form

Colonies of the massive coral *G. fascicularis* (n = 12) were interpreted as hemispheres. Maximum and minimum horizontal diameters of each colony were measured, and the average radius was calculated. The height of the colony was assessed from the reference plane to the highest tip. Thereafter, colony surface area was calculated by the use of the surface area formula for hemispheres.

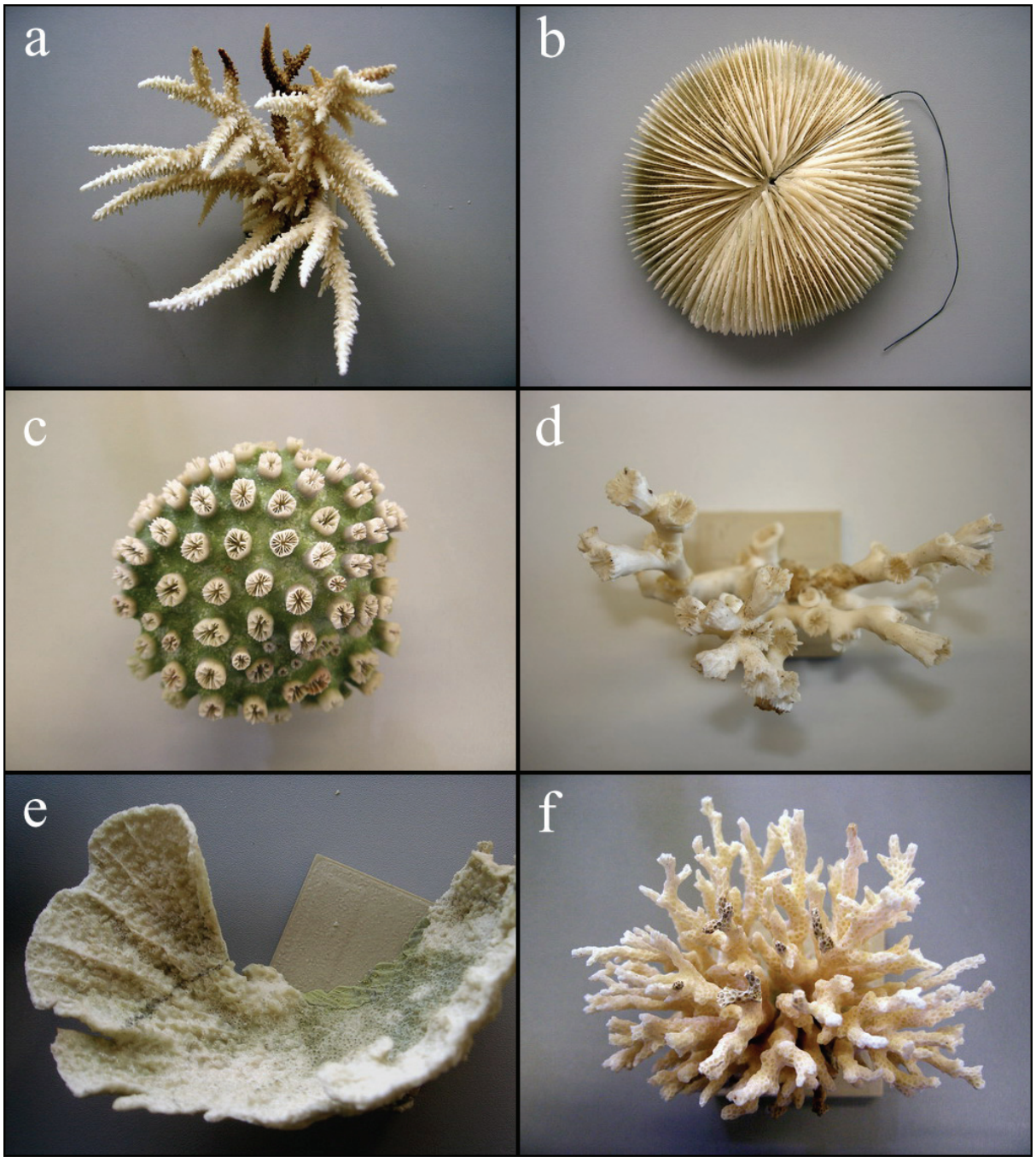

Fig. 1 *Top view of specimens assessed in coral surface area measurements. Panels a: Acropora spp., b: Fungia spp. with attached thread used for Wax Coating, c: Galaxea fascicularis, d: Lophelia pertusa, e: Montipora sp. and f: Pocillopora damicornis.*

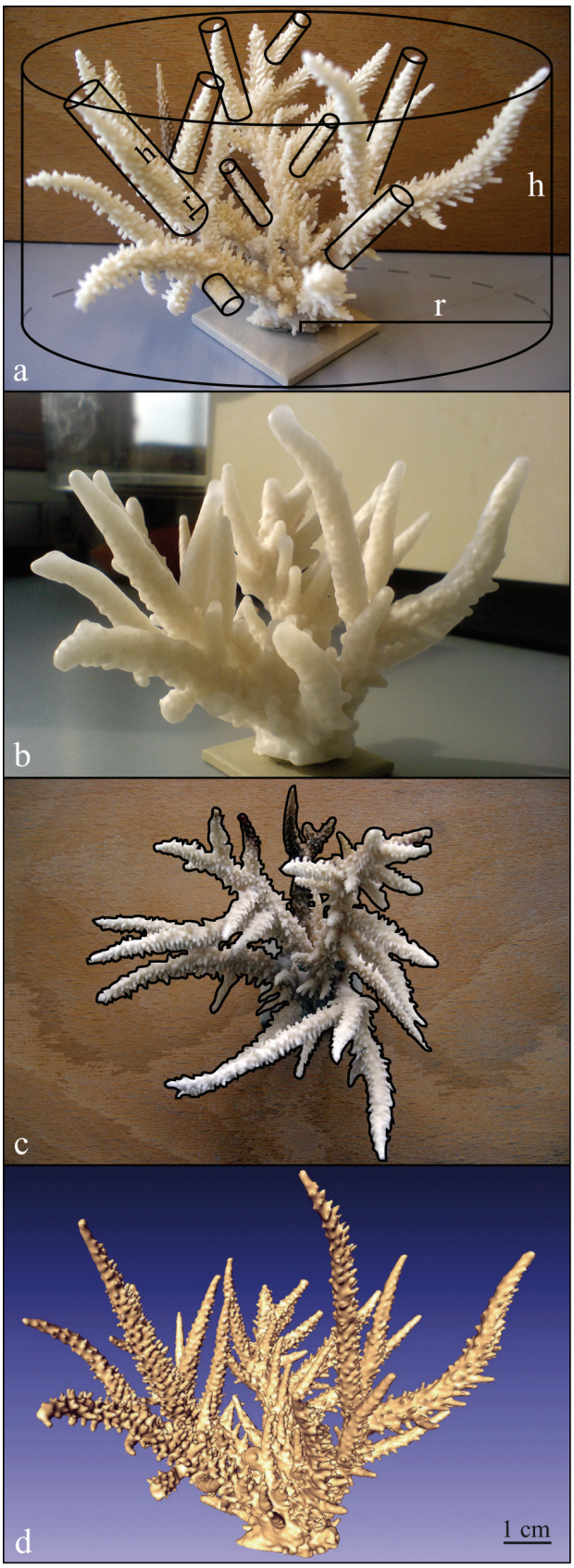

Fig. 2 *Techniques for coral surface area quantification applied in this study. (Panels a - d: Acropora coral specimen #10); Panel a: Simple Geometry and Advanced Geometry, b: Wax Coating, c: Planar Projection Photography, d: 3-D surface reconstruction of computer tomography-derived data.*

Foliose growth form

Colonies of a foliose species of *Montipora* (n = 12) were measured as rectangular plates. Side lengths and the overall perimeter were recorded with a flexible tape measure, taking into account curving skeleton characteristics. Average height (thickness) of the plate was measured with callipers at 4 points. The area of a rectangle was calculated by the side lengths and the result was doubled to represent both sides of the plate. The side of the corals was calculated as a rectangle from the perimeter and the plate thickness, and subsequently added.

Disc-like growth form

A *Fungia* polyp resembles a disc, which is composed of 2 circular sides and a rectangular side. The maximum and minimum horizontal diameters of each polyp (n = 11) were measured, from which the average radius was calculated. Oral and aboral disc surface areas were then calculated as circles. The average height (thickness) of the disc was measured with callipers at 4 points. After the polyp perimeter was assessed with a flexible tape measure, the side could be calculated as a rectangle and was finally added to gain the total area of the disc.

Table 1 *Area equations for geometric shapes and forms used in geometric approximation calculations (Simple and Advanced Geometry). Abbreviations: A = Area, π = pi, h = height, r = radius*

Geometry	Area equation
Cylinder shell surface	$A = 2\,\pi\,h\,r$
Hemisphere	$A = 2\,\pi\,r^2$
Circle	$A = \pi\,r^2$
Rectangle	$A = x\,y$ (a)
Right angle triangle	$A = \frac{1}{2}(m\,n)$ (b)

a: where x and y are side-length of a rectangle; b: where m and n are side-length of a triangle

Advanced Geometry

Branching growth form

The surface area of the entirety of branches of *Acropora* spp., *L. pertusa,* and *P. damicornis* colonies was calculated as cylinder shell surfaces, which showed closer approximation to CT reference in comparison to cone surfaces. For complexly branched colonies of *P. damicornis*, the total number of branches was counted and more than 10 % of all branches were measured; these surface areas were then extrapolated to the total number of branches. The radius and height of branches longer than 1 cm were assessed by measuring the branch diameter at the base of each branch and the height from branch base to tip. The cylinder cover area was only calculated and added for the main branch of each colony. Calculated surface areas from all branches were added to gain the total colony surface area estimate.

Foliose growth form

Montipora colonies were divided into several 2-D shapes (i.e., rectangles, right angle triangles, semi-circles and quarters of circles) and the respective parameters (side-lengths and radii) were measured by flexible tape measure or callipers (where suitable). The surface areas of all shapes were calculated and doubled before the rectangular area (side of the coral) calculated by the perimeter and plate thickness, was added.

Wax Coating

Surface estimates were determined by a paraffin wax coating technique modified from Stimson and Kinzie (1991) and Vytopil and Willis (2001). Paraffin wax (Merck, paraffin powder, melting point: 55 – 57°C) was melted at 58°C in a 5 l glass beaker inside a water bath. Coral colonies were dipped into the melted paraffin wax for 2 s and carefully shaken to remove drops. Through this procedure, the surface was sealed and equal adhesion could be ensured while preserving approximate skeleton topography. The initial weight of the wax coated colonies was measured after 5 min; they were then re-dipped for 5 s and weighed again 5 min later in order to determine the mass increment caused by the second wax coating (Fig. 2, panel b). Geometric bodies of different known surface areas (4 metal cubes and 4 wooden spheres, 5.9 – 77.5 cm^2) were treated accordingly for calibration purposes. The use of different calibration body materials resulted in negligible differences concerning surface adhesion during the initial coating step. The regression relationship between mass increment and surface area of the calibration bodies ($y = 0.0008x – 0.0909$, $r^2 = 0.9974$) was used to determine the surface area of all assessed coral colonies.

Planar Projection Photography

The planar projection of coral fragments was photographed using a digital camera (Casio® QV-R40; resolution: 4.0 megapixels) at a vertical position relative to the natural growth orientation of the colonies. Callipers were held in-plane with top extensions of the fragments for image scaling. Processing of photographs was carried out using image analysis software (*ImageJ*, V. 1.37m, National Institutes of Health, USA). The *Straight line* tool and *Set scale* function were applied to transform the depicted callipers scale into pixel dimension. The perimeter of each fragment was then digitally encircled using the *Polygon* tool at 50 % zoom (Fig. 2, panel c). The enclosed area was subsequently calculated (in square millimetres) using the integrated *Measure* function. Measurements at different zoom levels (50, 75, and 100 %) of three selected colonies representing the largest, smallest, and average size classes from three growth forms, resulted in an error of 0.3 ± 0.3 %. The methodological error determined by repeated digitising of one fragment was 0.4 %, which results in a negligible total error of less than 1 %.

Computer tomography and 3-D surface reconstruction

Tomographic records of corals and calibration bodies (3 polyvinyl chloride cubes; accuracy: 0.01 mm; fixed onto ceramic tiles) were produced in air by conventional medical CT, using a Siemens Somatom Sensation 64® tomograph. CT tube voltage of 120 kV (Eff mAs 341) and a 310 mm field of view were applied. The integrated Somaris software (Syngo CT 2006A, Siemens, Germany) was used for data acquisition. Resulting stacks of image slices (DICOM image format; slice dimensions:

512 * 512 pixels; voxel size: 0.605 * 0.605 * 0.6 mm) were further processed in a computer-aided surface reconstruction procedure using the software *Amira*® (V. 4.1.1; Mercury Computer Systems SAS, France). Image stacks of the calibration bodies were loaded to *Amira*® and regular 3-D isosurfaces (object surface rendering within a 3-D scalar field with regular Cartesian coordinates) were created by application of the integrated *Isosurface* tool. The *Isosurface* tool combined all image slice data of an object and generated a polygonal surface model composed of triangles using a specific threshold value (Hounsfield Unit corresponding to X-ray attenuation values) defining the distinct boundary between object surface and the surrounding air. Different threshold values (75, 0, -100, -150, -200, -300, -400, -500 and -600) were tried for isosurface generation of all calibration bodies to determine the closest fit to the actual known surface area, followed by the creation of a new *Surface,* including a 3-D *Surface View* (option: vertex normal) within the *Surface Editor* tool. The *Surface Editor* tool was used to remove the ceramic tiles and remaining artefacts not belonging to the calibration bodies originating from background noise. Thereafter, a new *Surface* was computed to measure the surface area through the application of the *Surface Area* tool. Surface area values and threshold parameters in isosurface creation of calibration bodies showed a strong polynomial correlation (r^2=0.9998), which allowed for the calculation of a closest-fit threshold value (-354). This threshold value was used in the subsequent isosurface computation of all measured coral colonies.

Data acquisition and processing for coral colonies were carried out as described above using the closest-fit threshold value (-354) derived from calibration bodies for isosurface generation. The *Surface Editor* tool was applied to extract the reconstructed coral surface precisely along the reference plane lining the base of each colony. Critical visual inspections of generated 3-D coral models in comparison to the actual coral colonies were performed to ensure optimal settings for image processing (Kruszynski et al. 2006, 2007) and thus, realistic results of surface area measurements (Fig. 2, panel d).

Data analysis

Percentage accuracy of established techniques

Compiled surface area estimates derived from the four different established techniques were compared to CT-derived reference values for all coral specimens to calculate the percentage accuracy using the equation:

$$\%\ \text{of CT} = \text{Area value of established technique} / \text{Area value of CT} * 100$$

From these results, the genus-specific average percentage accuracy was computed for all established techniques. Differences found for surface area values between established techniques and CT reference were analysed by Wilcoxon signed ranks tests (2-tailed).

Approximation factors (AF)

Surface area values for all coral specimens derived by established techniques were subsequently compared to the respective CT reference values to generate AF (AF = ratio of CT-derived area to area from established techniques). Genus-specific average AF were subsequently calculated from

ratios of all assessed specimens. The term AF was chosen in this study to prevent confusion with Dahl's (1973) term SI (surface index).

Results

Comparative analyses of area estimates by established techniques to CT reference showed significant surface area over- and underestimations for the majority (82 %) of analysed established techniques and coral genera assessed (Table 2). An example of the different surface area values obtained by the use of different techniques for *Acropora* specimen # 10 is given in Table 3. The highest accuracy to CT reference values including all coral genera was found for geometric approximations; except for *Acropora* spp., for which Wax Coating nearly replicated area values obtained by CT (Table 2). Simple Geometry performed most accurate with branching *P. damicornis* and foliose *Montipora* sp. corals, while Advanced Geometry showed highest accuracy in assessing branching *Acropora* spp.. In the case of *G. fascicularis*, Simple Geometry accuracy was identical to Wax Coating (55 %). Similar accuracy resulting from under- and overestimation of CT reference was also found for Advanced Geometry and Wax Coating assessing *P. damicornis* (Table 2). Except for *Acropora* spp., Wax Coating reached intermediate accuracy, ranging from 47 to 74 %. Planar Projection Photography displayed low accuracy in the measurement of all genera (12 – 36 %). The branching cold water coral *L. pertusa* was most accurately assessed by Advanced Geometry (141 %), closely followed by Wax Coating (57 %); while Simple Geometry delivered the highest accuracy to CT reference for *Fungia* spp. (78 %). AF computed by comparison of established techniques and CT area values reflected the results obtained for percentage accuracy of established techniques, indicated by established techniques of high accuracy showing AF values equal to, or closely approaching, 1 (Table 4).

Table 2 *Accuracy of established techniques in comparison to CT reference. Values are given as percentage accuracy of CT. Significant differences in surface area values found for the respective established techniques are indicated by asterisks: * p<0.05; ** p<0.005; n.d. indicates comparisons where no significant difference was found. Abbreviations: SG = Simple Geometry; AG = Advanced Geometry; WA = Wax Coating; PP = Planar Projection Photography; CT = Computer Tomography and 3-D surface reconstruction*

Growth form			Branching				Massive		Foliose		Disc	
Coral	*Acropora* spp.		*L. pertusa*		*P. damicornis*		*G. fascicularis*		*Montipora* sp.		*Fungia* spp.	
SG	258	n= 12 **	168	n = 13 **	116	n = 12 n.d.	55	n = 8 *	104	n = 12 n.d.	78	n = 11 **
AG	108	n = 12 n.d.	141	n = 13 *	127	n = 12 **	–		76	n = 12 **	–	
WA	101	n = 11 n.d.	57	n = 13 **	74	n = 12 **	55	n = 8 *	70	n = 12 **	47	n = 11 **
PP	19	n = 12 **	21	n = 13 **	21	n = 12 **	36	n = 8 *	12	n = 12 **	27	n = 11 **

Table 3 *Surface area values for Acropora specimen #10 obtained by different techniques. Abbreviations: SG = Simple Geometry; AG = Advanced Geometry; WA = Wax Coating; PP = Planar Projection Photography; CT = Computer Tomography and 3-D surface reconstruction*

	Method				
Acropora coral #10	SG	AG	WA	PP	CT
Surface area (cm^2)	537	333	327	41	361

Table 4 *Approximation factors (AF) for conversion of surface area values derived by established techniques in relation to CT accuracy. Values are given as average AF calculated from all specimens of the respective genus (n = 8 - 13) ± standard error. Abbreviations: SG = Simple Geometry; AG = Advanced Geometry; WA = Wax Coating; PP = Planar Projection Photography; CT = Computer Tomography and 3-D surface reconstruction*

Growth form		Branching		Massive	Foliose	Disc
Coral	*Acropora* spp.	*L. pertusa*	*P. damicornis*	*G. fascicularis*	*Montipora* sp.	*Fungia* spp.
SG	0.44 ± 0.05	0.62 ± 0.03	0.94 ± 0.08	1.86 ± 0.09	1.00 ± 0.06	1.74 ± 0.32
AG	0.95 ± 0.04	0.75 ± 0.05	0.83 ± 0.05	–	1.37 ± 0.09	–
WA	1.00 ± 0.03	1.79 ± 0.07	1.36 ± 0.04	1.86 ± 0.10	1.44 ± 0.03	2.32 ± 0.27
PP	9.04 ± 1.28	5.41 ± 0.64	6.11 ± 1.12	2.82 ± 0.16	11.58 ± 1.99	4.59 ± 0.85

Discussion

The majority of comparisons between established techniques and CT reference reveal significantly different results when quantifying the surface area of identical coral colonies. This demonstrates a need for standardisation, as many past and present studies use different approaches for coral surface area quantification to standardise equivalent parameters (Meyer and Schultz 1985; Tanner 1995; Goffredo et al. 2004). Computer tomography in combination with 3-D reconstruction offers accurate surface area quantification (Laforsch et al. 2008) and can therefore serve as a reference for standardisation.

The genera-wide identified high accuracy of geometric approximations indicates the appropriateness of these non-invasive and practical techniques for coral surface area quantification. Simple Geometry results for the finely branched *P. damicornis* and the foliose *Montipora* sp. show no significant differences to CT reference values. Furthermore, application of Advanced Geometry increases accuracy of Simple Geometry (by 27 – 150 %) for 50 % (branching corals: *Acropora* spp. and *L. pertusa*) of all coral genera assessed by both geometric approaches. Wax Coating demonstrates higher accuracy (101%) in the assessment of *Acropora* spp. compared to Advanced Geometry (108%), which may be explained by the sealing of the intricate skeleton topography of *Acropora* spp. colonies (e.g., protruding corallites) by the first coating step of Wax Coating and by CT scanning at a resolution of 0.6 mm, potentially resulting in the generation and subsequent measurement of similar surface topographies. For the majority of corals, application of Wax Coating delivers accuracy comparable to geometric approximations (e.g., Simple Geometry and

Wax Coating with *G. fascicularis*), thus qualifying Wax Coating as a passable substitute for geometry. However, Simple Geometry and Advanced Geometry provide in addition to accuracy, low cost and ubiquitous applicability the deciding advantage of non-invasive application with living coral specimens, not feasible by Wax Coating. Low accuracy found for Planar Projection Photography (12 – 36 %) emphasises its apparent 2-D limitation. For all measured coral colonies, surface area values obtained by Planar Projection Photography underestimate CT reference by a factor ranging between 2.6 and 27.2. Overall lowest accuracy (12%) derived by Planar Projection Photography for the foliose coral *Montipora* sp. may result from the natural growth orientation (diagonal upright) of the corals during recording of photographs. Accuracy delivered by Planar Projection Photography for surface area estimates of rather horizontal coral growth forms (e.g., plate forms), not assessed by this study, may however stay within an acceptable range.

Improved accuracy of surface area values derived from established techniques can be achieved by mathematical approximation in relation to CT accuracy using AF values, presented here. Consequently, data from various studies can be transformed by application of AF to improved surface area estimates and facilitate standardised comparison. As this work represents the first comparison between a variety of established techniques and the contemporary most accurate CT-based method, AF values for Planar Projection Photography represent the only category comparable to existing literature data (i.e., SI). SI ratios developed by previous studies (Dahl 1973; Alcala and Vogt 1996) are lacking an accurate reference. Holmes (2008) presented SI ratios of surface area estimates from 3-D laser scanning and planar projection data using laser scanning accuracy as reference. As the resolution of laser scanning still differs considerably from the reference of the present study (laser: 2.5 mm; CT: 0.6 mm) only rough comparisons are feasible. Nonetheless, SI values presented by Holmes (2008) for *Open branching* and *Complex branching* (6.16 and 6.43, respectively) are in the same range as AF values found here for the branching *P. damicornis* (6.11). AF values computed for the massive *G. fascicularis* are lower (2.82) than the SI for massive corals shown by Holmes (2008) (3.20), which may be explained by the difference in resolution of reference methods. Due to the distinct skeleton topography (i.e., large protruding corallites; see Fig. 1, panel c), causing a substantial increase in surface area, AF values calculated for the massive *G. fascicularis* by the present study should only find application in the work with this species, as species specific growth characteristics alter AF values considerably, thus becoming unsuitable for massive coral species lacking similar skeleton topography (e.g., *Porites* spp.). For surface area quantification of massive corals exhibiting rather smooth surface topography, Courtney et al. (2007) have presented log-linear models applicable for in situ as well as for laboratory studies.

Accuracy of established techniques and specific AF values, presented here, are derived from comparison, and thus dependent on the accuracy of the reference technique (i.e., CT). As CT is limited to detection of the coral carbonate skeleton only, accurate CT measurements of the intricate skeleton topography may cause under- or overestimation of the actual tissue surface area composed by the polyps and the coenosarc. Therefore, further studies are necessary to find possible solutions for this limitation, e.g., by application of different tomographic imaging techniques (Frahm et al. 1986), supporting the possibility for detection and discrimination of organic and inorganic coral components. In addition, solely combination of the latter with measurements of living corals under

natural conditions (i.e., submerged in seawater, extended polyps and tentacles), will finally provide high accuracy quantification of coral tissue surface area.

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References

Alcala MLR, Vogt H (1996) Approximation of coral reef surfaces using standardised growth forms and video counts. Proc 8th Int Coral Reef Symp 2:1453-1458

Babcock RC (1991) Comparative demography of three species of scleractinian corals using age- and size-dependent classifications. Ecol Monogr 61:225-244

Bak RPM, Meesters EH (1998) Coral population structure: the hidden information of colony size-frequency distributions. Mar Ecol Prog Ser 162:301-306

Benzion M, Achituv Y, Stambler N, Dubinsky Z (1991) A photographic, computerized method for measurements of surface-area in *Millepora*. Symbiosis 10:115-121

Bessat F, Boiseau M, Leclerc AJ, Buigues D, Salvat B (1997) Computerized tomography and oxygen stable isotopic composition of *Porites lutea* skeleton at Mururoa (French Polynesia): application to the study of solar radiation influence on annual coral growth. Compt Rendus Acad Sci III Sci Vie 320:659-665

Bohnsack JA (1979) Photographic quantitative sampling of hardbottom communities. Bull Mar Sci 29:242-252

Bosscher H (1993) Computerized tomography and skeletal density of coral skeletons. Coral Reefs 12:97-103

Bythell JC, Pan P, Lee J (2001) Three-dimensional morphometric measurements of reef corals using underwater photogrammetry techniques. Coral Reefs 20:193-199

Chancerelle Y (2000) Methodes d'estimation des surfaces developpees de coraux scleractiniaires a l'echelle d'une colonie ou d'un peuplement. Oceanol Acta 23:211-119

Cocito S, Sgorbini S, Peirano A, Valle M (2003) 3-D reconstruction of biological objects using underwater video technique and image processing. J Exp Mar Biol Ecol 297:57-70

Courtney LA, Fisher WS, Raimondo S, Oliver LM, Davis WP (2007) Estimating 3-dimensional colony surface area of field corals. J Exp Mar Biol Ecol 351:234-242

Dahl AL (1973) Surface area in ecological analysis: quantification of benthic coral reef algae. Mar Biol 23:239-249

Done TJ (1981) Photogrammetry in coral ecology: a technique for the study of change in coral communities. Proc 4th Int Coral Reef Symp 2:315-320

Fagoonee I, Wilson HB, Hassell MP, Turner JR (1999) The dynamics of zooxanthellae populations: a long-term study in the field. Science 283:843-845

Falkowski PG, Dubinsky Z (1981) Light-shade adaptation of *Stylophora pistillata*, a hermatypic coral from the Gulf of Eilat. Nature 289:172-174

Fisher W, Davis W, Quarles R, Patrick J, Campbell J, Harris P, Hemmer B, Parsons M (2007) Characterizing coral condition using estimates of three-dimensional colony surface area. Environ Monit Assess 125:347-360

Frahm J, Haase A, Matthaei D (1986) Rapid three-dimensional MR imaging using the FLASH technique. J Comput Assist Tomogr 10:363-368

Glynn PW, D'Croz L (1990) Experimental evidence for high temperature stress as the cause of El Nino-coincident coral mortality. Coral Reefs 8:181-191

Goffredo S, Mattioli G, Zaccanti F (2004) Growth and population dynamics model of the Mediterranean solitary coral *Balanophyllia europaea* (Scleractinia, Dendrophylliidae). Coral Reefs 23:433-443

Hoegh-Guldberg O (1988) A method for determining the surface area of corals. Coral Reefs 7:113-116

Holmes G (2008) Estimating three-dimensional surface areas on coral reefs. J Exp Mar Biol Ecol 365:67-73

Hounsfield GN (1973) Computerized transverse axial scanning (tomography): Part I. Description of system. Br J Radiol 46:1016-1022

Hughes TP, Jackson JBC (1985) Population dynamics and life histories of foliaceous corals. Ecol Monogr 55:141-166
Jokiel PL, Morrissey JI (1986) Influence of size on primary production in the reef coral *Pocillopora damicornis* and the macroalga *Acanthophora spicifera*. Mar Biol 91:15-26
Jones AM, Cantin NE, Berkelmans R, Sinclair B, Negri AP (2008) A 3D modeling method to calculate the surface areas of coral branches. Coral Reefs 27:521-526
Kaandorp JA, Sloot PMA, Merks RMH, Bak RPM, Vermeij MJA, Maier C (2005) Morphogenesis of the branching reef coral *Madracis mirabilis*. Proc R Soc Biol Sci Ser B 272:127-133
Kanwisher JW, Wainwright SA (1967) Oxygen balance in some reef corals. Biol Bull 133:378-390
Ketcham RA, Carlson WD (2001) Acquisition, optimization and interpretation of X-ray computed tomographic imagery: applications to the geosciences. Comput Geosci 27:381-400
Kruszynski KJ, van Liere R, Kaandorp JA (2006) An interactive visualization system for quantifying coral structures. In: Ertl T, Joy K, Santos B (eds) Eurographics/ IEEE-VGTC Symposium on Visualization, pp 1-8
Kruszynski KJ, Kaandorp JA, van Liere R (2007) A computational method for quantifying morphological variation in scleractinian corals. Coral Reefs 26:831-840
Laforsch C, Christoph E, Glaser C, Naumann M, Wild C, Niggl W (2008) A precise and non-destructive method to calculate the surface area in living scleractinian corals using X-ray computed tomography and 3D-modeling Coral Reefs 27:811-820
Marsh JA (1970) Primary productivity of reef-building calcareous red algae. Ecology 51:255-263
Mergner H, Schuhmacher H (1979) Quantitative ökologische Analyse eines Rifflagunenareals bei Aqaba (Golf von Aqaba, Rotes Meer). Helgoländer wiss Meeresunters 32:476-507
Meyer JL, Schultz ET (1985) Tissue condition and growth rate of corals associated with schooling fish. Limnol Oceanogr 30:157-166
Muscatine L, Porter JW, Kaplan IR (1989) Resource partitioning by reef corals as determined from stable isotope composition. Mar Biol 100:185-193
Odum EP, Kuenzler EJ, Blunt MX (1958) Uptake of P^{32} and primary productivity in marine benthic algae. Limnol Oceanogr 3:340-345
Odum HT, Odum EP (1955) Trophic structure and productivity of a windward coral reef community on Eniwetok Atoll. Ecol Monogr 25:291-320
Pichon M (1978) Problems of measuring and mapping coral reef colonies. In: Stoddart DR, Johannes RE (eds) Coral reefs: research methods. United Nations Educational, Scientific and Cultural Organization, Paris, pp 219-230
Rahav O, Benzion M, Achituv Y, Dubinsky Z (1991) A photographic, computerized method for insitu growth measurements in reef-building cnidarians. Coral Reefs 9:204-204
Roberts CM, Ormond RFG (1987) Habitat complexity and coral-reef fish diversity and abundance on Red-Sea fringing reefs. Mar Ecol Prog Ser 41:1-8
Stimson J, Kinzie RA (1991) The temporal pattern and rate of release of zooxanthellae from the reef coral *Pocillopora damicornis* (Linnaeus) under nitrogen-enrichment and control conditions. J Exp Mar Biol Ecol 153:63-74
Szmant-Froelich A (1985) The effect of colony size on the reproductive ability of the Caribbean coral *Montastraea annularis* (Ellis and Solander). Proc 5th Int Coral Reef Symp 4:295-300
Tanner JE (1995) Competition between scleractinian corals and macroalgae - An experimental investigation of coral growth, survival and reproduction. J Exp Mar Biol Ecol 190:151-168
Vollmer SV, Edmunds PJ (2000) Allometric scaling in small colonies of the scleractinian coral *Siderastrea siderea* (Ellis and Solander). Biol Bull 199:21-28
Vytopil E, Willis BL (2001) Epifaunal community structure in *Acropora* spp. (Scleractinia) on the Great Barrier Reef: implications of coral morphology and habitat complexity. Coral Reefs 20:281-288
Wegley L, Yu Y, Breitbart M, Casas V, Kline DI, Rohwer F (2004) Coral-associated archaea. Mar Ecol Prog Ser 273:89-96
Wild C, Woyt H, Huettel M (2005) Influence of coral mucus on nutrient fluxes in carbonate sands. Mar Ecol Prog Ser 287:87-98

3

Coral sand O_2 uptake and benthic-pelagic 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 these sands using benthic chambers and sediment traps during several seasonal expeditions between May 2004 and May 2008 in a fringing reef on the Jordanian Red Sea coast. Twelve 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 in the reef and back reef lagoon. Resulting 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 nutrient-deprived 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, therefore, may rather be a function of lateral transport of labile organic particles produced by reef organisms (e.g. benthic algae and corals) than sedimentation of water column production.

Introduction

Coral reefs are characterised 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, Wild et al. 2004c, Wild et al. 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 re-oxidation 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 the sedimentary organic matter mineralization processes, and the spatial and temporal changes in water-sediment 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 world-wide 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 were nonrecurring investigations exclusively focusing on one or a few shallow reef locations (Boucher et al. 1994, Boucher et al. 1998, Rasheed et al. 2004, Reimers et al. 2004, Wild et al. 2005). To our knowledge, only Clavier & Garrigue (1999) studied sedimentary O_2 uptake in the lagoon of a barrier reef in New Caledonia on temporal and spatial scales, and found pronounced differences between seasons, locations and sediment 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 fractions of reef lagoons are often 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 the coral sands permits water flow through the pore space of the upper sediment layers (Wild et al. 2004b, Wild et al. 2004c), and waves and bottom flows, thereby, can affect spatial and temporal O_2 distribution in the sea bed (Booij et al. 1991, Ziebis et al. 1996, Falter & Sansone 2000). As the intensity of wave-generated 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 the 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 the permeable sands for the cycling of matter in the reef and led to the initiation of this study.

Main objectives were to a) 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 b) investigate the O_2 uptake of sandy sediments located at a deeper site in the lagoon. Working hypotheses were that a) sedimentary O_2 uptake shows temporal and spatial variations caused mainly by changes in organic matter supply and water column conditions (e.g. temperature, nutrient concentrations), and b) 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 more than three years.

Material and Methods

Study site

The study was conducted in a fringing reef located near the Marine Science Station (MSS) in Aqaba, Jordan (latitude 29° 27', longitude 34° 58'). During three 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. Three stations at 2.5, 5.5, and 9.5 m water depth were established in the lagoon sand area (in the following text termed "lagoon sands") and two stations at 7.0 and 16.5 m depth in the reef area on small sand patches between the coral colonies (termed "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 x 10^{-11} m^2 (Wild et al. 2005). Water temperatures at the study site range from 20 °C in February to 28 °C in August. Due to strong evaporation, salinity year round is relatively high (40.3 to 40.8) (Manasrah et al. 2006). Water currents in this region 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.

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), Wild et al. (2004b/c), Wild et al. (2005) were used in order 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 this study). A plastic lid containing a sampling port for water

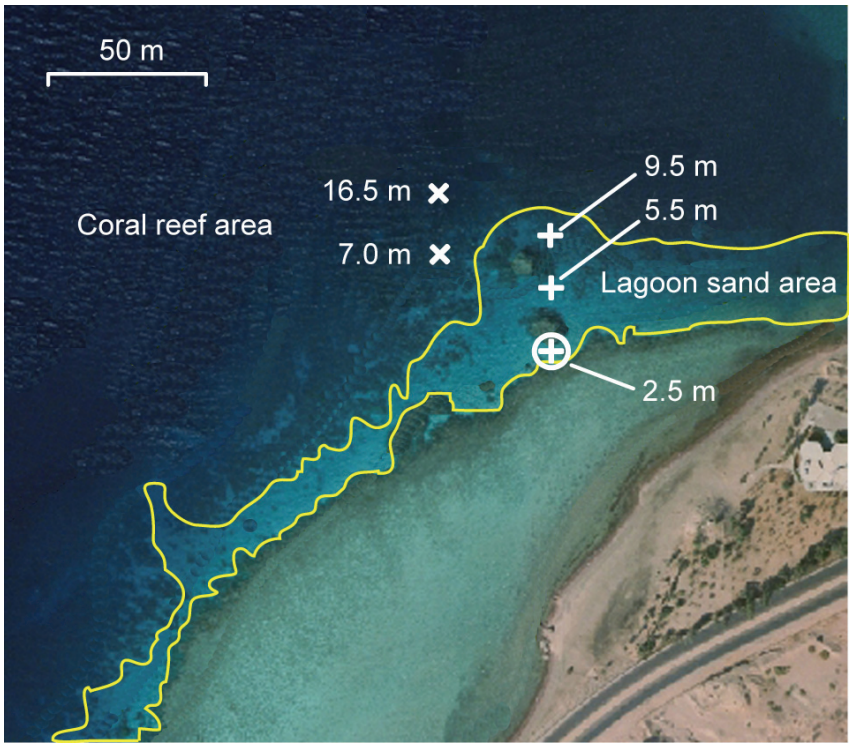

Fig. 1 *Aerial photograph of the study area with the locations of the chamber measurements on reef sands (white x) and lagoon sands (white +). The long term monitoring station is indicated by the circle around the + marking the station. The yellow line delineates the back reef lagoon. Picture generated by ASEZA GIS Unit (R. Mobiedeen), Aqaba, Jordan.*

extraction and a second port permitting replacement of the sampled water volume covered each chambers. The water inside the chambers was circulated by a horizontally rotating disk of 17 cm 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, Huettel et al. 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 at our sites 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 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.. More details of the functioning of these chambers are given in Huettel & Gust (1992) and Cook et al. (2007).

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 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 x 3 m (9 m^2).

Water samples (60 ml) were extracted at pre-set time intervals (30 to 120 min) from the chambers for later analyses of O_2 concentrations using plastic syringes. 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

The 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) from 9 m water depth (1 m above the reef) in replicates of n = 4 in clean 5 litre plastic containers using SCUBA. Water samples were then processed within 30 min or kept at 4 °C for less than 12 h before processing. Sub-samples 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, circa 10 ml of the sample solutions were filtered through 0.2 µm sterile syringe filters (polyethersulfone membrane, *VWR Collection*). The first 4 ml of the filtrate were discarded and the following 6 ml were collected in new, pre-combusted glass ampoules, which were instantly frozen at -20 °C and kept frozen until

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

Expedition	Date	Location	Water depth (m)	No of replicate chambers
Spring	27.05.2004	lagoon sands	2.5	4
	31.05.2004	lagoon sands	2.5	2
	04.06.2004	lagoon sands	2.5	1
	05.06.2004	lagoon sands	2.5	1
	14.06.2004	lagoon sands	2.5	2
Autumn	22.11.2006	lagoon sands	9.5	2
	26.11.2006	lagoon sands	2.5	3
	29.11.2006	lagoon sands	5.5	4
	02.12.2006	reef sands	16.5	3
	04.12.2006	reef sands	7.0	2
	06.12.2006	lagoon sands	9.5	3
Summer	21.08.2007	lagoon sands	2.5	2

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%).
Sub-samples from each container were used in order to determine the microbial oxygen consumption rate. For this purpose the initial dissolved oxygen concentration of each sub-sample was measured using a Optical Dissolved Oxygen Sensor (HACH LANGE HQ10, accuracy ± 0,05 % of the effective range). The sub-samples were then incubated in 60 ml Winkler glas bottles in the dark at in-situ temperature for 16 – 24 h. Oxygen concentrations at the end of the period were measured again as described and the difference used to calculate microbial oxygen consumption rates.

Sub-samples for particulate organic carbon (POC) and 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^{TM} NC 2500 elemental analyser. Peptone, Atropine and cyclohexanone-2,4-dinitrophenylhydrazone were used as standard, and standard deviations of replicate measurements were < 3%.

Sub-samples for chlorophyll a analysis were obtained identical to POC and PN sub-samples but stored 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*.

Inorganic nutrient concentrations (nitrate and phosphate) were monthly measured 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.

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

Expedition	Temperature (°C)	Nitrate concentration (µM)	Phosphate concentration (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	0.03 - 0.03
Winter 2008	20.6 - 21.5	0.76 - 0.90	0.06 - 0.07
Spring 2008	22.0 - 25.7	0.37	0.04

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 triplicates, 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 weighed 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 in situ carefully washed into the container using a 60 ml syringe using SCUBA. 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 three times with sea water 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 less than 12 h before processing. Aliquots of the trapped material were prepared for the analysis of POM (particulate organic carbon (POC) and particulate nitrogen (PN)) content by filtering the particulate material onto pre-combusted 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 and converted to m^2.

Sub-samples for chlorophyll a analysis in the trapped material were obtained identical to POC and PN sub-samples, but stored 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 above.

Results

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

Temporal variability of sedimentary O_2 uptake

Over the observation period of more than 3 years, 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 statistically not significant (U-test after Mann, Wilcoxon and Whitney, $p > 0.05$). Sedimentary O_2 uptake showed no correlation with water temperature, which for our sampling campaigns ranged from 23 to 27 °C (Fig. 3).

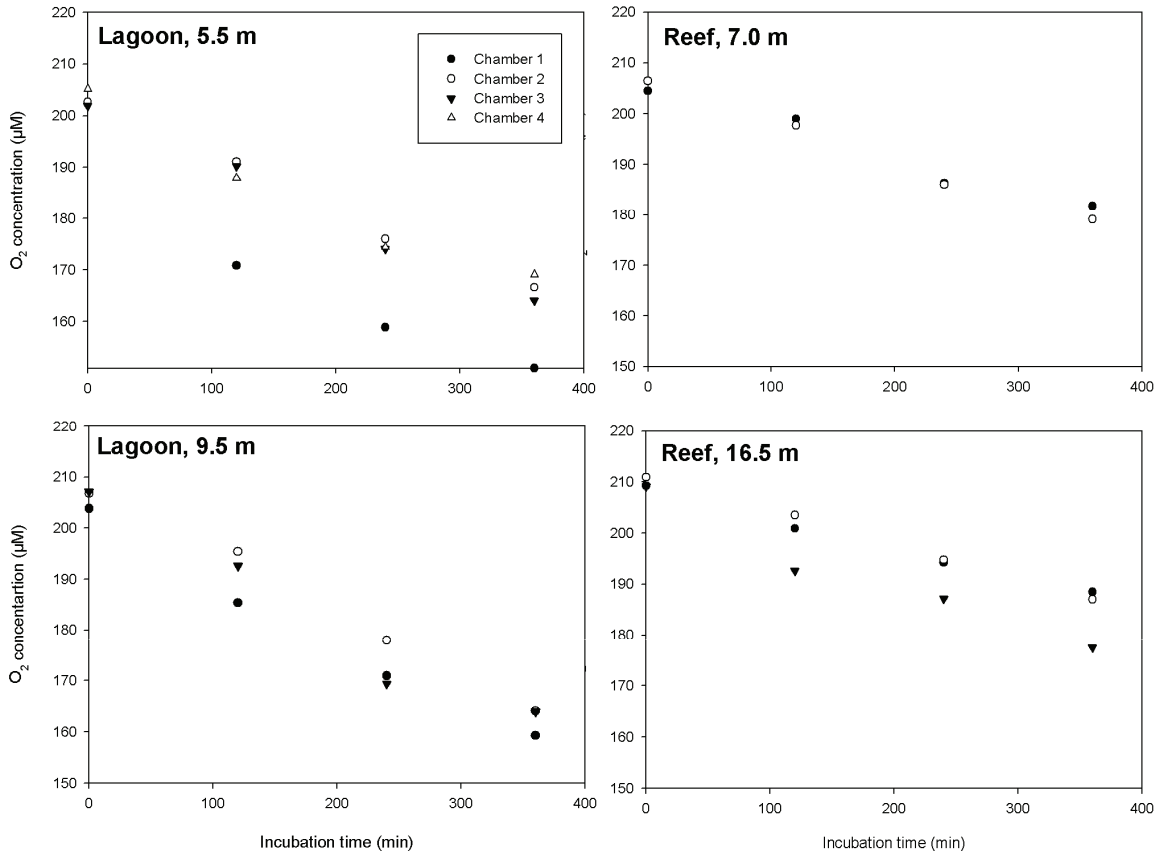

Fig. 2 *O_2 concentration development during 4 independent benthic chamber experiments on lagoon and reef sands at different water depths.*

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 very similar (around 20 mmol m^{-2} d^{-1}) to those rates measured at the reference station at 2.5 m water depth (Fig. 3).

Organic matter sedimentation

POC and PN supply to the reef sediments as measured by the sediment traps in seasonal resolution 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 the water depth of 10 m 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 highest POC sedimentation rates were observed in summer (86 ± 40 mg POC m^{-2} d^{-1}) and 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, chlorophyll contents in sediment traps were highly variable and thus displayed no significant temporal differences (Fig. 6).

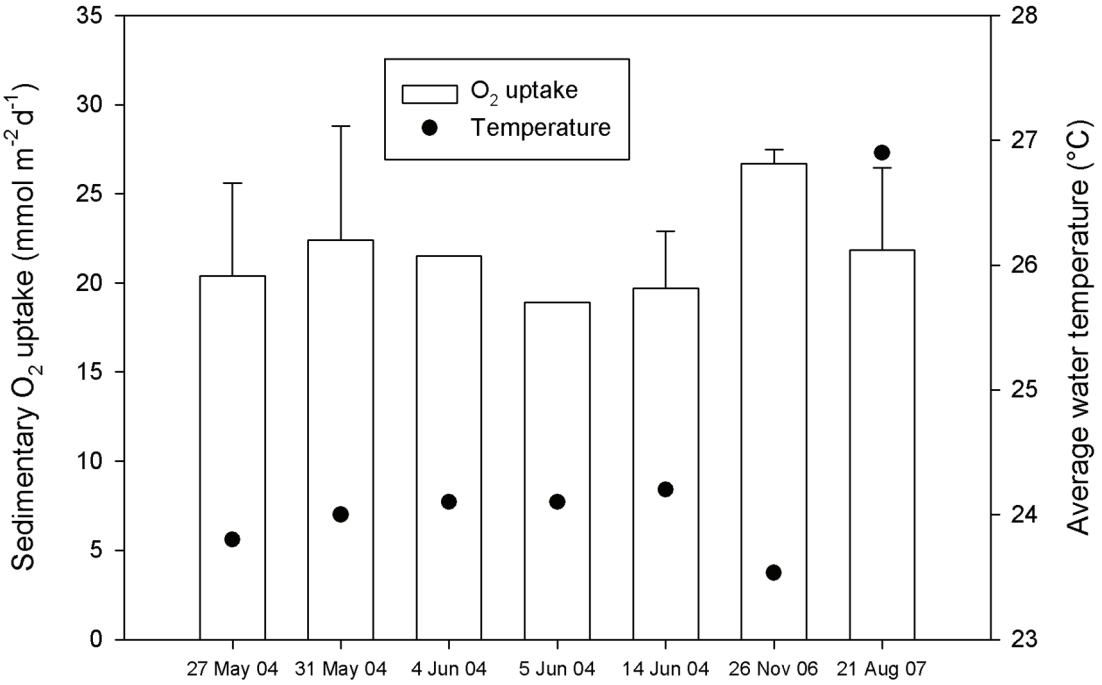

Fig. 3 *Temporal changes of sedimentary O_2 uptake measured with the chambers (averages ± SD) and mean water temperatures at the long-term monitoring station at a water depth of 2.5 m. These chamber measurements were carried out between spring expedition 2004 and summer expedition 2007.*

Discussion

Temporal changes of sedimentary O_2 uptake and organic matter supply

The study of 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 that 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 of the present study at 2.5 m water depth (19 – 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, Wild et al. 2004b, 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 land-derived influence occurred at the study site of the present study 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 year study period. This was unexpected as

inorganic nutrient (Table 2) and chlorophyll concentrations (Table 3) in the water column at our study site showed clear temporal variations with low values from May to October and higher values from November to April. The temporal differences in chlorophyll concentrations in the water column, with 2 times higher values in winter and spring compared to summer and autumn, were significant (one-way ANOVA, $p < 0.001$), (Table 3). These observations agree with those of (Rasheed et al. 2002) and (Rasheed et al. 2003b). Measurements conducted during the present study also revealed higher dissolved organic carbon (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 O_2 consumption measured parallel to the benthic chamber deployments.*

Expedition	POC (mg l^{-1})	PN (mg l^{-1})	DOC (mg l^{-1})	Chl a (µg l^{-1})	O_2 (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	n.m.	n.m.	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. 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, e.g. (Wild et al. 2004c, Glud et al. 2008) observed that the sudden organic matter supply to coral reef sands in the Great Barrier Reef caused by the 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 then is degraded over a longer time period) despite their function as biocatalytical filter systems (Wild et al. 2004a, Wild et al. 2004c, Wild et al. 2008). This is supported by the study of (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 is 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 oxygen consumption rates between periods of increased organic matter input to the sediment.

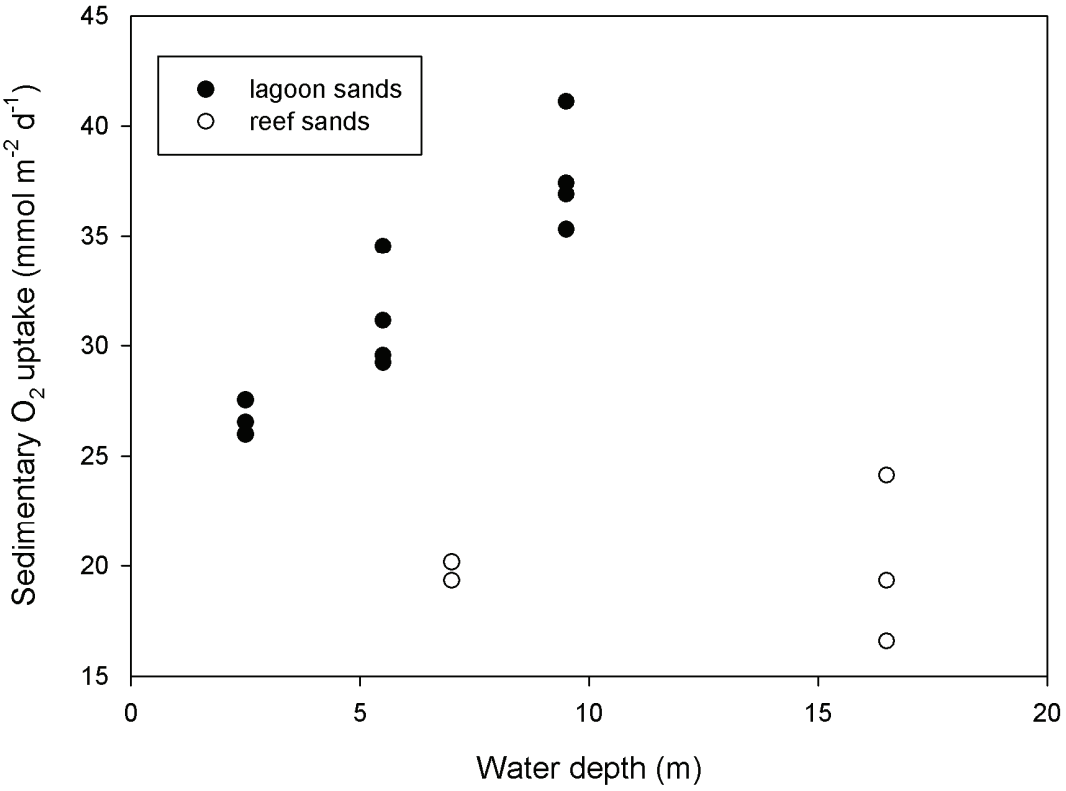

Fig. 4 *Sedimentary O_2 uptake at lagoon and reef sands versus water depth as measured with the benthic chambers. All chamber measurements for these investigations were carried out during the autumn expedition in November/December 2006.*

Sedimentary O2 uptake and organic matter supply at the different water depths

While the temporal changes in O2 flux were relatively small, larger increases in O2 flux were observed with increasing water depths in the lagoon sands. This positive correlation may have been caused by a) the larger 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, b) lateral transport processes providing more organic matter to the deeper sites, and c) 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 typically clear oligotrophic waters where coral reefs grow, high light intensities can penetrate relatively deep permitting photosynthesis at water depth 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 particulate organic carbon integrated over the entire water

column above the reef thus can 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 e.g. 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 have been resuspended. A clear distinction of the two sources is difficult as in the wave swept reef environment, as 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 theses sediments can lead to oxygen depletion in the sediment, which leads to the build up of sulphides resulting from the microbial sulphate reduction activities.

Nevertheless, the trend of increasing sedimentary O_2 uptake with increasing depth could not be observed in the reef sand patches embedded between living coral colonies. A 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 of 29 to 67 % seafloor coverage (Naumann et al. unpublished data). The intense feeding of particulate organic matter by corals has been demonstrated by Anthony (1999), but also 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) filter particles from the water column, thereby reducing organic matter flux to the reef sand patches between the corals. 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 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 reflect the 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 an organic matter production that is not consumed in the water column or by benthic reef organisms and thus can settle to the sediments. In an organic matter limited ecosystem like 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 rather represents 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, 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, Mayer et al. submitted).

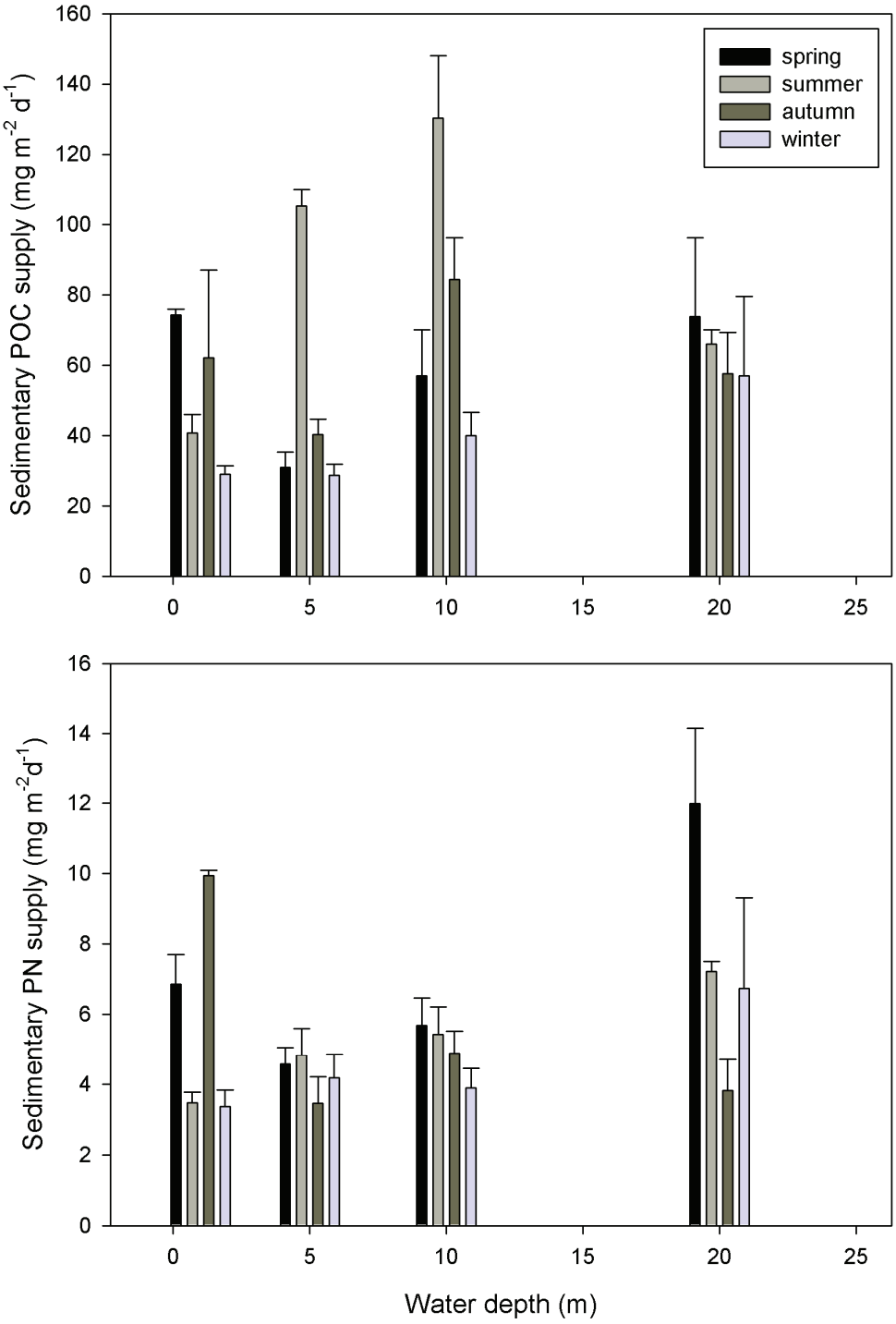

Fig. 5 *Particulate organic carbon (POC) and Particulate Nitrogen (PN) supply to reef sediments over time measured with sediment traps. Values are averages ± SD. All traps were deployed in the reef, except traps at 5 m water depth, which were deployed in the lagoon.*

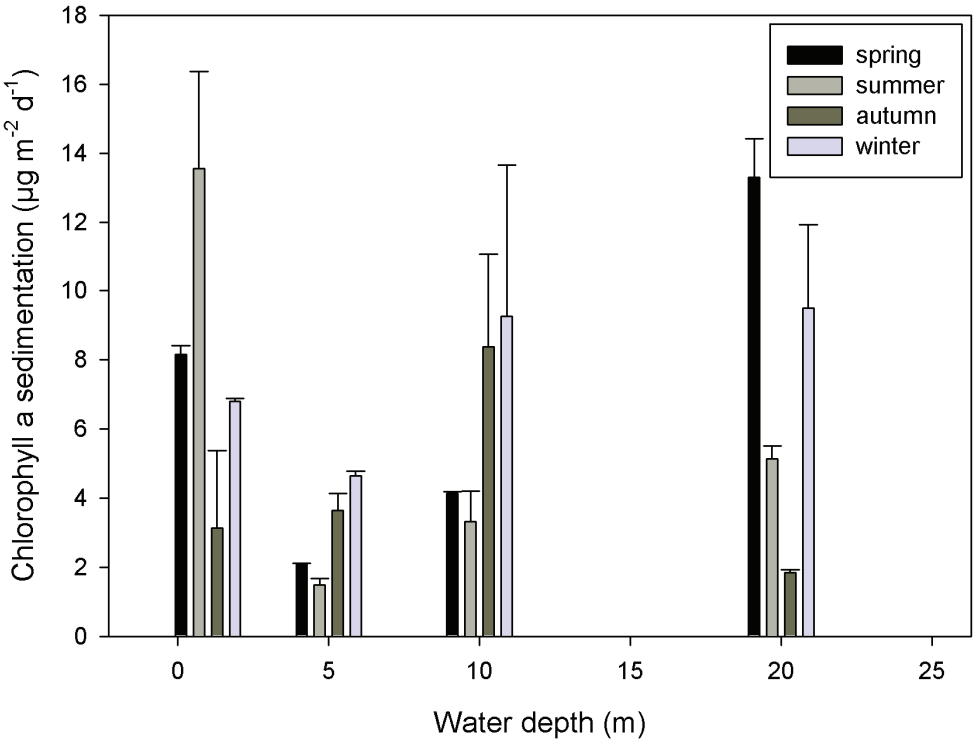

Fig. 6 *Chlorophyll a sedimentation over time measured with sediment traps. Values are averages ± SD. All traps were deployed in the reef, except traps at 5 m water depth, which were deployed in the lagoon.*

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References

Anthony KRN (1999) Coral suspension feeding on fine particulate matter. J Exp Mar Biol Ecol 232:85-106

Booij K, Helder W, Sundby B (1991) Rapid redistribution of oxygen in a sandy sediment induced by changes in the flow velocity of the overlying water. Netherlands J Sea Res 28:149-165

Boucher G, Clavier J, Garrigue C (1994) Oxygen and carbon dioxide fluxes at the water - sediment interface of a tropical lagoon. Mar Ecol Prog Ser 107:185-193

Boucher G, Clavier J, Hily C, Gattuso JP (1998) Contribution of soft-bottoms to the community metabolism (primary production and calcification) of a barrier reef flat (Moorea, French Polynesia). J Exp Mar Biol Ecol 225:269-283

Canfield DE, Jørgensen BB, Fossing H, Glud R, Gundersen JK, Ramsing NB, B T, Hansen JW, Nilsen LP, Hall POJ (1993) Pathways of organic carbon oxidation in three continental margin sediments. Mar Geol 113:27-47

Chabanet P, Ralambondrainy H, Amanieu M, Faure G, Galzin R (1997) Relationships between coral reef substrata and fish. Corals Reefs 16:93-102

Clavier J, Garrigue C (1999) Annual sediment primary production and respiration in a large coral reef lagoon. Mar Ecol Prog Ser 191:79-89

Coffroth MA (1984) Ingestion and incorporation of coral mucus aggregates by a Gorgonian soft coral. Mar Ecol Prog Ser 17:193-199

Cook PLM, Wenzhöfer F, Glud RN, Janssen F, Huettel M (2007) Benthic solute exchange and carbon mineralization in two shallow shallow subtidal sandy sediments: Effect of advective pore-water exchange. Limnol Oceanogr 52:1943-1963

Crossland CJ, Barnes DJ (1983) Dissolved nutrients and organic particulates in water flowing over coral reefs at Lizard-Island. Austr J Mar Freshw Res 34:835-844

Eyre B, Glud RN, Patten N (2008) Mass coral spawning: A natural large-scale nutrient addition experiment. Limnol Oceanogr 53:997-1013

Falter JL, Sansone FJ (2000) Hydraulic control of pore water geochemistry within the oxic-suboxic zone of a permeable sediment. Limnol Oceanogr 45:550-557

Gattuso JP, Gentili B, Duarte CM, Kleypas JA, Middelburg JJ, Antoine D (2006) Light availability in the coastal ocean: impact on the distribution of benthic photosynthetic organisms and their contribution to primary production. Biogeosciences 3:489-513

Glud RN, Eyre B, Patten N (2008) Biogeochemical responses on mass coral spawning at the Great Barrier Reef:Effects on respiration and primary production. Limnol Oceanogr 53:1014-1024

Hansen JA, Klumpp DW, Alongi DM, Dayton PK, Riddle MJ (1992) Detrital pathways in a coral reef lagoon II. Detritus deposition, benthic microbial biomass and production. Mar Biol 113:363-372

Hatcher BG (1988) Coral-reef primary productivity - a beggars banquet. Trends Ecol Evol 3:106-111

Hatcher BG (1997) Coral reef ecosystems: how much greater is the whole than the sum of the parts? Corals Reefs 16:S77-S91

Hoegh-Guldberg O, Mumby PJ, Hooten AJ, Steneck RS, Greenfield P, Gomez E, Harvell CD, Sale PF, Edwards AJ, Caldeira K, Knowlton N, Eakin CM, Iglesias-Prieto R, Muthiga N, Bradbury RH, Dubi A, Hatziolos ME (2007) Coral reefs under rapid climate change and ocean acidification. Science 318:1737-1742

Huettel M, Gust G (1992) Solute release mechanisms from confined sediment cores in stirred benthic chambers and flume flows. Mar Ecol Prog Ser 82:187-197

Huettel M, Roy H, Precht E, Ehrenhauss S (2003) Hydrodynamical impact on biogeochemical processes in aquatic sediments. Hydrobiologia 494:231-236

Huettel M, Ziebis W, Forster S (1996) Flow-induced uptake of particulate matter in permeable sediments. Limnol Oceanogr 41:309-322

Hughes T, Rodrigues M, Bellwood D, Ceccarelli D, Hoegh-Guldberg O, McCook L, Moltschaniwskyj N, Pratchett M, Steneck R, Willis B (2007) Phase shifts, herbivory, and the resilience of coral reefs to climate change Current Biol 17:360-365

Jarrett BD, Hine AC, Halley RB, Naar DF, Locker SD, Neumann AC, Twichell D, Hu C, Donahue BT, Jaap WC, Palandro D, Ciembronowicz K (2005) Strange bedfellows - a deep-water hermatypic coral reef superimposed on a drowned barrier island; Southern Pulley Ridge, SW Florida platform margin. Mar Geol 214:295-307

Johannes R (1967) Ecology of organic aggregates in the vicinity of a coral reef. Limnol Oceanogr 12:189-195

Johnstone RW, Koop K, Larkum AWD (1990) Physical aspects of coral reef lagoon sediments in relation to detritus processing and primary production. Mar Ecol Prog Ser 66:271-283

Jordana E, Duchêne JC, Charles F, Grémare A, Amouroux JM (2000) Experimental study of suspension-feeding activity in the serpulid polychaete *Ditrupa arietina* (O.F. Müller). J Exp Mar Biol Ecol 252:57-74

Kappner I, Al-Moghrabi SM, Richter C (2000) Mucus-net feeding by the vermetid gastropod *Dendropoma maxima* in coral reefs. Mar Ecol Progr Ser 204:309-313

Kayanne H, Suzuki A, Saito H (1995) Diurnal changes in the partial pressure of carbon dioxide in coral reef water. Science 269:214-216

Manasrah RS, Al-Horani F, Rasheed MY, Al-Rousan SA, Khalaf MA (2006) Patterns of summer vertical and horizontal currents in coastal waters of the northern Gulf of Aqaba, Red Sea. Estu Coast Shelf Sci 69:567-579

Marshall M (1968) Observations on organic aggregates in the vicinity of coral reefs. Mar Biol 2:50-55

Mayer FW, Manasrah R, Mayr C, Wild C (submitted) Coral mucus via particle trapping initiates short linked element cycles in fringing reefs of the Northern Red Sea. Coral Reefs

McManus GB, Dawson R (1994) Phytoplankton pigments in the deep chlorophyll maximum of the Caribbean Sea and the Western Tropical Atlantic Ocean. Mar Ecol Prog Ser 113:199-206

Monismith SG, J.R. K, Thompson JK, O'Riordan CA (1990) A study of model bivalve siphonal currents. Limnol Oceanogr 35:680-696

Petersen JK (2007) Ascidian suspension feeding. J Exp Mar Biol Ecol 342:127-137

Precht E, Huettel M (2004) Rapid wave-driven advective pore water exchange in a permeable coastal sediment. J Sea Res 51:93-107
Rasheed M, Badran M, Huettel M (2003a) Influence of sediment permeability and mineral composition on organic matter decomposition in three sediments from the Gulf of Aqaba, Red Sea. Estu Coast Shelf Sci 57:369-384
Rasheed M, Badran M, Huettel M (2003b) Particulate matter filtration and seasonal nutrient dynamics in permeable carbonate and silicate sands of the Gulf of Aqaba, Red Sea. Corals Reefs 22:167-177
Rasheed M, Badran MI, Richter C, Huettel M (2002) Effect of reef framework and bottom sediment on nutrient enrichment in a coral reef of the Gulf of Aqaba, Red Sea. Mar Ecol Prog Ser 239:277-285
Rasheed M, Wild C, Franke U, Huettel M (2004) Benthic photosynthesis and oxygen consumption in permeable carbonate sediments at Heron Island, Great Barrier Reef, Australia. Estuar Coast Shelf Sci 59:139-150
Rathbun C, Doyle A, Waterhouse T, Sorensen J (1997) Measurement of Algal Chlorophylls and Carotenoids by HPLC. In: BATS Methods, p 81-87
Reimers CE, Stecher HA, Taghon GL, Fuller CM, Huettel M, Rusch A, Ryckelynck N, Wild C (2004) In-situ measurements of advective solute transport in permeable shelf sands. Cont Shelf Res 24:183-201
Ribak G, Heller A, Genin A (2005) Mucus-net feeding on organic particles by the vermetid gastropod Dendropoma maximum in and below the surf zone. Mar Ecol Prog Ser 293:77-87
Richter C, Wunsch M (1999) Cavity-dwelling suspension feeders in coral reefs-a new link in reef trophodynamics. Mar Ecol Prog Ser 188:105-116
Richter C, Wunsch M, Rasheed M, Kotter I, Badran MI (2001) Endoscopic exploration of Red Sea coral reefs reveals dense populations of cavity-dwelling sponges. Nature 413:726-730
Riddle MJ, Alongi DM, Dayton PK, Hansen JA, Klumpp DW (1990) Detrital pathways in a coral reef lagoon. Mar Biol 104:109-118
Skyring GW (1985) Anaerobic microbial processes in coral reef sediments. Proc 5th Int Coral Reef Congr 3:421-425
Skyring GW, Chambers LA (1976) Biological sulfate reduction in carbonate sediments of a coral reef. Austr J Mar Freshw Res 27:595-602
Vooren CM (1981) Photosynthetic rates of benthic algae from the deep coral reef of Curacao. Aquatic Botany 10:143-159
Werner U, Bird P, Wild C, Ferdelman T, Polerecky L, Eickert G, Johnstone R, Hoegh-Guldberg O, deBeer D (2006) Spatial variability of aerobic and anaerobic mineralization in coral reef sediments (Heron Island, Australia). Mar Ecol Prog Ser 309:93-105
Wild C, Huettel M, Klueter A, Kremb SG, Rasheed M, Jørgensen BB (2004a) Coral mucus functions as an energy carrier and particle trap in the reef ecosystem. Nature 428:66-70
Wild C, Jantzen C, Struck U, Hoegh-Guldberg O, Huettel M (2008) Biogeochemical responses on coral mass spawning at the Great Barrier Reef: Pelagic-benthic coupling. Corals Reefs 27:123-132
Wild C, Laforsch C, Huettel M (2006) Detection and enumeration of microbial cells in highly porous carbonate reef sands. Mar Freshw Res 57:415-420
Wild C, Rasheed M, Jantzen C, Cook P, Struck U, Huettel M, Boetius A (2005) Benthic metabolism and degradation of natural particulate organic matter in silicate and carbonate sands of the Northern Red Sea. Mar Ecol Prog Ser 298:69-78
Wild C, Rasheed M, Werner U, Franke U, Johnstone R, M. H (2004b) Degradation and mineralization of coral mucus in reef environments. Mar Ecol Prog Ser 267:159-171
Wild C, Tollrian R, Huettel M (2004c) Rapid recycling of coral mass spawning products in permeable reef sediments. Mar Ecol Prog Ser 271:159-166
Ziebis W, Huettel M, Forster S (1996) Impact of biogenic sediment topography on oxygen fluxes in permeable seabeds. Mar Ecol Prog Ser 140:227-237

4

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

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Abstract

Here we report on particulate organic matter (POM) and dissolved organic carbon (DOC) release by 6 dominant hermatypic coral genera (*Acropora*, *Fungia*, *Goniastrea*, *Millepora*, *Pocillopora* and *Stylophora*) measured under submerged conditions 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 hydrozoan 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. Genera-wide average POC:PN ratio of released POM was 12 ± 1 indicating high carbon / low nitrogen content of coral-derived organic matter. Stable isotope signatures of POM in coral incubated seawater ($\delta^{13}C$: –18.1‰ and $\delta^{15}N$: 1.0‰) hint to photosynthates translocated by endosymbiotic zooxanthellae as the predominant source for organic compounds released by corals. POM release showed little seasonal variation, but 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 component 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 lipid compounds transferred by the phototrophic endosymbiotic zooxanthellae (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), 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 key 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 1998) or only presented total organic matter release (e.g., Johannes 1967; Richman et al. 1975), thereby disregarding the fractionation of mucus into POM and DOM by dissolution in seawater.

Only two studies so far have quantified coral-derived organic matter release in correlation with environmental factors. The recent study by Wild et al. (2005b) showed that POM release rates by two *Acropora* coral species from the Great Barrier Reef (Australia) increased under air exposed conditions, and the study of Crossland (1987) revealed 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.

This study therefore aims to generate a comprehensive dataset on organic matter release rates (POC, PN and DOC) for dominant hermatypic corals of a Northern Red Sea fringing reef in a high spatiotemporal resolution under submerged conditions. In addition, correlation between coral-derived organic matter release and environmental key factors (water temperature, light availability

and ambient inorganic nutrient concentrations) was evaluated. In order to achieve these goals, identical measurements were carried out during four independent seasonal expeditions to the Northern Red Sea between fall 2006 and spring 2008.

Material and Methods

Study site

The investigations presented in this study were carried out during four expeditions (November–December 2006 (fall), August–September 2007 (summer), February–March 2008 (winter) and May 2008 (spring)) at the Marine Science Station (MSS) Aqaba, Jordan (latitude: 29°27′N, longitude: 34°58′E), 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.

Environmental monitoring

Environmental monitoring was carried out during the entire study period (November 2006 to May 2008) to investigate the influence of environmental factors on organic matter release rates by hermatypic corals. 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 for inorganic nutrient concentrations of in situ surface seawater samples (water depth: 1 m) were conducted according to Grasshoff et al. (1999). Light availability was assessed in triplicates in water depths of 1, 5, 10 and 20 m during spring, summer and winter seasons, and at 5 m water depth during fall season, using data loggers (Onset HOBO® Pendant UA-002-64).

Identification of dominant hermatypic coral genera

To identify the dominant hermatypic coral genera out of 68 described genera in the study area (Veron and Stafford-Smith 2002), line-point intercept (LPI) transect approaches modified from Loya (1978) and Nadon and Stirling (2006) were carried out. Duplicate transects of 50 m length with regular 0.5 m point intervals were conducted in 0.5, 1.0, 5.0, 10.0 and 20.0 m water depth parallel to the reef crest at a northern (latitude: 29°27′26″ N, longitude: 34°58′29″E) and southern location (latitude: 29°27′09″N, longitude: 34°58′16″E) within the MSS fringing reef using SCUBA. Coordinates of locations are shore line positions situated at right angle to line transects. In total, 44 transects were carried out during the 4 seasonal expeditions. LPI data were analysed to derive the percentage coverage for hermatypic coral genera. This revealed that the anthozoans *Acropora*, *Fungia*, *Goniastrea*, *Pocillopora* and *Stylophora*, and the hydrozoan *Millepora*, were among the top 10 dominant coral genera at all investigated water depths, together accounting for 24–66% of total live coral coverage depending on water depth (Table 1). Contribution to total live coral coverage was genus-specific and depth-dependent, e.g. genus *Acropora* accounted for 29 and 6%, and genus *Stylophora* for 3 and 12%, at 1 and 20 m water depth, respectively.

Table 1 *Percentage contribution of investigated coral genera to total live coral coverage in vertical resolution. Investigated coral genera (6 out of 68 locally occurring genera): Acropora, Fungia, Goniastrea, Millepora, Pocillopora, Stylophora; Abbreviation: LCC = Live coral coverage.*

Depth (m)	LCC (%)	Investigated coral genera (% of LCC)
0.5	20	49
1.0	59	66
5.0	8	48
10.0	37	39
20.0	55	24
Average	36	45

Coral samples

During each of 4 seasonal expeditions, 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 by SCUBA divers 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. Special care was taken to exclude coral colonies infested by endolithic bioeroders (e.g. boring bivalves; Lazar and Loya 1991) that could have possibly affected experimental results by release or uptake of organic matter, and to include different species for each investigated genus (i.e. sampling of clones was definitely avoided). Coral samples from 5 m water depth were transported to the laboratory without air-exposure and kept in a 1000 l cultivation tank supplied with fresh seawater (flow-through rate ~ 20 l min^{-1}) at in situ seasonal temperature (21–29°C), salinity (43 ± 1) and light availability (daylight average: 216–400 µmol m^{-2} s^{-1}). Light availability was adjusted to in situ conditions by comparative measurements of seasonal in situ light availability at 5 m water depth using Onset HOBO® Pendant Temperature and Light Data Loggers UA-002-64 and application of variable layers of black plastic gauze covering the cultivation tanks. With the exception of free-living *Fungia* polyps (diameter: 6–9 cm), all coral samples of branching (*Acropora*, *Millepora*, *Pocillopora* and *Stylophora*; length: 4–8 cm) and massive (*Goniastrea*; diameter: 5–8 cm) growth forms 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. Corals were subsequently left for healing and acclimatisation in flow-through tanks for 2 weeks, during which algal overgrowth on ceramic tiles and glue junction was removed regularly using a soft tooth brush. *Acropora* colony fragments (length: 4–8 cm) sampled from 1, 10 and 20 m depth were treated accordingly, but were subsequently transported back to the MSS reef for healing and acclimatisation at their original water depth, from where they were returned on the evening before the day of experiments. *Fungia* polyps (diameter: 6–9 cm) from 1, 10 and 20 m depth were likewise sampled from the reef during dusk of the day prior to incubation. The skeleton surface area of all corals was quantified applying geometric techniques (*Advanced Geometry* for *Acropora*, *Millepora*, *Pocillopora* and *Stylophora*; *Simple Geometry* for *Fungia* and *Goniastrea*) and respective *Approximation Factors* as described in

Naumann et al. (2009b), which resulted in an overall average skeleton surface area of 122 ± 63 cm^2 (average ± SD) for all incubated corals.

Quantification of organic matter release

Laboratory experiments regarding the quantification of organic matter release by the 6 investigated coral genera were conducted using the established beaker incubation method (Herndl and Velimirov 1986; Wild et al. 2005b) during all 4 seasonal expeditions. Beaker incubations were carried out during daylight hours (10:00–16:00 hrs), except for 2 additional night time incubations (20:00–02:00 hrs) of *Acropora* and *Fungia* corals originating from 5 m water depth. On each experimental day, one single coral genus was incubated. Immediately before the respective experiment, ceramic tiles and glue junctions were cleaned using a soft tooth brush before coral colony surfaces were exposed to a smooth stream of seawater inside the cultivation tanks to remove attached organic and inorganic particles. Subsequently, the corals were transferred without air-exposure, by touching only the ceramic tiles, into clean (acetone and seawater pre-washed) 1000 ml glass beakers filled with 830–987 ml (beaker content excluding coral volume) fresh in situ seawater fully submerging the corals. Beakers containing corals (n = 5), accompanied by control beakers (n = 5), containing only fresh seawater (1000 ml) of identical origin, were placed at in situ temperature into a water bath flushed with seawater freshly pumped from the reef. Control beakers were not equipped with ceramic tiles and coral glue junctions, as preliminary studies attested a non-detectable influence on particulate and dissolved organic matter concentrations in seawater by those materials. The beaker openings were covered with transparent cellophane foil to protect open beakers from input of airborne particles, leaving 2 peripheral openings for air exchange. Light availability and temperature comparable to in situ conditions (water depths: 1, 5, 10 and 20 m) was ensured during incubations by comparative measurements using data loggers (Onset HOBO® Pendant UA-002-64). Light availability was adjusted for each incubation experiment by application of black plastic gauze. To ensure comparability to previous beaker incubation studies (Herndl and Velimirov 1986; Wild et al. 2005b) and to rule out the influence of water current on organic matter release by reef organisms (Niggl et al. unpublished data), coral as well as control beaker contents were not stirred during incubations. With further respect to the low water current velocity prevailing in the study area (Manasrah et al. 2006; Naumann et al. unpublished data), the incubation procedure without stirring represented a close approximation to in situ conditions. Powder-free gloves were used during all experimental procedures to prevent contamination of the incubation water.

After 6 h of incubation, corals were removed from the beakers using clean tweezers and transferred back to the cultivation tank. Samples for DOC were immediately taken from the incubation water. Sterile syringes were used to thoroughly homogenise the content of coral and control incubation beakers and to collect 10 ml incubation water samples from each beaker for later DOC quantification. 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 volume (5 ml) was filtered at low pressure into pre-combusted (450°C; 4 h) amber glass vials. 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 sample acidification with orthophosphoric acid (20%) to pH<2 and sparging with oxygen, using potassium hydrogen phthalate as elemental standard (standard deviation <3%). Following DOC sample collection, dissolved O_2 concentration in all beakers was measured using an O_2 optode (Hach Lange, HQ 10) attesting oxic conditions (>92% O_2 saturation) in coral incubations and controls of all conducted experiments. POM samples (770 – 900 ml for coral treatments and 900 ml for controls) were prepared no later than 3 h after incubation, by filtration onto 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, coupled with a THERMO/Finnigan Conflo II-interface to a THERMO/Finnigan MAT Delta plus isotope ratio mass spectrometer. Contents of POC and PN were derived from calculation using certified elemental standards (atropine, cyclohexanone-2,4-dinitrophenylhydrazone; Thermo Quest; standard deviation <3%). Stable C and N isotopic ratios were expressed in the conventional delta notation ($\delta^{13}C$, $\delta^{15}N$) relative to the standards Vienna PeeDee Belemnite (VPDB) (Coplen 1995) and atmospheric nitrogen (Mariotti 1983), respectively. A standard deviation of <0.15‰ for C and N was derived from repeated measurements of laboratory standard (Peptone) of various initial weights. As the presence of particulate inorganic carbon could be ruled out by test measurements of the incubation water, samples were not treated with HCl to ensure validity of $\delta^{15}N$ values.

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. Statistical tests were performed applying the Mann and Whitney U-test (2-tailed) inside SPSS© software packages and results were regarded as statistically significant at $p<0.05$, if not mentioned differently.

Results

Environmental monitoring

Seawater temperature during the study period ranged from 21–29°C with highest values during summer season (July–August) and minimum values during winter and early spring (January–March). Average daytime (10:00–16:00 hrs) light availability at 5 m water depth varied between 216–400 µmol m^{-2} s^{-1} from winter to summer season, respectively. Depth-mediated light availability ranged from 527 (1 m depth) to 78 (20 m depth) µmol m^{-2} s^{-1} during winter, and 946 (1 m depth) to 144 (20 m depth) µmol 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

Measurement of POM concentrations from coral and control beakers revealed significantly increased POC and PN concentrations in coral incubation beakers compared 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 in comparison to seawater from control beakers (0.13 ± 0.03 and 0.015 ± 0.003 mg l^{-1}, average ± SE, n = 120, respectively). Consequently, organic matter release in form of POM was detectable for all investigated corals originating from 5 m water depth during all seasons. The majority of POM released by all corals was composed of relatively carbon-rich compounds, as evidenced by POC:PN ratios ranging from 5–20 (Table 2). POC:PN ratios for coral-derived organic matter were significantly elevated in comparison to POM in seawater controls (4–12), indicating higher carbon / lower nitrogen contents of POM released by hermatypic corals. POC release rates of all genera, averaged over all investigated seasons, were significantly different, except for *Goniastrea* in comparison to *Fungia* corals (Table 2). Branching scleractinian coral genera (*Acropora*, *Pocillopora* and *Stylophora*) could be identified as the dominant POC and PN exuding growth forms, followed by massive *Goniastrea* corals. Genera-wide average POC release accounted for 2.4 ± 0.9 mg POC m^{-2} coral surface area h^{-1} (average ± SE) on an annual basis. *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* corals 19 and 11-fold, for POC and PN respectively. Seasonal-averaged PN release also showed significant differences for the majority of measured corals, resulting in a genera-wide average release rate of 0.23 ± 0.07 mg PN m^{-2} coral surface area h^{-1} (average ± SE).

Corals investigated during all seasons (*Acropora*, *Fungia* and *Stylophora*) showed no significant seasonal variation in POC and PN release (Fig. 1, panel a–d), except for *Acropora*, which displayed increased PN release rates during summer (Fig. 1, panel b). However, linear regression analysis confirmed a correlation of average POC and PN release rates to variable seasonal parameters (temperature ($r^2 \geq 0.923$) and daytime average light availability at 5 m depth ($r^2 \geq 0.969$), as depicted for *Acropora* and *Fungia* in Fig. 2, panel a, b. Seasonal fluctuations in ambient nitrate concentrations showed a significant correlation to POC and PN release rates for *Acropora*, *Fungia* and *Stylophora* corals (Spearman rank-order correlation, 2-tailed, $p<0.001$). Increase of nitrate concentrations resulted in an exponential decrease in POC and PN release (Fig. 2, panel c); while seasonally variable concentrations of all other inorganic nutrients (i.e., nitrite, ammonium and phosphate) showed no correlation to POM release (Spearman rank-order correlation, 2-tailed, $p>0.05$).

Average POC and PN release rates of *Acropora* and *Fungia* corals originating from different water depth (1–20 m) showed a positive correlation (Spearman rank-order correlation, 2-tailed, $p<0.05$) to depth-mediated light availability (Fig. 3, panel a). However, including all replicate

measured release rates, 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 light availability for both corals (Fig. 3, panel a), as reflected by POC:PN ratios ranging from 17 to 8 and 11 to 8 for light intensity representative of 1 m water depth and night time incubation for *Acropora* and *Fungia*, respectively (data not shown). Carbon stable isotope signature ($\delta^{13}C$) of bulk POM from coral incubations deviated significantly from seawater controls on genera-wide annual average ($p<0.001$). Values for $\delta^{13}C$ from coral incubations were significantly ^{13}C-enriched relative to POM in seawater controls, accounting for –18.1 ± 0.3‰ compared to –20.6 ± 0.3‰ (average ± SE), respectively. Analysis of bulk POM $\delta^{15}N$ values resulted in no significant differences between coral incubations (1.0 ± 0.4‰) and seawater controls (0.7 ± 0.7‰).

Table 2 *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	6.3 ± 0.8	0.52 ± 0.03	14 ± 3	5
	Summer	7.0 ± 0.8	0.55 ± 0.14	12 ± 3	4
	Fall	6.6 ± 1.6	0.52 ± 0.10	17 ± 1	4
	Winter	6.0 ± 2.5	0.47 ± 0.16	15 ± 1	5
	Mean	6.5 ± 2.1	0.52 ± 0.02	15 ± 1	
Goniastrea	Summer	0.9 ± 0.1	0.07 ± 0.01	14 ± 1	5
	Fall	1.8 ± 0.7	0.22 ± 0.11	11 ± 3	5
	Winter	1.5 ± 1.2	0.08 ± 0.15	11 ± 1	5
	Mean	1.4 ± 0.3	0.12 ± 0.05	12 ± 1	
Pocillopora	Summer	3.9 ± 0.3	0.24 ± 0.01	20 ± 1	5
	Fall	2.8 ± 0.4	0.32 ± 0.11	14 ± 1	5
	Mean	3.3 ± 0.5	0.28 ± 0.04	17 ± 3	
Millepora	Summer	0.3 ± 0.1	0.03 ± 0.01	10 ± 2	5
	Fall	0.3 ± 0.3	0.04 ± 0.03	11 ± 2	5
	Mean	0.3 ± 0.1	0.04 ± 0.01	11 ± 1	
Mean		2.4 ± 0.9	0.23 ± 0.07	13 ± 1	∑ = 91

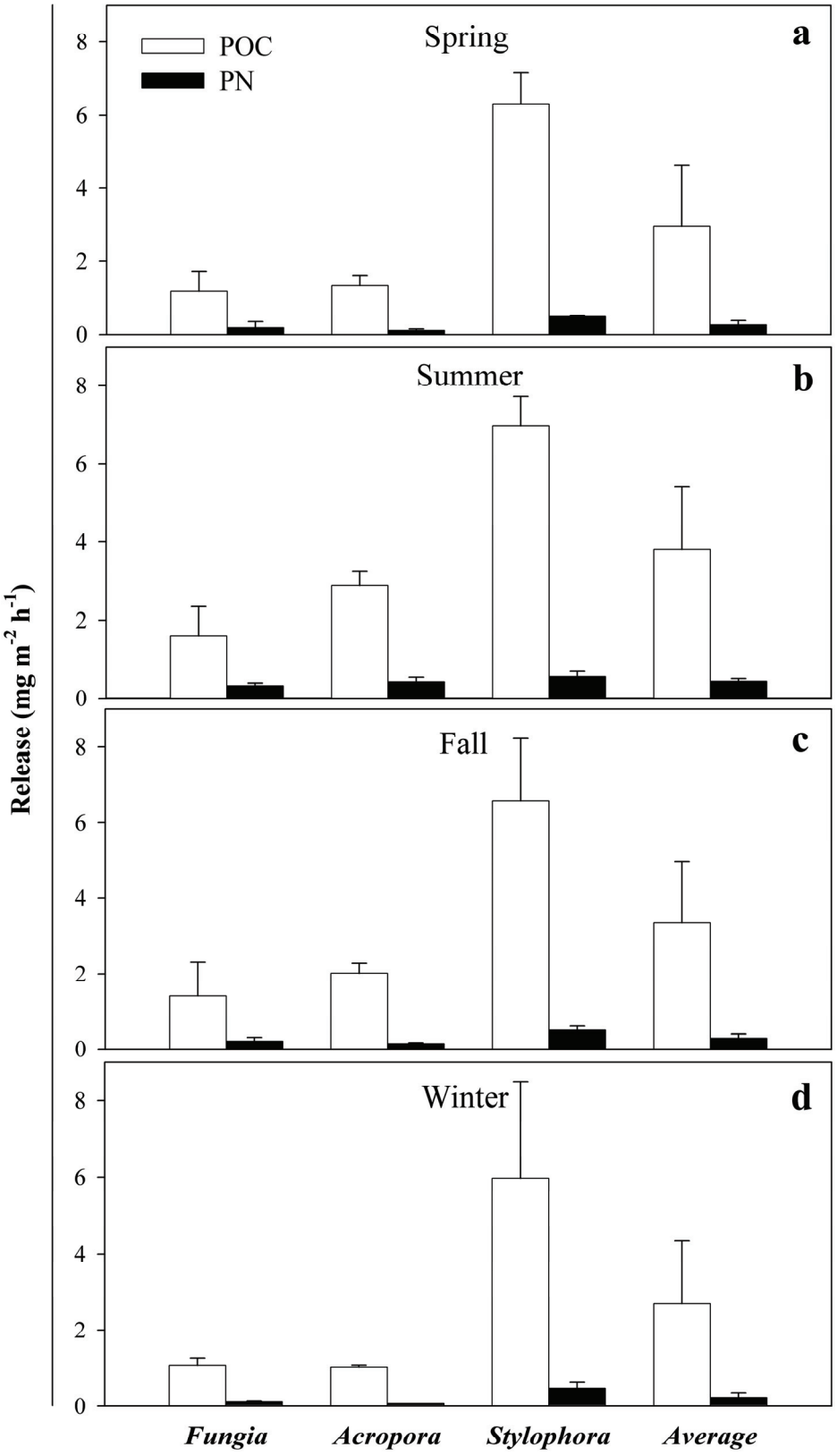

Fig. 1 *Genus-specific average seasonal particulate organic matter release rates (POC and PN) for Acropora, Fungia and Stylophora corals from the investigated Red Sea fringing reef. Panel a: spring; panel b: summer; panel c: fall; panel d: winter. Values are given as average ± SE for POC and PN release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

DOC release

DOC concentrations measured from coral and control beakers of all conducted incubation experiments ranged from 0.8–29 mg l^{-1} and were significantly higher than the respective POC concentrations (0.04–0.84 mg l^{-1}). On overall average DOC concentrations were 12 and 38-fold higher than POC concentrations in coral and control beakers, respectively. However, overall average DOC concentration in control beakers (4.8 ± 3.0 mg l^{-1} (average ± SE)) was increased in comparison to coral beakers (4.0 ± 2.3 mg l^{-1}) which consequently resulted in the calculation of negative DOC fluxes, i.e. DOC uptake, for 50% of the investigated coral 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) corals. *Pocillopora* corals showed DOC uptake throughout all investigated seasons (-263.4 ± 105.2 mg DOC m^{-2} coral surface area h^{-1}), which was significantly increased in comparison to all other genera ($p<0.01$), while *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 investigated coral genera, significant seasonal variation was only detectable for *Fungia* corals, which showed increased DOC uptake during fall (-38.5 ± 5.3 mg DOC m^{-2} coral surface area h^{-1}) in comparison to summer and winter seasons (-5.4 ± 1.1 and 1.0 ± 0.2 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 ranging from 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 light intensity (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 an exponential saturation level reached at around 1 m water depth (Fig. 3, panel b). The determined DOC fluxes of all investigated coral 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 in this study provide the first comprehensive overview of organic matter release by hermatypic corals in warm water coral reef ecosystems. Organic matter release rates (POC, PN and DOC) are presented for 6 dominant coral genera (*Acropora*, *Fungia*, *Goniastrea*, *Millepora*, *Pocillopora* and *Stylophora*), together representing 45% of total live coral coverage between 0.5 and 20 m water depth in a fringing coral reef of the Northern Red Sea. In addition, the influence of variable environmental factors (water temperature, light availability and ambient inorganic nutrient concentrations) on coral-derived organic matter release was investigated by seasonal and manipulative incubation experiments.

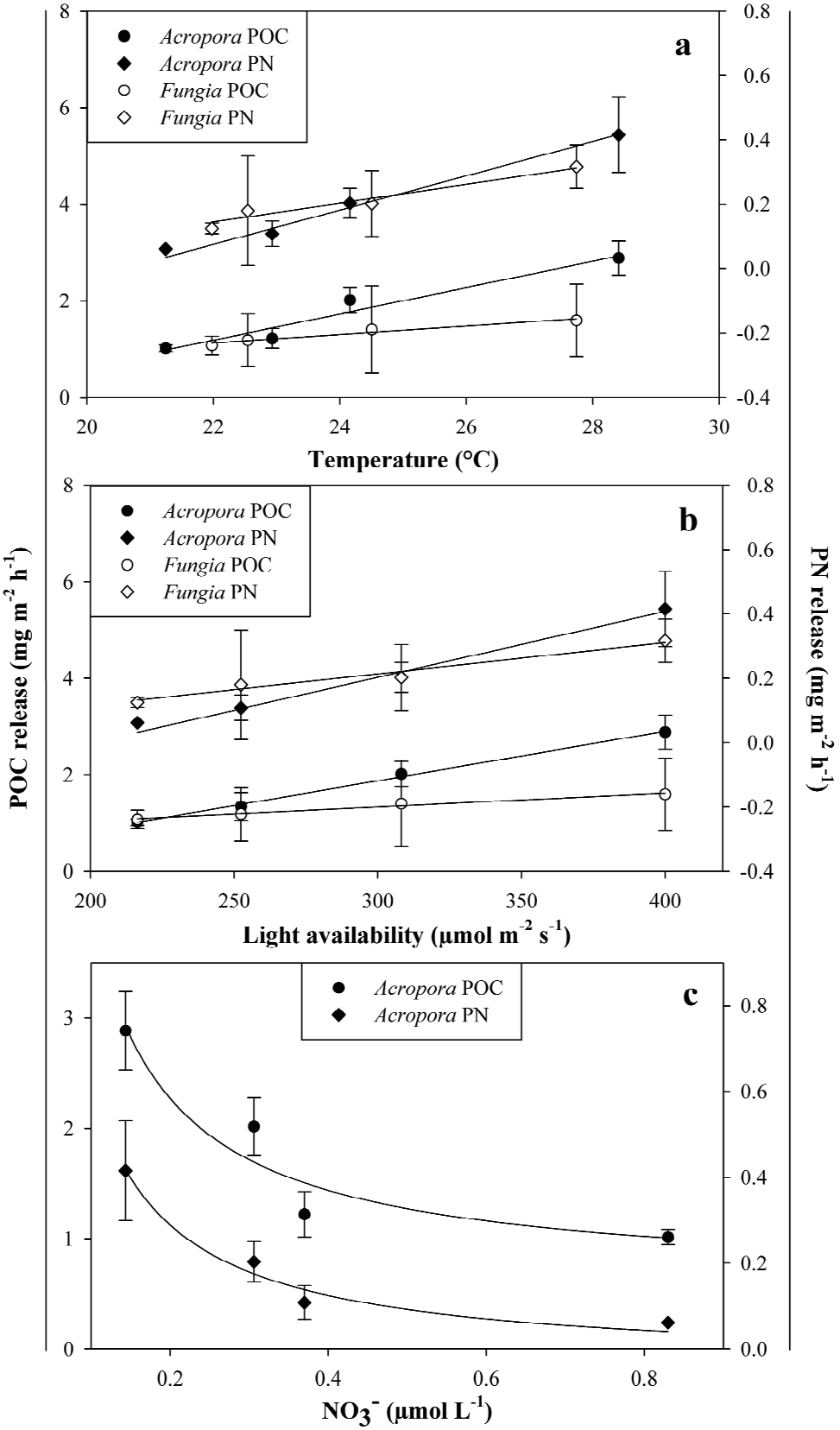

Fig. 2 *POM release rates by corals from 5 m water depth influenced by seasonal fluctuations in some environmental parameters. Panel a: temperature; panel b: light availability; panel c: in situ seawater nitrate concentration.*

Genus-specific POM release

All coral genera investigated by the present study released POM to the surrounding seawater, thereby importantly contributing to reef trophodynamics by input of energy-rich carbon and nitrogen compounds (Benson and Muscatine 1974; Wild et al. 2004a, b). The released POM was predominantly composed of POC, as shown by the high resulting 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 cylindrical*, respectively.

The finding that branching anthozoan coral genera (*Acropora*, *Pocillopora* and *Stylophora*) show higher POM release than the massive genus (*Goniastrea*), contradicts the results of Richman et al. (1975), who described enhanced mucus release rates for several massive genera, and further presented very similar values for *Goniastrea pectinata, Acropora variabilis* and *Stylophora pistillata* from the same study region. Unfortunately, mucus release rates by Richman et al. (1975) were not normalised to coral surface area, but to "coral head", thus inhibiting direct comparison to values obtained by the present study. Nevertheless, the comparably invasive quantification technique of enclosing coral heads in situ using plastic bags, utilised by 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.

Genera-wide POM release rates were in the range of results obtained by Wild et al. (2005b), who also used the beaker incubation technique. In particular, POC and PN release by *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}) 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 genera-wide conformity. 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). This large variation may, however, account for methodological differences in the applied quantification techniques. POC release rates of *Acropora* corals from 5 m depth were comparable to values generated 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}). 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 variable *Stylophora* species composition assessed in the present study, as some *Stylophora* specimens demonstrated lower POC release ranging from 2.9 to 3.3 mg C m^{-2} coral surface area h^{-1} (data not shown).

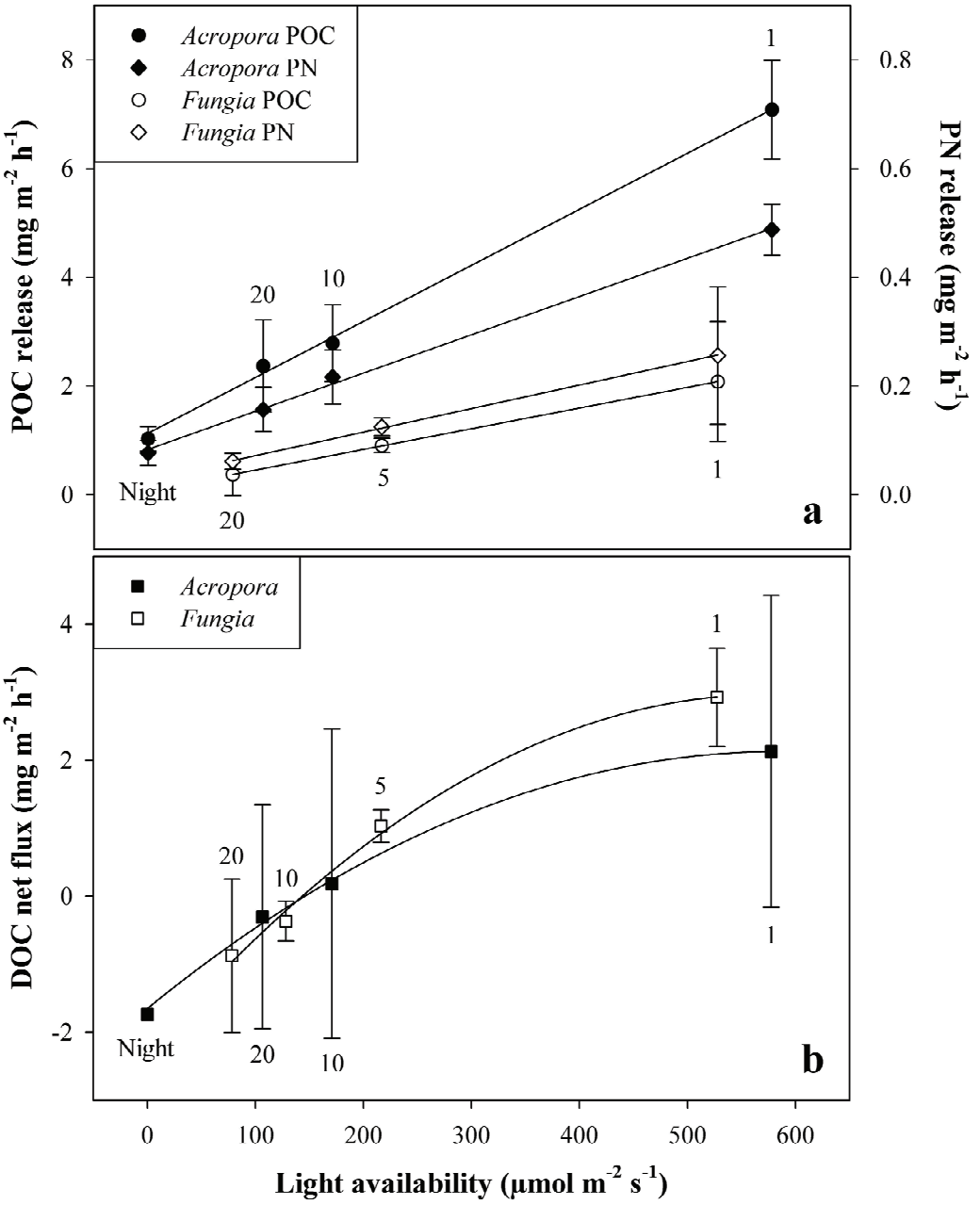

Fig. 3 *POC, PN and dissolved organic carbon (DOC) release rates for Acropora and Fungia corals at different light regimes. Panel a: POC and PN release; panel b: DOC net flux. Numbers above and below data points represent the original water depth (m) of incubated corals and the corresponding light availability applied during incubations.*

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 attached particulate mucus strings on the surface of *Millepora* colonies (72% of inspected corals). Surprisingly, resulting POC as well as PN release rates of *Millepora* were relatively low. This could likely be caused by the genera-wide highest dissolution ratio of organic matter released by *Millepora* (DOC:POC ratio: 50 ± 9), or possibly by a lower abundance of energy-providing zooxanthellae in hydrozoan *Millepora* tissues (0.72 x 10^6 cells cm^{-2}; Drew 1972)

compared to their abundance in anthozoan coral tissues (e.g., *Stylophora*: 1.6 x 10^6 cells cm^{-2}; Falkowski and Dubinsky 1981).

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 release of coral-derived POM. However, Hoegh-Guldberg et al. (1987) showed that carbon loss by expulsion of zooxanthellae represents a minor part of the daily budget of photosynthetically-fixed carbon by *Stylophora* corals (0.01%). Further, zooxanthellae release rates measured by Stimson and Kinzie (1991) for *Pocillopora* corals attest only a minor contribution (<5%) by zooxanthellae carbon to the overall *Pocillopora* POC release presented here, thus confirming coral mucus as the bulk component of organic matter released by tropical reef corals (Crossland 1987).

Relevant environmental factors for POM release

Constant year-round POM release rates found for all 3 seasonally investigated coral genera (*Acropora*, *Fungia* and *Stylophora*) together representing 30% of total live coral coverage in the MSS reef, confirm a steady input of hermatypic coral-derived POC and PN into organic matter cycles of Red Sea reef environments. Although no statistically significant differences were observed between seasonal POC and PN release rates, strong correlation between average release values of POC and PN and seasonally variable temperature and light availability suggests an influence of these parameters. Change of temperature, a known stress factor for hermatypic corals (Hoegh-Guldberg 1999a), can lead to increased release of coral mucus (Kato 1987) and may, even by variation within an accustomed annual range, affect temperature-sensitive metabolic processes regulating extracellular organic matter release, as shown for other components of coral metabolism (i.e., calcification and tissue carbon incorporation) (Crossland 1984). The important influence of light availability on POC release rates is confirmed by Crossland (1987), who studied organic carbon release under different light regimes and found a substantial decrease in POC release with reduced irradiance. The significant proportion of nitrogen-containing compounds (glycoproteins and peptides) in coral-derived POM (Krupp 1985; Meikle et al. 1988) may further explain the increased PN release observed for *Acropora* at higher light intensity during summer. The effect of depth-mediated light availability is additionally highlighted by incubation experiments with *Acropora* corals from different water depths carried out in the present study. 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. With the reduction of light availability between 1 and 20 m water depth, POC release also reduced 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 the quantity and composition of coral-derived POM. Consequently, the results of this study emphasise the important role of the light-dependent phototrophic zooxanthellae by the supply of photosynthates required for synthesis and release of POM by the coral host in warm water reefs.

Significant seasonal differences of ambient inorganic nutrient concentrations in the study area have already been described by previous studies (Rasheed et al. 2002). Corals are able to take up inorganic nutrients from ambient seawater to cover specific metabolic demands (Hoegh-Guldberg 1999b; Grover 2002). Except for nitrate, seasonal concentration differences of inorganic nutrients showed no significant influence on POM release rates, which highlights that nitrate represents another key factor for coral-derived organic matter release. Increased nitrate concentrations during winter and spring season caused a decrease in coral-derived POM release, as shown here for *Acropora* corals. Nitrate represents an important source of nitrogen for corals and is predominantly assimilated by the zooxanthellae (Grover et al. 2003). In nitrate-poor reef waters, zooxanthellae experience nutrient-limitation (Muscatine et al. 1989a) and 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 lead to a nutrient-demanding increase in zooxanthellae abundance, and thus to a decreased transfer of carbon-rich photosynthates to the coral host, thereby impairing the release of POM by hermatypic corals, as shown for decreasing coral calcification rates (Marubini and Davies 1996). Reduced supply of nitrogen-rich compounds 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 PN release during winter season seems, at least for *Acropora*, to be uncoupled from heterotrophic nitrogen sources.

Stable isotope signatures of coral-derived POM

Stable carbon isotopic signatures of bulk POC in coral incubations and seawater controls deviated significantly by more than 2‰ on genera-wide annual average. Seawater control POC $\delta^{13}C$ values found here (~ –20‰) are consistent with findings of previous studies (Yamamuro et al. 1995; Swart et al. 2005), while POC $\delta^{13}C$ values in coral incubations (–18.1 ± 0.3‰) represent a mixture of seawater and coral-derived POC. This mixture becomes enriched in $\delta^{13}C$ in comparison to seawater, due to the introduction of coral-derived POC (average: 58% of total POC) with higher $\delta^{13}C$ contents (coral mucus: –16.2 ± 0.4‰; Naumann et al. unpublished data). This reflects specific carbon isotope partitioning during photosynthesis (Muscatine et al. 1989b). Similarity of PN $\delta^{15}N$ values in seawater (0.7 ± 0.7‰) and coral incubations (1.0 ± 0.4‰), can be interpreted as a mixture of seawater PN and coral-derived PN (average: 53% of total PN) by substitution of $\delta^{15}N$ values for coral mucus (1.5 ± 0.7‰; Naumann et al. unpublished data). Similar $\delta^{15}N$ values for seawater and coral-derived POM, without an indication of heterotrophic partitioning, further indicate the predominant autotrophic origin of coral-derived POM compounds (Muscatine et al. 2005). Finally, $\delta^{13}C$ and $\delta^{15}N$ values of POM in coral-incubated seawater verify the minor contribution of released zooxanthellae ($\delta^{13}C$: –12.1 ± 1.2‰, $\delta^{15}N$: 4.8 ± 0.8‰, Swart et al. 2005) to POM release measured by the present study, further indicating a comparably low stress impact by the beaker incubation approach.

DOC flux

Only half of the investigated coral genera (*Acropora*, *Goniastrea* and *Millepora*) showed DOC net release. The other coral genera displayed a negative DOC balance after quantitative subtraction of seawater control contents, consequently concluding DOC uptake during coral incubation. The study of Ferrier-Pages et al. (1998) demonstrates the role of coral-associated bacteria for substantial DOC uptake, measured during incubations of the coral *Galaxea fascicularis*. Similar processes likely explain the DOC uptake observed for *Fungia*, *Pocillopora* and *Stylophora* corals investigated in the present study. 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 coral genera (e.g. *Pocillopora*). In addition, different 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 coral genera 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 found 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. DOC net release rates determined for *Acropora* corals during summer season (data not shown), are only half (4.0 ± 0.3 mg DOC m^{-2} coral surface area h^{-1}) of the release rates measured 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), indicating high variability in DOC release by different *Acropora* species. Depth-mediated light availability influences DOC release rates of *Acropora* and *Fungia* corals, resulting in increased DOC release with increased light availability and DOC uptake at low light conditions (10, 20 m water depth and during the night). Decreased DOC release with increasing water depth is confirmed by Crossland (1987), who described a comparable relationship for *Acropora variabilis* and *Stylophora pistillata*, 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 studies are necessary to fully resolve DOC flux dynamics within the coral holobiont (animal – zooxanthellae – bacteria) and its exchange between corals and a variable environment.

In conclusion, organic matter release rates generated by this study provide a comprehensive overview of POM and DOC release (-uptake) for 6 genera of dominant hermatypic Red Sea corals in high spatiotemporal resolution. In addition, combined analysis by comparison with environmental parameters reveals light availability, temperature and ambient nitrate concentrations as significant key factors for their organic matter release. This comprehensive data set, together with the determined benthic community structure, provides essential information required for the understanding of element cycles and ecosystem functioning in warm water coral reefs.

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References

Al-Moghrabi S, Allemand D, Jaubert J (1993) Valine uptake by the scleractinian coral *Galaxea fascicularis*: characterization and effect of light and nutritional status. J Comp Physiol B 163:355-362

Benson A, Muscatine L (1974) Wax in coral mucus – energy transfer from corals to reef fishes. Limnol Oceanogr 19:810-814

Brown BE, Bythell JC (2005) Perspectives on mucus secretion in reef corals. Mar Ecol Prog Ser 296:291-309

Coffroth MA (1990) Mucous sheet formation on poritid corals – an evaluation of coral mucus as a nutrient source on reefs. Mar Biol 105:39-49

Coplen TB (1995) Reporting of stable hydrogen, carbon, and oxygen isotopic abundances. Geothermics 24:707-712

Crossland CJ (1984) Seasonal variations in the rates of calcification and productivity in the coral *Acropora formosa* on a high-latitude reef. Mar Ecol Prog Ser 15:135-140

Crossland CJ (1987) In situ release of mucus and DOC-lipid from the corals *Acropora variabilis* and *Stylophora pistillata* in different light regimes. Coral Reefs 6:35-42

Drew EA (1972) The biology and physiology of alga-invertebrate symbiosis. II. The density of symbiotic algal cells in a number of hermatypic hard corals and alcyonarians from various depths. J Exp Mar Biol Ecol 9:71-75

Drollet JH, Glaziou P, Martin PMV (1993) A study of mucus from the solitary coral *Fungia fungites* (Scleractinia: Fungiidae) in relation to photobiological UV adaptation. Mar Biol 115:263-266

Falkowski PG, Dubinsky Z (1981) Light-shade adaptation of *Stylophora pistillata*, a hermatypic coral from the Gulf of Eilat. Nature 289:172-174

Ferrier-Pages C, Leclercq N, Jaubert J, Pelegri SP (2000). Enhancement of pico- and nanoplankton growth by coral exudates. Aquat Microb Ecol 21:203-209

Ferrier-Pages C, Gattuso JP, Cauwet G, Jaubert J, Allemand D (1998) Release of dissolved organic carbon and nitrogen by the zooxanthellate coral *Galaxea fascicularis*. Mar Ecol Prog Ser 172:265-274

Grasshoff K, Ehrhardt M, Kremling K (1999) Methods of seawater analysis, 3rd ed. Verlag Chemie.

Grover R, Maguer JF, Reynaud-Vaganay S, Ferrier-Pages C (2002) Uptake of ammonium by the scleractinian coral *Stylophora pistillata*: Effect of feeding,light, and ammonium concentrations. Limnol Oceangr 47:782-790

Grover R, Maguer JF, Allemand D, Ferrier-Pages C (2003) Nitrate uptake in the scleractinian coral *Stylophora pistillata*. Limnol Oceangr 48:2266-2274

Herndl GJ, Velimirov B (1986) Microheterotrophic utilization of mucus released by the Mediterranean coral *Cladocora cespitosa*. Mar Biol 90:363-369

Hoegh-Guldberg O (1999a) Climate change, coral bleaching and the future of the world's coral reefs. Mar Freshw Res 50:839-866

Hoegh-Guldberg O, Williamson J (1999b) Availability of two forms of dissolved nitrogen to the coral *Pocillopora damicornis* and its symbiotic zooxanthellae. Mar Biol 133:561-570

Hoegh-Guldberg O, McCloskey LR, Muscatine L (1987) Expulsion of zooxanthellae by symbiotic cnidarians from the Red Sea. Coral Reefs 5:201-204

Huettel M, Wild C, Gonelli S (2006) Mucus trap in coral reefs: formation and temporal evolution of particle aggregates caused by coral mucus. Mar Ecol Prog Ser 307:69-84

Johannes R (1967) Ecology of organic aggregates in the vicinity of a coral reef. Limnol Oceanogr 12:189-195

Kato M (1987) Mucus-sheet formation and discoloration in the reef-building coral *Porites cylindrica*: effect of altered salinity and temperature. Galaxea 6:1-16

Krupp DA (1984) Mucus production by corals exposed during an extreme low tide. Pac Sci 38:1-11

Krupp DA (1985) An immunochemical study of the mucus from the solitary coral *Fungia scutaria* (Scleractinia, Fungiidae). Bull Mar Sci 36:163-176

Lazar B, Loya Y (1991) Bioerosion of coral reefs – A chemical approach. Limnol Oceanogr 36:377-383

Loya Y (1978) Plotless and transect methods. In: Stoddart DR, Johannes RE (eds) Coral reefs: research methods. United Nations Educational, Scientific and Cultural Organization, Paris, pp 197-217

Manasrah RS, Al-Horani FA, Rasheed MY, Al-Rousan SA, Khalaf MA (2006) Patterns of summer vertical and horizontal currents in coastal waters of the northern Gulf of Aqaba, Red Sea. Estuar Coast Shelf Sci 69: 567-579

Mariotti A (1983) Atmospheric nitrogen is a reliable standard for natural ^{15}N abundance measurements. Nature 303:685-687

Marshall M (1968) Observations on organic aggregates in the vicinity of coral reefs. Mar Biol 2:50-55

Marshall AT, Wright OP (1993) Confocal laser scanning light microscopy of the extra-thecal epithelia of undecalcified scleractinian corals. Cell Tissue Res 272:533-543

Marubini F, Davies PS (1996) Nitrate increases zooxanthellae population density and reduces skeletogenesis in corals. Mar Biol 127:319-328

Meikle P, Richards GN, Yellowlees D (1987) Structural determination of the oligosaccharide side-chains from a glycoprotein isolated from the mucus of the coral *Acropora formosa*. J Biol Chem 262:16941-16947

Meikle P, Richards G, Yellowlees D (1988) Structural investigations on the mucus from 6 species of coral. Mar Biol 99:187-193

Muscatine L, Porter JW, Kaplan IR (1989b) Resource partitioning by reef corals as determined from stable isotope composition, I. Mar Biol 10:185-193

Muscatine L, Falkowski PG, Dubinsky Z, Cook PA, McCloskey LR (1989a) The effect of external nutrient resources on the population dynamics of zooxanthellae in a reef coral. Proc R Soc Lond B 236:311-324

Muscatine L, Goiran C, Land L, Jaubert J, Cuif JP, Allemand D (2005) Stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) of organic matrix from coral skeleton. Proc Natl Acad Sci USA 102:1525-1530

Nadon MO, Stirling G (2006) Field and simulation analyses of visual methods for sampling coral cover. Coral Reefs 25:177-185

Naumann MS, Richter C, el-Zibdah M, Wild C (2009a) Coral mucus as an efficient trap for picoplanktonic cyanobacteria – implications for pelagic-benthic coupling in the reef ecosystem. Mar Ecol Prog Ser 385:65-76

Naumann MS, Niggl W, Laforsch C, Glaser C, Wild C (2009b) Coral surface area quantification – evaluation of established methods by comparison with computer tomography. Coral Reefs 28:109-117

Rasheed M, Badran MI, Richter C, Huettel M (2002) Effect of reef framework and bottom sediment on nutrient enrichment in a coral reef of the Gulf of Aqaba, Red Sea. Mar Ecol Prog Ser 239:277-285

Richman S, Loya Y, Slobodkin L (1975) Rate of mucus production by corals and its assimilation by the coral reef copepod *Acartia negligens*. Limnol Oceanogr 20:918-923

Ritchie KB (2006) Regulation of microbial populations by coral surface mucus and mucus-associated bacteria. Mar Ecol Prog Ser 322:1-14

Rohwer F, Seguritan V, Azam F, Knowlton N (2002) Diversity and distribution of coral-associated bacteria. Mar Ecol Prog Ser 243:1-10

Schuhmacher H (1977) Ability of fungiid corals to overcome sedimentation. Proc 3rd Int Coral Reef Symp 1:503-509

Sebens KP, Vandersall KS, Savina LA, Graham KR (1996) Zooplankton capture by two scleractinian corals, *Madracis mirabilis* and *Montastrea cavernosa*, in a field enclosure. Mar Biol 127:303-317

Stimson J, Kinzie RA (1991) The temporal pattern and rate of release of zooxanthellae from the reef coral *Pocillopora damicornis* (Linnaeus) under nitrogen-enrichment and control conditions. J Exp Mar Biol Ecol 153:63-74

Swart PK, Saied A, Lamb K (2005) Temporal and spatial variation in the $\delta^{15}N$ and $\delta^{13}C$ of coral tissue and zooxanthellae in *Montastrea faveolata* collected from the Florida reef tract. Limnol Oceangr 50:1049-1058

Tanaka Y, Miyajima T, Koike I, Hayashibara T, Ogawa H (2008) Production of dissolved and particulate organic matter by the reef-building corals *Porites cylindrica* and *Acropora pulchra*. Bull Mar Sci 82:237-245

Veron JEN, Stafford-Smith MG (2002) Coral ID. Australian Institute of Marine Science.

Wild C, Woyt H, Huettel M (2005b) Influence of coral mucus on nutrient fluxes in carbonate sands. Mar Ecol Prog Ser 287:87-98

Wild C, Huettel M, Klueter A, Kremb SG, Rasheed M, Jørgensen BB (2004a) Coral mucus functions as an energy carrier and particle trap in the reef ecosystem. Nature 428:66-70

Wild C, Rasheed M, Werner U, Franke U, Johnstone R, Huettel M (2004b) Degradation and mineralization of coral mucus in reef environments. Mar Ecol Prog Ser 267:159-171

Wild C, Rasheed M, Jantzen C, Cook P, Struck U, Boetius A (2005a) Benthic metabolism and degradation of natural particulate organic matter in carbonate and silicate reef sands of the Northern Red Sea. Mar Ecol Prog Ser 298:69-87

Wild C, Mayr C, Wehrmann LM, Schöttner S, Naumann M, Hoffmann F, Rapp HT (2008) Organic matter release by cold water corals and its implication for fauna-microbe interaction. Mar Ecol Prog Ser 372:67-75

Wolf-Vecht A, Paldora N, Brenner S (1992) Hydrographic indications of advection/convection effects in the Gulf of Eilat. Deep-sea Res 39:1393-1401

Yahel R, Yahel G, Genin A (2005) Near-bottom depletion of zooplankton over coral reefs: I: diurnal dynamics and size distribution. Coral Reefs 24:75-85

Yamamuro M, Kayanne H, Minigawa M (1995) Carbon and nitrogen stable isotopes of primary producers in coral reef ecosystems. Limnol Oceanogr 40:617-621

5

Contribution of coral-derived organic carbon to biogeochemical processes in a fringing reef of the Northern Red Sea

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Abstract

The release of organic C-rich material by hermatypic (reef-building) corals can represent an important link in biogeochemical coral reef processes by substantially contributing to element cycles and concomitant rapid nutrient recycling. However, relative share of coral-derived organic C to reef-wide organic C pools and the possible fate of this material in fringing reef ecosystems, the most common reef type world-wide, are unresolved. Thus, quantitative investigations of organic C release by hermatypic corals were conducted in high temporal and spatial resolution in a typical fringing reef of the Northern Red Sea. In addition, a series of parallel laboratory and in situ studies involving water column sampling and deployment of benthic chambers as well as sediment traps was carried out in order to estimate the contribution of coral-derived organic C to element cycles and recycling processes. Findings showed that total organic carbon (TOC) release by the coral community of the investigated reef (projected area = 0.19 km^2) in 0-20 m water depth accounted for 12.8 kmol C d^{-1}, thereby contributing between 3 and 8 % to seawater TOC content. This release is further representative of 30 – 39 % of pelagic- and 8-fold of benthic net primary productivity in sandy reef sediments. Degradation of coral-derived TOC in reef waters represented 0.1 – 4.6 % of total planktonic microbial O_2 consumption. Coral-derived organic matter reaching the benthic environment covered between 34 and 41 % of the C demand of the reef sand communities and approximately 4 % of the daily coral-derived TOC was recycled there. This confirms the important role of coral-derived organic matter as a carrier of C also for fringing reef environments and further indicates a significant contribution of the reef framework to the cycling and retention of coral-derived organic material within the fringing reef ecosystem.

Introduction

Warm water coral reef ecosystems thriving in oligotrophic marine environments are nevertheless characterised by high rates of primary productivity, which is primarily attributed to efficient utilization, recycling and conservation of the sparsely available nutritious organic components suspended or dissolved in reef waters (Muscatine & Porter 1977, Richter et al. 2001, Wild et al. 2004a). Hermatypic corals are usually dominant taxa in warm water coral reef ecosystems and can contribute importantly to the organic matter pool in reef waters, because they continuously release particulate (POM) and dissolved (DOM) organic matter, e.g. as mucus (Johannes 1967, Crossland 1987, Wild et al. 2004a, Naumann et al. submitted). Coral mucus is a transparent organic exopolymer principally composed of C-rich components (carbohydrates, glycoproteins and lipids), which is synthesised and exuded by ectodermal cells as a protective mucoid layer covering the coral's tissue surface (Schuhmacher 1977, Krupp 1985, Meikle et al. 1987, Marshall and Wright 1993, Ritchie 2006). The process of ectodermal mucus secretion is accompanied by the immediate dissolution of a significant mucus fraction and a successive ablation of particulate mucus components from the coral surface, consequently resulting in the entry of this material into the DOM and POM pool of reef waters (Wild et al. 2004a). Coral mucus can be released in such quantities that it dominates the suspended matter around reefs (Johannes 1967, Marshall 1968), significantly influences the growth and metabolism of pelagic microbial communities (Ferrier et al. 2000, Wild et al. 2004a) and acts as a particle trap importantly supporting benthic-pelagic coupling processes by rapid sedimentation of enriched mucus aggregates (Coles & Strathmann 1973, Wild et al. 2004a, Wild et al. 2005, Huettel et al. 2006, Naumann et al. 2009a).

While several studies have investigated organic C dynamics of coral reefs in general with emphasis on ecosystem primary productivity and organic matter cycling (e.g. Odum & Odum 1955, Gordon 1971, Charpy & Charpy-Roubaud 1991, Hata et al. 2002), only few workers (Wild et al. 2004a, b, Huettel et al. 2006) have focussed on the contribution and related function of organic C contained in coral mucus for organic C dynamics in reef-ecosystem. These studies describe the important role of coral mucus in nutrient cycling for the platform reef system of Heron Island (Australia), thereby emphasizing the role of coral mucus in nutrient conservation and regeneration via the efficient trapping of suspended particles and initiation of relatively short-linked element cycles significantly influencing benthic and pelagic microbial reef metabolism. Further, these studies provide evidence for the important contribution of organic C contained in coral mucus to bulk sedimentary and planktonic decomposition processes, thus highlighting coral mucus as a substantial source of degradable C in coral reef environments.

However, our current understanding of the contribution and related function of coral-derived organic C in reef ecosystem-wide organic C dynamics is still limited to platform reef systems indicating a potentially similar importance for different reef types, such as fringing coral reefs. In addition, previous studies on coral-derived organic C cycling have focussed on organic matter release rates by only few hermatypic coral species (Wild et al. 2004a, 2005a), possibly misjudging the actual contribution by the usually diverse hermatypic coral community using extrapolation approaches.

In consideration and comparison of the above mentioned findings for platform reef systems, the present study was conducted in a fringing coral reef of the Northern Red Sea, the globally most common reef type, principally aiming (1) to determine the contribution of organic material released by hermatypic corals to bulk ecosystem organic C dynamics and reef metabolism and (2) to investigate the possible fate of coral-derived organic C in trophic pathways of fringing reef ecosystems.

Material and Methods

Study site

The present study was conducted in a fringing reef of the Northern Red Sea (Gulf of Aqaba) during 4 seasonal expeditions (November/December 2006 (fall), August/September 2007 (summer), February/March 2008 (winter) and May 2008 (spring)) to the Marine Science Station (MSS) Aqaba, Jordan (latitude: 29° 27′ N, longitude: 34° 58′ E). According to earlier investigations regarding morphology and zonation, this fringing reef can be partitioned in a reef flat divided into back reef (depth: 0.0–1.8 m), reef crest (0.5–1.0 m) and reef slope (1–6 m) joined by a fore reef facing the open sea (Mergner & Schuhmacher 1974). The fore reef consists of an upper (4–8 m depth), middle (8–20 m) and lower (20–40 m) part distinguished by morphological features and species composition. The fringing reef system extends approximately 1.1 km along its reef crest bordering the coastline in a half-ellipsoid shape (Fig. 1). Investigations carried out by the present study focussed on the reef area framed by the reef flat and the middle fore reef (depth range: 0–20 m; hereinafter called: reef-wide).

Ecosystem assessment

Benthic coverage

To assess the benthic reef assemblage by substrate types and to identify the dominant hermatypic coral genera in the study area, Line-point intercept surveys (total: n = 44), as described in Naumann et al. (submitted), were carried out in 0.5, 1.0, 5.0, 10.0 and 20.0 m water depth during the seasonal expeditions. Analysis of LPI data yielded the percentage coverage by main substrate types including the total live coral coverage and the contribution of dominant hermatypic coral genera (i.e. anthozoans: *Acropora*, *Fungia*, *Goniastrea*, *Pocillopora* and *Stylophora*, and the hydrozoan *Millepora*) (Table 1).

Seawater organic C content

To investigate organic C concentrations in the reef-overlying water column, seawater samples (3000–5000 ml; n = 4) were collected from the fore reef area in 10 m water depth on three dates during each of the seasonal expeditions. Sampling containers were immediately transferred to the laboratory where samples for dissolved organic carbon (DOC) analysis were prepared. Sterile syringes were used to collect 10 ml subsamples from each sampling container. These samples were

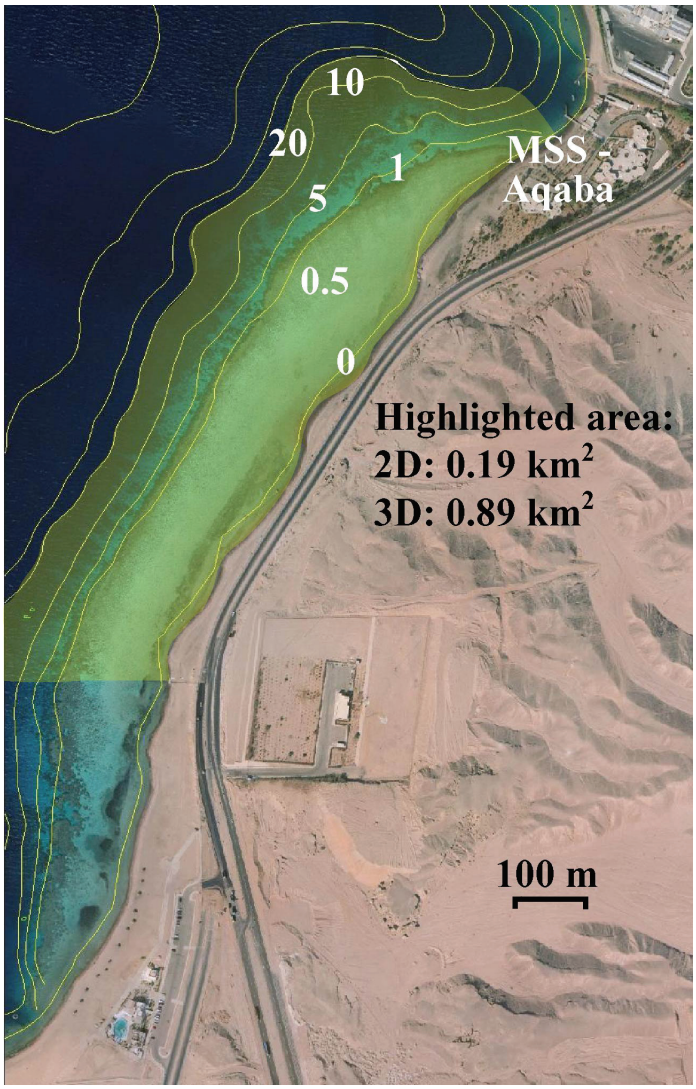

Fig. 1 *Air-borne photography of the study area (highlighted in yellow) representing the area between 0 – 20 m water depth of a fringing reef close to the Marine Science Station Aqaba (MSS) bordering the Jordanian Red Sea coast line. Numbers indicate the respective water depths. Photograph generated by ASEZA GIS Unit (R. Mobiedeen), Aqaba, Jordan.*

Table 1 *Percentage coverage by reef substrates types within the study site. Abbreviation: LCC = Live hermatypic coral coverage.*

Substrate	**Benthic coverage (%)**
Live hermatypic coral coverage	40
Dominant genera (n = 6)	*45 (of LCC)*
Non-dominant genera (n = 62)	*55 (of LCC)*
Macro-algae	16
Bare coral rock	12
Sand	28
Other	4

subsequently filtered through 0.2-µm-pore-sized sterile polyethersulfone membrane filters. The initial 5 ml of sample volume were used to wash the filter membrane discarding the filtrate, while the remaining sample volume (5 ml) was filtered at low pressure into pre-combusted (450°C; 4 h) amber glass vials. DOC samples were kept frozen at –20°C until analysis by high-temperature catalytic oxidation (HTCO) using a Rosemount Dohrmann DC-190 TOC analyser. The concentration of non-purgable organic C was measured, after sample acidification with orthophosphoric acid (20 %) to pH < 2 and O_2 sparging, using potassium hydrogen phthalate as an elemental standard (standard deviation <3%).

Subsamples (1000 ml) for particulate organic carbon (POC) analysis were filtered onto pre-combusted (450°C; 4 h) GF/F filters (Whatman™, 25 mm diameter), subsequently dried for at least 48 h at 40°C and analysed for POC content using a Thermo™ NA 2500 elemental analyser (elemental standards: atropine, cyclohexanone-2,4-dinitrophenylhydrazone; Thermo Quest; standard deviation < 3 %).

Pelagic metabolism

Three subsamples from each seawater sampling container were used to determine primary production (PP) and microbial oxygen consumption in reef-overlying waters. The initial dissolved oxygen concentration was measured in the first subsample using an optical dissolved O_2 sensor (Hach Lange HQ10, accuracy ± 0.05 %). The second and third subsamples were filled into 60 ml gas-proof Winkler glass bottles, of which one was incubated during the day light period (8 to 12 h) at in-situ light and temperature conditions monitored by data loggers, while the other was kept in the dark at in-situ temperature for 16 to 24 h. O_2 concentration was measured at the end of the incubation periods as described above and the difference was used to calculate PP and microbial O_2 consumption rates.

Benthic metabolism of reef sand communities

Benthic PP and microbial O_2 consumption in calcareous reef sands was assessed during 2 expeditions (fall and summer) by measuring O_2 dynamics in seawater enclosed by stirred benthic chambers as described in Wild et al. (2005b, 2009). Briefly, between 1 and 4 stirred benthic chambers were used in 7 independent chamber experiments carried out at water depths of 2.5 to 16.5 m. Transparent (PP measurements) and opaque (respiration measurements) acrylic chambers were inserted into calcareous reef sands to a depth of about 12 cm. The volume inside the chambers was 5.7 l and incubations lasted for 5 to 8 h.

At pre-set time intervals (30 to 120 min), water samples (60 ml) were collected from the chambers through sampling ports for later analyses of oxygen concentrations. Fixation of the samples took place within 15 min after collection. O_2 concentration was measured by Winkler titration within 1 h after fixation. PP and O_2 consumption rates by the benthic communities were determined by linear regression of O_2 concentrations over time (4 data points per chamber) and finally related to the water volume and sediment surface enclosed by the chambers.

Sedimentation of particulate organic carbon

To determine the amount of POC settling to the seafloor, sediment traps were deployed in triplicates at 1, 5, 10 and 20 m water depth during each of the seasonal expeditions. Traps were designed and used as described in Wild et al. (2009). All traps (total: n = 48) were placed on reef sands with their opening at a height of 7.5 cm above the seafloor.

Traps were deployed for 48 h, after which the collected contents were prepared for POC analysis by filtering the particulate material onto pre-combusted GF/F filters. Samples were subsequently dried for 48 h at 40°C and exposed to a fuming HCl (1 N) atmosphere for 24 h to remove inorganic C contents, before they were analysed using a Thermo™ NA 2500 elemental analyser. POC contents of all sediment traps were finally related to the trapping area (i.e. trap opening in m^2) and time of deployment to generate POC sedimentation rates.

Organic C release by hermatypic corals

Quantification of organic C release by the identified 6 dominant hermatypic coral genera was carried out using the established laboratory beaker incubation technique (Herndl & Velimirov 1986, Wild et al. 2005a) following the modified procedure described in Naumann et al. (submitted).

Briefly, fragments from 5 different coral colonies of each of the dominant coral genera or 5 individual *Fungia* polyps were collected from 5 m water depth using SCUBA during each seasonal expedition. Additionally, specimens of the genera *Acropora* and *Fungia* were sampled from 1, 10 and 20 m water depth in replicates of 5 during spring and winter expeditions, respectively.

Corals (n = 5) were incubated in 1000 ml beakers filled with fresh seawater, in parallel with seawater controls (n = 5), for 6 h during daylight (10:00–16:00). Two additional night time incubations (20:00–02:00) of *Acropora* and *Fungia* corals originating from 5 m water depth were conducted to assess organic C release during the night. In situ conditions (light availability and temperature) comparable to the original water depth of the incubated corals were ensured during incubations by comparative measurements using data loggers (Onset HOBO® Pendant UA-002-64) and application of variable layers of black plastic gauze.

Following incubation, corals were removed from the beakers and water samples for POC and DOC analysis were prepared and processed as described above for seawater samplings. Quantification of the skeleton surface area of all incubated corals was carried out by application of geometric techniques and specific approximation factors as described in Naumann et al. (2009b).

POC and DOC release rates per coral surface area and incubation time were calculated by subtraction of control beaker contents from those measured in the incubation water of the beakers containing corals. The resulting net POC and DOC concentrations of coral incubations were subsequently normalised using the measured coral specific skeleton surface area and incubation time to generate POC and DOC release rates.

Degradation of coral-derived organic C

Pelagic microbial degradation

Microbial degradation of coral-derived organic C in reef waters was measured as described by Wild et al. (in press) using dark incubation of seawater subsamples (ca. 140 ml) taken from each individual beaker at the end of all conducted coral beaker incubations. For every subsample, 2 gas-proof glass bottles (volume: 60 ml) were filled. The initial O_2 concentration in one of the bottles was measured using Winkler titration or a Hach HQ 10 optode. The second bottle was incubated for at least 16 h in the dark at in-situ temperature, after which the O_2 concentration of the enclosed water was determined as described above. O_2 consumption by microbial activity in the incubation water was calculated by subtracting final from initial concentration.

Pelagic microbial degradation of the added coral-derived organic C was calculated using the respective POC + DOC amount and the increase in O_2 consumption in coral-derived organic C containing bottles relative to controls (assuming that 1 mol added organic C was oxidized by 1 mol O_2).

Benthic degradation in reef sands

Degradation of coral-derived organic C in calcareous reef sands was studied in-situ using opaque stirred benthic chambers (see above) following the procedure detailed in Wild et al. (in press). Briefly, experiments were carried out at a sandy reef site (water depth: 2.5 m) described in Wild et al. (2005b). At the beginning of 2 independent experiments, coral-derived organic C (60 and 77 µmol, respectively) was added to half of the opaque chambers via a sampling port, while the remaining chambers served as controls for bulk organic C degradation in reef sands. The duration of the individual chamber experiments ranged between 5 to 8 h. Water samples (60 ml) were taken from each chamber at least every 2 h. O_2 concentrations in the chamber water were measured by Winkler titration and benthic degradation rates of the added coral-derived organic C were calculated as described above after subtraction of bulk benthic degradation rates derived from control chambers.

Calculations

Reef surface area and water column volume

The projected area coverage of the studied reef area between 0 and 20 m water depth (ca. 0.19 km^2) was derived from digital analysis of an air-borne photograph including a bathymetric profile (Fig. 1) using the software *ImageJ* (V. 1.37m, National Institutes of Health, USA). From this, the 3D outer reef surface area was estimated applying an averaged approximation factor (6.59) for 2D to 3D coral surface area conversion (Naumann et al. 2009b). The approximation factor was only applied to the projected area covered by the reef framework, which had priory been derived from the percentage benthic coverage assessed by LPI surveys as described above. The amount of sand covered areas assessed by benthic survey data was subsequently added as 2D area to gain the total 3D outer reef surface area estimate (ca. 0.89 km^2), which was 4.8-fold increased compared to the projected area.

The total volume of the water column overlying the reef (ca. 935 x 10^6 l) was estimated stepwise by multiplying the 2D area enclosed by 2 alternating depths of the bathymetric profile (Fig. 1) with the average of the enclosing water depths. The resulting water volumes above the particular reef sections (0–1, 1–5, 5–10 and 10–20 m) were subsequently summed to yield the total water column volume in between 0 and 20 m water depth.

Reef-wide organic C release by hermatypic corals

Daytime depth-mediated differences and variations in POC release rates during day and night found for *Acropora* and *Fungia* corals (Naumann et al. submitted) were used to generate conversion factors, which were applied to estimate POC release of the remaining investigated dominant coral genera (sampled from 5 m depth) for day and night time conditions at 1, 10 and 20 m water depth during all seasons. As seasonal POC release rates showed no significant differences (Naumann et al. submitted), release rates calculated for all investigated water depths and seasons were averaged and an overall POC release rate coral surface area^{-1} and h^{-1} was determined for each investigated genus from day and night time values. The mean of all genus-specific overall POC release rates was subsequently used as an estimate for the release of by non-dominant coral genera occurring in the study area.

As simultaneous DOC uptake during coral beaker incubations could not be ruled out (Ferrier et al. 1998), DOC release rates for all dominant and non-dominant coral genera were derived from POC release rates by calculation applying the DOC:POC dissolution factor for released coral mucus (i.e. 2.1) described in Wild et al. (2004a). All above specified POC release rates (genus- depth- and daytime specific) were multiplied by this factor and the resulting DOC release rates were further averaged to overall DOC release coral surface area^{-1} and h^{-1} as described for POC (see above). Finally, genus-specific overall POC and DOC release rates were summed to yield the total organic carbon (TOC) release rates for the respective dominant and non-dominant coral genera.

To estimate the overall organic C (POC, DOC and TOC) release by all hermatypic corals in the study area, the depth-specific 2D coverage of each dominant coral taxon and the non-dominant coral taxa was averaged to a reef-wide mean and subsequently converted to 3D using growth form specific approximation factors (Naumann et al. 2009b). For the non-dominant genera, the percentage contribution by different growth forms was considered. This reef-wide 3D surface area for each coral taxon was multiplied with the priory generated specific overall POC, DOC and TOC release rates and finally summed including all coral taxa to yield the reef-wide overall organic C release by hermatypic corals.

Results

Ecosystem organic C dynamics

TOC (POC + DOC) concentrations in seawater were highly variable ranging from 67 – 490 µmol C l^{-1} during the seasonal samplings (Table 2). Taking into account the approximate total water column volume, the reef-wide amount of seawater TOC was determined as 62 – 458 kmol C with an

estimated average TOC pool of 162 ± 118 kmol. Pelagic net PP was on average 35 ± 19 µmol C l^{-1} d^{-1} (mean ± SE), while planktonic microbial O_2 consumption in reef waters ranged from 4 – 15 µmol l^{-1} d^{-1} (Table 2). Considering the total water column volume, this corresponds to a daily reef-wide estimated pelagic net PP and microbial O_2 consumption of 33 ± 18 and 9 ± 3 kmol C, respectively. Pelagic organic C turnover rates calculated from TOC contents and planktonic microbial O_2 consumption rates ranged from 0.1 – 0.7 % h^{-1}.

Reef sands showed bulk microbial O_2 consumption rates of 18.6 – 26.6 mmol m^{-2} sediment surface area d^{-1} (Table 2), which under consideration of the total reef sand area accounted for 1.4 ± 0.1 kmol C on daily reef-wide scale. Net PP in reef sands equally accounted for 1.4 ± 0.7 kmol C d^{-1} reef-wide with seasonal rates ranging from 4.4 – 44.8 mmol m^{-2} sediment surface area d^{-1}. Sedimentation of bulk POC to the reef floor was 0.7 – 1.5 mmol POC m^{-2} 3D reef area d^{-1} (Table 2) corresponding to 0.6 – 1.3 kmol POC d^{-1} (mean: 0.9 ± 0.2) regarding the reef-wide 3D reef floor area.

Table 2 *Pelagic and benthic ecosystem organic C dynamics. Values are given as means ± SE. Abbreviations: POC = particulate organic carbon, TOC = total organic carbon.*

	Pelagic				**Benthic (reef sands)**		
	TOC content (µmol l^{-1})	**Primary production** (µmol l^{-1} d^{-1})	**Planktonic microbial O_2 consumption** (µmol l^{-1} d^{-1})	**TOC turnover** (% h^{-1})	**Primary production** (mmol m^{-2} d^{-1})	**O_2 consumption** (mmol m^{-2} d^{-1})	**POC sedimentation** (mmol m^{-2} d^{-1})
	67 – 490	8 – 74	4 – 15	0.1 – 0.7	4.4 – 44.8	18.6 – 26.6	0.7 – 1.5
Mean	173 ± 127	35 ± 19	10 ± 3	0.3 ± 0.2	22.8 ± 11.7	23.5 ± 2.3	1.1 ± 0.3

Organic C release by hermatypic corals

Genus-specific organic C release rates (POC, DOC and TOC) by the dominant and non-dominant hermatypic coral genera are presented in Table 3. As described in detail by Naumann et al. (submitted), significant differences in POC release rates exist on a genera-wide basis. These differences consequently also concern the presented DOC and TOC release rates shown in Table 3, which are derived from calculation using POC values. Based on these taxon-specific data, Table 4 displays the results from reef-wide daily organic C release estimations for the dominant and non-dominant hermatypic corals in the study area. The genera *Acropora* and *Stylophora* can be identified as the main contributors of coral-derived organic C by releasing 16 to 90-fold more than the remaining dominant genera. Non-dominant coral genera are responsible for approximately 50 % of the overall release reflecting their 55 % area share of live coral coverage (see Table 1). The calculation derived overall daily estimate for organic C release by hermatypic corals accounts for 4.1 ± 1.9 and 8.7 ± 4.0 kmol C, for POC and DOC respectively, consequently adding up to a reef-wide daily TOC release of 12.8 ± 6.0 kmol C (mean ± SE).

Table 3 *Organic C release rates (POC, DOC and TOC), normalized to coral surface area and time, for the dominant and non-dominant hermatypic coral genera occurring within the study site. Abbreviations: POC = particulate organic carbon, DOC = dissolved organic carbon, TOC = total organic carbon.*

Coral	**POC (µmol m^{-2} coral surface area h^{-1})**	**DOC (µmol m^{-2} coral surface area h^{-1})**	**TOC (µmol m^{-2} coral surface area h^{-1})**
Dominant			
Acropora	377 ± 288	801 ± 611	1178 ± 899
Fungia	141 ± 65	300 ± 139	441 ± 204
Goniastrea	227 ±147	482 ± 312	709 ± 458
Millepora	54 ± 35	115 ± 74	169 ± 109
Pocillopora	540 ± 349	1147 ± 742	1687 ± 1091
Stylophora	1041 ± 673	2213 ± 1431	3254 ± 2104
Non-dominant / Mean	397 ± 360	843 ± 765	1240 ± 1125

Table 4 *Reef-wide daily organic C (POC, DOC and TOC) release estimates for the dominant and non-dominant hermatypic coral genera in the study area. Abbreviations: POC = particulate organic carbon, DOC = dissolved organic carbon, TOC = total organic carbon.*

Coral	**POC release (mol d^{-1})**	**DOC release (mol d^{-1})**	**TOC release (mol d^{-1})**
Dominant			
Acropora	999 ± 762	2,122 ± 1,619	3,121 ± 2,380
Fungia	12 ± 6	26 ± 12	38 ± 17
Goniastrea	42 ± 27	89 ± 58	131 ± 85
Millepora	59 ± 38	125 ± 81	183 ± 118
Pocillopora	11 ± 7	23 ± 15	34 ± 22
Stylophora	971 ± 628	2,064 ± 1,334	3,035 ± 1,962
Non-dominant	2,015 ± 1,828	4,281 ± 3,885	6,269 ± 5,713
Sum	**4,108 ± 1,904**	**8,703 ± 4,046**	**12,839 ± 5,950**

Coral-derived organic C dynamics

Calculation-based results revealed that coral-derived TOC contributes 3 – 8 % (12.8 ± 6.0 kmol) to the reef-wide pelagic TOC pool (162 ± 118 kmol) on a daily basis. This contribution accounts for 30 – 39 % of daily pelagic net PP and exceeds reef-wide benthic net PP in reef sand communities by more than 8-times (Fig. 2). Pelagic microbial degradation measurements showed a rapid turnover of coral-derived organic C in reef waters ranging from 0.2 – 21.5 % h^{-1} (mean: 8 ± 6 %), accounting for 0.1 – 4.6 % of total planktonic microbial O_2 consumption. Considering the reef-wide organic C release by hermatypic corals, this turnover rate results in the pelagic degradation of approximately 0.7 kmol C d^{-1} (5 % of overall release) (Fig. 2).

Benthic degradation of coral-derived organic C in reef sands calculated from microbial O_2 consumption rates accounted for 6.3 – 10.8 mmol C m^{-2} sediment surface area d^{-1} with degradation rates averaging 9.9 ± 0.1 % h^{-1}. From this, a daily reef-wide turnover of coral-derived organic C in

reef sands ranging from 0.4 – 0.6 kmol C (4 % of overall release) could be extrapolated. This turnover of coral-derived organic C represents a substantial contribution of 34 to 41 % (0.5 kmol C d^{-1}) to the bulk daily O_2 consumption within the reef sands (Fig. 2). Taking into account results from sediment trap analyses conducted by Mayer et al. (submitted) on reef sands in the study area, the contribution of coral-derived POC (5 – 67 %) to bulk POC sedimentation (0.9 ± 0.2 kmol d^{-1}) was calculated as 0.5 kmol C d^{-1} on reef-wide scale (Fig. 2). This accounts for approximately 12 and 4 % of the daily coral-derived POC and TOC release, respectively.

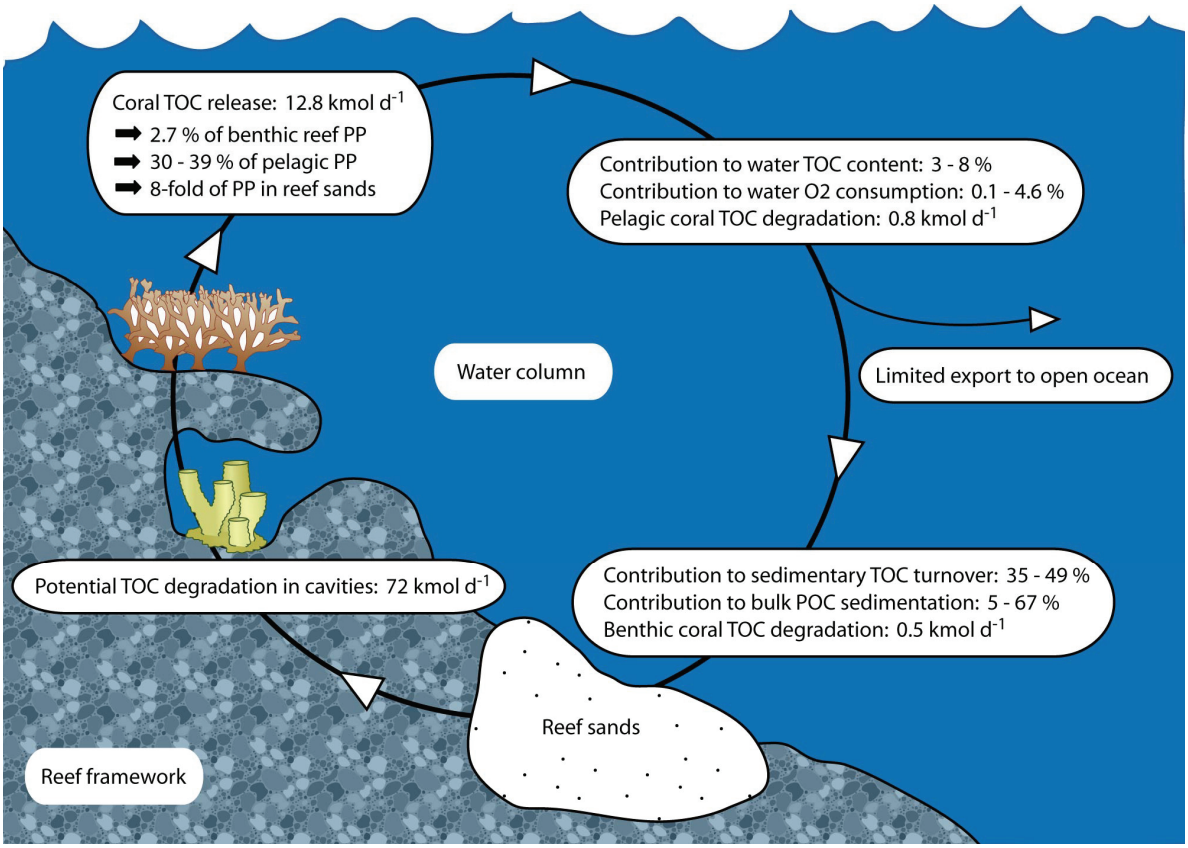

Fig. 2 *Schematic description of coral-derived organic C cycling in a Red Sea fringing reef ecosystem. Abbreviations: PP = Primary production, TOC = total organic carbon*

Discussion

Contribution of coral-derived C to reef ecosystem organic C dynamics

This study provides the first quantitative estimation of reef ecosystem-wide organic C release by hermatypic corals based on a comprehensive data set of organic C release rates by the dominant coral genera. Further, results from coral-derived organic C degradation experiments and concomitant assessment of pelagic and benthic organic C metabolism allow for estimations regarding its reef-wide metabolic contribution. The results show that organic C release by hermatypic corals can contribute substantially (up to 8 %) to the organic C pool of the investigated

fringing reef system (Fig. 2). Notably, this substantial contribution reflects the comparably high live coral coverage (ca. 40 %) in the study area. Taking into account average benthic net PP rates of coral reefs (reviewed in Hatcher 1990), the estimated reef-wide TOC release by hermatypic corals represents 2.7 % of benthic net PP. These findings are in line with Johannes (1967), who found that the export of coral-derived material was 2 % of reef community production at Eniwetok Atoll. This suggests a similar contribution of coral-derived organic material to organic C cycling in different hermatypic coral dominated Indo-Pacific reef types.

The mentioned idea is further supported by the contribution of coral-derived organic C to bulk seawater O_2 consumption (0.1 – 4.6 %), which is in the range of results by Wild et al. (2004a) who found a contribution of 0.1 – 2.5 % by coral mucus in lagoon waters of the platform reef system at Heron Island. For this site, Wild et al. (2004a) measured a contribution of 10 – 20 % to microbial O_2 consumption in reef lagoon sediments via particulate coral mucus aggregates. The higher contribution by coral-derived organic C found here (34 – 41 %) possibly reflects the inclusion of coral-derived DOC compounds by the present study and highlights the general validity of rapid degradation of coral-derived organic C in calcareous reef sands as already indicated by the studies of Wild et al. (2005a, b).

Calcareous reef sands thus represent important locations for the microbial degradation of coral-derived organic C due to their high abundance of associated heterotrophic bacteria (Wild et al. 2006). The results of the present study indicate that up to 4 % of the daily estimated 12.8 kmol coral-derived organic C released can be degraded within the reef sands (Fig. 2). Wild et al. (2005b) and Mayer et al. (submitted), working in the study site, described the predominant (95 % of all coral-derived mucus aggregates) short-range (< 120 cm distance to producing coral colony) sedimentation of coral-derived POC aggregates onto reef sediments with subsequent rapid benthic microbial degradation. These findings suggest that the fraction of coral-derived organic C degraded in sandy reef sediments (0.5 kmol C d^{-1}) is principally composed of coral-derived POC, which is supported by the reef-wide average coral-derived POC sedimentation rate of 0.5 kmol C d^{-1}.

Results from pelagic microbial degradation measurements revealed a daily turnover of approximately 5 % (0.7 kmol C d^{-1}) of the organic C released by hermatypic corals reef-wide, adding a similar amount compared to reef sediments (4 %) to total coral-derived organic C degradation. Consequently, the fate of a large proportion (about 91 %) of the released organic C remains to be explained by other processes prevailing within the study area. This will be discussed in the following.

Fate of coral-derived organic C in a fringing reef ecosystem

Besides the reef-overlying water column and sandy reef sediments, the reef framework harbours a divers community of organisms highly specialized in the capture and consumption of POC and DOC compounds (Fabricius et al. 1995, Ferrier et al. 2000, Houlbrèque et al. 2004). The bioactive surface area of the reef framework is composed of the outer exposed part and the area contributed by inner cavities within the framework (Richter et al. 2001). Hermatypic corals themselves, while dominantly dwelling on the outer exposed reef surface, are also able to take up POC (Anthony 1999) and DOC (Ferrier et al. 1998) to cover their metabolic demands. However, as organic C

release calculations carried out here are based on net POC and DOC release rates by corals measured in closed incubation systems, this potential uptake of organic C has already been considered.

The inner cavity surface of coral reef frameworks is densely populated by a divers community of suspension feeding organisms including cavity-dwelling sponges (Richter et al. 1999; Wunsch et al. 2002), covering up to 60 % of crevice surfaces (Richter et al. 2001). These communities remove substantial amounts of suspended organic material, which is constantly transported from free-stream waters into the cavity system by rapid water exchange rates (Richter et al. 2001). Sponges as dominant cavity-dwelling taxa have been identified as efficient POC and DOC consumers (Richter & Wunsch 1999; de Goeij et al. 2007, 2008a, b) providing an explanation for the finding of reef framework cavities as major sinks for organic C. Applying the factor (6.375) derived from comparison of the projected reef area to measured 3D cavity surface area provided by Richter et al. (2001) for the study area, results show that cavities represent 97 % of the 3D outer reef surface area estimated here. Further taking into account TOC removal rates measured by de Goeij et al. (2007) in Indo-Pacific reef cavities (90 ± 45 mmol m^{-2} cavity area d^{-1}) exhibiting a comparable sponge coverage compared to the site of the present study (de Goeij personal communication), calculations arrive at an estimated TOC uptake of 72 kmol C d^{-1} by the cavity framework of the investigated fringing reef (Fig. 2). This uptake accounts for approximately 44 % of the entire water column TOC pool and is more than 5-fold of the daily reef-wide coral-derived organic C release, thereby clearly demonstrating the potential of the inner reef framework community for the efficient uptake of coral-derived organic C from reef waters and the associated contribution to the retention of this material within the ecosystem.

Export of coral-derived organic C to oceanic waters by currents within the reef-overlying water column represents a possible antagonistic aspect to the retention of coral-derived organic C by the reef framework community. However, with respect to low water current velocities (mean: 5 cm s^{-1}) in the study area (Manasrah et al. 2006) and the calculated residence time of water over the reef (mean: 3 h), the comparably rapid transport of water through framework cavities (within minutes; Richter et al. 2001) combined with substantial removal rates for organic matter (64 % of chlorophyll *a*; Richter et al. 2001) suggests only a limited export of coral-derived TOC to oceanic waters.

The efficient uptake by cavity-dwelling sponges and framework cavity communities in general, may thus provide a substantial sink for coral-derived organic C and lead to a complete retention of this material within the reef ecosystem. Additional investigations, e.g. applying stable isotope labelling of coral mucus (Naumann et al. submitted) in in-situ tracer experiments, are necessary to elucidate the possible contribution of cavity-dwelling sponges to coral-derived organic C degradation and other potential trophic pathways describing the fate of this material in fringing reef ecosystems.

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References

Anthony KRN (1999) Coral suspension feeding on fine particulate matter. J Exp Mar Biol Ecol 232:85–106

Charpy L, Charpy-Roubaud CJ (1991) Particulate organic matter fluxes in a Tuamotu atoll lagoon (French Polynesia). Mar Ecol Prog Ser 71:53–63

Coles S, Strathman R (1973) Observations on coral mucus "flocs" and their potential trophic significance. Limnol Oceanogr 18:673–678

Crossland CJ (1987) In situ release of mucus and DOC-lipid from the corals *Acropora variabilis* and *Stylophora pistillata* in different light regimes. Coral Reefs 6:35-42

de Goeij JM, van Duyl FC (2007) Coral cavities are sinks of dissolved organic carbon (DOC). Limnol Oceangr 52: 2608–2617

de Goeij JM, van den Berg H, van Oostveen MM, Epping EHG, van Duyl FC (2008a) Major bulk dissolved organic carbon (DOC) removal by encrusting coral reef cavity sponges. Mar Ecol Prog Ser 357:139–151

de Goeij JM, Moodley L, Houtekamer M, Carballeira NM, van Duyl FC (2008b) Tracing ^{13}C-enriched dissolved and particulate organic carbon in the bacteria-containing coral reef sponge *Halisarca caerulea*: Evidence for DOM feeding. Limnol Oceangr 53: 1376–1386

Fabricius KE, Genin A, Benayahu Y (1995) Flow-dependent herbivory and growth in zooxanthellae-free soft corals. Limnol Oceanogr 40:1290–1301

Ferrier-Pages C, Leclercq N, Jaubert J, Pelegri SP (2000). Enhancement of pico- and nanoplankton growth by coral exudates. Aquat Microb Ecol 21:203–209

Ferrier-Pages C, Gattuso JP, Cauwet G, Jaubert J, Allemand D (1998) Release of dissolved organic carbon and nitrogen by the zooxanthellate coral *Galaxea fascicularis*. Mar Ecol Prog Ser 172:265–274

Gordon DC (1971) Organic carbon budget of Fanning Island lagoon. Pac Sci 25:222-227

Hata H, Kudo S, Yamano H, Kurano N, Kayanne H (2002) Organic carbon flux in Shiraho coral reef (Ishigaki Island, Japan). Mar Ecol Prog Ser 232: 129–140

Hatcher BG (1990) Coral reef primary productivity: a hierarchy of pattern and process. Tree 5:149–155

Herndl GJ, Velimirov B (1986) Microheterotrophic utilization of mucus released by the Mediterranean coral *Cladocora cespitosa*. Mar Biol 90:363–369

Houlbrèque F, Tambutté E, Richard C, Ferrier-Pagès C (2004) Importance of a micro-diet for scleractinian corals. Mar Ecol Prog Ser 282:151–160

Huettel M, Wild C, Gonelli S (2006) Mucus trap in coral reefs: formation and temporal evolution of particle aggregates caused by coral mucus. Mar Ecol Prog Ser 307:69–84

Johannes R (1967) Ecology of organic aggregates in the vicinity of a coral reef. Limnol Oceanogr 12:189–195

Krupp DA (1985) An immunochemical study of the mucus from the solitary coral *Fungia scutaria* (Scleractinia, Fungiidae). Bull Mar Sci 36:163–176

Manasrah RS, Al-Horani FA, Rasheed MY, Al-Rousan SA, Khalaf MA (2006) Patterns of summer vertical and horizontal currents in coastal waters of the northern Gulf of Aqaba, Red Sea. Estuar Coast Shelf Sci 69: 567 579

Marshall AT, Wright OP (1993) Confocal laser scanning light microscopy of the extra-thecal epithelia of undecalcified scleractinian corals. Cell Tissue Res 272:533–543

Meikle P, Richards GN, Yellowlees D (1987) Structural determination of the oligosaccharide side-chains from a glycoprotein isolated from the mucus of the coral *Acropora formosa*. J Biol Chem 262:16941–16947

Mergner H, Schuhmacher H (1974) Morphologie, Ökologie and Zonierung von Korallenriffen bei Aqaba, (Golf von Aqaba, Rotes Meer). Helgol Wiss Meeresunters 26:238–358

Muscatine L, Porter JW (1977) Reef corals-mutualistic symbioses adapted to nutrient-poor environments. BioScience 27:454–460

Naumann MS, Richter C, el-Zibdah M, Wild C (2009a) Coral mucus as an efficient trap for picoplanktonic cyanobacteria – implications for pelagic-benthic coupling in the reef ecosystem. Mar Ecol Prog Ser 385:65–76

Naumann MS, Niggl W, Laforsch C, Glaser C, Wild C (2009b) Coral surface area quantification – evaluation of established methods by comparison with computer tomography. Coral Reefs 28:109–117

Odum HT, Odum EP (1955) Trophic structure and productivity of a windward coral reef community on Eniwetok atoll. Ecol Monogr 25:291–320
Richter C, Wunsch M (1999) Cavity-dwelling suspension feeders in coral reefs a new link in reef trophodynamics. Mar Ecol Prog Ser 188:105–116
Richter C, Wunsch M, Rasheed M, Kötter I, Badran MI (2001) Endoscopic exploration of Red Sea coral reefs reveals dense populations of cavity-dwelling sponges. Nature 413:726–730
Ritchie KB (2006) Regulation of microbial populations by coral surface mucus and mucus-associated bacteria. Mar Ecol Prog Ser 322:1–14
Schuhmacher H (1977) Ability of fungiid corals to overcome sedimentation. Proc 3rd Int Coral Reef Symp 1:503–509
Wild C, Huettel M, Klueter A, Kremb SG, Rasheed M, Jørgensen BB (2004a) Coral mucus functions as an energy carrier and particle trap in the reef ecosystem. Nature 428:66–70
Wild C, Rasheed M, Werner U, Franke U, Johnstone R, Huettel M (2004b) Degradation and mineralization of coral mucus in reef environments. Mar Ecol Prog Ser 267:159–171
Wild C, Woyt H, Huettel M (2005a) Influence of coral mucus on nutrient fluxes in carbonate sands. Mar Ecol Prog Ser 287:87–98
Wild C, Rasheed M, Jantzen C, Cook P, Struck U, Boetius A (2005b) Benthic metabolism and degradation of natural particulate organic matter in carbonate and silicate reef sands of the Northern Red Sea. Mar Ecol Prog Ser 298:69–87
Wild C, Laforsch C, Huettel M (2006) Detection and enumeration of microbial cells within highly porous calcareous reef sands. Mar Freshw Res 57:415–420
Wild C, Naumann MS, Haas A, Struck U, Mayer FW, Rasheed MY, Huettel M (2009) Coral sand O_2 uptake and pelagic–benthic coupling in a subtropical fringing reef, Aqaba, Red Sea. Aquat Biol 6:133–142
Wunsch MS, Al-Moghrabi M, Kötter I (2002) Communities of coral reef cavities in Jordan, Gulf of Aqaba (Red Sea). Proc 9th Int Coral Reef Symp 1:595–600

6

Coral mucus as an efficient trap for picoplanktonic cyanobacteria - implications for pelagic-benthic coupling in the reef ecosystem

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Abstract

Although the planktonic community of coral reefs is dominated by picoplankton (e.g. the cyanobacterium *Synechococcus*), it was long believed to play only a marginal role in pelagic-benthic coupling, as its minute size (0.2 – 2.0 µm) and negligible sinking rate render it largely unavailable for the filter-feeding reef benthos. However, scleractinian corals have been shown to continuously release mucus that functions as an efficient trap and important carrier for particulate matter within the reef ecosystem. This study investigates the trapping potential of coral mucus for picoplankton in the laboratory and in the field. Freshly released mucus of *Fungia* corals already contained background levels of pelagic and/or associated synechococcoid cyanobacteria ($1.0 \pm 0.2 \times 10^4$ cells ml^{-1}). Mesocosm experiments in flow-through tanks revealed up to 46-fold picoplankton enrichment in aged mucus aggregates, while laboratory experiments with rotated chambers confirmed the pelagic source of these mucus enrichments. Addition of coral mucus resulted in a significant increase in clearance rates (32 – 52% h^{-1}) of the initial *Synechococcus* population compared to clearance found in non-mucus chambers (6 – 18% h^{-1}). Drifting mucus aggregates originating from *Acropora* corals collected *in situ*, exhibited high *Synechococcus* enrichment (up to 4.6×10^6 cells ml^{-1}) compared to the surrounding seawater ($2.1 \pm 0.8 \times 10^4$ cells ml^{-1}), indicating efficient picoplankton enrichment by two orders of magnitude. The ensuing rapid sedimentation (0.5 – 1 cm s^{-1}) of enriched aggregates highlights the importance of coral mucus as a so far overlooked vector enhancing the flux of pelagic picoplankton to the coral reef benthos.

Introduction

Most of the world's pelagic ocean regimes are dominated by unicellular picoplankton organisms (0.2 – 2.0 µm cell size) (Sieburth et al. 1978) known to constitute a substantial fraction of planktonic biomass and primary production (Li et al. 1983, Owens et al. 1993, Tremblay & Legendre 1994). Oligothrophic waters around coral reefs are characterised by a picoplankton community, principally composed of phototrophic cyanobacteria, heterotrophic bacteria and a diverse group of picoeukaryotes (e.g. Ribes et al. 2003). Of those, heterotrophic bacteria generally dominate by one order of magnitude in terms of cell abundance (Weisse 1989, Gradinger et al. 1992, Ribes et al. 2003), while unicellular cyanobacteria dominate picoplankton biomass (Ducklow 1990). In Red Sea waters, picoplanktonic cyanobacteria (*Synechococcus* and *Prochlorococcus* (Johnson & Sieburth 1979)) represent 69 % of planktonic chlorophyll a and 77 % of planktonic primary production (Pillen & Moigis, cited in Lenz et al. 1988) reaching maximum abundances in the range of 2.6 x 10^5 *Synechococcus* cells ml^{-1} during bloom seasons (Lindell & Post 1995). In spite of the widely recognised importance of picoplankton within marine ecosystems as biomass pool and energy source, the role of picoplankton in coral reef pelagic-benthic coupling is so far little understood. Due to their minute size, picoplankton cells contribute little to the vertical particle flux (Pedros-Alio et al. 1989, Richardson & Jackson 2007) and thus remain largely inaccessible to the benthos. Although active suspension feeders (e.g. sponges) are known to thrive on this minute particulate food (Richter et al. 2001), there is growing evidence that also passive suspension feeders such as scleractinian (Ribes et al. 2003, Houlbrèque et al. 2004) and soft corals (Fabricius et al. 1995) are able to tap the stream of picoplankton across the reef, yet a plausible mechanistic explanation for picoplankton uptake by passive suspension feeders is still wanting. Significant removal of picoplankton from water flowing across coral reefs has been reported in several studies (Ayukai 1995, Ribes et al. 2003, Houlbrèque et al. 2006). Ayukai (1995) suggested in this context, that the observed decrease in picoplankton might partially be affected by aggregate forming transparent exopolymers (i.e. mucoid exudates) released by the benthic reef community. Several benthic reef species are known to utilize mucoid exudates for feeding purposes, e.g. the vermetid gastropod *Dendropoma maxima* (Kappner et al. 2000) or the scleractinian coral *Mycetophyllis reesi* (Goldberg 2002).

Mucoid exudates of scleractinian corals and their aged contaminated aggregation stages represent a potential trophic- and nutrient-recycling pathway in coral reef ecosystems acting as efficient traps and aggregation media for suspended particulate matter including small plankton organisms (Johannes 1967, Wild et al. 2004a, Wild et al. 2004b, Wild et al. 2005a). However, microscopic investigations concerning particle enrichment of coral mucus aggregates carried out thus far disregarded the possible enrichment with entrapped picoplankton. Other authors have reported on the entrapment of picoplankton-sized particles and cells into organic aggregates, followed by a rapid vertical transport to greater depth (Noji et al. 1997). Organic aggregates sampled from 120 m depth within the Subtropical Front off New Zealand show high contents of embedded picophytoplankton and high vertical mass fluxes to this depth (Waite et al. 2000). As shown for deep epipelagic environments, entrapment of picoplankton and subsequent vertical transport of picoplankton-enriched coral mucus aggregates may represent a potential trophodynamic

pathway linking the picoplankton-rich pelagic compartment to the benthos in shallow reef ecosystems.

The goal of this study was to investigate the potential of scleractinian coral mucus to act as an efficient trap for picoplankton, thus forming picoplankton-enriched mucus aggregates enhancing the availability of this otherwise elusive biomass for reef dwelling organisms. For this purpose, trapping efficiency of, and picoplankton enrichment in, coral mucus aggregates was tested in three different experimental approaches (rotated chamber incubations, mesocosm experiments and *in situ* samplings of coral mucus aggregates) utilising the picoplanktonic cyanobacterium *Synechococcus* as a representative of picoplankton.

Material and Methods

Study site

The present study was conducted at the Marine Science Station Aqaba, Jordan (latitude: 29° 27′ N, longitude: 34° 58′ E), situated approximately 10 km south of the city of Aqaba with exclusive access to a marine reserve featuring a typical Red Sea fringing reef. All laboratory and field work was carried out in local facilities and adjacent reef sites in the study period October 2004 until March 2005.

Coral samples

Individuals of the solitary mushroom coral *Fungia* (n = 21) were collected over several days from 4 different locations within the fore reef in 5 to 10 m depth. The coral polyps were transported to the laboratory and kept in 1000 l flow-through tanks with a constant supply of fresh seawater at *in situ* temperature (21 – 22°C) and salinity (41 – 43). *Fungia* polyps were selected because of their high abundance and strong mucus production, as observed in a previous study (Wild et al. 2005b). Additionally, as *Fungia* polyps are free-living, they can be removed and returned to the reef without any mechanical damage to the coral and the surrounding environment. The skeleton surface area of all corals was quantified applying a geometric approximation protocol in combination with a specific AF (approximation factor) for *Fungia* corals, derived from comparative analysis with a computer tomography based surface area measurement technique (Naumann et al. 2009). Briefly, each *Fungia* polyp was considered as a disc composed of two circular sides and a rectangular side. The average disc radius was calculated from the measured maximum and minimum horizontal polyp diameters. From this, the oral and aboral polyp surface areas were calculated as circles. The surface area of the rectangular side was calculated from measurements of the disc height and perimeter, and finally added to gain the total surface area of the disc. Subsequently, the geometrically assessed surface area was multiplied with the above mentioned AF to improve accuracy.

Rotated chamber incubations

Cultivation of picoplankton

Clonal cultures of *Synechococcus* strain RS9909, isolated from the Northern Red Sea by Fuller et al. (2003), were obtained through the Roscoff Culture Collection, France. Cultures were grown in liquid ASN III+ medium, which had been adjusted to local surface seawater salinity values (42). Cultivation was carried out in 100 ml Erlenmeyer flasks at a constant temperature of 21°C and light intensity of approximately 50 µmol m^{-2} s^{-1} (12/12 h light/dark cycle).

Coral mucus collection

Mucus collection was carried out according to the technique described in Wild et al. (2005b). *Fungia* polyps were exposed to air, holding the disk-like coral polyps vertically. Mucus release started immediately and was observed by long gel-like mucus threads dripping from the coral surface. The first 30 s of mucus production were discarded to prevent contamination and dilution through seawater. Subsequently, the released mucus (volumes: 2 to 25 ml) was collected for 2 min in a clean glass dish and processed immediately or stored at 4 °C in a sealed glass container until further use. The abundance of synechococcoid cyanobacteria (*Synechococcus*, but possibly also coral-associated cyanobacteria; cf. Lesser et al. (2006)) in freshly released coral mucus served as a reference to calculate the mucoidal entrapment rates of *Synechococcus* in subsequent experiments. Preliminary research revealed that the volume of mucus released by *Fungia* polyps after 30 s of air exposure was variable and showed a positive correlation to polyp surface area ($r^2 = 0.869$, Naumann unpubl.). Considering this, and to additionally account for polyp-specific variability, possibly caused by differing life histories, 4 random replicates from 4 differently sized *Fungia* polyps (surface areas: 132, 178, 228 and 294 cm^2) and reef locations were selected. Abundance of synechococcoid cyanobacteria was assessed in 1 ml aliquots of freshly released mucus using epifluorescence microscopy (EFM) as described below.

Rotated chamber incubations

To investigate trapping efficiency of coral mucus for natural as well as for a cultured (RS9909) *Synechococcus* strain, independent triplicate rotated chamber experiments (hereafter: I, II, and III) were carried out. To discriminate between the contribution of coral mucus and other possible factors influencing *Synechococcus* clearance, chamber incubations with different control characteristics were run in parallel. Acid-washed cylindrical transparent acrylic chambers (C1 – C5, diameter = 11.5 cm, height = 11.5 cm) were filled with freshly collected seawater and chamber-specific additives (see also Fig. 1), to a final volume of 555 ml (headspace ≈ 445 ml air). All chambers contained fresh Red Sea seawater including natural *Synechococcus* strains (mean abundance ≈ 2.6 x 10^4 cells ml^{-1}). Chamber C1 always contained untreated seawater with natural concentrations of *Synechococcus*. Chamber C2 was additionally spiked with *Synechococcus* strain RS9909 (mean abundance ≈ 2.1 x 10^5 cells ml^{-1}, corresponding to *Synechococcus* concentrations during the bloom season (Lindell & Post 1995)). Chambers C3 and C4 contained natural and spiked concentrations of *Synechococcus*, respectively, with freshly collected *Fungia* mucus (15 ml for experiment I and 50 ml for experiments II and III). Chambers C1 – C4 were placed on a roller table

apparatus as described in Shanks & Edmondson (1989) and rotated for 1 h at a constant rotation velocity of 2 (experiment I) or 1 (experiments II and III) cm s^{-1} (equivalent to ≈ 3 or 1.5 rpm, respectively). Chambers C1 and C2 were used to account for clearance caused by rotation-induced cell aggregations and grazing. As all chamber incubations were carried out under dimmed light intensity (≈ 5 µmol m^{-2} s^{-1}), picoplankton growth was regarded as negligible within the short experimental duration, and cell aggregation caused by intense illumination (Koblizek et al. 2000) could be ruled out. Chambers C3 and C4, combining all possible factors for picoplankton clearance, served to identify the contribution of mucus trapping to picoplankton clearance by consideration of all other factors observed in parallel-run chambers. The increased *Synechococcus* concentration in C4 was used to investigate mucus trapping and enrichment at elevated picoplankton abundances, i.e. during bloom season. Chamber C5, treated equally to C4, was placed without rotation next to the roller table (equal temperature and light conditions) as a control for rotation independent picoplankton clearance processes (cell aggregation, mucus trapping and grazing on *Synechococcus*). Mucus volume added was converted to particulate organic carbon (POC) using POC contents of *Fungia* mucus (Naumann unpubl.), mucus dissolution ratios (Wild et al. 2004a) and *in situ* POC concentrations during the study period (92 – 279 mg l^{-1}; Naumann unpubl.), leading to corresponding values of 81 and 269 mg C l^{-1} for 15 and 50 ml mucus added, respectively. The latter value was equivalent to a doubling of the POC concentrations in chambers C3 – C5.

Synechococcus clearance was assessed by measurements of cell abundance, at initial conditions (before chamber rotation) and after 1 h of continuous rotation, in all chambers of the independent replicate experiments (n = 3). Chambers C1 – C5 were placed on a table and left for 5 min without disturbance. After this preliminary settling period, one 10 ml sample was drawn from the uppermost surface water of each container using sterile syringes and transferred into a 15 ml settling vial (height = 13 cm). To each settling vial, 1 ml of a fixative solution (final concentration = 0.1 % paraformaldehyde and 0.05 % glutaraldehyde), as described in Marie et al. (2000), was added to inhibit cell growth, and to ensure preservation of *Synechococcus* accessory pigments (i.e. phycoerythrins, chlorophylls) for later EFM analyses. Subsequently, the settling vials were left for a 48 h sedimentation period in the dark, leading to a separation of cells between the suspended (homogenous distribution, no gravitational settling) and aggregated fraction (gravitational settling) with time. After the separation period, a 4 ml sample was taken from the upper surface water of each settling vial. *Synechococcus* abundance was quantified in these solutions using EFM in the way described below. Samples from rotated chambers C2, C4 and non-rotated chamber C5 were investigated for cell abundance of cultured *Synechococcus* strain RS9909, while C1 and C3 samples were checked for cell abundance of natural *Synechococcus* strains.

Mesocosm experiments

Trapping efficiency of coral mucus for natural strains of *Synechococcus* was examined by 6 independent identical experiments in a 50 l flow-through aquarium flushed with seawater freshly pumped from the reef. Each experiment was conducted during one daylight period and involved the incubation of 2 – 5 *Fungia* polyps (total: n = 21). Water temperature, measured by using mercury thermometers, was constant during all conducted experiments (21 – 22 °C), and salinity measured

with a handheld refractometer ranged between 41 and 43. Water flow-through rates were 1.8 – 4.2 l min^{-1}, as derived by quantification of aquarium outflow. *Fungia* polyps were briefly air-exposed (5 s) to initiate enhanced mucus production and thereafter placed on the bottom of the aquarium filled with fresh seawater. Air-exposure, as a natural incident for scleractinian corals in the study area (Loya 1976), was used to generate a distinct starting point for *Synechococcus* trapping by coral mucus that was subsequently included in the calculation of exposure periods for mucus aggregates, and finally applied in calculations of *Synechococcus* trapping rates. The aquarium inflow was directed horizontally at half aquarium height creating a unidirectional flow of ca. 4 cm s^{-1} (comparable to *in situ* conditions), as derived from visual tracing of particles over the coral surface. The water stream carried air bubbles of various size classes, simulating conditions identical to those observed in the natural shallow reef environment. *Fungia* polyps exuded mucus that formed webs drifting in the water stream, thereby becoming continuously enriched with air bubbles and suspended particulate matter. These mucus webs were subsequently compacted to mucus strings by the water stream and finally ascended to the water surface, where they accumulated to mucus floats. As the substantial part of particle trapping took place during mucus web and mucus string formation periods (Wild et al. 2004a, Wild et al. 2005b), the transition from 3-dimensional string morphology to 2-dimensional float morphology consequently represented the end point of effective mucus particle trapping.

After 30 or 60 min of incubation, mucus float accumulation was sufficient for quantitative sampling, and a sample was taken by syringe from the aggregated mucus float at the water surface. Sampling was carried out by establishing contact between the opening of the syringe tip and the mucus float to initiate suction of the sample. Shortly after, the syringe was lifted approximately 2 cm up from the water surface, while suction of the sample was continued; taking advantage of cohesion strength and viscosity of the mucus float material. By this gravitational separation procedure, sample contamination/dilution by *Synechococcus* from ambient seawater was minimised. Dilution ratio was approximately 100:1 (mucus float sample : seawater). Immediately after suction, the sample volume was measured using the syringe volume scale, and mucus floats were stored in the dark at 4 °C until further processing no later than 6 h after sampling. Seawater samples (100 ml, n = 3) were taken between individual coral incubations within each experiment to determine ambient versus mucus aggregate abundances of *Synechococcus*. Mucus float (5 – 10 ml) and seawater samples (100 ml) were filtered and analysed by EFM as described below. EFM results were related to the initially measured volume of sample material, avoiding bias caused by thermal mucus volume compaction during storage (4 °C), and expressed as *Synechococcus* cells ml^{-1} mucus float or seawater. To account for possible bias due to synechococcoid cyanobacteria already present in freshly released *Fungia* mucus (i.e. to provide trapping rates on a "per volume of freshly released mucus" basis) we subtracted these concentrations from *Synechococcus* concentrations in mucus floats in the calculation of *Synechococcus* trapping rates. To correct for possible bias by mucus volume compaction and mucus dissolution during incubations, compaction factors (CFs) were generated 3-fold for each experiment. For this purpose, a syringe was filled with 100 ml of fresh gel-like mucus and subsequently used to spread this mucus onto the skeleton surface of a dead *Fungia* polyp placed in the flow-through aquarium. A dead clean *Fungi*a skeleton was used as a

substitute simulating morphological features of a living *Fungia* polyp, thus providing the mucus with a substrate allowing for similar mucus aggregate formation and compaction conditions. After an incubation period of 30 and 60 min, respectively, CFs were determined, as defined by the relation between the volume of detectable mucus float and the volume of the initially introduced mucus. To correct for the dissolution of fresh gel-like mucus in seawater, the initial volume of introduced gel-like mucus was corrected by a dissolution factor of 0.32 (Wild et al. 2004a).

In situ sampling of coral mucus aggregates

Mucus aggregates were sampled from the water column in close vicinity to the reef crest to explore the trapping efficiency of coral mucus for *Synechococcus* under *in situ* conditions. At each of 4 sampling events (comparable environmental conditions; summary given in Table 1), coral colonies of the genus *Acropora* growing in 0.5 – 1.0 m water depth were visually inspected for attached mucus strings using SCUBA. Mucus strings originating from 5 – 10 coral colonies were collected separately, after final detachment from the coral and drifting through the water column for 8 to 15 min, using 10 ml syringes (total: 27 string samples). Inside sampling syringes, mucus string samples were clearly distinguishable from supernatant seawater, introduced during sampling, by entrapped particulate matter inducing gravitational settlement of the strings. Separation of mucus string material from supernatant seawater was carried out to avoid spurious increases of *Synechococcus* in the mucus string material. This was accomplished by holding the sampling syringe vertically (opening pointing upward) and draining of the supernatant seawater to a ratio of 100:1 (mucus string : seawater). After elimination of seawater, the volume of the sampled mucus strings was measured inside the sampling syringe using the volume scale, before strings were filtered for subsequent EFM analysis. At each sampling event, triplicate seawater samples (100 ml) were taken in the vicinity of the reef crest to determine ambient *Synechococcus* abundance. Further processing of mucus string samples and determination of *Synechococcus* abundance in strings and seawater was carried out using EFM as described below. The EFM-resulting *Synechococcus* abundance in mucus strings and seawater was related to the volume of the filtered material and expressed as *Synechococcus* cells ml^{-1} mucus string or seawater.

Table 1 *Environmental conditions and sedimentation rates during in situ mucus sampling events (abbreviations: Temp. = temperature, Bft = Beaufort, n.a. = not available).*

Date	Temp. (°C)	Wind (Bft)	Current (cm s^{-1})	Cloud cover	Sampling water depth (m)	Visibility (m)	Mean sedimentation (cm s^{-1})	n
19 Feb 2005	23	1 – 2	n.a	clear	0.5 – 2.0	10	0.5	10
23 Feb 2005	23	2	5	partially cloudy	0.5 – 2.0	10	0.5	6
27 Feb 2005	23	2	4 - 5	clear	0.5 – 2.0	12	0.5	6
04 Mar 2005	23	3	5	partially cloudy	0.5 – 2.0	8	0.5	5
Total								**27**

Microscopic analysis

Mucus aggregate samples were diluted with 0.2 µm filtered seawater and homogenized in the sample vial by vigorous shaking for 1 min. A hand tissue grinder (100 strokes per sample) was used to further disintegrate aggregates, followed by another 1 min of vigorous shaking. Preliminary research indicated no substantial *Synechococcus* cell loss following this procedure. Samples from all experiments to be analysed by EFM were filtered onto black polycarbonate membrane filters (Millipore™ Isopore GTBP, 0.2 µm pore size) using a vacuum of not more than 100 mm of Hg (Passow & Alldredge 1995). To achieve random distribution of cells, filters were soaked in deionised water before use (Crumpton 1987) and sucked dry after placing on the filtration unit. A membrane filter (Millipore™ HA; 0.4 µm pore-size) was used as a backing filter. Filtered samples were either examined within 1 h by EFM for *Synechococcus* abundance or stored at -20°C in the dark for later analysis. Samples were examined for *Synechococcus* autofluorescence of phycoerythrin using a standard Zeiss™ epifluorescence microscope (Axioskop 2) equipped with a HBO 100 W mercury vapour lamp and Zeiss™ fluorescence filter sets (488015 and 488009). More than 100 cells from randomly selected fields (ocular grid: 100 x 100 µm) were counted in the dark from each filter (Li & Wood 1988) at 1000x magnification using a Zeiss™ Plan-Neofluar 100x objective in combination with a 10x ocular. *Synechococcus* strain RS9909 cells were detected by their bright red fluorescence following green light excitation (filter set: 488015) and by orange-red fluorescence after blue light excitation (filter set: 488009) (Booth 1987). Natural strains showed a bright yellow light emission after blue excitation and fluoresced orange-red at green excitation wavelengths. Additionally, for *Synechococcus* strain RS9909, the more elongated coccoid cell shape was used for identification, as all natural strains in contemporary seawater samples showed spherical coccoid cell morphology. Abundance of *Synechococcus* in clusters formed by mucus scavenging was determined by scanning of the whole filter area (at 100x) and counting of all cells inside clusters (at 1000x). Cell contents of clusters were added quantitatively and related to the filtered sample volume.

Results

Rotated chamber incubations

The occurrence of synechococcoid cyanobacteria in freshly released *Fungia* mucus ($1.0 \pm 0.2 \times 10^4$ cells ml^{-1}) showed that pelagic *Synechococcus* and/or coral associated synechococcoid cyanobacteria were consistently present in considerably constant concentrations (coefficient of variation $V = 20$ %) within the corals' surface mucus layer (SML). Addition of mucus and chamber rotation (C3 and C4) resulted in the formation of mucus-particle aggregates of various size classes (diameter < 5 mm – 2 cm) from which the majority accumulated to one macroaggregate (diameter = 3.5 cm) after 1 h of incubation. By contrast, no aggregate formation was observed in non-mucus (C1 and C2) and non-rotated (C5) chambers. Clearance was detectable in all chambers after 1 h (Fig. 1). *Synechococcus* clearance rates were significantly increased in rotated chambers with mucus addition (Wilcoxon signed rank test, 2-tailed, $p < 0.005$), accounting for 32 – 52 % h^{-1} of the

initial cell content (C3 and C4), exceeding those due to cell aggregations and/or picoplankton grazing in non-mucus and non-rotated chambers (C1, C2 and C5) by a factor of 4 (mean of experiments I, II and III) ranging only between 6 – 18 % h^{-1} (Fig. 1). Taking into account clearance caused by cell aggregations and picoplankton grazing, coral mucus added to chambers C3 and C4 was responsible for 15 – 43 % of *Synechococcus* clearance h^{-1}. Rotation-induced cell aggregations (C1 and C2) accounted for 3 – 8 % clearance h^{-1} as calculated with consideration of results from non-rotated chamber C5 combining clearance rates caused by rotation independent cell aggregation, rotation independent mucus trapping and picoplankton grazing. No significant differences were observed in percentage clearance between C3 and C4 with regard to different initial cell abundances (1 – 2 orders of magnitude) of natural (C3) and artificially added (C4) *Synechococcus* strains (experiments II and III, Fig. 1). However, different initial cell abundances resulted in a significant variation in mucus trapping rates found per volume of added mucus and rotation time, constituting $2.3 \pm 1.8 \times 10^3$ and $1.9 \pm 0.8 \times 10^4$ cells ml^{-1} mucus min^{-1} for C3 and C4, respectively (Mann and Whitney U-test, 2-tailed, $p < 0.01$). In comparison of experiments I and II/III, increased rotation velocity (experiment I) resulted in a higher cell clearance rate in the chamber with mucus addition (C4), in contrast to the non-mucus chamber (C2), where no velocity dependent differences were observed. Cell clearance in chamber C5 was slightly elevated in experiment II, but remained constant between experiments I and III.

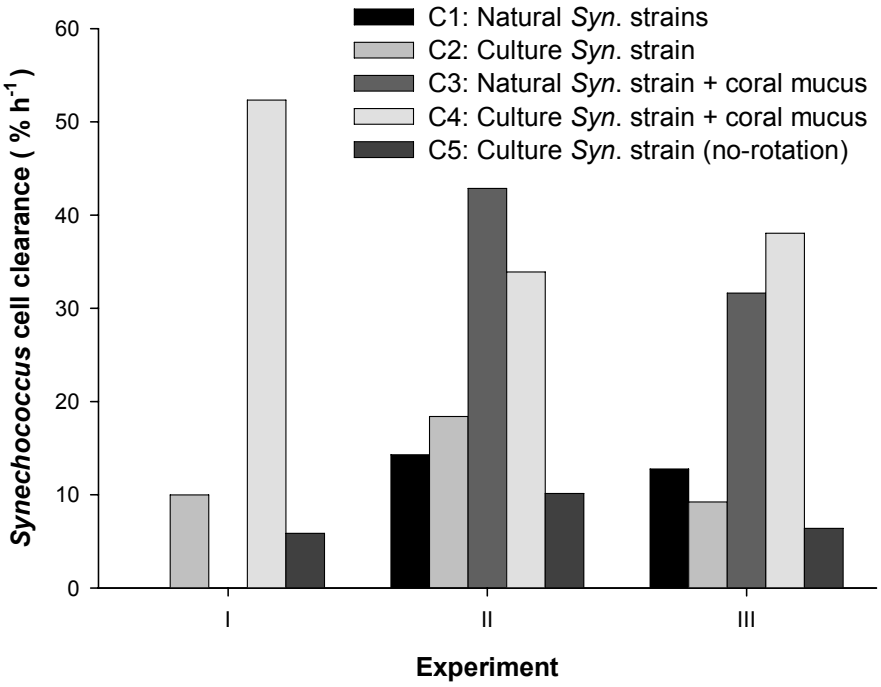

Fig. 1 *Synechococcus clearance rates for different treatments from 3 independent rotated chamber incubations. Chambers C1 – C5 from left to right; results for C1 and C3 not available for experiment I; rotation velocity: 2 and 1 cm s^{-1} (experiment I; experiment II and III); abbreviation: Syn. = Synechococcus.*

Mesocosm experiments

Experiments in a large flow-through aquarium allowed for a close follow-up on the successive stages of coral mucus aggregate formation, during which extensive particle trapping occurs. Shortly (1 – 3 min) after a polyp was placed in the aquarium, mucus webs of 15 – 50 cm length, made visible by entrapped air bubbles, were observed reaching from the coral surface into the water column. Driven by the water stream and ongoing mucus production the webs gained size and spread through the entire tank volume with slightly positive buoyancy. After 3 – 4 min, the aquarium was almost entirely filled with mucus webs reaching maximum sizes of 50 x 30 x 20 cm. In 90 % of all replications, webs stayed attached to the coral surface forming compacted string-like structures, while the rest of the mucus web drifted through the tank volume unfolded. Under the influence of the water stream, mucus webs consequently compacted to mucus strings in which the amount of trapped particles (e.g. pieces of macro algae) became visible. The increasing load of entrapped air bubbles transported mucus strings to the water surface after a mean time of 8 min (interval from incubation start until first detected float) forming thick mucus floats successively accumulating the ascending string material. At sampling time, a main portion of the water surface was covered with contaminated mucus floats (up to 5 cm thick) from which mucus strings extended downwards connecting the floats with the coral. Results from EFM analysis of mucus float material displayed significantly higher *Synechococcus* abundance (Wilcoxon signed rank test, 2-tailed, $p < 0.05$) in all mucus float samples (mean: $1.3 \pm 0.7 \times 10^5$ cells ml^{-1}) compared to seawater samples (mean: $1.2 \pm 1.0 \times 10^4$ cells ml^{-1}) on each respective experimental day, resulting in 3 to 46-fold picoplankton enrichment during the mucus aggregate formation process (Fig. 2). *Synechococcus* abundance in seawater showed significant variability, within the local seasonal fluctuation range (Lindell & Post 1995), between experimental days (One-way ANOVA, $p < 0.05$), but was constant in the course of each experimental day (paired-samples T-test, 2-tailed, $p > 0.05$). *Synechococcus* abundance in seawater was not correlated to *Synechococcus* enrichment found in mucus float samples on the respective experimental days (Spearman rank-order correlation, 2-tailed, $p > 0.05$). Trapping rates of *Synechococcus* per volume of fresh mucus during exposure time (8 min) accounted for $0.3 \pm 0.2 \times 10^3$ cells ml^{-1} min^{-1}. Spurious enrichment of mucus floats by *Synechococcus* from ambient seawater could be regarded as negligible due to insignificant contribution to sample volume and significant differences in cell abundance (Wilcoxon signed rank test, 2-tailed, $p < 0.05$). Mucus CFs determined for the respective experiments ranged from 17 to 160-fold.

In situ sampling of coral mucus aggregates

At all sampling events, mucus aggregates from various size classes and aging stages were abundant in the water column above the reef flat and in close seawards vicinity of the reef crest. The vast majority of *Acropora* colonies showed extensive mucus production in the form of mucus webs (Fig. 3 a) that compacted on the coral surface and remained attached to lateral or top edges of the colonies as mucus strings (Fig. 3 b and c). Strings reached lengths of between 2 – 25 cm and displayed positive buoyancy caused by entrapped air bubbles. Due to low current velocity (4 – 5 cm

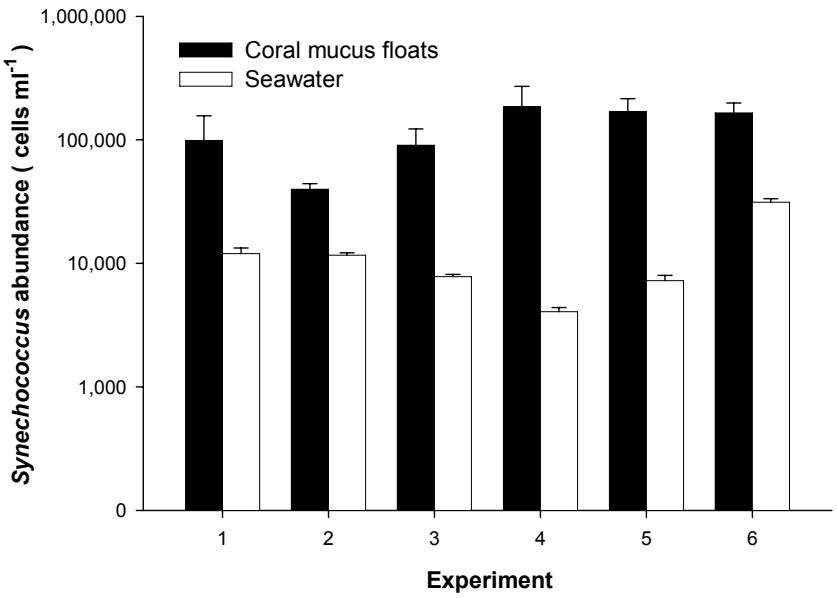

Fig. 2 *Synechococcus abundance (mean ± SD) in coral mucus floats and seawater from mesocosm experiments. Coral mucus floats and seawater samples were collected in replicates of at least n = 3 per experiment.*

s^{-1}), mucus strings exhibited slow compaction and remained attached to the coral for an undefined period of time. After final detachment, mucus strings drifted with neutral to positive buoyancy continuously compacting to smaller aggregates (>1 cm) accompanied by subsequent loss of entrapped air bubbles (Fig. 3 d). Horizontal currents (2 – 3 m min^{-1}) transported mucus strings for more than 25 m over deeper waters of the lower fore reef (20 – 30 m depth), where all strings showed negative buoyancy and descended to the seafloor with an average sedimentation velocity of 0.5 cm s^{-1} (Table 1). *Synechococcus* abundance in mucus strings ranged from 8.8 x 10^4 – 4.6 x 10^6 cells ml^{-1} and was found to be significantly higher than in ambient seawater (Wilcoxon signed rank test, 2-tailed, $p < 0.05$) by statistical comparison for each experimental day. Seawater *Synechococcus* abundance showed significant variability (1.2 – 3.1 x 10^4 cells ml^{-1}; One-way ANOVA, $p < 0.05$) within the seasonal fluctuation range during the study period (Fig. 4), but remained constant in the course of each sampling event (paired-samples T-test, 2-tailed, $p > 0.05$). On average, *Synechococcus* abundance in mucus strings (8.2 x 10^5 cells ml^{-1}) was 40-fold higher than in the surrounding seawater (2.1 x 10^4 cells ml^{-1}). In contrast to mesocosm experiments, *Synechococcus* abundance in seawater showed correlation to *Synechococcus* enrichment found in mucus strings on the respective experimental days (Spearman rank-order correlation, 2-tailed, $p < 0.01$). As for mucus float samples in mesocosm experiments, enrichment of mucus strings by *Synechococcus* from ambient seawater during sample processing was regarded as negligible due to insignificant contribution to sample volume and significant differences in *Synechococcus* abundance (Wilcoxon signed rank test, 2-tailed, $p < 0.05$).

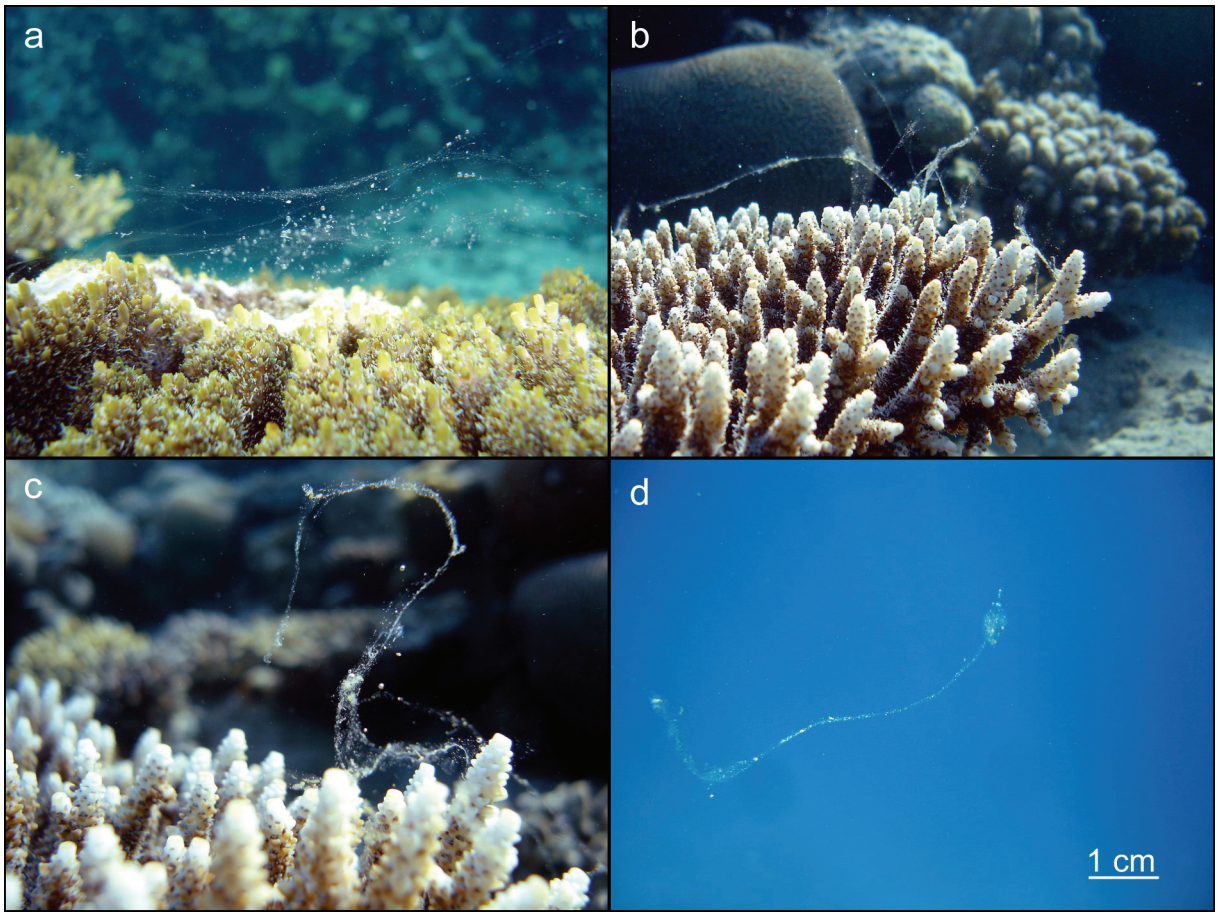

Fig. 3 *Stages of coral mucus aggregate formation observed in situ. Panels a – d show mucus aggregates produced by Acropora colonies in successive aggregation. Panel a: mucus web over colony surface; panel b: mucus string accumulation; panel c: mucus string after continued compaction; panel d: mucus string drifting in water column after detachment.*

Discussion

Occurrence of *Synechococcus* within the surface mucus layer

The interesting finding that freshly produced mucus from *Fungia* corals already contained background levels of synechococcoid cyanobacteria ($1.0 \pm 0.2 \times 10^4$ cells ml^{-1}), may be explained by 1) introduction of coral-associated cyanobacteria or 2) entrapment of pelagic cells by the adhesive SML, as described for larger suspended particles by Wild et al. (2005b). Previous studies have identified synechococcoid cyanobacteria to be associated with corals (Rohwer et al. 2002, Bourne & Munn 2005, Lesser et al. 2007, Kvennefors & Roff 2009), indicating the possible introduction of those cells into freshly produced mucus during sample collection by air-exposure. Continuous enrichment of pelagic *Synechococcus* cells via particle trapping in the attached SML remains suggestive, in particular since the mucus released during the first 30 s was discarded during sample collection for this study. Although the SML of corals holds a dynamic microbial community (Johnston & Rohwer 2007), recent studies have reported on the different microbial community composition found in the SML compared to the surrounding seawater (Guppy & Bythell 2006).

Ritchie (2006) showed a distinct antibiotic activity regulated by coral mucus and mucus associated bacteria, presumably only allowing for short term retention of entrapped pelagic cells. Furthermore, Koh (1997) observed the production of specific allelochemicals inside coral tissues targeting pelagic *Synechococcus* (100 % of 100 tested species), which seems to rule out a long-term association of *Synechococcus* with corals.

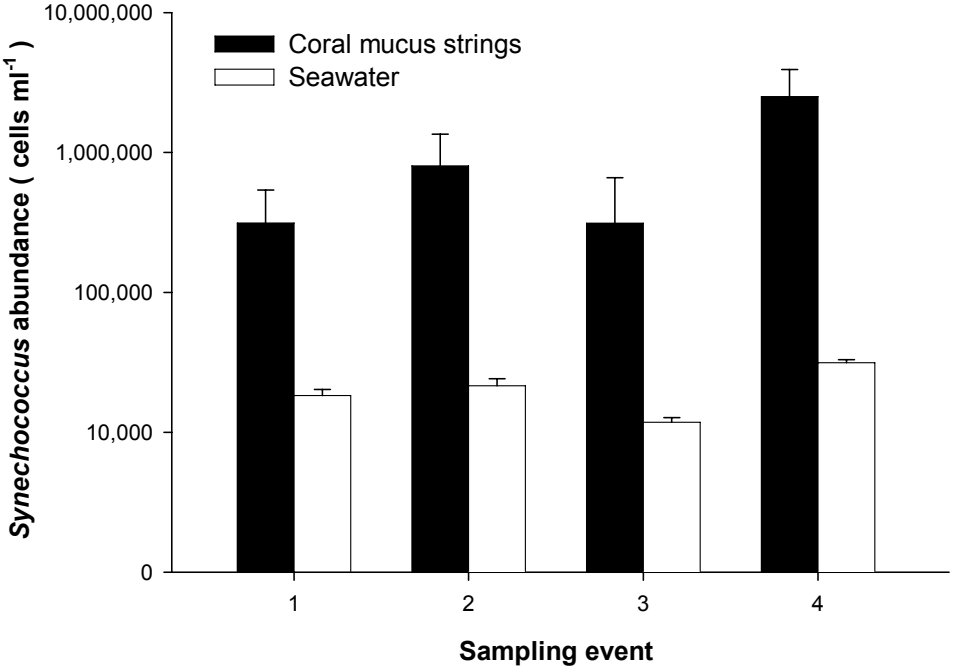

Fig. 4 *Synechococcus abundance (mean ± SD) in coral mucus strings and local seawater at four in situ sampling events as described in Table 1. Coral mucus strings and seawater reference samples were collected in replicates of at least n = 5 (total: n = 27) and n = 3 respectively, during each sampling event.*

Picoplankton trapping by coral mucus and relevant factors

The results of the present study show the efficient trapping and enrichment function of coral mucus for picoplanktonic cyanobacteria (i.e. *Synechococcus*), providing a potentially important vector for pelagic picoplankton to the reef benthos. This confirms the mucus trapping efficiency for suspended particulate organic matter (POM) demonstrated by previous studies (Wild et al. 2004a, Huettel et al. 2006) and emphasizes the validity for minute suspended particles: picoplankton. Aggregate formation processes of picoplankton-enriched mucus aggregates recorded *in situ* and during experimental approaches of the present study closely correspond to previous observations (Johannes 1967, Coles & Strathman 1973, Wild et al. 2004a, Wild et al. 2005b, Huettel et al. 2006), indicating uniformity among different reef types and oceanic regions. In rotated chamber incubations, simulation of *in situ* current velocity by constant rotation, created coral mucus aggregates comparable in size, shape and texture to mucus strings sampled *in situ* at the study location. This demonstrates the applicability of the rotated chamber technique for investigations on coral mucus trapping and aggregation properties under laboratory conditions (Shanks & Edmondson 1989). The

added coral mucus trapped up to 43 % of the initial *Synechococcus* cell content within 1 h of chamber rotation, thereby characterising coral mucus as an efficient scavenger for picoplankton cells, exceeding trapping efficiency for picoplankton-sized particles previously described for mucus web-feeding pteropods (Noji et al. 1997).

Relevant factors defining the extent of picoplankton enrichment in mucus aggregates appear to be current velocity, time of exposure and cell abundance, which likewise control attachment probability of larger POM (Alldredge & McGillivary 1991). Dependency on water movement is displayed by the comparably low clearance rates in stagnant incubation chambers (C5), presumably mainly caused by grazers such as copepods and protozoa (Koehl & Strickler 1981, Caron et al. 1991). Slight differences observed in clearance of C5 between experiments I and II likely indicate the variability of picoplankton predator abundances in seawater samples at the respective days of experiments. Water movement as a relevant factor for intensified clearance by mucus aggregates is additionally promoted by elevated clearance rates found in C4 of experiment I (increased rotation velocity) in comparison to experiments II and III. The influence of different exposure periods (60 and 8 min exposure, respectively) is highlighted by the elevated *Synechococcus* trapping rates per volume of fresh mucus in rotated chamber incubations ($2.3 \pm 1.8 \times 10^3$ cells ml^{-1} fresh mucus min^{-1}) compared to mesocosm experiments ($0.3 \pm 0.2 \times 10^3$). However, this comparison may be falsified by the disputable use of a dead *Fungia* skeleton for the determination of compaction factors during mesocosm experiments, possibly leading to an underestimation of the respective *Synechococcus* trapping rates. The significant difference of *Synechococcus* enrichment in mucus aggregates from *in situ* samplings and mesocosm experiments (Wilcoxon signed rank test, 2-tailed, $p < 0.001$) (Fig. 5), may also be explained by prolonged exposure of coral-attached *in situ* mucus webs and strings before initial string observation. However, higher *Synechococcus* abundances of seawater and mucus aggregates *in situ* compared to mesocosm experiments (Mann and Whitney U-test, 2-tailed, $p < 0.01$, cf. Fig. 2 and 3), and correlation of *in situ* mucus string enrichment to seawater abundance may suggest ambient cell abundance as an additional influencing factor. This is supported by clearance rates in rotated chamber incubations, where increased *Synechococcus* abundance results in a higher removal concerning cell numbers.

Coral mucus as a carrier for picoplankton from the pelagic to the benthic reef environment

Organic aggregates represent important hot-spots for the transfer of particulate material in aquatic ecosystems (e.g. Simon et al. 2002). Particle trapping by organic aggregates and successive enrichment of suspended matter serves as a vector in the energy transport to higher trophic levels, thereby enhancing access to otherwise elusive picoplankton biomass (Ling & Alldredge 2003). In Red Sea waters, planktonic chlorophyll a and primary production are dominated by picoplanktonic cyanobacteria (Pillen & Moigis, cited in Lenz et al. 1988). Efficient picoplankton trapping by coral mucus and subsequent formation of enriched mucus aggregates, as demonstrated by the present study, may provide enhanced access of planktonic biomass to reef dwelling organisms by particle enlargement. In the pelagic realm, entrapment of picoplankton in mucus aggregates may add to the nutrition of mucus-feeding zooplankton (Richman et al. 1975, Gottfried & Roman 1983) and fish

(Benson & Muscatine 1974), while subsequent sedimentation of picoplankton-enriched mucus aggregates to the reef benthos implies a potential trophodynamic link in reef pelagic-benthic coupling. Integration of picoplankton into the spectrum of suspended particulates trapped by coral mucus may, in addition to the reported significant nutritious value of the mucus itself (Ducklow & Mitchell 1979, Wild et al. 2004b), provide relevant biomass input to pelagic and benthic microbial degradation. Entrapment of picoplankton in coral mucus provides further understanding for the substantial removal of picoplankton over reef communities described by previous studies (Ayukai 1995, Ribes et al. 2003, Houlbrèque et al. 2006). This represents a plausible mechanistic explanation for the proposed uptake of picoplankton by the benthos via picoplankton enrichment in seizable mucus aggregates. The capture and consumption of picoplankton-enriched mucus aggregates may thus be of nutritional value for a wide variety of benthic reef taxa (e.g. soft corals, gorgonians and sponges). In particular passive suspension feeders that depend on ambient supply with seizable particulate food items may benefit from this enhanced availability of planktonic biomass, as they are not known to possess anatomical or physiological adaptations for the bulk capture of picoplankton. For instance, the pinnules on the tentacles of the azooxanthellate soft coral *Dendronephthya* spp. are too widely spaced (60 – 80 µm; Fabricius et al. (1995)) to allow for an effective capture of minute picoplankton cells. Remarkably, Fabricius et al. (1995) reported the detection of picoplankton cells in the gastrovascular cavities of *Dendronephthya hemprichi* polyps, leaving thereby in question how these minute particles are intercepted by the tentacles and transferred to the interior of the polyps. As conceivable for *Dendronephthya* spp., which still presents unresolved gaps within its metabolic energy budget (Widdig & Schlichter 2001), facilitated capture of larger picoplankton-enriched mucus aggregates may also provide an important trophic source for other passive suspension feeding reef organisms.

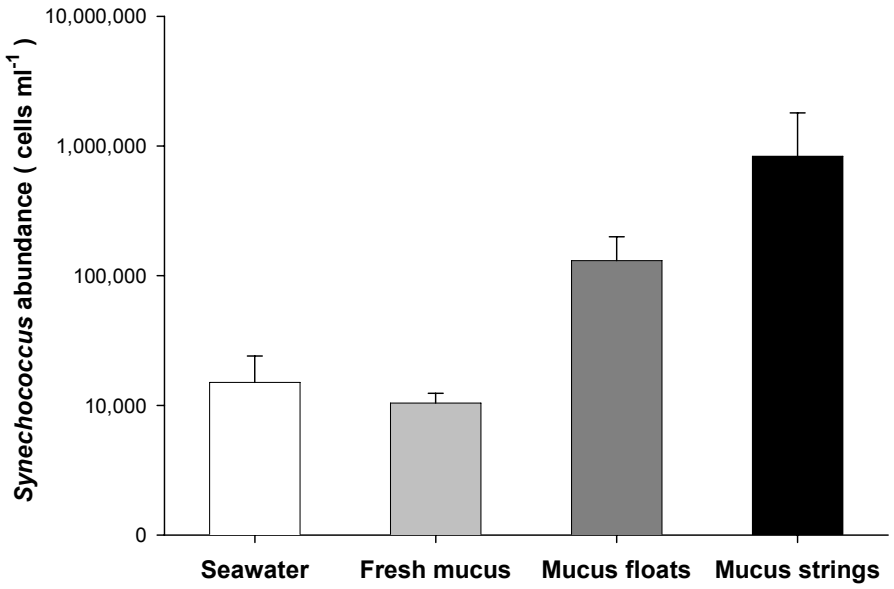

Fig. 5 *Synechococcus abundance (mean ± SD) in seawater, freshly released coral mucus, coral mucus floats, and coral mucus strings investigated in this study. Seawater values are average values from mesocosm experiments and in situ samplings.*

Conclusions

This study demonstrates the efficient trapping and enrichment abilities of scleractinian coral mucus for picoplankton-sized organisms using the cyanobacterium *Synechococcus* as a model organism. *Synechococcus* abundance in seawater measured here ($1.2 – 3.1 \times 10^4$ cells ml^{-1}) was in the range of results from previous studies ($2.5 \pm 2.1 \times 10^4$ cells ml^{-1}; Gradinger et al. 1992) and indicates the dominance of unicellular cyanobacteria within Red Sea picoplankton biomass. However, as another important picoplankton group, heterotrophic bacteria have been found in significantly higher cell abundances ($5.2 – 8.8 \times 10^5$ cells ml^{-1}; Weisse 1989, Grossart & Simon 2002), although contributing a disproportionately smaller fraction to overall picoplankton biomass, at least in terms of nitrogen (Ribes et al. 2003). An assumed similar mucus trapping efficiency for all groups within the picoplankton community may emphasize the role of coral mucus as a carrier of trapped picoplankton biomass to the benthic communities.

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References

Alldredge A, McGillivary P (1991) The attachment probabilities of marine snow and their implications for particle coagulation in the ocean. Deep-Sea Res Part A 38:431–443

Ayukai T (1995) Retention of phytoplankton and planktonic microbes on coral reefs within the Great Barrier Reef, Australia. Coral Reefs 14:141–147

Benson A, Muscatine L (1974) Wax in coral mucus: Energy transfer from corals to reef fishes. Limnol Oceanogr 19:810–814

Booth BC (1987) The use of autofluorescence for analysing oceanic phytoplankton communities. Bot Mar 30:101–108

Bourne DG, Munn CB (2005) Diversity of bacteria associated with the coral *Pocillopora damicornis* from the Great Barrier Reef. Environ Microbiol 7:1162–1174

Caron DA, Lim EL, Miceli G, Waterbury JB, Valois FW (1991) Grazing and utilization of chroococcoid cyanobacteria and heterotrophic bacteria by protozoa in laboratory cultures and a coastal community. Mar Ecol Prog Ser 76:205–217

Coles S, Strathman R (1973) Observations on coral mucus "flocs" and their potential trophic significance. Limnol Oceanogr 18:673–678

Crumpton WG (1987) A simple and reliable method for making permanent mounts of phytoplankton for light and fluorescence microscopy. Limnol Oceanogr 32:1154–1159

Ducklow HW, Mitchell R (1979) Bacterial populations and adaptations in the mucus layers on living corals. Limnol Oceanogr 24: 715–725

Ducklow HW (1990) Coral Reefs. In: Dubinsky Z (ed) Ecosystems of the World. Elsevier, Amsterdam

Fabricius KE, Genin A, Benayahu Y (1995) Flow-dependent herbivory and growth in zooxanthellae-free soft corals. Limnol Oceanogr 40:1290–1301

Fuller NJ, Marie D, Partensky F, Vaulot D, Post AF, Scanlan DJ (2003) Clade-specific 16S ribosomal DNA oligonucleotides reveal the predominance of a single marine *Synechococcus* clade throughout a stratified water column in the Red Sea. Appl Environ Microbio 69:2430–2443

Goldberg WM (2002) Feeding behaviour, epidermal structure and mucus cytochemistry of the scleractinian *Mycetophyllis reesi*, a coral without tentacles. Tissue Cell 34:232–245

Gottfried M, Roman MR (1983) Ingestion and incorporation of coral mucus detritus by reef zooplankton. Mar Biol 72:211–218

Gradinger R, Weiss T, Pillen T (1992) Significance of picocyanobacteria in the Red Sea and the Gulf of Aden. Bot Mar 35:245–250

Grossart HP, Simon M (2002) Bacterioplankton dynamics in the Gulf of Aqaba and the northern Red Sea during early spring. Mar Ecol Prog Ser 239: 263–276

Guppy R, Bythell JC (2006) Environmental effects on bacterial diversity in the surface mucus layer of the reef coral *Montastraea faveolata*. Mar Ecol Prog Ser 328:133–142

Houlbrèque F, Delesalle B, Blanchot J, Montel Y, Ferrier-Pagès C (2006) Picoplankton removal by the coral reef community of La Prévoyante, Mayotte Island. Aquat Microb Ecol 44:59–70

Houlbrèque F, Tambutté E, Richard C, Ferrier-Pagès C (2004) Importance of a micro-diet for scleractinian corals. Mar Ecol Prog Ser 282:151–160

Huettel M, Wild C, Gonelli S (2006) Mucus trap in coral reefs: formation and temporal evolution of particle aggregates caused by coral mucus. Mar Ecol Prog Ser 307:69–84

Johannes R (1967) Ecology of organic aggregates in the vicinity of a coral reef. Limnol Oceanogr 7:189–195

Johnson PW, Sieburth JM (1979) Chroococcoid cyanobacteria in the sea: A ubiquitous and diverse phototrophic biomass. Limnol Oceanogr 24:928–935

Johnston IS, Rohwer F (2007) Microbial landscapes on the outer tissue surfaces of the reef-building coral *Porites compressa*. Coral Reefs 26:375–383

Kappner I, Al-Moghrabi SM, Richter C (2000) Mucus-net feeding by the vermetid gastropod *Dendropoma maxima* in coral reefs. Mar Ecol Prog Ser 204:309–313

Kvennefors ECE, Roff G (2009) Evidence of cyanobacteria-like endosymbionts in Acroporid corals from the Great Barrier Reef. Coral Reefs [doi 10.1007/s00338-009-0473-0]

Koblizek M, Komenda J, Masojidek J, Pechar L (2000) Cell aggregation of the cyanobacterium *Synechococcus elongatus*: Role of the electron transport chain. J Phycol 36:662–668

Koehl MAR, Strickler JR (1981) Copepod feeding currents: food capture at low Reynolds number. Limnol Oceanogr 26:1062–1073

Koh EGL (1997) Do scleractinian corals engage in chemical warfare against microbes? J Chem Ecol 23:379–398

Lenz J, Schneider G, El Hag AGD, Gradinger R, Fritsche P, Moigis A, Pillen T, Rolke M, Weisse T (1988) Planktological data from the central Red Sea and the Gulf of Aden. Ber Inst Meereskd Christian-Albrechts Univ Kiel, p 1–200

Lesser MP, Falcón LI, Rodríguez-Román A, Enríquez S, Hoegh-Guldberg O, Iglesias-Prieto R (2007) Nitrogen fixation by symbiotic cyanobacteria provides a source of nitrogen for the scleractinian coral *Montastraea cavernosa*. Mar Ecol Prog Ser 346:143–152

Li WKW, Rao DVS, Harrison WG, Smith JC, Cullen JJ, Irwin B, Platt T (1983) Autotrophic picoplankton in the tropical ocean. Science 219:292–295

Li WKW, Wood AM (1988) Vertical distribution of North Atlantic ultraphytoplankton: analysis by flow cytometry and epifluorescence microscopy. Deep-Sea Res 35:1615–1638

Lindell D, Post AF (1995) Ultraphytoplankton succession is triggered by deep winter mixing in the Gulf of Aqaba (Eilat), Red Sea. Limnol Oceanogr 40:1130–1141

Ling SC, Alldredge AL (2003) Does the marine copepod *Calanus pacificus* consume transparent exopolymer particles (TEP)? J Plankton Res 25:507–515

Loya Y (1976) Recolonization of Red Sea corals affected by natural catastrophes and man-made perturbations. Ecology 57: 278-289

Marie D, Partensky F, Simon N, Guillou L, Vaulot D (2000) Flowcytometry analysis of marine picoplankton. In: Diamond RA, DeMaggios (eds) In living colors: protocols in flowcytometry and cell sorting. Springer Verlag, Heidelberg

Naumann M S, Niggl W, Laforsch C, Glaser C, Wild C (2009) Coral surface area quantification – evaluation of established methods by comparison with computer tomography. Coral Reefs 28:109–117

Noji TT, Bathmann UV, Bodungen BV, Voss M, Antia A, Krumbholz M, Klein B, Peeken I, Noji CI-M, Rey F (1997) Clearance of picoplankton-sized particles and formation of rapidly sinking aggregates by the pteropod, *Limacina retroversa*. J Plankton Res 19:863–875

Owens NJP, Burkill P, Mantoura R, Woodward E, Bellan I, Aiken J, Howland R, Llewellyn C (1993) Size-fractionated primary production and nitrogen assimilation in the Northwestern Indian Ocean. Deep-Sea Res 240:697–709

Passow U, Alldredge AL (1995) A dye-binding assay for the spectrophotometric measurement of transparent exopolymer particles (TEP). Limnol Oceanogr 40:1326–1335

Pedros-Alio C, Mas J, Gasol JM, Guererro R (1989) Sinking speeds of free-living phototrophic bacteria determined with covered and uncovered sediment traps. J Plankton Res 11:887–905
Ribes M, Coma R, Atkinson MJ, Kinzie III RA (2003) Particle removal by coral reef communities: picoplankton is a major source of nitrogen. Mar Ecol Prog Ser 257:13–23
Richardson TL, Jackson GA (2007) Small phytoplankton and carbon export from the surface ocean. Science 315:838–840
Richman S, Loya Y, Slobodkin LB (1975) The rate of mucus production by corals and its assimilation by the coral reef copepod *Acartia negligens*. Limnol Oceanogr 20:918–923
Richter C, Wunsch M, Rasheed M, Kötter I, Badran MI (2001) Endoscopic exploration of Red Sea coral reefs reveals dense populations of cavity-dwelling sponges. Nature 413:726–730
Ritchie KB (2006) Regulation of microbial populations by coral surface mucus and mucus-associated bacteria. Mar Ecol Prog Ser 322:1–14
Rohwer F, Seguritan V, Azam F, Knowlton N (2002) Diversity and distribution of coral- associated bacteria. Mar Ecol Prog Ser 243:1–10
Shanks A, Edmondson EW (1989) Laboratory-made artificial marine snow: a biological model of the real thing. Mar Biol 101:463–470
Sieburth JM, Smetacek V, Lenz J (1978) Pelagic ecosystem structure: Heterotrophic compartments of the plankton and their relationship to plankton size fractions. Limnol Oceanogr 23:1256–1263
Simon M, Grossart H-P, Schweitzer B, Ploug H (2002) Microbial ecology of organic aggregates in aquatic ecosystems. Aquat Microb Ecol 28:175–211
Tremblay JE, Legendre L (1994) A model for the size-fractionated biomass and production of marine phytoplankton. Limnol and Oceanogr 39:2004–2014
Waite AM, Safi KA, Hall JA, Nodder SD (2000) Mass sedimentation of picoplankton embedded in organic aggregates. Limnol Oceanogr 45:87–97
Weisse T (1989) The microbial loop in the Res Sea – Dynamics of pelagic bacteria and heterotrophic nanoflagellates. Mar Ecol Prog Ser 55:241–250
Widdig A, Schlichter D (2001) Phytoplankton: a significant trophic source for soft corals? Helgol Mar Res 55:198–211
Wild C, Huettel M, Klueter A, Kremb S, Rasheed M, Joergensen BB (2004a) Coral mucus functions as an energy carrier and particle trap in the reef ecosystem Nature 428:66–70
Wild C, Rasheed M, Werner U, Franke U, Johnstone R, Huettel M (2004b) Degradation and mineralization of coral mucus in reef environments. Mar Ecol Prog Ser 267:159–171
Wild C, Woyt H, Huettel M (2005a) Influence of coral mucus on nutrient fluxes in carbonate sands. Mar Ecol Prog Ser 287:87–98
Wild C, Rasheed M, Jantzen C, Cook P, Struck U, Boetius A (2005b) Benthic metabolism and degradation of natural particulate organic matter in carbonate and silicate reef sands of the Northern Red Sea. Mar Ecol Prog Ser 298:69–87

7

Stable isotope labeling of coral mucus reveals its uptake by epizoic *Waminoa* worms

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Abstract

Mucus released by scleractinian corals can act as an important energy and nutrient carrier in coral reef ecosystems, and a distinct isotopic signature would allow following the fate of this material. Therefore, this study presents the natural C and N stable isotopic signatures of mucus released by four scleractinian coral genera (*Acropora*, *Fungia*, *Pocillopora* and *Stylophora*) in comparison to those of suspended particulate organic matter (POM) in seawater of a Northern Red Sea fringing coral reef near Aqaba, Jordan. The natural $\delta^{13}C$ and $\delta^{15}N$ signatures of coral mucus differed significantly from POM, but were inappropriate for explicit tracing of mucus in the coral reef food web. Thus, a labeling technique using stable isotope tracers (^{13}C and ^{15}N) was developed that produced $\delta^{13}C$ values of up to 122 ± 5‰ (mean ± SE) and $\delta^{15}N$ of up to 2100 ± 151‰ in mucus exuded by *Fungia*. ^{13}C and ^{15}N-enriched compounds were rapidly (within 3 h) and light-dependently transferred from the endosymbiotic zooxanthellae to the mucus-producing coral host. The traceability of ^{15}N-labeled mucus was examined by evaluating its uptake and potential utilization by epizoic acoelomorph *Waminoa* worms naturally occurring in high abundances on several scleractinian coral taxa. This tracer experiment resulted in the uptake of coral mucus by coral-associated acoelomorphs, and further demonstrated the applicability of coral mucus stable isotope labeling by revealing a new trophic pathway in coral reef ecosystems.

Introduction

Scleractinian corals in warm water coral reef ecosystems continuously synthesize and release organic mucoid exudates (i.e., coral mucus) onto their epidermal tissue surfaces (Ducklow and Mitchell 1979a). Released mucus principally functions as protection layer against ambient environmental stressors (Schuhmacher 1977; Krupp 1984; Ritchie 2006). Coral mucus is a transparent exopolymer synthesized by mucus gland cells located in the coral ectoderm (Marshall and Wright 1993) and is principally composed of glycoproteins and lipids (Meikle et al. 1988), which provide a high energy content of significant nutritious value (Ducklow and Mitchell 1979b; Wild et al. 2004a). Previous studies have shown that the continuous release of scleractinian coral mucus in dissolved and particulate forms can result in a dominance of coral-derived organic material in reef-surrounding waters (Johannes 1967; Marshall 1968). There, coral mucus can act as an important energy and nutrient carrier in benthic-pelagic coupling processes (Wild et al. 2004b; Wild et al. 2005b; Naumann et al. 2009). Coral mucus significantly contributes to reef ecosystem functioning by influencing planktonic and benthic metabolism (Wild et al. 2005a; Huettel et al. 2006) and facilitation of fast nutrient recycling via the initiation of element cycles (Ferrier-Pages et al. 2000; Wild et al. 2004b; Wild et al. 2005a). Despite the apparent trophic significance of scleractinian coral mucus in reef ecosystems, specific pathways and roles of coral mucus in the cycles of matter remain poorly understood. Its transparent property and strong dissolution affinity (Wild et al. 2004b) present significant obstacles for visual tracing to potential mucus consuming organisms. Consequently, specific properties of coral mucus (e.g., its stable isotope signatures) may prove as effective tools to follow the paths of this material through the reef ecosystem.

The stable isotope signature of living organisms contains information on their nutrition, metabolism and life strategy (DeNiro and Epstein 1981). For instance, the $\delta^{13}C$ value can be used as an indicator for the relative autotrophic or heterotrophic character of scleractinian corals, as intense photosynthetic activity leads to a more positive $\delta^{13}C$ due to fractionation and fixation of the heavier C isotope (Muscatine et al. 1989). On a larger scale, the $\delta^{13}C$ signature was used by Fry et al. (1999) to follow the migration of zooplankton. Measurements of $\delta^{15}N$ proved to be robust indicators for trophic positions and food web structures (Schoeninger and DeNiro 1984), as nitrogen isotope fractionation during heterotrophic consumption results in $\delta^{15}N$ differences of about 3‰ per trophic level (Minagawa and Wada 1984; Schoeninger and DeNiro 1984), compared to less than 1‰ for $\delta^{13}C$ (Fry and Sherr 1984). Stable isotope labeling (artificial enrichment with ^{13}C and/or ^{15}N) of living organisms can be applied to produce isotopic signatures, which differ significantly from natural signatures and enable researchers to study the ecological pathways of organic matter contained in and produced by these organisms through the food web (e.g., Boschker et al. 1998, 1999; Herman et al. 2000; Middelburg et al. 2000). To investigate whether the natural isotopic signature of scleractinian coral mucus can be used to trace the pathway of mucus in the reef ecosystem we measured the stable isotopic composition of coral mucus and contrasted it to that of particulate organic matter (POM) found suspended in coral reef waters. In addition, we examined the transfer of inorganic artificial label (^{13}C and ^{15}N) into scleractinian coral mucus to develop a labeling technique that generates traceable mucus.

The feasibility to trace stable isotope labeled coral mucus was investigated in the following by focusing on the epizoic association of acoelomorph *Waminoa* worms to corals (Ogunlana et al. 2005; Barneah et al. 2007; Haapkylä et al. 2009). Worms of the genus *Waminoa* live attached to the external coral surface and infest coral colonies of different growth forms in variable numbers (Ogunlana et al. 2005). While the worms themselves form an association with endosymbiotic dinoflagellates zooxanthellae (*Symbiodinium*, and presumably *Amphidinium*), detrimental effects to the coral host, except for shading from ambient light, have not been reported to date (Barneah et al. 2007). Likewise, an explanation for the causality of this association and possible benefits for both involved organism groups is still missing. Barneah et al. (2007) observed the removal of the external surface mucus layer of the soft coral *Stereonephthya cundabiluensis* by associated *Waminoa* worms and suggested a possible metabolic utilization of this mucus by the worms. However, direct evidence for this possible trophic relationship is still wanted. Here, we demonstrate the use of ^{15}N-labeling to provide first evidence for the uptake of coral mucus by epizoic *Waminoa* worms.

Material and Methods

Study site

The investigations presented within the present study were carried out during four seasonal expeditions (November–December 2006 (fall), August–September 2007 (summer), February–March 2008 (winter) and May 2008 (spring)) to the Northern Gulf of Aqaba, Red Sea. All experiments and preparation of samples were carried out in the facilities of the Marine Science Station (MSS) Aqaba, Jordan (latitude: 29°27′N, longitude: 34°58′E).

Collection of natural particulate organic substrates

Seawater POM

Weekly seawater samples of 3000–5000 ml (n = 4) were collected from the MSS reef (10 m water depth) in clean plastic containers on three dates during each of 4 seasonal expeditions. These samples were immediately transferred to the laboratory and within 2 h prepared for stable isotopic analysis of suspended POM. Subsamples (1000 ml) were filtered onto pre-combusted (450°C; 4 h) GF/F filters (Whatman™, 25 mm diameter), subsequently dried for at least 48 h at 40°C and analyzed for stable isotopic signature as described below.

Coral mucus

Colonies of the dominant Red Sea scleractinian coral genera *Acropora* (n = 4; diameter: 32–45 cm), *Pocillopora* (n = 4; diameter: 21–34 cm), *Stylophora* (n = 4; diameter: 23–32 cm) and individual *Fungia* coral polyps (n = 12; diameter: 18–28 cm) were collected from the fore reef area in 10 m water depth using SCUBA. Corals were directly transported to the aquarium facilities of the MSS without air-exposure and kept in two 1000 l tanks supplied with identical seawater directly pumped from the reef (flow-through rate ~20 l min^{-1}). Prior to mucus collection, corals were left to recover

from possible stress during sampling for at least 24 h at in situ seasonal temperatures (21–29°C), salinity range (43 ± 1) and depth-specific light availability (daylight average: 216–400 µmol photons m^{-2} s^{-1}). Light availability was adjusted to in situ conditions by comparative measurements of seasonal in situ light availability at 10 m water depth using Onset HOBO® Pendant Temperature and Light Data Loggers UA-002-64 and application of black plastic gauze covering the cultivation tanks. Mucus collection was carried out according to the technique described in Wild et al. (2005b). Air-exposure, as a natural incident for scleractinian corals in the study area (Loya 1976) was used to initiate increased mucus production. Corals were exposed to air by holding them upside-down to collect the exuded mucus. Mucus release started immediately and was observed by long gel-like mucus threads dripping from the coral surface. The first 30 s of mucus production were discarded to prevent contamination and dilution through seawater. Subsequently, the released gel-like mucus (volumes: 5–25 ml) was collected for 2 min in a clean glass dish and immediately processed or frozen (–20°C) in until further use. From each coral 5–10 ml of mucus were filtered onto pre-combusted (450°C; 4 h) GF/F filters (Whatman™, 25 mm diameter). In the following, filters were dried for at least 48 h at 40°C and analyzed for stable isotopic composition as described below. In addition, subsamples (n = 4; 2 ml) of mucus obtained from *Acropora* corals were filtered through sterile 0.2-µm pore-sized polyethersulfone membrane filters into clean Eppendorf® reaction vials to remove potential particulate contents (e.g., microbes and expelled zooxanthellae) for later stable isotopic analysis of the filtered mucus matrix. These samples were subsequently frozen, lyophilized and analysed by mass-spectrometry like described below.

Mucus stable isotope labeling

Labeling time series

Stable isotope labeling of coral mucus was investigated to follow inorganic label incorporation over distinct time intervals. Individual *Fungia* coral polyps (n = 4; diameter 11–21 cm) were incubated in a 86 l flow-through aquarium supplied with fresh seawater (flow-through rate ~70 ml min^{-1}) with a single addition of 24 mg l^{-1} $NaH^{13}CO_3$ and 1 mg l^{-1} $Na^{15}NO_3$ (Cambridge Isotope Laboratories, 98 atom %) at the beginning of the incubation. After 3, 6, 24 and 31 h incubation time, mucus was collected from each of the polyps according to the following procedure: Polyps were separately taken out of the aquarium and washed within another aquarium filled with fresh label-free seawater. Mucus was subsequently collected as described above. From each coral polyp, 10 ml of mucus for each sampling were filtered onto pre-combusted (450°C; 4 h) GF/F filters (Whatman™, 25 mm diameter) and subsequently dried at 40°C for at least 48 h. Stable isotopic composition measurements of these filters were conducted like described below. In order to assess potential differences in label incorporation during light and dark periods, mucus samples were taken after periods of illumination with a 100W fluorescent tube (3, 6 and 31 h samplings; last sampling with 7 h previous illumination) and darkness (24 h sampling; 18 h previous darkness).

An additional labeling experiment was conducted to investigate the incorporation and transfer of inorganic ^{13}C and ^{15}N label within different compartments of corals (i.e., endosymbiotic zooxanthellae, tissue and mucus). To this end, freshly collected branching *Acropora* coral colonies (n = 3; diameter: 30–42 cm) were exposed to ^{13}C and ^{15}N label following the general mucus

labeling procedure (see below), after which mucus samples were collected and processed as described therein. Subsequently, terminal branches (n = 3; length = 3–4 cm) were sampled from each coral colony, of which the overlying tissue was removed using an artist's airbrush (Szmant and Gassman 1990) and collected in clean sealable plastic bags. The resulting tissue slurry was separated in zooxanthellae and coral tissue fractions by centrifugation following the technique described in Swart et al. (2005b). Zooxanthellae and coral tissue fractions (volume: 2 ml) were filtered onto pre-combusted (450°C; 4 h) GF/F filters (Whatman™, 25 mm diameter), subsequently dried at 40°C for at least 48 h and analysed for $\delta^{13}C$ and $\delta^{15}N$ signatures as described below. For direct comparison to the natural $\delta^{13}C$ and $\delta^{15}N$ signatures of zooxanthellae and coral tissue, material from freshly collected *Acropora* colonies (n = 4) was sampled and processed accordingly.

General mucus labeling procedure

As preliminary results from the labeling time series revealed only a relatively weak incorporation of the applied ^{13}C label, the general labeling procedure was limited to ^{15}N-labeling of coral mucus in subsequent experiments. Results from the labeling time series resulted in the formation of the following standard protocol for the ^{15}N-labeling of coral mucus: Fungiid *Fungia* (n = 12; diameter: 21–35 cm) and *Ctenactis* (n = 6; diameter: 30–43 cm) coral polyps, sampled from 10 m depth within the fore reef area, were incubated in four 50 l flow-through aquaria, supplied with fresh seawater directly pumped from the reef, under in situ temperature and light conditions adjusted as described above. Over a period of 3 d, the water inflow was stopped at 08:00 hrs and each aquarium was treated with $Na^{15}NO_3$ (Cambridge Isotope Laboratories, 98 atom %) to a final concentration of 1 mg l^{-1}. After 4 h, the water flow-through was re-established to 100 ml min^{-1}. Before label additions on the second and third day, each aquarium was flushed for 1 h at a flow-through rate of ~10 l fresh label-free seawater min^{-1}. Labeled mucus was collected at the end of the day-light period (17:00–19:00 hrs) of the third incubation day according to the following procedure: Coral polyps were individually taken out of the labeling setup and washed for 1 min in a stream of fresh label-free seawater. Subsequently, corals were transferred individually into opened sealable plastic bags. By the use of the plastic bags, increased mucus production due to air-exposure was ensured, while the released mucus was entirely collected. After 10 min, corals were removed from the bags and placed back into the labeling setup, while the plastic bags containing the exuded mucus were instantly sealed. In the following, the collected mucus was either processed as described below or immediately used in mucus tracer experiments. Mucus subsamples (5–10 ml) from each coral polyp were either directly filtered onto pre-combusted (450°C; 4 h) GF/F filters (Whatman™, 25 mm diameter), or previously filtered through sterile 0.2-µm pore-sized polyethersulfone membrane filters, and subsequently dried at 40°C for at least 48 h to determine the resulting ^{15}N label.

Incubation experiment with *Waminoa* worms

To investigate the proposed uptake of coral mucus by epizoic acoelomorph *Waminoa* worms, *Acropora* (n = 2) and *Stylophora* (n = 2) coral colonies (diameter: 20–25 cm) densely infested with *Waminoa* were sampled from the fore reef area in 6 m water depth using SCUBA and immediately transported without air-exposure to the laboratory using air-tight sealable plastic bags. Under

submerged conditions, approximately 200 worms (individual diameter: 1.0–1.9 mm) were removed from the surface of each coral colony by the gentle water jet of a plastic Pasteur pipette and subsequently collected in a glass container filled with fresh seawater. To isolate *Waminoa* from the ambient planktonic community, individual worms were washed 5-fold in 0.2 µm freshly filtered seawater (FSW) by single transfers through 5 FSW-filled glass dishes using clean plastic pipettes. To investigate the natural N isotopic signature of *Waminoa*, groups of 10 worms (n = 5) were filtered onto pre-combusted GF/F filters (Whatman™, 25 mm diameter), which were subsequently processed and analysed like described below. A total of 180 *Waminoa* worms were placed in groups of 10 each into 18 round glass dishes (diameter: 4.5 cm) filled with 10 ml of FSW. Freshly collected ^{15}N-labeled gel-like coral mucus (5 ml; $\delta^{15}N$ = 2131 ± 37‰, mean ± SE; mixture of *Ctenactis* and *Fungia* mucus) was added to 6 randomly selected glass dishes, while 6 other glass dishes were treated with an equal volume of a 0.2 µm filtrate of the same ^{15}N-labeled coral mucus material (5 ml; $\delta^{15}N$ = 1969 ± 18‰). The remaining 6 dishes served as controls without coral mucus addition. *Waminoa* worms were incubated in a time series for 7 or 28 h in the laboratory at in situ temperature and light availability adjusted by comparable measurements of in situ temperature and light availability at 6 m water depth using Onset HOBO® Pendant Temperature and Light Data Loggers UA-002-64 and application of variable layers of black plastic gauze covering the incubation (12:12 h, light:dark cycle). After 7 and 28 h, three glass dishes for each treatment and control were removed from the incubation and the contained worms were sampled by clean pipettes and individually washed 5-fold using FSW like described above. Preliminary studies proved this washing procedure being effective to remove remaining ^{15}N-labeled external mucus material (Niggl et al. unpublished data). Washed worms were filtered onto 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 by mass spectrometry like described below.

Mass spectrometric measurements

All samples for stable isotope analysis were measured using a Thermo™ NA 2500 elemental analyzer, coupled with a THERMO/Finnigan Conflo II-interface to a THERMO/Finnigan MAT Delta plus isotope ratio mass spectrometer. Stable C and N isotopic ratios were expressed in the conventional delta notation ($\delta^{13}C$, $\delta^{15}N$) relative to the standards Vienna PeeDee Belemnite (VPDB) (Coplen 1995) and atmospheric nitrogen (AIR) (Mariotti 1983), respectively. A standard deviation of <0.15‰ for C and N was derived from repeated measurements of laboratory standard (Peptone) of various initial weights.

Data analysis

Statistical tests were performed applying the Mann and Whitney U-test (2-tailed) inside SPSS© software packages and results were regarded as statistically significant at $P < 0.05$, if not mentioned differently.

Results

Natural isotopic signature of coral mucus

The carbon stable isotopic signature of gel-like mucus from all investigated coral genera ($\delta^{13}C$ = -16.2 ± 0.4; mean ± SE) showed significant differences in comparison to seawater POM values ($\delta^{13}C$ = -20.9 ± 0.9) generated during the respective seasonal samplings (Table 1). Seasonal differences in $\delta^{13}C$ of mucus were apparent for *Acropora* and *Fungia* corals, which displayed significantly lower values during fall and winter seasons, respectively. Significant genus-specific differences in $\delta^{13}C$ were observed during winter season, where only *Fungia* and *Stylophora* corals showed a similar carbon isotopic signature. Coral mucus $\delta^{15}N$ differed significantly from seawater POM for the majority of tested coral genera on a seasonal basis (Table 1). Genus-specific differences in $\delta^{15}N$ were significant for *Acropora* and *Fungia* in comparison to *Pocillopora* and *Stylophora* during winter season, where the latter pair displayed nearly identical values (Table 1). Filtered mucus (mucus matrix) of *Acropora* corals showed similar $\delta^{13}C$ values (-15.1 ± 0.2, mean ± SE) in comparison to gel-like mucus (-15.4 ± 0.2), but was significantly depleted in $\delta^{15}N$ (-0.6 ± 0.2, mean ± SE) compared to its non-filtered counterpart (0.7 ± 0.2).

Table 1 *Natural stable carbon and nitrogen isotopic signatures of gel-like coral mucus (means ± SE) and seawater POM collected during different seasons.*

Season	Material	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	n
Spring (2008)	*Fungia* mucus	-15.7 ± 0.3	1.4 ± 0.3	8
	Seawater POM	-18.9 ± 0.4	5.2 ± 0.5	12
Summer (2007)	*Acropora* mucus	-15.7 ± 0.2	0.9 ± 0.2	4
	Fungia mucus	-15.2 ± 0.2	0.2 ± 0.1	10
	Seawater POM	-20.0 ± 0.3	-2.1 ± 0.5	12
Fall (2006)	*Fungia* mucus	-19.4 ± 1.1	4.1 ± 1.6	4
	Seawater POM	-22.5 ± 0.3	1.0 ± 1.0	12
Winter (2008)	*Acropora* mucus	-17.2 ± 0.2	3.4 ± 0.7	4
	Fungia mucus	-15.5 ± 0.2	4.9 ± 0.4	4
	Pocillopora mucus	-16.9 ± 0.4	-1.0 ± 0.3	4
	Stylophora mucus	-15.0 ± 0.9	-1.0 ± 0.6	4
	Seawater POM	-22.1 ± 0.3	4.1 ± 1.0	8
Mean	Coral mucus	-16.2 ± 0.4	1.5 ± 0.7	∑ = 42
	Seawater POM	-20.9 ± 0.9	2.1 ± 1.6	∑ = 44

Mucus stable isotope labeling

During illumination periods of the labeling time series experiment, inorganic ^{13}C and ^{15}N labels were rapidly (3 h) incorporated into *Fungia* mucus (Fig. 1). During periods of darkness, concentrations of both labels in exuded mucus decreased over time. Maximum recorded labeling of released gel-like mucus during the labeling time series experiment was 122 ± 5‰ (mean ± SE) for $\delta^{13}C$ and 2100 ± 151‰ for $\delta^{15}N$.

The additional labeling experiment with *Acropora* corals revealed that the supplied ^{13}C and ^{15}N labels were detectable in all investigated parts of the coral (i.e., tissue, zooxanthellae, gel-like mucus and filtered mucus), as $\delta^{13}C$ and $\delta^{15}N$ values in corals from the labeling experiment were significantly higher than in natural corals (Fig. 2). Values for $\delta^{13}C$ and $\delta^{15}N$ were similar, i.e. not statistically different, for coral tissue and zooxanthellae in both natural and labeled coral samples, respectively. Regarding $\delta^{13}C$, values for gel-like mucus corresponded to $\delta^{13}C$ of coral tissue and zooxanthellae for labeled and natural corals. This was also true for $\delta^{13}C$ of filtered mucus originating from natural corals, but not for the labeled counterpart, which was significantly depleted in ^{13}C compared to the remaining investigated labeled coral components (Fig. 2). The $\delta^{15}N$ of gel-like and filtered mucus differed significantly from results for coral tissue and zooxanthellae in labeled and natural corals. Natural gel-like and filtered mucus were significantly depleted in ^{15}N compared to natural coral tissue and zooxanthellae, while labeled gel-like mucus was significantly enriched in ^{15}N in comparison to labeled tissue and zooxanthellae (Fig. 2). However, $\delta^{15}N$ values for filtered labeled mucus were significantly lower than for all investigated labeled coral components.

The general mucus labeling procedure applying ^{15}N-label to *Fungia* and *Ctenactis* corals resulted in similar maximum $\delta^{15}N$ values for gel-like mucus of *Fungia* corals (2187 ± 137‰; mean ± SE) after 3 d of incubation compared to the preliminary time series experiment (2100 ± 151‰). In comparison to *Fungia*, gel-like *Ctenactis* mucus showed a significantly lower ^{15}N-label (1674 ± 143‰). As found for labeled *Acropora* mucus, $\delta^{15}N$ values of filtered mucus were significantly depleted for *Fungia* (1350 ± 99‰; mean ± SE) and *Ctenactis* (775 ± 60‰) in comparison to the gel-like mucus material.

Incubation experiment with *Waminoa* worms

The time series incubation experiment with epizoic *Waminoa* worms revealed a significant transfer of ^{15}N-labeled coral mucus compounds into worms treated with gel-like as well as with filtered coral mucus. After 7 and 28 h of incubation, $\delta^{15}N$ values of *Waminoa* worms in both mucus treatments were significantly elevated in comparison to worms in controls (Fig. 3). Addition of ^{15}N-labeled filtered coral mucus resulted in an increase of $\delta^{15}N$ in *Waminoa* with a maximum of 26 ± 4‰ (mean ± SE) after 28 h of incubation. Gel-like and filtered ^{15}N-labeled coral mucus caused a similar $\delta^{15}N$ signature in *Waminoa* after 7 h incubation, whereby treatment with gel-like ^{15}N-labeled mucus resulted in a significantly higher $\delta^{15}N$ in *Waminoa* (44 ± 7‰; mean ± SE) at the end of the incubation experiment (28 h). Worms in control treatments showed a significant depletion in ^{15}N after 7 h which remained stable after 28 h of incubation (Fig. 3).

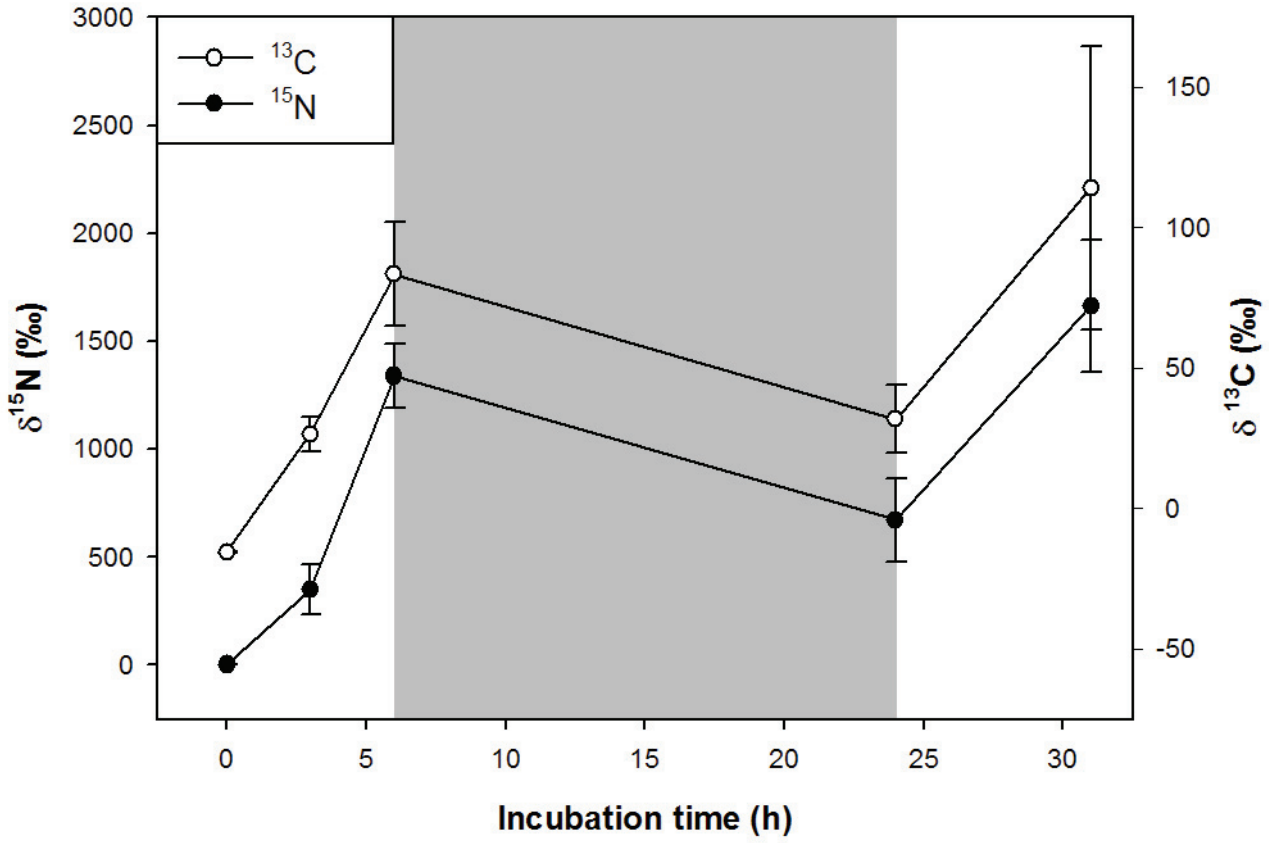

Fig. 1 *Change of $\delta^{13}C$ and $\delta^{15}N$ in coral mucus released from Fungia polyps during the time series incubation experiment. Shaded area shows the period when illumination of the tank was switched off. Values are given means ± SE of n = 4 Fungia coral polyps*

The percentage contribution of coral mucus N to total *Waminoa* biomass was estimated using the following linear mass balance equation derived from an isotope mixing model (c.f., Fry 2006):

$$x = \left(\delta^{15}N_{w,tn} - \delta^{15}N_{w,t_0}\right) / \left(\delta^{15}N_{m} - \delta^{15}N_{w,t_0}\right) \times 100 \quad (a)$$

(a): where x is the resulting percentage contribution of mucus N; $\delta^{15}N_{w,tn}$ is the signature of *Waminoa* after n hours incubation; $\delta^{15}N_{w,t0}$ is the natural signature of *Waminoa*; $\delta^{15}N_{m}$ is the signature of provided ^{15}N-labeled mucus

According to this calculation, the resulting percentage contribution of ^{15}N-labeled coral mucus to total *Waminoa* biomass ranged from 0.5–0.6% and 1.4–2.4% after 7 and 28 h of incubation, respectively.

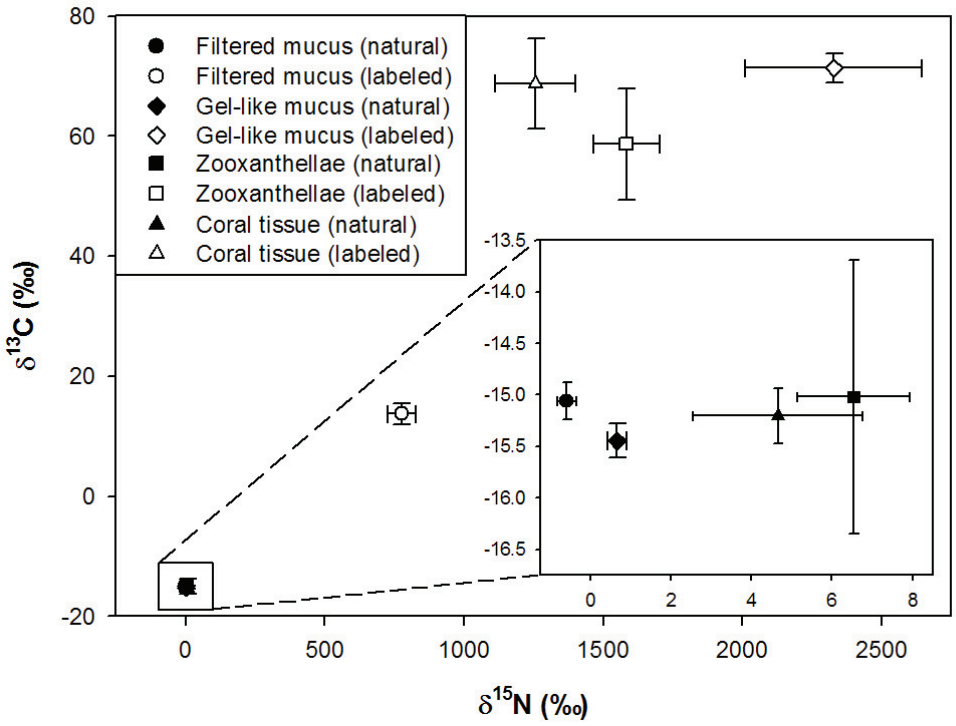

Fig. 2 *Stable isotope signatures ($\delta^{13}C$ and $\delta^{15}N$) of natural and artificially labeled (^{13}C and ^{15}N-enriched) Acropora coral mucus (filtered and gel-like mucus), coral tissue and endosymbiotic zooxanthellae. Values are given as means ± SE*

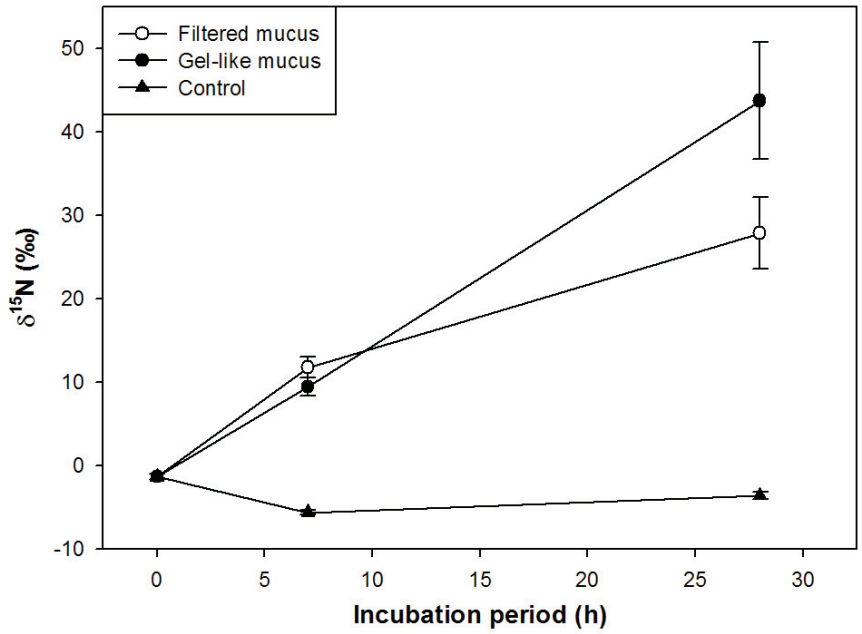

Fig. 3 *$\delta^{15}N$ signatures of epizoic Waminoa worms after 7 and 28 h of incubation with ^{15}N-labeled coral mucus and under control conditions. Uptake of mucus-N is indicated by continuous increase of $\delta^{15}N$ in Waminoa worms exposed to ^{15}N-enriched mucus (filtered and gel-like mucus). Values are given means ± SE of n = 3 replicate incubations including 10 Waminoa worms each.*

Discussion

Natural isotopic signature of coral mucus

The $\delta^{13}C$ signature of POM suspended in Red Sea waters measured in the present study was similar to that observed in coral reefs of the Western Pacific (Yamamuro et al. 1995), and is consistent with $\delta^{13}C$ values of organic matter derived from phytoplankton, which generally range from -24 to -18‰ (Heip et al. 1995). Coral mucus $\delta^{13}C$ was significantly higher than seawater POM which, surprisingly, may indicate a relatively minor contribution of coral-derived organic matter to total POM suspended in reef waters of the Northern Red Sea. This finding may be explained by the reported high dissolution ratio of coral mucus (56–80%, Wild et al. 2004b) possibly resulting in an underestimation of coral-derived organic matter content by seawater POM sampling. Natural coral tissue and zooxanthellae $\delta^{13}C$ values are in the range reported by Muscatine et al. (1989) for corals originating from the same water depth (10 m), while $\delta^{15}N$ values are comparable to results obtained by Swart et al. (2005a). Zooxanthellae provide a large proportion of the organic carbon used for the synthesis of coral mucus (Crossland et al. 1980, Davies 1984), which is reflected here by the natural $\delta^{13}C$ signatures of mucus (gel-like mucus and filtered mucus matrix) of *Acropora* corals showing similar values in comparison to zooxanthellae and coral tissue. Further, similarity of $\delta^{13}C$ in zooxanthellae and coral tissue indicates a minor contribution of heterotrophy, as also reported by Muscatine et al. (1989), and emphasizes the functional autotrophic character of scleractinian corals. The important role of the autotrophic endosymbionts in mucus synthesis is also reflected by seasonal differences of $\delta^{13}C$ in mucus of *Acropora* corals, where higher values during summer season (higher irradiance; Naumann et al. unpublished data) indicate decreased isotopic fractionation of photosynthetically fixed carbon. Genus-specific differences in $\delta^{13}C$ observed here during winter season may further suggest genus-specific strategies regarding the contribution of heterotrophy during periods of lower irradiance, as shown for decreasing $\delta^{13}C$ in zooxanthellae in relation to increasing water depth (Muscatine et al. 1989).

As for $\delta^{13}C$, the significant difference in $\delta^{15}N$ found for coral mucus and seawater POM reflects the major contribution of zooxanthellae-derived photosynthates in coral mucus. Genus-specific differences, as found during winter samplings, may however suggest genus-specific feeding strategies under comparable environmental conditions. A possible influence of seasonal feeding strategies is also reflected in the mucus obtained from *Fungia* corals with lower $\delta^{15}N$ values during oligotrophic, high irradiance summer and spring seasons in comparison to fall and winter seasons known for elevated inorganic nutrient levels and lower irradiance (Rasheed et al. 2002; Naumann et al. unpublished data). Significant differences in $\delta^{15}N$ for gel-like and filtered mucus likely reflect the removal of zooxanthellae cell material by sterile filtration. Mucus $\delta^{15}N$ values close to 0‰ found for different coral genera during different seasons may additionally indicate a differential contribution of N_2 fixation under variable seasonal environmental conditions in this sub-tropical region. The extent to which N_2 fixation contributes to the N requirements of corals in the oligotrophic reef environment remains uncertain; however it is generally assumed that a large fraction is met by N fixation through cyanobacteria endosymbionts (Lesser et al. 2004).

Mucus stable isotope labeling

The results presented here provide first evidence that coral mucus can be efficiently labeled using inorganic ^{13}C and ^{15}N compounds. Transfer of ^{13}C and ^{15}N into mucus of *Fungia*, *Ctenactis* and *Acropora* corals was achieved by incubation in ^{13}C and ^{15}N-enriched seawater. In addition, both labels were detectable in the coral tissue and zooxanthellae endosymbionts indicating a transfer of labeled compounds within the coral holobiont. Uptake of both labels was already detectable in the mucus after 3 h of incubation suggesting a rapid transfer of fixed C and N-containing metabolites, like amino acids or peptides from the zooxanthellae to the mucus producing coral host. This transfer pathway is confirmed by previous studies using radio isotope labeling (e.g., Schlichter et al. 1983).

We also could demonstrate that incorporation of both labels into coral mucus depends on the light history of the incubated corals. The labeling intensity increased during light and decreased during dark incubation of the mucus producing corals. For the carbon label, this is caused by the photosynthetic activity of the zooxanthellae when illuminated, delivering up to 90% of the fixed C to the coral host (Barnes and Hughes 1999; Goreau et al. 1979, Muscatine et al. 1981) that releases up to 50% of the C fixed by its zooxanthellae as mucus (Crossland et al. 1980, Davies 1984). In the dark, transfer of labeled C to the coral host is minimal (Black and Burries 1983; Muscatine et al. 1984) and direct respiration of photosynthates (e.g., glycerol; Schlichter et al. 1983) translocated during light conditions may be responsible for the observed decrease in ^{13}C labeling intensity. The $\delta^{15}N$ decrease in coral mucus after dark incubation of the corals was most likely caused by the light-dependent zooxanthellae uptake of $^{15}NO_3^+$ under high concentrations of dissolved inorganic N (DIN) (Grover et al. 2003) and the respiring of N-rich compounds to DIN by the coral holobiont. It is therefore recommended to incubate corals for at least 30 h with illumination in order to gain strongly labeled mucus. The significant difference in $\delta^{15}N$ observed for labeled gel-like and filtered mucus of all tested corals most probably results from the removal of highly ^{15}N-enriched zooxanthellae cells from gel-like mucus following sterile filtration.

Uptake of coral mucus by *Waminoa* worms

Based on observations describing the removal of the external surface mucus layer in *Waminoa*-infested soft corals, previous studies were able to make assumptions on the possible metabolic utilization of coral mucus by epizoic *Waminoa* worms (Barneah et al. 2007). Results from the present study clearly indicate that ^{15}N-labeled mucus compounds are transferred into *Waminoa* worms, while the continuous increase of $\delta^{15}N$ by incorporation of mucus-N within the worms (up to 2.4% of *Waminoa* N-biomass after 28 h exposure) provides first evidence for the utilization of coral mucus as a food source by *Waminoa*. The significantly increased $\delta^{15}N$ of worms treated with gel-like mucus in comparison to filtered mucus further highlights the heterotrophic utilization of coral mucus by *Waminoa*, as this increase was probably caused by the parallel uptake of highly ^{15}N-enriched zooxanthellae cells trapped within the mucus. In addition, depletion of $\delta^{15}N$ in *Waminoa* from mucus-free controls after 7 and 28 h of incubation supports the established role of coral mucus N sources in *Waminoa* nutrition; likely compensated here by a shift to an increased metabolic contribution by photosynthates generated by the worm's endosymbionts. During the present study, *Waminoa* were predominantly observed on *Acropora* and *Stylophora* corals, which represent

dominant scleractinian coral taxa in the study area (Naumann et al. unpublished data). *Acropora* and *Stylophora* corals have additionally been identified as dominant POM producers amongst hermatypic Red Sea coral taxa (Naumann et al. unpublished data), which may provide an explanation for the preferred association of *Waminoa* with these genera. However, *Waminoa* originating from *Acropora* and *Stylophora* corals fed on mucus released by *Fungia* and *Ctenactis* corals, where no association was found to date (Barneah et al. 2007; Haapkylä et al. 2009; Naumann, personal observation), suggesting that *Waminoa* may be able to utilize mucus compounds from a variety of coral species. The question why *Waminoa* only occur on specific coral taxa remains unanswered. Additional research will be required to gain knowledge about the proportional contribution of heterotrophic mucus feeding and energy supply through photosynthesis by the worm's endosymbionts; accompanied by investigations on possible associations of different epizoic acoelomorph worm species with specific coral taxa.

Although there are no indications for *Waminoa* directly damaging the coral host by feeding on tissue components (Ogunlana et al. 2005), feeding on the surface mucus layer and thus removal of this important protective barrier, may lead to a weakening of the coral's resistance to environmental impacts (e.g., sedimentation) and further enhance susceptibility to potential diseases (Ritchie et al. 2006). Dense infestations by *Waminoa* worms, as observed on *Turbinaria*, *Acropora* and *Stylophora* corals in the Northern Red Sea (Barneah et al. 2007; Naumann, personal observation), may therefore have at least two potential indirect detrimental effects on corals, physical shading of ambient light and reduction of the coral's defense potential. Nevertheless, as no detrimental effects on the coral host have been described to date, the association of epizoic *Waminoa* worms with scleractinian corals will require further investigation to fully resolve its potential function, including the possible role of *Waminoa* as a pest in corals.

Why label coral mucus?

Previous research has shown that coral mucus may be a carrier of energy and nutrients in the reef ecosystem (Johannes 1967; Ducklow and Mitchell 1979a, b; Coffroth 1990; Ferrier-Pages et al. 2000; Wild et al. 2004a, b). However, the conclusions derived from this research attest coral mucus a contribution in the cycles of matter in the reef ranging from insignificant (Coffroth 1990; Vacelet and Thomassin 1991) to important (Wild et al. 2004a). Consensus is, however, that mucus contributes a dominant fraction to the pools of particulate and dissolved organic matter in reef environments (Johannes 1967; Marshall 1968). Accepted is also that coral mucus contains significant amounts of proteins, lipids and nutrients (Benson and Muscatine 1974; Crossland et al. 1980, Coffroth 1990, Wild et al. 2005a), and further research is necessary to investigate the fate and role of this material in the cycles of matter in the reef. Coral bleaching, as observed more frequently in recent years (e.g., Hoegh-Guldberg et al. 1999) may lead to enhanced release of mucus by corals (Glynn 1993; Niggl et al. in press). Investigating the dynamics and pathways of mucus in coral reefs may provide insights into the consequences of bleaching events for the reef ecosystem and the rapid decay of coral reefs associated with these events. In this study, we show that the mucus stable isotope signature can differ significantly from seawater POM. However, these differences may not be sufficient to trace coral mucus within the reef ecosystem. Nevertheless, this study also shows

that coral mucus can be labeled with stable isotopes and that the labeled mucus can be successfully traced to mucus-consuming organisms. Coral mucus labeling with stable isotopes, thus, may be a powerful tool to investigate pathways of organic material contained in coral mucus through the detrital food chain of coral reef ecosystems. In contrast to labeling of coral-derived organic matter using potentially harmful radioactive isotopes (Richman et al. 1975), stable isotope labeling of coral mucus provides the advantage to be applicable within in situ investigations and mesocosm experiments, which may already predict a regular application of this approach in future coral reef research.

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References

Barneah O, Brickner I, Hooge M, Weis VM, LaJeunesse TC, Benayahu Y (2007) Three party symbiosis: acoelomorph worms, corals and unicellular algal symbionts in Eilat (Red Sea). Mar Biol 151:1215–1223

Barnes R, Hughes R (1999) An introduction to marine ecology. Blackwell Scientific Publications, Oxford

Benson A, Muscatine L (1974) Wax in coral mucus - energy transfer from corals to reef fishes. Limnol Oceanogr 19: 810–814

Black CC, Burris JE (1983) Diurnal carbon-14 partitioning between zooxanthellae and the coral animal tissue of intact *Seriatopora hystrix* colonies. Mar Biol 75:117–120

Boschker HTS, Nold SC, Wellsbury P, Bos D, deGraaf W, Pel R, Parkes RJ, Cappenberg TE (1998) Direct linking of microbial populations to specific biogeochemical processes by ^{13}C-labelling of biomarkers. Nature 392:801–805

Boschker HTS, deBrouwer JFC, Cappenberg TE (1999) The contribution of macrophyte derived organic matter in microbial biomass in salt marsh sediments: stable carbon-isotope analysis of microbial biomarkers. Limnol Oceanogr 44:309–319

Coffroth MA (1990) Mucous sheet formation on poritid corals - an evaluation of coral mucus as a nutrient source on reefs. Mar Biol 105:39–49

Crossland C, Barnes D, Borowitzka M (1980) Diurnal lipid and mucus production in the staghorn coral *Acropora acuminata*. Mar Biol 60:81–90

Davies PS (1984) The role of zooxanthellae in the nutritional energy requirements of *Pocillopora eydouxi*. Coral Reefs 2:181–186

DeNiro MJ, Epstein S (1981) Influence of diet on the distribution of nitrogen isotopes in animals. Geochim Cosmochim Acta 45:341–351

Ducklow HW, Mitchell R (1979a) Composition of mucus released by coral reef coelenterates. Limnol Oceanogr 24:706–714

Ducklow H, Mitchell R (1979b) Bacterial populations and adaptations in the mucus layers on living corals. Limnol Oceanogr 24:715–725

Ferrier-Pages C, Leclercq N, Jaubert J, Pelegri SP (2000) Enhancement of pico- and nanoplankton growth by coral exudates. Aquat Microb Ecol 21:203–209#

Fry B (2006) Stable isotope ecology. Springer, Berlin

Fry B, Sherr E (1984) δ^{13}C measurements as indicators of carbon flow in marine and freshwater ecosystems. Contrib Mar Sci 27:13–47

Fry B, Mumford PL, Robblee MB (1999) Stable isotope studies of pink shrimp (*Farfantepenaeus duorarum* Burkenroad) migrations on the southwestern Florida shelf. Bull Mar Sci 65:419–430

Glynn PW (1993). Coral reef bleaching-ecological perspectives. Coral Reefs 12:1–17
Goreau TF, Goreau NI, Goreau TJ (1979) Corals and coral reefs. Sci Am 241:124–135
Grover R, Maguer JF, Allemand D, Ferrier-Pages C (2003) Nitrate uptake in the scleractinian coral *Stylophora pistillata*. Limnol Oceangr 48:2266–2274
Haapkylä J, Seymour AS, Barneah O, I Brickner, Hennige S, Suggett D, Smith D (2009) Association of *Waminoa* sp. (Acoela) with corals in the Wakatobi Marine Park, South-East Sulawesi, Indonesia. Mar Biol 156:1021–1027
Heip CHR, Goosen NK, Herman PMJ, Kromkamp J, Middelburg JJ, Soetart K (1995) Production and consumption of biological particles in temperate tidal estuaries. Oceanogr Mar Biol Annu Rev 33:1–150
Herman PMJ, Middelburg JJ, Widdows J, Lucas CH, Heip CHR (2000) Stable isotopes as trophic tracers: combining field sampling and manipulative labeling of food resources for macrobenthos. Mar Ecol Prog Ser 204:79–92
Hoegh-Guldberg O (1999) Climate change, coral bleaching and the future of the world's coral reefs. Mar Freshw Res 50:839–866
Huettel M, Wild C, Gonelli S (2006) Mucus trap in coral reefs: formation and temporal evolution of particle aggregates caused by coral mucus. Mar Ecol Prog Ser 307:69–84
Johannes R (1967) Ecology of organic aggregates in the vicinity of a coral reef. Limnol Oceanogr 12:189–195
Krupp DA (1984) Mucus production by corals exposed during an extreme low tide. Pac Sci 38:1–11
Lesser MP, Mazel CH, Gorbunov MY, Falkowski PG (2004) Discovery of symbiotic nitrogen-fixing cyanobacteria in corals. Science 305:997–1000
Loya Y (1976) Recolonization of Red Sea corals affected by natural catastrophes and man-made perturbations. Ecology 57:278–289
Mariotti A (1984) Atmospheric nitrogen is a reliable standard for natural ^{15}N abundance measurements. Nature 303:685–687
Marshall M (1968) Observations on organic aggregates in the vicinity of coral reefs. Mar Biol 2:50–55
Marshall AT, Wright OP (1993) Confocal laser scanning light microscopy of the extra-thecal epithelia of undecalcified scleractinian corals. Cell Tissue Res 272:533–543
Meikle P, Richards G, Yellowlees D (1988) Structural investigations on the mucus from 6 species of coral. Mar Biol 99:187–193
Middelburg JJ, Barranguet C, Boschker HTS, Herman MJ, Moens T, Heip CHR (2000) The fate of intertidal microphytobenthos carbon: an in situ ^{13}C-labeling study. Limnol Oceanogr 45:1224–1234
Minagawa M, Wada E (1984) Stepwise enrichment of ^{15}N along food chains. Further evidence and the relation between $\delta^{15}N$ and animal age. Geochim Cosmochim Acta 48:1135–1140
Muscatine L, McCloskey LR, Marian RE (1981) Estimating the daily contribution of carbon from zooxanthellae to coral animal respiration. Limnol Oceanogr 26:601–611
Muscatine L, Falkowski P, Porter J, Dubinsky Z (1984) Fate of photosynthetically-fixed carbon in light and shade-adapted colonies of the symbiotic coral *Stylophora pistillata*. Proc R Soc London B 222:181–202
Muscatine L, Porter JW, Kaplan IR (1989) Resource partitioning by reef corals as determined from stable isotope composition I. $\delta^{13}C$ of zooxanthellae and animal tissue versus depth. Mar Biol 100:185–193
Naumann MS, Richter C, el-Zibdah M, Wild C (2009) Coral mucus as an efficient trap for picoplanktonic cyanobacteria – implications for pelagic-benthic coupling in the reef ecosystem. Mar Ecol Prog Ser 385:65–76
Niggl W, Glas M, Laforsch C, Mayr C, Wild C (in press) First evidence of coral bleaching stimulating organic matter release by reef corals. In: Proceedings of the 11th international coral reef symposium, Fort Lauderdale, USA
Ogunlana M, Hooge MD, Tekle YI, Benayahu Y, Barneah O, Tyler S (2005) *W. brickneri* n. sp. (Acoela: Acoelomorpha) associated with corals in the Red Sea. Zootaxa 1008:1–11
Rasheed M, Badran MI, Richter C, Huettel M (2002) Effect of reef framework and bottom sediment on nutrient enrichment in a coral reef of the Gulf of Aqaba, Red Sea. Mar Ecol Prog Ser 239:277–285
Richman S, Loya Y, Slobodkin L (1975) Rate of mucus production by corals and its assimilation by the coral reef copepod *Acartia negligens*. Limnol Oceanogr 20:918–923
Ritchie KB (2006) Regulation of microbial populations by coral surface mucus and mucus-associated bacteria. Mar Ecol Prog Ser 322:1–14
Schlichter D, Svoboda A, Kremer BP (1983) Functional autotrophy of *Heteroxenia fuscescens* (Anthozoa: Alcyonaria): carbon assimilation and translocation of photosynthates from symbionts to host. Mar Biol 78:29–38
Schoeninger MJ, DeNiro MJ (1984) Nitrogen and carbon isotopic composition of bone collagen from marine and terrestrial animals. Geochim Cosmochim Acta 48:625–639
Schuhmacher H (1977) Ability of fungiid corals to overcome sedimentation. In: Proceedings of the 3rd international coral reef symposium Miami, USA 1:503-509
Schwarcz HP (1991) Some theoretical aspects of isotope paleodiet studies. J Archeol Sci 18:261–275
Swart PK, Saied A, Lamb K (2005a) Temporal and spatial variation in the $\delta^{15}N$ and $\delta^{13}C$ of coral tissue and zooxanthellae in *Montastrea faveolata* collected from the Florida reef tract. Limnol Oceanogr 50:1049–1058

Swart PK, Szmant A, Porter JW, Dodge RE, Tougas JI, and Southam JR (2005b) The isotopic composition of respired carbon dioxide in scleractinian corals: Implications for cycling of organic carbon in corals. Geochim Cosmochim Acta 69:1495–1509

Szmant AM, Gassman NJ (1990) The effects of prolonged "bleaching" on the tissue biomass and reproduction of the reef coral *Montastrea annularis*. Coral Reefs 8:217–224

Vacelet E, Thomassin B (1991) Microbial utilization of coral mucus in long term in situ incubation over a coral reef. Hydrobiologia 211:19–32

Wild C, Rasheed M, Werner U, Franke U, Johnstone R, Huettel M (2004a) Degradation and mineralization of coral mucus in reef environments. Mar Ecol Prog Ser 267:159–171

Wild C, Huettel M, Klueter A, Kremb SG, Rasheed M, Jørgensen BB (2004b) Coral mucus functions as an energy carrier and particle trap in the reef ecosystem. Nature 428:66–70

Wild C, Woyt H, Huettel M (2005a) Influence of coral mucus on nutrient fluxes in carbonate sediments. Mar Ecol Prog Ser 287:87–98

Wild C, Rasheed M, Jantzen C, Cook P, Struck U, Huettel M, Boetius A (2005b) Benthic metabolism and degradation of natural particulate organic matter in silicate and carbonate sands of the Northern Red Sea. Mar Ecol Prog Ser 298:69–78

Yamamuro M, Kayanne H, Minagawa M (1995) Carbon and nitrogen stable isotopes of primary producers in coral reef ecosystems. Limnol Oceanogr 40:617–621

8

Organic matter release by the benthic upside-down jellyfish *Cassiopea* sp. fuels pelagic food webs in coral reefs

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Abstract

Recent studies have demonstrated that organic matter released by hermatypic corals can play an important role as carrier of energy, thereby initiating element cycles in coral reef systems. However, although another commonly occurring cnidarian, the scyphozoan upside-down jellyfish *Cassiopea* sp., can reach high abundances in such reef systems, its potential contribution to cycles of matter remains unresolved. Therefore this study aimed to quantify organic matter release by *Cassiopea* from the Northern Red Sea and evaluate whether this material is transferred to planktonic microbes and zooplankton. Mean particulate organic matter release was 21.2 ± 9.4 mg POC and 2.3 ± 1.1 PN m^{-2} jellyfish surface area h^{-1}, which exceeds release rates reported for hermatypic corals by factors of 2 to 15. Labelling experiments using stable N isotopes demonstrated uptake of *Cassiopea*-derived organic matter by the jellyfish-associated zooplanktonic mysids *Idiomysis tsurnamali*. Incubation experiments revealed that O_2 consumption by microbes and zooplankton was 5.9 and 3.8-fold higher compared to seawater controls, respectively, when *Cassiopea*-derived organic matter was present, which demonstrates fast mineralization of *Cassiopea*-derived organic matter. These findings indicate that *Cassiopea*-derived organic matter may function as a newly discovered trophic pathway for organic C and N from the benthic environment to pelagic food chains in coral reef and other marine ecosystems.

Introduction

Release of organic matter is common among marine plants and animals. Extracellular release, mainly of dissolved organic matter, has been studied most extensively for phytoplankton (e. g. Anderson and Zeutschel, 1970; Thomas, 1971; Berman and Holm-Hansen, 1974; Sharp, 1977), but it has also been demonstrated that marine macrophytes release organic matter in remarkable amounts (e. g. Khailov and Burlakova, 1969; Wetzel, 1969; Brylinsky, 1977; Carlson and Carlson, 1984; Sondergaard, 1990). Reasons for such release depend on various factors, but include active disposal of excess carbon (Fogg, 1983) and/or passive permeation through the cell membrane (Bjornsen, 1988). Furthermore, animals such as fish (Denny, 1989; Coello and Khan, 1996; Ebran et al., 2000), gastropods (Kappner et al., 2000), bivalves (Morton, 2000), zooplankton (Webb and Johannes, 1967; Von Vaupel Klein and Koomen, 1994; Steinberg et al., 2004) and several cnidarian species (e.g. Ducklow and Mitchell, 1979b; Hansson and Norrman, 1995; Ferrier-Pages et al., 1998) are also known to produce and release organic exudates e. g. for protection, feeding or locomotion (summarized by Wotton, 2004). Following secretion, exudates eventually become detached from the producing organism and fulfil important roles for aquatic ecosystem functioning (Wotton, 2004). The importance of dissolved organic matter (DOM) for pelagic food webs was addressed by Pomeroy (1974), who stated that microorganisms mediate a large fraction of the energy flow by processing DOM. Later studies (e. g. Cole et al., 1982; Baines and Pace, 1991) confirmed Pomeroy's statement and the importance of DOM for pelagic food webs was generally accepted. As well as dissolved organic matter, particulate organic matter (POM) is also an important component of marine ecosystem functioning.

In coral reef environments, organic matter is released as mucus by hard, soft and fire corals (e.g. Richman et al., 1975; Crossland et al., 1980; Meikle et al., 1988; Wild et al., 2004a). These adhesive mucus exudates function as particle traps and energy carriers, thereby supporting benthic life, while reducing loss of energy and nutrients from the oligotrophic reef ecosystem (Wild et al., 2004a). It is also known that mucoid coral exudates act as trophic vectors to microbes (Herndl and Velimirov, 1986; Wild et al., 2004b) and reef animals such as fish (Benson and Muscatine, 1974), bivalves (Goreau et al., 1970; Shafir and Loya, 1983), crabs (Knudsen, 1967; Rinkevich et al., 1991), and zooplankton (Richman, et al., 1975; Gottfried and Roman, 1983). Thus, coral-derived organic matter fulfils several important ecological functions in the reef ecosystem. However, the respective role of other common benthic reef organisms in organic matter cycling is still poorly resolved.

Whereas organic matter release by anthozoan corals has traditionally been the focus of study in the phylum cnidaria, pelagic scyphozoan jellyfish are also known to release organic matter (Hansson and Norrman, 1995). *Cassiopea* sp., the benthic scyphozoan upside-down jellyfish, commonly occurs in coral reef environments (e.g. Mergner and Svoboda, 1977; Ducklow and Mitchell, 1979b; Fitt and Hofmann, 1985; Schuhmacher and Mergner, 1985) where it can reach high abundances. In coral reef environments of the Northern Red Sea, *Cassiopea* sp. can locally dominant the benthic community reaching abundances of up to 31 animals m^{-2} and a benthic coverage of up to 20 % (Niggl and Wild unpublished). Milking experiments, in which specimens were exposed to air, have demonstrated release of potentially nutritious mucoid exudates containing

proteins, lipids and carbohydrates by *Cassiopea* sp. (Ducklow and Mitchell, 1979b). However, no data is available on in-situ release rates of organic matter by *Cassiopea* sp. and the ecological role of benthic jellyfish-derived organic matter in coral reef environments. The present study therefore aims to investigate whether *Cassiopea* sp. release significant quantities of dissolved and particulate organic matter into the surrounding waters and whether this released organic matter can function as a carrier of energy and nutrients to fuel pelagic food webs.

For this purpose, organic matter release was quantified by incubating *Cassiopea* sp. in seawater filled beakers, under in-situ conditions. Released organic matter was labelled with stable isotopes and provided to *Idiomysis tsurnamali*, a zooplanktonic mysid commensalistically associated with *Cassiopea* sp. (Bacescu, 1973), in order to investigate the potential uptake of *Cassiopea*-derived organic matter. O_2 consumption experiments were conducted to determine degradation of *Cassiopea*-derived organic matter by planktonic microbes and *Idiomysis tsurnamali* in the Northern Red Sea.

Material and Methods

Description of study site and collection of organisms

The experiments presented in this study were conducted during two field expeditions in February/March and May 2008 to the Marine Science Station (MSS), Aqaba (29° 27′ N, 34° 58′ E) situated at the Northern Gulf of Aqaba (Red Sea, Jordan).

Cassiopea jellyfish (n_{total} = 37, size: 5 to 8 cm in diameter) were collected from directly in front of the MSS jetty at water depths of 5 to 16 m using SCUBA. *Cassiopea* sp. specimens were collected by carefully lifting them from the seafloor and transferring them into seawater filled polyethylene zip locked plastic bags (ca. 500 ml volume). Both, jellyfish exhibiting an association with *Idiomysis tsurnamali* and jellyfish without mysid-association were collected, whereby associated mysids were removed. *Cassiopea* specimens were subsequently transported to two 40 L flow-through tanks supplied with in-situ seawater at exchange rates of approximately 1.5 l min^{-1}, providing in-situ water temperatures of 20.8 to 21.3 °C in February/March (23 Feb – 08 Mar 2008) and 21.8 to 24.5 °C in May (10 May - 30 May 2008). All *Cassiopea* specimens were kept in the flow-through tanks for 2 to 14 days, with jellyfish that exhibited mysid-association in-situ being kept separately from those that did not.

Specimens of the zooplanktonic mysid *Idiomysis tsurnamali*, in-situ occurring in swarms hovering above *Cassiopea*, were collected in-situ using 100 ml plastic syringes with large entry opening (ca. 1 cm in diameter). Mysids (n = approximately 30 animals per syringe, total: approximately 150 animals) were carefully sucked into the syringes and immediately transported to the laboratory, where they were kept in a 2 l glass beaker filled with fresh seawater at in-situ temperatures (22.0 to 23.3 °C) no longer than 1 h prior to the start of subsequent experiments.

In addition, solitary scleractinian mushroom corals *Fungi*a (n = 9) and *Ctenactis* (n = 6) were collected as reference organisms from the fore reef directly in front of the MSS, at water depths

from 5 to 10 m. Corals were transferred to the laboratory and kept in a 1000 L flow-through tank supplied with fresh seawater directly pumped from the reef (flow-through approximately 20 L min^{-1}) at in-situ water temperature.

Light intensity in all tanks was reduced to in-situ conditions by covering the tanks with various layers of black fishing nets until in-situ conditions were achieved (128 – 267 µmol photons m^{-2} s^{-1}; range of daily means from 10 am to 4 pm in winter and summer) and monitored using light data loggers (HOBO Pendant Temperature/Light Data Logger).

Quantification of organic matter release by *Cassiopea* sp.

The procedure for quantification of organic matter released by *Cassiopea* sp. described in the following was carried out identically three times on three different days.

Eleven 1 L glass beakers were first washed with acetone, then with distilled water and subsequently flushed and filled with approximately 900 ml of fresh seawater from the flow through tank. Six *Cassiopea* sp. specimens (5 – 8 cm in diameter), thereof 3 formerly in-situ associated with *Idiomysis tsurnamali* and 3 without mysids association were each transferred (without air exposure) from the maintenance tank into one of the 1 L glass beakers. Five additional beakers were filled with fresh seawater from the flow-through tank only and served as controls. All beakers were randomly placed in two additional 40 L flow-through tanks, protruding 2 cm above the tanks' water surface in order to avoid water exchange. Additionally, the beakers were covered with a thin transparent cellophane foil to protect open beakers from input of airborne particles, leaving 2 peripheral openings for air exchange. Light and temperature conditions were adjusted to in-situ conditions as described above. After 6 h of incubation, the *Cassiopea* specimens were carefully removed from the beakers using laboratory gloves and a bent spoon. Before and between each removal, the spoon was cleaned with acetone, flushed with distilled water and dried in air. Following jellyfish removal, the incubation water of all 11 beakers was instantly processed.

In order to analyse the concentration of dissolved organic carbon (DOC), 10 ml of incubation water from each beaker was extracted with a 10 ml sterile syringe and filtered through a sterile syringe filter (VWR®, polyethersulfone membrane, 0.2 µm pore size) no longer than 20 min after the removal of *Cassiopea* specimens. The first 4 ml of the filtrate was discarded, and the following 6 ml discharged into pre-combusted (450 °C; 4 h) brown glass ampoules, which were instantly sealed and frozen at -20 °C.

Approximately 200 ml of the incubation water of each beaker was dissipated and instantly processed for feeding experiments (see below).

For POM analysis, the volume of the remaining incubation water (590 ml - 760 ml) was determined using a graduate glass cylinder (accuracy ± 10 ml) and filtered onto pre-combusted GF/F filter (Whatman®, 25 mm diameter). The filters were dried for at least 48 hours at 40 °C in a cabinet dryer and kept dry until further processing.

For release rates calculation, POM and DOM concentrations in the control beakers were subtracted from the concentrations in *Cassiopea* incubation beakers.

After the incubation, the diameter of the aboral side of all animals was determined using a ruler (accuracy ± 0.1 cm). The surface area of the oral side of all specimens was calculated with the formula

$$A = r^2 \times \pi \quad (A = \text{surface area; } r = \text{radius; } \pi = \text{circular constant})$$

and used as a reference unit for organic matter release rate calculations.

Mineralization of *Cassiopea*-derived organic matter by *Idiomysis tsurnamali* and planktonic microbes

Two Winkler glass bottles of 60 ml and 120 ml volume were filled with aliquots of incubation water from each of the 11 beakers of each organic matter release quantification experiment - 6 enriched with organic matter released by incubated *Cassiopea* specimens and 5 control beakers without *Cassiopea* incubation. The initial O_2 concentration in each of the resulting 22 bottles was determined using an O_2 optode (HACH, HQ 10). Afterwards, 5 medium sized (body length: approximately 3 - 4mm) *Idiomysis tsurnamali* specimens were added to each of the eleven 120 ml Winkler glass bottles, while the 60 ml bottles served as controls. All bottles were closed gas-tight and stored in the dark at in-situ temperature.

After 4.0 – 4.5 h of incubation, the 120 ml Winkler bottles containing both planktonic microbes and mysids were opened and the O_2 concentration was measured. After 19.0 – 20.2 h, the 60 ml control bottles were opened and the O_2 concentrations were measured. O_2 consumption rates were calculated by subtracting end from start concentration values and related to the incubation time. Organic carbon degradation was calculated assuming that 1 mol of added organic C was mineralized by 1 mol of consumed O_2. In order to calculate carbon degradation rates of solely *Cassiopea*-derived POC, the POC concentrations and O_2 consumption rates of microbes and mysids of the control incubation waters were subtracted from the *Cassiopea* incubation waters. The entire experiment described above was repeated three times on three different days.

Replicate experiments where checked for significant inter-experimental differences. Microbial O_2 consumption and carbon degradation rates of the control water samples of the third experiment significantly differed ($p < 0.001$, independent samples t-test) from the first and the second experiment. However, results of the third experiment were not excluded as the mean microbial O_2 consumption rates of all experiments, including the third experiment, were in the same range as those measured in the water column at similar water depths of the study area (data not shown).

Labelling of *Cassiopea*- and coral-derived organic matter using stable N isotopes

Thirteen *Cassiopea* sp. specimens were kept in a 40 L flow-through tank (exchange rate: approximately 1.5 L min^{-1}) at in-situ conditions. In order to label *Cassiopea*-derived organic material, sodium nitrate enriched in ^{15}N (98% ^{15}N, Cambridge Isotope Laboratories, Inc.) was dissolved in distilled water and added to the tank water. Before each addition, the tank volume was completely exchanged by increased water flow-through. Immediately before addition of $Na^{15}NO_3$, the water flow was stopped in order to retain the label inside the tank. Over a nine-day period, in the

morning of days 1, 2, 3, 8 and 9, 40 mg of $Na^{15}NO_3$, pre-dissolved in distilled water, was added, resulting in a start concentration of 1 mg $Na^{15}NO_3$ L^{-1}. After 4 h of incubation, the water flow was re-established and adjusted to approximately 100 ml min^{-1} until the next addition of $Na^{15}NO_3$. Although no visible symptoms of stress were observed, the jellyfish were given time to recover from the labelling procedure between days 3 and 8, with a water flow-through of approximately 500 ml min^{-1}.

Individual *Fungia* (n = 9) and *Ctenactis* (n = 6) polyps were incubated accordingly in four 40 L flow through tanks for a total labelling period of 3 days with addition of $Na^{15}NO_3$ occurring in the morning of each day. The corals served as reference organisms for results obtained from *Cassiopea*-labelling and -feeding experiments, as previous studies had demonstrated the efficient labelling of coral-derived organic matter using stable N isotopes (Naumann et al., unpublished).

Sampling of labelled Cassiopea- and coral-derived organic matter

For sampling of mucus released by the jellyfish, specimens were placed in a common kitchen sieve with the bell to the bottom and exposed to air for approximately 3 min. Immediately after exposure to air, *Cassiopea* specimens started to release mucus. In order to prevent contamination of released mucus with draining seawater, the mucus of the first 60 seconds was discarded and only subsequently released mucus was collected in a clean glass dish (cleaned with acetone, flushed with distilled water). Mucus released by all 13 *Cassiopea* sp. (volumes: 1.5 to 7.0 ml per individual) was pooled and stored for no longer than 20 h in 50 ml Falcon tubes at 4 °C until use in subsequent experiments.

Collection of mucus released by the scleractinian mushroom corals (i.e. coral mucus) was carried out according to a modified technique described in Wild et al. (2005b). Briefly, *Fungia* and *Ctenactis* polyps were initially washed in fresh running seawater for 1 min and subsequently exposed to air, holding the disk-like coral polyps vertically. Coral mucus release started immediately and was observed by gel-like mucus threads dripping from the coral surface. The initial 30 s of mucus release were discarded to prevent contamination and dilution through seawater. Subsequently, the released mucus of each individual coral (volumes: 15 to 75 ml) was collected for 15 min in clean polyethylene zip-lock bags. Coral mucus released by all *Fungia* and *Ctenactis* corals was pooled and stored at 4 °C in sealed glass containers until further use in subsequent experiments.

In order to prepare collected mucus for later combined isotope and organic matter analysis, triplicate sub-samples (*Cassiopea*: 9 ml, *Fungia*/*Ctenactis*: 8 ml) were filtered through sterile syringe filters (VWR®, polyethersulfone membrane, 0.2 µm pore size) and subsequently filtered on GF/F filters (Whatman®, 25 mm diameter). The filters were then dried in a cabinet dryer at 40 °C for at least 48 hours and kept dry until further analysis, as described below.

Incubation experiments with labelled organic matter and *Idiomysis tsurnamali*

Cassiopea- and coral-derived organic matter enriched with the heavy isotope ^{15}N were provided to specimens of *Idiomysis tsurnamali* in order to examine the potential uptake by the mysids. To this end, 24 glass Petri dishes (diameter: 4.5 cm) were first washed with acetone, flushed with distilled

water and finally flushed with sterile filtered (0.2 µ) seawater (FSW). Three of these glass Petri dishes were filled with ca. 20 ml FSW. Prior to experiment start, all mysids (n = 105) were washed 3 times in FSW by carefully transferring them from one Petri dish into the next using a plastic pipette (1 ml). Without any further treatment, five *Idiomysis tsurnamali* were filtered on pre-combusted GF/F filters (Whatman®, 25 mm diameter; n = 3) to obtain the natural N isotopic signature. The remaining 21 glass Petri dishes were filled with 10 ml FSW and 5 mysids were transferred into each glass dish. Three ml of sterile filtered (VWR®, polyethersulfone membrane, 0.2 µm pore size), labelled ($\delta\ ^{15}N$ = 177 ± 2.0 ‰), *Cassiopea*-derived organic matter (jellyfish organic matter = JOM) was added to 9 glass dishes. Another 6 dishes were provided with 3 ml of sterile-filtered (VWR®, polyethersulfone membrane, 0.2 µm pore size), labelled ($\delta\ ^{15}N$ = 925.2 ± 67.0 ‰), coral-derived organic matter (coral organic matter = COM) as reference. The remaining six glass dishes without any supplement served as controls. Experiments were conducted in sterile seawater and 0.2 µ filtered JOM and COM was used in order to minimize transfer of matter via the microbial loop (Azam et al., 1983).

During the experiment, temperature and light intensity in the laboratory was adjusted to in-situ conditions using data loggers (HOBO Pendant Temperature/Light Data Logger). Total incubation time was 25 h. Mysids were removed from the glass dishes in triplicates in a time series after 0.5 (only JOM treatment samples), 4.0 and 25.0 h (JOM treatment, COM treatment and control samples). All sampled mysids were washed and filtered as described above. All filters were dried at 40 °C for at least 48 h and kept dry until further analysis.

O_2 concentration of the incubation waters was monitored using an O_2 optode (HACH, HQ 10) in order to ensure sufficient O_2 supply during the experiment. After 25 h of incubation, O_2 concentrations were lowest in the JOM samples (187 – 193 µmol O_2 L^{-1}) followed by the COM samples (206 – 207 µmol O_2 L^{-1}) and the controls (216 – 218 µmol O_2 L^{-1}). Hence, all O_2 concentrations were well above critical values.

Analyses of samples

DOC concentrations were determined by high-temperature catalytic oxidation (HTCO) using a Rosemount Dohrmann DC-190 total organic carbon (TOC) analyser and potassium hydrogenphtalat as standard. Each sample was acidified by adding 100 µl of 20 % phosphoric acid and purged for 5 min using O_2 in order to remove dissolved inorganic carbon. The DOC concentration of each sample was measured five times. An outlier test was conducted and DOC concentrations of the remaining sub-measurements ($n \geq 3$) averaged.

POC and PN contents were determined using a THERMO™ NA 2500 elemental analyser (standard deviation < 3%) and atropine and cyclohexanone-2,4-dinitrophenylhydrazone as elemental standards. Nitrogen isotopic signatures were determined using a Thermo™ NA 2500 elemental analyser, coupled with a THERMO/Finnigan Conflo II-interface to a THERMO/Finnigan MAT Delta plus isotope ratio mass spectrometer. Stable N isotopic ratios were expressed using the conventional delta notation ($\delta\ ^{15}N$) relative to atmospheric nitrogen.

Results

Release of organic matter by *Cassiopea* sp.

Table 1 gives an overview on POM and DOC release rates measured in the respective experiments. POC and PN concentrations in all *Cassiopea* incubation waters exceeded the controls, thus revealing net POM release by all *Cassiopea* specimens (n = 18) ranging from 10.4 to 50.3 mg POC m^{-2} jellyfish area h^{-1} and from 1.1 to 5.0 mg PN m^{-2} jellyfish area h^{-1}. In contrast, DOC release was highly variable with 45 % of all incubated jellyfish exhibiting net DOC uptake. The POC/PN ratio of the released organic matter of 11.0 ± 0.3 (mean ± standard error; n = 18) was significantly ($p < 0.01$, Mann-Whitney U test) elevated compared to the controls (10.2 ± 0.1). POC, PN and DOC release rates obtained from the second incubation series (Exp. 2) were slightly elevated compared to the other incubation series. However, statistical analysis (Mann-Whitney U test) revealed no significant differences. *Cassiopea* specimens formerly associated with *Idiomysis tsurnamali* and those specimens without mysids-association exhibited similar POC, PN and DOC release rates.

Table 1 *POM and DOC release rates (controls already subtracted) by Cassiopea from the Northern Red Sea. Values are averages ± SD.*

Exp.	N	POC release (mg m^{-2} h^{-1})	PN release (mg m^{-2} h^{-1})	DOC release (mg m^{-2} h^{-1})	POC/PN	POC/DOC
1	6	18.5 ± 4.2	1.9 ± 0.5	4.3 ± 12.5	11.5 ± 0.7	-1.0 ± 3.8
2	6	27.8 ± 13.3	3.1 ± 1.5	6.9 ± 5.1	10.6 ± 1.8	4.7 ± 2.0
3	6	17.3 ± 5.1	1.8 ± 0.5	- 16.0 ± 23.0	11.0 ± 0.6	2.1 ± 4.6
Mean	18	21.2 ± 9.4	2.3 ± 1.1	-1.2 ± 17.6	11.0 ± 1.1	0.4 ± 4.5

Transfer of *Cassiopea*- and coral-derived organic matter to zooplanktonic mysids

Cassiopea- and coral-derived organic matter was successfully labelled. Labelled *Cassiopea* mucus exhibited a N isotopic signature of $\delta^{15}N$ = 177 ± 2.0 ‰ (n = 3) which was significantly elevated compared to unlabelled controls ($\delta^{15}N$ = 0.8 ± 0.1 ‰; n = 3). Labelling of coral mucus resulted in N isotopic signature of $\delta^{15}N$ = 925.2 ± 67.0 ‰ (n = 3), which was also significantly elevated compared to unlabelled controls (*Fungia*: $\delta^{15}N$ = 4.9 ± 0.4 ‰; *Ctenactis*: 9.0 ± 1.4 ‰).

Natural $\delta^{15}N$ of freshly collected mysids was 3.9 ± 0.1 ‰ (n = 15). Mysids removed from JOM Petri dishes after less than 30 min incubation exhibited a $\delta^{15}N$ of 3.9 ± 0.3 ‰ (n = 15), which was similar to the natural isotope signature, thus verifying the washing procedure being effective to remove external particulate matter from the mysids.

During the course of the experiment, $\delta^{15}N$ of all mysids increased (Figure 1). After 25 h of incubation, mysids provided with ^{15}N-labelled jellyfish- and coral-derived organic matter exhibited a significantly ($p < 0.001$, One-way ANOVA) higher $\delta^{15}N$ signature compared to unfed control mysids. Addition of ^{15}N-labelled coral-derived organic matter resulted in a more rapid ^{15}N increase than addition of labelled *Cassiopea*-derived organic matter. However, results verify uptake of both labelled *Cassiopea*- and coral-derived organic matter by *Idiomysis tsurnamali*.

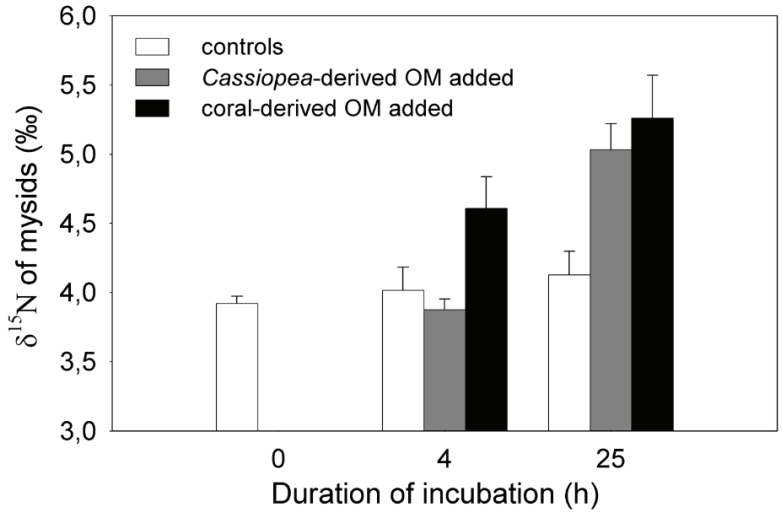

Fig. 1 *Stable nitrogen isotopic signature of mysids provided with ^{15}N-labelled Cassiopea- and coral-derived organic matter.*

Microbial degradation of *Cassiopea*-derived organic matter

POC concentrations in all *Cassiopea* incubation water samples ranged from 0.25 to 2.00 µg ml^{-1} (n = 18) and thus were higher than those in the control water samples ranging from 0.08 to 0.13 µg ml^{-1} (n = 15). In contrast, DOC concentrations in the *Cassiopea* incubation water samples (0.91 to 2.11 µg C ml^{-1}) were similar to DOC concentrations in the control water samples (0.97 to 2.08 µg C ml^{-1}). Microbial O_2 consumption rates (Table 2) in *Cassiopea* incubation water samples ranged from 28.4 to 94.6 µM O_2 d^{-1} (n = 18) and were significantly elevated ($p < 0.001$, Mann-Whitney U test) compared to microbial O_2 consumption rates in the controls ranging from 1.7 to 21.9 µM O_2 d^{-1} (n = 15). On average, *Cassiopea*-incubation water samples exhibited 6.1 fold increased POC concentrations and 5.9-fold increased microbial O_2 consumption rates compared to the control incubation waters. This indicates degradation of *Cassiopea*-derived organic matter by planktonic microbes. Statistical analysis revealed no correlation between DOC concentration and microbial O_2 consumption ($p = 0.275$, Spearman rank-order correlation), but showed that POC concentration was significantly correlated to microbial O_2 consumption rates ($p < 0.001$, Spearman rank-order correlation). Therefore, carbon degradation rates were established by using POC concentration data. In the *Cassiopea* incubation waters, POC was degraded by planktonic microbes with rates from 0.7 to 13.7 % POC h^{-1}. In the control incubation water samples, microbial POC degradation rates were similar and ranged from 0.7 to 13.1 % POC h^{-1}. Solely *Cassiopea*-derived POC was degraded at rates of 5.1 ± 2.7 % POC h^{-1}, whereas microbial POC degradation in the control incubation water samples were highly variable with values of 4.2 ± 4.8 % POC h^{-1} (all means ± SD).

The results of the 3 replicate experiments were similar. Only microbial O_2 consumption rates in the control water samples of the third experiment were significantly elevated ($p< 0.001$, independent samples t-test) compared to the other experiments. Elevated O_2 consumption rates led to a high mean carbon degradation rate of 11.3 % POC h^{-1}, whereby POC and DOC concentrations as well as the POC/PN ratio remained similar compared to experiments 1 and 2.

Table 2 *POC and DOC concentrations, microbial O_2 consumption and carbon degradation rates for incubation water samples with previous Cassiopea incubation and controls for all experiments (mean ± SD).*

Exp.	Controls				Organic matter addition			
	POC (µg C ml^{-1})	DOC (µg C ml^{-1})	O_2 (µM d^{-1})	Carbon degradat. (% POC h^{-1})	POC conc. (µg C ml^{-1})	DOC conc. (µg C ml^{-1})	O_2 consump. (µM d^{-1})	Carbon degradat. (% POC h^{-1})
1	0.12 ± 0.01	1.40 ± 0.37	3.6 ± 2.4	1.6 ± 1.1	0.58 ± 0.14	1.53 ± 0.32	56.3 ± 19.4	5.0 ± 1.8
2	0.10 ± 0.01	1.51 ± 0.23	5.2 ± 3.2	2.6 ± 1.8	1.06 ± 0.54	1.75 ± 0.20	71.9 ± 16.5	9.8 ± 2.6
3	0.09 ± 0.01	1.67 ± 0.33	20.1 ± 2.2	11.3 ± 1.6	0.38 ± 0.11	1.43 ± 0.46	44.9 ± 18.0	2.6 ± 1.4
Mean	0.10 ± 0.01	1.52 ± 0.29	9.8 ± 8.1	4.2 ± 4.3	0.67 ± 0.42	1.57 ± 0.35	57.7 ± 20.4	5.8 ± 3.6

Degradation of *Cassiopea*-derived organic material by zooplankton

During the second incubation experiment, all *Idiomysis tsurnamali* specimens in two Winkler bottles died and very small mysids, likely recently born, appeared in three Winkler bottles. Thus, all O_2 concentration measurements from this experiment were excluded from further analysis.

Mysids in *Cassiopea*-incubation water samples consumed 0.19 to 0.78 µmol O_2 $mysid^{-1}$ d^{-1} (n = 12) whereas O_2 consumption of mysids in the control incubation water samples ranged from 0.03 to 0.16 µmol O_2 $mysid^{-1}$ d^{-1} (n = 10). In both experiments, O_2 consumption rates of mysids in *Cassiopea*-incubation water samples were significantly ($p < 0.01$, independent samples t-test) elevated compared to mysids in control incubation water samples (Table 3). This indicates mineralization of *Cassiopea*-derived organic matter by *Idiomysis tsurnamali*.

Cassiopea-derived organic matter was mineralized at a rate of 0.16 ± 0.08 µg C h^{-1} $mysid^{-1}$ (n = 12). In control seawater samples, carbon was mineralized at a rate of 0.06 ± 0.02 µg C h^{-1} $mysid^{-1}$ (n = 10).

In *Cassiopea* incubation water samples, *Cassiopea*-derived organic matter contributed to 77 ± 6 % of bulk POC. Mysid degradation of *Cassiopea*-derived organic matter was calculated to contribute 70 ± 13 % of bulk carbon degradation.

Table 3 *O_2 consumption rates of mysids in incubation waters with previous Cassiopea incubation (input of Cassiopea-derived organic matter) and controls. Values are given as mean ± SD.*

Exp	Controls		Organic matter addition	
	N	O_2 consumption (µM O_2 $mysid^{-1}$ d^{-1})	N	O_2 consumption (µM O_2 $mysid^{-1}$ d^{-1})
1	5	0.12 ± 0.05	6	0.41 ± 0.13
2	5	N/A	6	N/A
3	5	0.12 ± 0.01	6	0.48 ± 0.18

Discussion

Release of organic matter by *Cassiopea* sp.

Release rates of DOC by *Cassiopea* sp. determined in the present study were highly variable with 44 % of all investigated specimens exhibiting net DOC uptake. This corresponds with previous studies, which demonstrated zooxanthellate medusae to take up and release dissolved organic matter (Webb and Johannes, 1967; Wilkerson and Kremer, 1992). However, the present study is the first one to quantify fluxes of DOC induced by *Cassiopea* sp. Both, net consuming and net releasing specimens were found. Whether "consumers" always consume and "releasers" always release DOC remains unresolved. Ferrier-Pagès et al. (1998) described DOC release rates of the zooxanthellate coral *Galaxea fascicularis* depending on the trophic status and size of the colony. The same likely applies for *Cassiopea* sp. On average, *Cassiopea* exhibited a net, albeit highly variable, uptake of 1.2 mg DOC m^{-2} h^{-1}, which is similar to mean DOC release rates of 1.1 ± 4.6 mg DOC m^{-2} h^{-1} found for several scleractinian coral species from the Northern Red Sea (Naumann et al. unpublished).

In contrast to DOC, all tested *Cassiopea* specimens released POM. This release was higher than reported for other tropical reef cnidaria under submersed conditions (Table 4). Only Herndl & Velimirov (1986) described higher POC release rates for the colony forming Mediterranean coral *Cladocera cespitosa.* In the study area, *Cassiopea* specimens exhibited substrate-dependant mean abundances of 0.4 – 7.9 animals m^{-2} seafloor area and individual diameters of 8.3 – 13.5 cm respectively (Niggl and Wild unpublished), resulting in organic matter release of 1 – 54 mg POC (0.1 – 5.9 mg PN) m^{-2} reef area d^{-1}. In an adjacent fringing reef environment close to the study area, *Acropora* sp., the most abundant coral covering 7 % of the seafloor in spring 2008, exhibited a mean POC release of 3.4 mg C m^{-2} coral surface area h^{-1} (Naumann et al. unpublished). Recalculating the two dimensional benthic cover by *Acropora* sp. to the three dimensional coral surface area using a conversion factor of 9.04 (Naumann et al., 2009) results in a release of 52 mg POC m^{-2} reef area d^{-1}. Herndl & Velimirov (1986) calculated a release of 32 mg mucus-derived C m^{-2} seafloor area d^{-1} by the Mediterranean coral *Cladocera cespitosa* taking benthic coverage into account. The dominant scleractinian corals of the genus *Acropora* were found to release 47 mg mucus-derived POC (4.8 mg PN) m^{-2} reef area d^{-1} at Heron Island, GBR, Australia (Wild et al., 2004a). Hence, *Cassiopea* release rates of POC and PN in the Northern Gulf of Aqaba related to the seafloor area are in a comparable range as described for other cnidarians.

As the seawater used for incubations was pumped from the study area, the POM concentrations of 0.105 mg POC l^{-1} and 0.012 mg PN l^{-1} of the controls resemble the POM concentration in the water column at the study area. *Cassiopea* specimens were sampled at water depths of approximately 10 m resulting in total POC and PN contents of 1050 mg and 120 mg respectively in the 10 m^3 water column above each m^2 of seafloor at the sampling site. *Cassiopea* daily organic matter release rates therefore accounted for up to 5.1 % of POC and 4.9 % of PN suspended in the overlying water column. In 10 m water depth of the neighbouring fringing reef corals of the dominating genus *Acropora* exhibited a benthic coverage of 6 % and a mean release of 2.8 mg POC m^{-2} coral surface area h^{-1} and 0.2 mg PN m^{-2} coral surface area h^{-1} (Naumann et al. unpublished). Using the conversion factor of 9.04 (Naumann et al., 2009) *Acropora* corals in 10 m water depth released 36.4 mg POC m^{-2} reef area d^{-1} and 2.6 mg PN m^{-2} reef area d^{-1} which accounts for 3.5 %

POC and 2.2 % PN of the overlying water column. These values are thus lower than estimated for *Cassiopea*.

Locally, *Cassiopea* exhibited maximum abundances of up to 31 specimens m^{-2} seafloor area (Niggl & Wild unpublished) resulting in a release of 185 mg POC (20.1 mg PN) m^{-2} reef area d^{-1}. Above these areas, *Cassiopea* daily organic matter release can therefore account for up to 17.6 % of POC and 16.8 % of PN concentrations in the overlying water column.

Table 4 *Release of particulate organic matter by various cnidarian taxa.*

Study Site	Organism	Cnidaria class	POC release (mg m^{-2} h^{-1})	PN release (mg m^{-2} h^{-1})	C : N ratio	Method	Reference
Heron Island	*Acropora* sp.	Anthozoa	7 – 10	0.8 - 1.3	7.9 – 13.8	Beaker	Wild et al. (2005a)
Gulf of Aqaba/ Eilat	*Millepora sp.*	Hydrozoa	0.3 ± 0.2	0.04 ± 0.01	11 ± 1	Beaker	Naumann et al. unpublished
Gulf of Aqaba/ Eilat	*Acropora variabilis*	Anthozoa	1.4 – 4.2	N/A	N/A	Perspex chambers	Crossland (1987)
Gulf of Aqaba/ Eilat	*Stylophora pistillata*	Anthozoa	2.7 – 4.0	N/A	N/A	Perspex chambers	Crossland (1987)
Laboratory	*Acropora* sp.	Anthozoa	7.8 ± 2.1	1.1 ± 0.3	8.7 ± 1.0	Beaker	Niggl et al. (in press)
Bight of Piran	*Cladocora cespitosa*	Anthozoa	58	N/A	N/A	Beaker	Herndl & Velimirov (1986)
Gulf of Aqaba/ Eilat	*Cassiopea* sp.	Scyphozoa	21 ± 9	2.3 ± 1.1	11.0 ± 1.1	Beaker	Present study

Uptake and degradation of *Cassiopea*-derived organic matter by microbes and zooplankton

Studies involving isotope labelling of phytoplankton organisms are frequently found in the literature (Mague et al., 1980; Coveney, 1982; Bronk and Glibert, 1991; Norrman et al., 1995). In contrast, studies using stable isotopes as trophic tracers to label organic matter released by marine animals are hardly available. In the present study, $Na^{15}NO_3$ was successfully used for the first time to label organic matter released by the jellyfish *Cassiopea* sp. Further, uptake of released organic nitrogen by zooplanktonic *Idiomysis tsurnamali* was demonstrated. Owing to the experimental setup, transfer of organic matter via the microbial loop (Azam et al., 1983) can be considered as minor, and direct uptake of organic matter by the mysids can be assumed.

The results of the present study thereby append the scyphozoan *Cassiopea* sp. to the list of coral reef cnidaria providing organic matter to reef organisms via the release of organic matter, particularly mucus. This study also demonstrates uptake of coral-derived nitrogen by *Idiomysis tsurnamali*, and thereby complements previous studies demonstrating uptake of cnidarian-derived organic matter by a variety of reef organisms (Knudsen, 1967; Goreau, et al., 1970; Benson and Muscatine, 1974; Richman, et al., 1975; Gottfried and Roman, 1983; Shafir and Loya, 1983; Rinkevich, et al., 1991).

In the present study, labelled nitrogen was provided as filtered mucus. Besides ^{15}N-rich compounds such as proteins, both *Cassiopea*- and coral-derived mucus contain less refractory energy-rich substances such as carbohydrates or lipids (Benson and Muscatine, 1974; Ducklow and Mitchell, 1979b; Crossland et al., 1980; Wild et al., 2005a). After uptake, mysids could use the energy-rich components for their metabolic demands. Mysids transferred to *Cassiopea*-incubation waters exhibited 3.8-fold elevated O_2 consumption rates compared to mysids in control waters, which indicates mineralization of *Cassiopea*-derived organic matter by *Idiomysis tsurnamali*.

Besides degradation by mysids, the present study demonstrated degradation of *Cassiopea*-derived organic matter by planktonic microbes. Similar carbon degradation rates of *Cassiopea*-derived organic matter and organic matter of the control incubations indicate either similar degradability of *Cassiopea*-derived organic matter and suspended POM at the study site, and/or a large proportion of *Cassiopea*-derived organic matter in the suspended POM. A comparison to microbial degradation of coral mucus, which can dominate suspended POM in coral reef waters (Johannes, 1967; Marshall, 1968), may prove interesting. Wild et al. (2004b) reported a microbial respiration of 334 µM O_2 d^{-1} in undiluted coral mucus with carbon concentrations of 10.8 – 51.6 µg C ml^{-1}. Thus, microbial respiration in undiluted coral mucus was almost 7-fold increased compared to O_2 consumption rates of microbes in *Cassiopea*-derived organic matter (48.1 µM O_2 d^{-1}) diluted in seawater with a concentration of 0.16 -1.89 µg C ml^{-1}. Higher microbial respiration in coral mucus may be attributed to higher carbon contents and/or differences in microbial community structures and abundance. Latter was found to be higher in coral mucus than in the surrounding seawater (Ducklow and Mitchell, 1979a; Wild et al., 2004b).

The results of all three replicate experiments were similar, with only O_2 consumption and carbon degradation rates of the seawater controls being elevated during experiment 3. As respective POC and DOC concentrations did not deviate from experiments 1 and 2 (Table 2), differences in the microbial community, higher microbial abundance and/or input of highly labile organic matter may explain these elevated O_2 consumption and carbon degradation rates.

Energy and nutrient pathways

The labelling experiments of this study revealed uptake of dissolved inorganic nitrogen by *Cassiopea* sp. and indicate assimilation to organic compounds by endosymbiotic zooxanthellae. These findings are supported by the results of Wilkerson and Kremer (1992), who demonstrated uptake of nitrate and ammonia by the zooxanthellate jellyfish *Linuche unguiculata*. Muscatine and Marian (1982) also found uptake of dissolved inorganic nitrogen by zooxanthellate jellyfish *Mastigias* sp., but in contrast to the present study they only found this in the form of ammonia. However, as medusae are unable to assimilate inorganic nutrients (Pitt et al., 2009), assimilation of inorganic nitrogen to organic matter was likely conducted by the zooxanthellae of *Cassiopea*. This is further supported by the study of Wilkerson and Kremer (1992), who reported assimilation of dissolved N and P from the water column by the zooxanthellae of *Linuche ungiculata*.

Release of ^{15}N labelled mucus by *Cassiopea* specimens indicates transfer of previously assimilated inorganic nitrogen as organic matter from the zooxanthellae to the jellyfish. Studies investigating transfer of assimilated organic matter from endosymbionts to cnidarian hosts have

mainly been undertaken on anthozoans (Pitt, et al., 2009). These studies indicate translocation of N as amino acids from zooxanthellae to the hosts (Swanson and Hoegh-Guldberg, 1998; Wang and Douglas, 1999). For *Cassiopea*, the mechanism of nitrogen translocation still remains unresolved. However, similar physiological mechanism as in anthozoans do also likely occur in their scyphozoan relatives (Pitt et al., 2009), which is supported by the results of the present study.

Cassiopea has already been reported to release mucus (Gohar and Eisawy, 1960; Ducklow and Mitchell, 1979b; Hofmann and Kremer, 1981). The present study delivers first evidence of zooxanthellae-derived particulate organic nitrogen in the released mucus. Labelling experiments with anthozoans and compositional analysis have demonstrated zooxanthellae-derived organic matter in released mucus (Crossland et al., 1980; Meikle et al., 1988), which now can also be confirmed for scyphozoans.

Dissolved organic carbon and dissolved free amino acids released by jellyfish have been described as easyly accessible C and N sources for bacteria and other marine sapotrophs (Webb and Johannes, 1967; Hansson and Norrman, 1995). In the present study, no correlation between DOC concentrations and microbial O_2 consumption rates could be found, but POC concentrations significantly correlated with microbial O_2 consumption. As suggested in a conceptual model of nutrient cycling by Pitt et al. (2009), the present study could confirm transfer of POM from zooxanthellate jellyfish to microbes. It further established an additional pathway of organic matter from the jellyfish directly to zooplankton.

Ecological Implications

The commensalistic relationship between *Cassiopea* sp. and *Idiomysis tsurnamali* has previously been attributed to the refuge the jellyfish offers to the mysids (Bacescu, 1973). In the event of danger, the mysids withdraw in between the jellyfish's tentacles. The results of the present study extend the knowledge by revealing ingestion and degradation of *Cassiopea*-derived organic matter by *Idiomysis tsurnamali*. A similar relationship has been suggested for corals and the acoel worms *Waminoa* sp., which live on the corals and are supposed to feed on mucus released by the corals (Barneah et al., 2007). The assumption, that the occurrence of *Cassiopea* sp. in association with mysids could be attributed to differences in organic matter release rates, could not be confirmed as there were no differences between release rates of jellyfish with or without a previous mysid-association.

Besides *Cassiopea* sp., the mysids are known to exhibit association to other reef coelenterates such as the sea anemone *Megalactis hemprichi* (Bacescu, 1973). Although results of the present study demonstrated uptake of coral exudates by *Idiomysis tsurnamali* (Fig. 1), no association of mysids with scleractinian corals could be observed during the field expeditions conducted for the present study and during several reef surveys conducted during an extensive reef monitoring program from 2006-2008. The socialization of mysids with reef cnidarians therefore may be based on two conditions: (i) the availability of organic matter and (ii) the provision of suitable refuge in the event of danger, with the latter possibly being better fulfilled by *Cassiopea* sp. and sea anemones than by scleractinian corals with often tiny polyps.

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References

Anderson, G.C., Zeutschel, R.P., 1970. Release of dissolved organic matter by marine phytoplankton in coastal and offshore areas of the Northeast Pacific Ocean. Limnol. Oceanogr. 15, 402-407.

Azam, F., Fenchel, T., Field, J.G., Gray, J.S., Meyerreil, L.A., Thingstad, F., 1983. The ecological role of water-column microbes in the sea. Mar. Ecol. Prog. Ser. 10, 257-263.

Bacescu, M., 1973. A new case of commensalism in the Red Sea: The mysid *Idiomysis tsurnamali* n.sp. with the Coelenterata *Megalactis* and *Cassiopea*. Rev. Roum. Biol., Zool. 18, 3-7.

Baines, S.B., Pace, M.L., 1991. The production of dissolved organic matter by phytoplankton and its importance to bacteria: Patterns across marine and freshwater systems. Limnol. Oceanogr. 36, 1078-1090.

Barneah, O., Brickner, I., Hooge, M., Weis, V.M., LaJeunesse, T.C., Benayahu, Y., 2007. Three party symbiosis: acoelomorph worms, corals and unicellular algal symbionts in Eilat (Red Sea). Mar. Biol. 151, 1215-1223.

Benson, A., Muscatine, L., 1974. Wax in coral mucus: Energy transfer from corals to reef fishes. Limnol. Oceanogr. 19, 810-814.

Berman, T., Holm-Hansen, O., 1974. Release of photoassimilated carbon as dissolved organic matter by marine phytoplankton. Mar. Biol. 28, 305-310.

Bjornsen, P.K., 1988. Phytoplankton Exudation of Organic Matter: Why Do Healthy Cells Do It? Limnol. Oceanogr. 33, 151-154.

Bronk, D.A., Glibert, P.M., 1991. A ^{15}N tracer method for the measurement of dissolved organic nitrogen release by phytoplankton. Mar. Ecol. Prog. Ser. 77, 171-182.

Brylinsky, M., 1977. Release of Dissolved Organic Matter by Some Marine Macrophytes. Mar. Biol. 39, 213-220.

Carlson, D.J., Carlson, M.L., 1984. Reassessment of exudation by fucoid macroalgae. Limnol. Oceanogr. 29, 1077-1087.

Coello, W.F., Khan, M.A.Q., 1996. Protection against heavy metal toxicity by mucus and scales in fish. Arch. Environ. Con. Tox. 30, 319-326.

Cole, J.J., Likens, G.E., Strayer, D.L., 1982. Photosynthetically produced dissolved organic carbon: An important carbon source for planktonic bacteria. Limnol. Oceanogr. 27, 1080-1090.

Coveney, M.F., 1982. Bacterial uptake of photosynthetic carbon from freshwater phytoplankton. Oikos 38, 8-20.

Crossland, C.J., 1987. *In situ* release of mucus and DOC-lipid from the coral *Acropora variabilis* and *Stylophora pistillata* in different light regimes. Coral Reefs 6, 35-42.

Crossland, C.J., Barnes, D.J., Borowitzka, M.A., 1980. Diurnal lipid and mucus production in the staghorn coral *Acropora acuminata*. Mar. Biol. 60, 81-90.

Denny, M.W., 1989. Invertebrate mucous secretions: functional alternatives to vertebrate paradigms. Symp. Soc. Exp. Biol. 43, 337-366.

Ducklow, H.W., Mitchell, R., 1979a. Bacterial populations and adaptations in the mucus layers on living corals. Limnol. Oceanogr. 24, 715-725.

Ducklow, H.W., Mitchell, R., 1979b. Composition of mucus released by coral reef coelenterates. Limnol. Oceanogr. 24, 706-714.

Ebran, N., Julien, S., Orange, N., Auperin, B., Molle, G., 2000. Isolation and characterization of novel glycoproteins from fish epidermal mucus: correlation between their pore-forming properties and their antibacterial activities. Biochim. Biophys. Acta 1467, 271-280.

Ferrier-Pages, C., Gattuso, J.P., Cauwet, G., Jaubert, J., Allemand, D., 1998. Release of dissolved organic carbon and nitrogen by the zooxanthellate coral Galaxea fascicularis. Mar. Ecol. Prog. Ser. 172, 265-274.

Fitt, W.K., Hofmann, D.K., 1985. Chemical induction of settlement and metamorphosis of the reef-dwelling coelenterate *Cassiopea andromeda*. Proc. 5th Int. Coral Reef Congr. 5, 239-244.

Fogg, G., 1983. The ecological significance of extracellular products of phytoplankton photosynthesis. Bot. Mar. 26, 3-14.

Gohar, H.A.F., Eisawy, A.M., 1960. The biology of *Cassiopea andromeda* (from the Red Sea). Publ. Mar. Biol. Stn. Ghardaqa 11, 4-42.
Goreau, T.F., Goreau, N.I., Yonge, C.M., Neuman, Y., 1970. On feeding and nutrition in Fungiacava eilatensis (Bivalvia, Mytilidae), a commensal living in fungiid corals. J. Zool. Lond. 160, 159-172.
Gottfried, M., Roman, M.R., 1983. Ingestion and Incorporation of Coral-Mucus Detritus by Reef Zooplankton. Mar. Biol. 721, 211-218.
Hansson, L.J., Norrman, B., 1995. Release of dissolved organic carbon (DOC) by the scyphozoan jellyfish *Aurelia aurita* and its potential influence on the production of planktic bacteria. Mar. Biol. 121, 527-532.
Herndl, G.J., Velimirov, B., 1986. Microheterotrophic utilization of mucus released by the Mediterranean coral *Cladocora cespitosa*. Mar. Biol. 90, 363-369.
Hofmann, D.K., Kremer, B.P., 1981. Carbon metabolism and strobilation in *Cassiopea andromedea* (Cnidaria: Scyphozoa): Significance of endosymbiotic dinoflagellates. Mar. Biol. 65, 25-33.
Johannes, R., 1967. Ecology of organic aggregates in the vicinity of a coral reef. Limnol. Oceanogr. 7, 189-195.
Kappner, I., Al-Moghrabi, S.M., Richter, C., 2000. Mucus-net feeding by the vermetid gastropod *Dendropoma maxima* in coral reefs. Mar. Ecol. Prog. Ser. 204, 309-313.
Khailov, K.M., Burlakova, Z.P., 1969. Release of dissolved organic matter by marine seaweeds and distribution of their total organic production to inshore communities. Limnol. Oceanogr. 14, 521-532.
Knudsen, J.W., 1967. Trapezia and Tetralia (Decapoda, Brachyura, Xanthidae) as obligate ectoparasites of pocilloporid and acroporid corals. Pacif. Sci. 21, 51-57.
Mague, T.H., Friberg, E., Hughes, D.J., Morris, I., 1980. Extracellular release of carbon by marine phytoplankton; a physiological approach. Limnol. and Oceanogr. 25, 262-279.
Marshall, M., 1968. Observations on organic aggregates in the vicinity of coral reefs. Mar. Biol. 2, 50-55.
Meikle, P., Richards, G., Yellowlees, D., 1988. Structural investigations on the mucus from six species of coral. Mar. Biol. 99, 187-193.
Mergner, H., Svoboda, A., 1977. Productivity and seasonal changes in selected reef areas in the Gulf of Aqaba (Red Sea). Helgol. Meeresun. 30, 383-399.
Morton, B., 2000. The Anatomy of *Callocardia hungerfordi* (Bivalvia: Veneridae) and the origin of its shell camouflage. J. Moll. Stud. 66, 21-30.
Muscatine, L., Marian, R.E., 1982. Dissolved inorganic nitrogen flux in symbiotic and nonsymbiotic medusae. Limnol. Oceanogr. 27, 910-917.
Naumann, M.S., Niggl, W., Laforsch, C., Glaser, C., Wild, C., 2009. Coral surface area quantification-evaluation of established techniques by comparison with computer tomography. Coral Reefs 28, 109-117.
Niggl, W., Glas, M., Laforsch, C., Mayr, C., Wild, C., in press. Stimulation of coral-derived organic matter release during bleaching. Proc. 11th Int. Coral Reef Symp.
Norrman, B., Zweifel, U.L., Hopkinson, C.S., Fry, B., 1995. Production and utilization of dissolved organic carbon during an experimental diatom bloom. Limnol. Oceanogr. 40, 898-907.
Pitt, K.A., Welsh, D.T., Condon, R.H., 2009. Influence of jellyfish blooms on carbon, nitrogen and phosphorus cycling and plankton production. Hydrobiol. 616, 133-149.
Pomeroy, L.R., 1974. The ocean's food web, a changing paradigm. BioScience 24, 499-504.
Richman, S., Loya, Y., Slobodkin, L.B., 1975. The rate of mucus production by corals and its assimilation by the coral reef copepod *Acartia negligens*. Limnol. Oceanogr. 20, 918-923.
Rinkevich, B., Wolodarsky, Z., Loya, Y., 1991. Coral crab association-a compact domain of a multilevel trophic system. Hydrobiol. 216, 279-284.
Schuhmacher, H., Mergner, H., 1985. Quantitative Analyse von Korallengemeinschaften des Sanganeb-Atolls (mittleres Rotes Meer). II. Vergleich mit einem Riffareal bei Aqaba (nördliches Rotes Meer) am Nordrande des indopazifischen Riffgürtels. Helgol. Meeresun. 39, 419-440.
Shafir, A., Loya, A., 1983. Consumption and assimilation of coral mucus by the burrowing mussel *Lithophaga lessepsiana*. Proc Int Conf Mar Sci Red Sea 9, 135-140.
Sharp, J.H., 1977. Excretion of Organic Matter by Marine Phytoplankton: Do Healthy Cells do it? Limnol. Oceanogr. 22, 381-399.
Sondergaard, M., 1990. Extracellular organic carbon (EOC) in the genus Carpophyllum (Phaeophyceae): diel release patterns and EOC lability. Mar. Biol. 104, 143-151.
Steinberg, D.K., Nelson, N.B., Carlson, C.A., Prusak, A.C., 2004. Production of chromophoric dissolved organic matter (CDOM) in the open ocean by zooplankton and the colonial cyanobacterium *Trichodesmium* spp. Mar. Ecol. Prog. Ser. 267, 45-56.
Swanson, R., Hoegh-Guldberg, O., 1998. Amino acid synthesis in the symbiotic sea anemone *Aiptasia pulchella*. Mar. Biol. 131, 83-93.
Thomas, J.P., 1971. Release of dissolved organic matter from natural populations of marine phytoplankton. Mar. Biol. 11, 311-323.
Von Vaupel Klein, J.C., Koomen, P., 1994. The Possible Origin of Mucus Jets Used for Immobilizing Prey in Species of *Euchirella* (Copepoda, Calanoida, Aetideidae). I. Theoretical Considerations in Relation To Swimming and Feeding Behaviour. Crustaceana 66, 184-204.

Wang, J.T., Douglas, A.E., 1999. Essential amino acid synthesis and nitrogen recycling in an alga-invertebrate symbiosis. Mar. Biol. 135, 219-222.
Webb, K.L., Johannes, R.E., 1967. Studies of the release of dissolved free amino acids by marine zooplankton. Limnol. Oceanogr. 12, 376-382.
Wetzel, R.C., 1969. Excretion of dissolved organic compounds by aquatic macrophytes. Bioscience 19, 539-540.
Wild, C., Woyt, H., Huettel, M., 2005a. Influence of coral mucus on nutrient fluxes in carbonate sands. Mar. Ecol. Prog. Ser. 287, 87-98.
Wild, C., Huettel, M., Klueter, A., Kremb, S., Rasheed, M., Joergensen, B., 2004a. Coral mucus functions as an energy carrier and particle trap in the reef ecosystem. Nature 428, 66-70.
Wild, C., Rasheed, M., Werner, U., Franke, U., Johnstone, R., Huettel, M., 2004b. Degradation and mineralization of coral mucus in reef environments. Mar. Ecol. Prog. Ser. 267, 159-171.
Wild, C., Rasheed, M., Jantzen, C., Cook, P., Struck, U., Boetius, A., 2005b. Benthic metabolism and degradation of natural particulate organic matter in carbonate and silicate reef sands of the Northern Red Sea. Mar. Ecol. Prog. Ser. 298, 69-87.
Wilkerson, F.P., Kremer, P., 1992. DIN, DON and PO_4 flux by a medusa with algal symbionts. Mar. Ecol. Prog. Ser. 90, 237-250.
Wotton, R.S., 2004. The ubiquity and many roles of exopolymers (EPS) in aquatic systems. Sci. Mar. 68, 13-21.

9

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

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Abstract

Mucus, a primarily carbohydrate complex, released by scleractinian warm and cold water reef corals can function as 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. 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 energy supply mechanisms.

Introduction

Both scleractinian warm and cold water corals continuously release mucus into their surrounding (Wild et al. 2005a, 2008) for various purposes (reviewed in Brown and 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 reef (Wild et al. 2008).

Previous studies confirmed the important function of warm water coral-derived mucus as 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. 2005b). For cold water coral-derived mucus, a pilot study also indicates fauna-microbe interaction via this material and its fast recycling by planktonic microbes (Wild et al. 2008).

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. (2005a) further analysed the carbohydrate composition of mucus released by six different 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 of the investigated coral species, which indicates differences in carbohydrate mucus composition between corals. 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.

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 (MSS) 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) and 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 at least 24 h prior to mucus sampling for regeneration purposes. Mucus was then collected from each coral genus by using the methodology described in Wild et al. (2005a). 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 allow healing. 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 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 water from the bottom and emptied from the top. A stir bar was employed to aid mixing. 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 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 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 and 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

Fatty acids could not be detected in any of the mucus samples analysed in the present study, which is in accordance with the study of Ducklow and Mitchell (1979), but contrasts the results of Benson and Muscatine (1974), Crossland et al. (1980) and Daumas et al. (1982), who found that up to 30 % of coral mucus dry mass can be composed out of lipids. Total carbohydrate contents of the analysed coral mucus samples were highly variable ranging from less than 1 to 22 mass %, which is in accordance with the literature review in the study of Coffroth (1990) demonstrating highly scattered carbohydrate contents in coral mucus samples likely caused by different purification techniques. Absolute carbohydrate contents should therefore be treated carefully so that the focus in the following lies onto the compositional analyses. These analyses shown in Table 1 revealed that C6 sugars (glucose, mannose, galactose) occurred 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 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 on the genus level. On the other hand, similarities compared to studies carried out in the Australian GBR (Richards et al. 1983; Meikle et al. 1988; Wild et al. 2005a) 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).

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

acetyl-glucosamine, 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 and 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 composition of coral mucus principally consists of 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. 2005b, 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).

Table 1 *Glycosyl 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

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 (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 and Hedges 1984) potentially associated to 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. 2005a) 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.

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References

Allers E, Niesner C, Wild C, Pernthaler J (2008) Microbes enriched in seawater after the addition of coral mucus. Appl Env Microbiol 74:3274-3278

Amon RMW, Fitznar HP, Benner R (2001) Linkages among the bioreactivity, chemical composition, and diagenetic state of marine dissolved organic matter. Limnol Oceangr 46:287-297

Arnosti C (2000) Substrate specificity in polysaccharide hydrolysis: contrasts between bottom water and sediments. Limnol Oceanogr 45:1112-1119

Benner R, Pakulski JD, McCarthy M, Hedges JI, Hatcher PG (1992) Bulk chemical characteristics of dissolved organic matter in the ocean. Science 255:1561-1564

Benson A, Muscatine L (1974) Wax in coral mucus - energy transfer from corals to reef fishes. Limnol Oceanogr 19:810-814

Brown BE, Bythell JC (2005) Perspectives on mucus secretion in reef corals. Mar Ecol Prog Ser 296:291-309

Coffroth MA (1990) Mucous sheet formation on poritid corals - an evaluation of coral mucus as a nutrient source on reefs. Mar Biol 105:39-49

Cowie GL, Hedges JI (1984) Carbohydrate sources in a coastal marine environment. Geochim Cosmochim Acta 48:2075-2087

Crossland C, Barnes D, Borowitzka M (1980) Diurnal lipid and mucus production in the staghorn coral *Acropora acuminata*. Mar Biol 60:81-90

Daumas R, Galois R, Thomassin BA (1982) Biochemical composition of soft and hard coral mucus on a New Caledonian lagoonal reef. Proc 4th Int Coral Reef Symp 2:59-68

Ducklow HW (1990) The biomass, production and fate of bacteria in coral reefs. In: Dubinsky Z (ed) Coral reefs, Ecosystems of the world. Elsevier, Amsterdam, pp 265-289

Ducklow HW, Mitchell R (1979) Composition of mucus released by coral reef coelenterates. Limnol Oceanogr 24:706-714

Johannes RE (1967) Ecology of organic aggregates in the vicinity of a coral reef. Limnol Oceanogr 12:189-195

Marshall M (1968) Observations on organic aggregates in the vicinity of coral reefs. Mar Biol 2:50-55

Meikle P, Richards G, Yellowlees D (1988) Structural investigations on the mucus from 6 species of coral. Mar Biol 99:187-193

Merkle RK, Poppe I (1994) Carbohydrate composition analysis of glycoconjugates by gas-liquid chromatography/mass spectrometry. Methods Enzymol 230:1-15

Ogawa H, Amagai Y, Koike I, Kaiser K, Benner R (2001) Production of refractory dissolved organic matter by bacteria. Science 292:917-920

Rich JH, Ducklow HW, Kirchman DL (1996) Concentrations and uptake of neutral monosaccharides along 140°W in the equatorial Pacific: Contribution of glucose to heterotrophic bacterial activity and the DOM flux. Limnol Oceangr 41:595-604

Richards GN, Meikle PJ, Yellowlees D (1983) Preliminary investigations into the chemical nature of mucus from the staghorn coral (*Acropora formosa*). Proc Inaug Great Barrier Reef Conf:353-359

Riemann L, Azam F (2002) Widespread N-Acetyl-D-Glucosamine uptake among pelagic marine bacteria and its ecological implications. Appl Environ Microbiol 68:5554-5562

Schöttner S, Hoffmann F, Wild C, Rapp HT, Boetius A, Ramette A (2009) Inter- and intra- habitat bacterial diversity associated with cold water corals. ISME J 3:756-759

Wild C, Woyt H, Huettel M (2005a) Influence of coral mucus release on nutrient fluxes in carbonate sands. Mar Ecol Prog Ser 287:87-98

Wild C, Huettel M, Klueter A, Kremb SG, Rasheed M, Jørgensen BB (2004a) Coral mucus functions as an energy carrier and particle trap in the reef ecosystem. Nature 428:66-70

Wild C, Rasheed M, Werner U, Franke U, Johnstone R, Huettel M (2004b) Degradation and mineralization of coral mucus in reef environments. Mar Ecol Prog Ser 267:159-171

Wild C, Rasheed M, Jantzen C, Cook P, Struck U, Huettel M, Boetius A (2005b) Benthic metabolism and degradation of natural particulate organic matter in silicate and carbonate sands of the Northern Red Sea. Mar Ecol Prog Ser 298:69-78

Wild C, Mayr C, Wehrmann L, Schöttner S, Naumann M, Hoffmann F, Rapp HT (2008) Organic matter release by cold water corals and its implication for fauna-microbe interaction. Mar Ecol Prog Ser 372:67-75

York WS, Darvill AG, McNeil M, Stevenson TT, Albersheim P (1985) Isolation and characterisation of plant cell walls and plant cell wall components Methods Enzymol 118:3-40

10

Organic matter release by cold water corals and its implication for fauna-microbe interaction

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Abstract

Particulate and dissolved organic matter (POM and DOM) released by the cold water corals *Lophelia pertusa* (L.) and *Madrepora oculata* (L.) was collected, analysed and quantitatively compared to that released by warm water reef-building corals. Particulate nitrogen (PN) and particulate organic carbon (POC) release rates of *L. pertusa* were 0.14 ± 0.07 mg N m^{-2} h^{-1} and 1.43 ± 1.22 mg C m^{-2} h^{-1}, respectively, which is in the lower range of POM release rates measured for warm water corals, while dissolved organic carbon (DOC) release was 47 ± 19 mg C m^{-2} h^{-1}. The resulting high DOC:POC ratio indicates that most cold water coral-derived organic matter immediately dissolved in the water column. Cold water corals, similar to their warm water counterparts, produced large amounts of nitrogen-rich coral mucus with C:N ratios of 5 - 7 for *Lophelia*- and 7 - 9 for *Madrepora*-derived mucus. Seven-fold increased oxygen consumption rates in cold-water coral mucus-amended seawater containing the natural microbial assemblage indicate that this organic matter provided an attractive food source for pelagic microbes. *In-situ* investigations at Røst Reef, Norway, showed that microbial activity in the sea water closest to the reef was 10 times higher than in the overlaying water column. This suggests that cold-water corals can stimulate microbial activity in the direct reef vicinity by the release of easily degradable and nutrient-rich organic matter, which may thereby function as a vector for carbon and nutrient cycling via the microbial loop in cold-water coral reef systems.

Introduction

Although only recently discovered and still far from completely explored, cold water coral reefs are highly endangered by direct anthropogenic threats such as bottom trawling, hydrocarbon drilling and seabed mining, but also by climate change consequences such as ocean acidification (Roberts et al. 2006). Taxonomic surveys in various cold water coral reefs showed that these ecosystems are hotspots for biodiversity providing habitat for more than 1300 benthic species (Jensen & Frederiksen 1992, Mortensen et al. 1995, Rogers 1999, Freiwald et al. 2004, Roberts et al. 2006). However, ecological studies focusing on interactions between corals and cold-water coral reef associated organisms, in particular on the role of reef associated planktonic microbial communities have not been conducted to our knowledge, although such investigations may result in fundamental insights of ecosystem functioning in cold-water coral reefs. This pilot study aims to contribute in this context.

It is known from warm water reefs that hard and soft corals do release organic matter in dissolved (Ferrier-Pages et al. 1998) or particulate form, comprising spawning products and mucus (e.g. Harrison et al. 1984, Crossland 1987) into their surroundings. The release of organic matter by corals importantly contributes to the ecological functioning of tropical coral reefs by controlling key processes such as the transport of organic matter. This in turn may influence planktonic and benthic metabolism as well as the associated recycling of essential elements. Mass release of eggs and sperm during the annual coral spawning event can have extensive biogeochemical consequences (Wild et al. 2004a, Eyre et al. 2008, Glud et al. 2008, Wild et al. 2008). Furthermore, mucus continuously released by tropical corals can act as an energy carrier and particle trap (Wild et al. 2004b) and consequently initiates element cycling and interaction between fauna and microorganisms in tropical reef ecosystems. Within this context, coral-derived mucus can strongly influence planktonic microbial metabolism (Ferrier-Pages et al. 2000), microbial abundance (Wild et al. 2004c) and microbial community composition (Allers et al. 2008).

Cold water coral reefs exhibit different environmental settings compared to their warm water counterparts (Freiwald et al. 2004, Roberts et al. 2006). Visual observations showed that cold water corals are also in principle able to release large amounts of organic matter as mucus into the surrounding water, at least under massive stress conditions (Reitner 2005). However, the need for nutrient conservation by possible trapping of suspended particles via coral-derived organic matter as suggested by Wild et al. (2004b) seems to be less important in these ecosystems, which are influenced by high current velocities and often nutrient rich waters (Roberts et al. 2006). The ecological function of organic matter release by cold water corals is entirely unknown. This study therefore provides the first data on the quantity of dissolved and particulate organic matter released by the cosmopolitan cold water coral *Lophelia pertusa*. Furthermore, pilot investigations at Røst Reef, Norway, the largest cold water coral reef system discovered to date (Freiwald et al. 2004), are presented, which target the microbial degradability of coral-derived organic matter and its possible implications for cold water coral reef-associated microbial metabolism and fauna-microbe interactions in the reef ecosystem.

Material and Methods

Study site

The different investigations described in this study were conducted at the aquarium facilities of University of Bergen, Norway, in October 2006 and during the RV Polarstern cruise ARK-XXII/1a to the Mid-Norwegian continental shelf in June 2007.

Quantification of organic matter release rates

Release rates of dissolved as well as particulate organic matter were quantified using the beaker incubation technique initially described by Herndl & Velimirov (1986). Prior to the experiments, a large colony (diameter: ca. 40 cm) of the cold water coral *Lophelia pertusa*, collected from Langenuen, a fjord area south of Bergen (position: 60° 25' N, 06º31' E; water depth: ca. 120 m), was kept in a flow-through aquarium with continuous inflow of in-situ seawater pumped from a water depth of ca. 100 m for more than 6 months. This resulted in stable salinities (32.5 – 33.8 PSU) and a constant water temperature of ca. 11 °C over the whole maintenance period as monitored from regular measurements using refractometer and thermometer. The colony was broken into fragments (length: 2 to 9 cm) using a wire cutter. These fragments were left in the maintenance aquarium for at least 3 days to recover prior to the experiment. For the experiment, 5 fragments were separately transferred (without air exposure) into 1 l glass beakers filled with 700 to 800 ml of untreated seawater from the aquarium. Three additional glass beakers, which served as controls, were filled with seawater from the aquarium only. All 8 beakers were kept in a flow-through aquarium in order to maintain an *in-situ* temperature of 11 °C during the entire 4 h dark incubation. During the beaker incubation it was observed that between 25 % and 40 % of the fragment polyps were protruded, which was well in the range of the mother colony and thus indicated an advanced recovery of the fragments, i.e. that duration of the healing period was sufficient.

Following incubation, coral fragments were removed from the beakers, the incubation water in the beakers was homogenized by gentle stirring for at least 30 s, and sub-samples were taken as follows. Samples for measurement of dissolved organic carbon (DOC) were prepared by filtering (0.2 µm *Millex* Nylon membrane filters) 2 ml incubation water aliquots into pre-combusted 3 ml brown glass vials (another 2 ml aliquot of each incubation volume was pre-filtered and discarded prior in order to clean the respective filter). Samples were immediately frozen at -20 °C and kept frozen until analysis. Samples for measurement of particulate organic C (POC) and N (PN) concentrations and their stable isotope signatures were prepared by filtering 200 ml incubation water aliquots onto pre-combusted GF/F filters (Whatman, 25 mm in diameter; combusted for 4-6 h at 500 °C). The filters were dried for at least 48 hours at 40 °C and kept dry until analysis as described below. Organic matter release rates were related to the coral surface area, which was assessed by application of a geometric approximation method in combination with a specific SAI (surface area approximation index) for *L. pertusa*, derived from comparative analysis with a computer tomography based surface area measurement technique (Naumann et al. submitted). Briefly, fragments were notionally divided into several branch sections with simple cylinder shape. Radius and height of each branch were assessed using a conventional caliper (accuracy: ± 0.05

mm), measuring the diameter of the branch base and the height from branch base to tip. Measured parameters were applied in the calculation of cylinder superficies surface area and cylinder cover area using conventional surface area formulas. Cylinder surface areas from all branches were added to gain the total fragment surface area, which was finally multiplied by the above mentioned SAI to improve accuracy.

Mucus composition and microbial degradation

During the *RV Polarstern* cruise, several large colonies (diameter > 30 cm) of the cold water corals *Lophelia pertusa* (L.) and *Madrepora oculata* (L.) were collected by the manned submersible JAGO (IFM-Geomar, Kiel) during 3 dives at Røst Reef (67° 31.22' N, 9° 28.43' E) from water depths of 310 m to 380 m. These coral colonies were immediately transferred into 300 l glass aquaria filled with fresh bottom water and kept at 6 °C in a cooling chamber on board the research vessel for less than 3 d before sample collection. Mucus was collected from 3 to 4 fragments of each of the following cold water corals colour morphotypes, a) white *Lophelia*, b) pink *Lophelia*, c) white *Madrepora* and d) orange *Madrepora* (see Fig. 1), by exposing the corals to air for 5 min and collecting the produced gel-like mucus in a clean glass dish. Depending on produced volume, between 2 and 20 ml of mucus from each coral fragment were filtered onto pre-combusted GF/F filters. In addition, bottom water samples from between 4 to 10 m height above Røst Reef were collected on 3 occasions (June 8, 9, 12; using CTD-attached 20 l Niskin bottles) and filtered in volumes of 250 ml or 500 ml onto pre-combusted GF/F filters in triplicates for each sampling occasion. All filters were dried at 40 °C for at least 48 h, and their POC as well as PN contents quantified as described below.

In order to measure microbial degradation of coral mucus, aliquots of freshly collected mucus from the orange *Madrepora* colonies in a dilution of 1:10 (mucus: bottom water) and pure bottom water as reference, were incubated for up to 200 h in 30 ml Winkler glass bottles under dark conditions at *in-situ* temperature (5 °C). The O_2 concentrations in both treatments were measured in parallel time series using the Winkler titration method, with each sampling point representing an individual Winkler bottle. O_2 consumption rates were calculated in µM l^{-1} using linear regression with at least 4 data points. Microbial C degradation was calculated by relating the oxygen consumption rates to the respective C content of each organic matter source (measured as described above in replicates of n = 4) assuming that 1 mol of organic C is oxidized by 1 mol of O_2.

Planktonic microbial oxygen demand above Røst reef

A CTD-attached water sampling device was used from the research vessel in order to collect water samples from various water depths above a small reef valley (exact coordinates: 67° 31.217' N, 9° 28.430' E) in 328 m water depth within Røst Reef. Water samples from 4, 11, 16, 21, 26, 32, 47 and 177 m above Røst Reef were retrieved using 20 l Niskin bottles. Aliquot water samples from each water depth were filled into two ca. 120 ml Winkler glass bottles for determination of oxygen concentration and planktonic microbial oxygen demand. One of the bottles filled with water from each depth was used for oxygen concentration determination using Winkler titration, and the other one was incubated for 134 h in the dark and at *in-situ* temperature in a cold-room on board. After

this period, O_2 concentration of the incubated water samples was measured using Winkler titration (Winkler 1888) and subtracted from the start values in order to calculate planktonic microbial oxygen demand. Replicate measurements (n = 4) of O_2 consumption rates in bottles filled with the same seawater were within 1.2 %.

In addition, 500 ml aliquots (n = 1-3 for each depth) from the water sampled in vertical resolution were filtered onto pre-combusted (4-6 h at 500 °C) Whatman GF/F filters. The filters were dried for at least 48 hours at 40 °C and kept dry until POM analyses as described below.

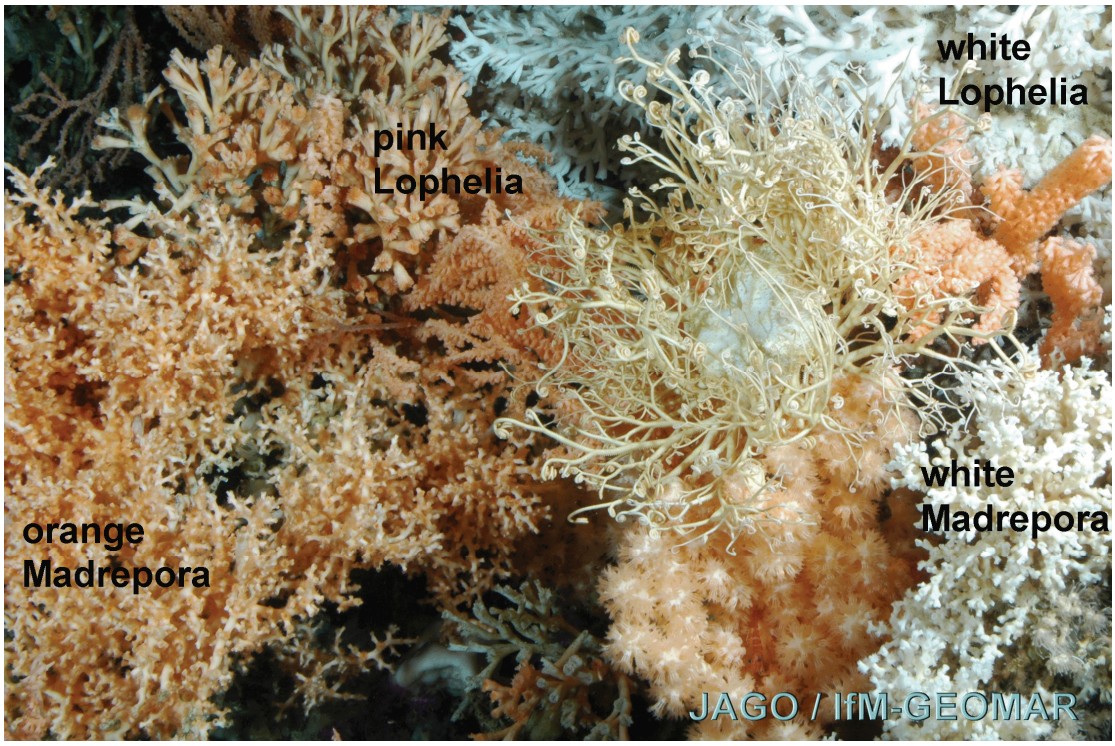

Fig. 1 *Digital image of a coral assemblage within Røst Reef, Norway (water depth: ca. 350 m) taken from manned submersible JAGO (IFM-GEOMAR) during RV Polarstern cruise ARK-XXII/1a. All four different colour morphotypes of Lophelia and Madrepora, investigated in the present study, are grouped around the gorgonian octocoral Primnoa resedaeformis with a basket star (Gorgonocephalus sp) on the top. Copyright: JAGO/IFM-GEOMAR 2007.*

Stable isotope and elemental analyses

DOC concentrations were quantified by high-temperature catalytic oxidation (HTCO) using a Rosemount Dohrmann DC-190 TOC analyzer. 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. C and N elemental concentration measurements were calculated from certified elemental standards (Atropine, Cyclohexanone-2,4-dinitrophenylhydrazone; Thermo Quest, Italy) analysed within each batch and typically showed standard deviations < 3 %. Stable isotope ratios are given in the conventional delta notation ($\delta\ ^{13}C$, $\delta\ ^{15}N$) 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) with a broad range of initial weights were better than 0.15 ‰ for nitrogen and carbon, respectively. Within each run, linearity was checked with isotope standards of different initial weights and data outside the linearity range were discarded or - if feasible - linearity corrected. Filters were not treated with HCl in order to prevent disturbance or falsification of the $\delta^{15}N$ signatures. Inorganic carbon was not present in the samples as evidenced from test measurements of the mucus and water samples.

Results

Organic matter release by cold water corals

The fragments of *Lophelia pertusa* released all investigated kinds of organic matter (POC, PN and DOC) in significant quantities (Table 1). The mean ratio between DOC and POC was greater than 30, which indicates that most of the coral-derived organic carbon entered the DOM pool in the water column close to cold water corals. POC and PN release rates of *Lophelia pertusa* (1.43 ± 1.22 and 0.14 ± 0.07 mg m^{-2} d^{-1} respectively) were in the lower range of rates reported from various warm water corals (POC: 0.8 – 88.8 and PN 0.04 – 1.30 mg m^{-2} d^{-1}), whereas *Lophelia* DOC release was always positive in contrast to DOC release rates reported for warm water corals, which were highly variable and often negative, indicating DOC uptake by the corals (Table 1).

Table 1 *Organic matter release rates of the investigated cold water coral in comparison with release rates reported for warm water corals. Values are means ± SD.*

Study site	Species	PN release (mg m^{-2} h^{-1})	POC release (mg m^{-2} h-1)	DOC release (mg m^{-2} h-1)	Method	Reference
Bergen, Norway	*Lophelia pertusa*	0.14 ± 0.07	1.43 ± 1.22	47 ± 19	Beaker incubation	This study
Aqaba, Jordan	various species	0.04 to 0.48	0.8 to 6.9	-351 to 75	Beaker incubation	Naumann et al. (unpubl. data)
Ishigaki Island, Japan	*Porites cylindrica*	not measured	70.8	40.8	Container incubation	Tanaka et al. (2008)
Ishigaki Island, Japan	*Acropora pulchra*	not measured	88.8	45.6	Container incubation	Tanaka et al. (2008)
Heron Island, Australia	*Acropora millepora*	1.30 ± 0.80	10.0 ± 5.0	not measured	Beaker incubation	Wild et al. (2005)
Eilat, Israel	*Acropora variabilis*	not measured	1.4 to 4.2	not measured	Chamber incubation	Crossland (1987)
Eilat, Israel	*Stylophora pistillata*	not measured	2.7 to 4.0	not measured	Chamber incubation	Crossland (1987)
Bight of Piran, Croatia	*Cladocora cespitosa*	not measured	6.0	not measured	Beaker incubation	Herndl & Velimirov (1986)

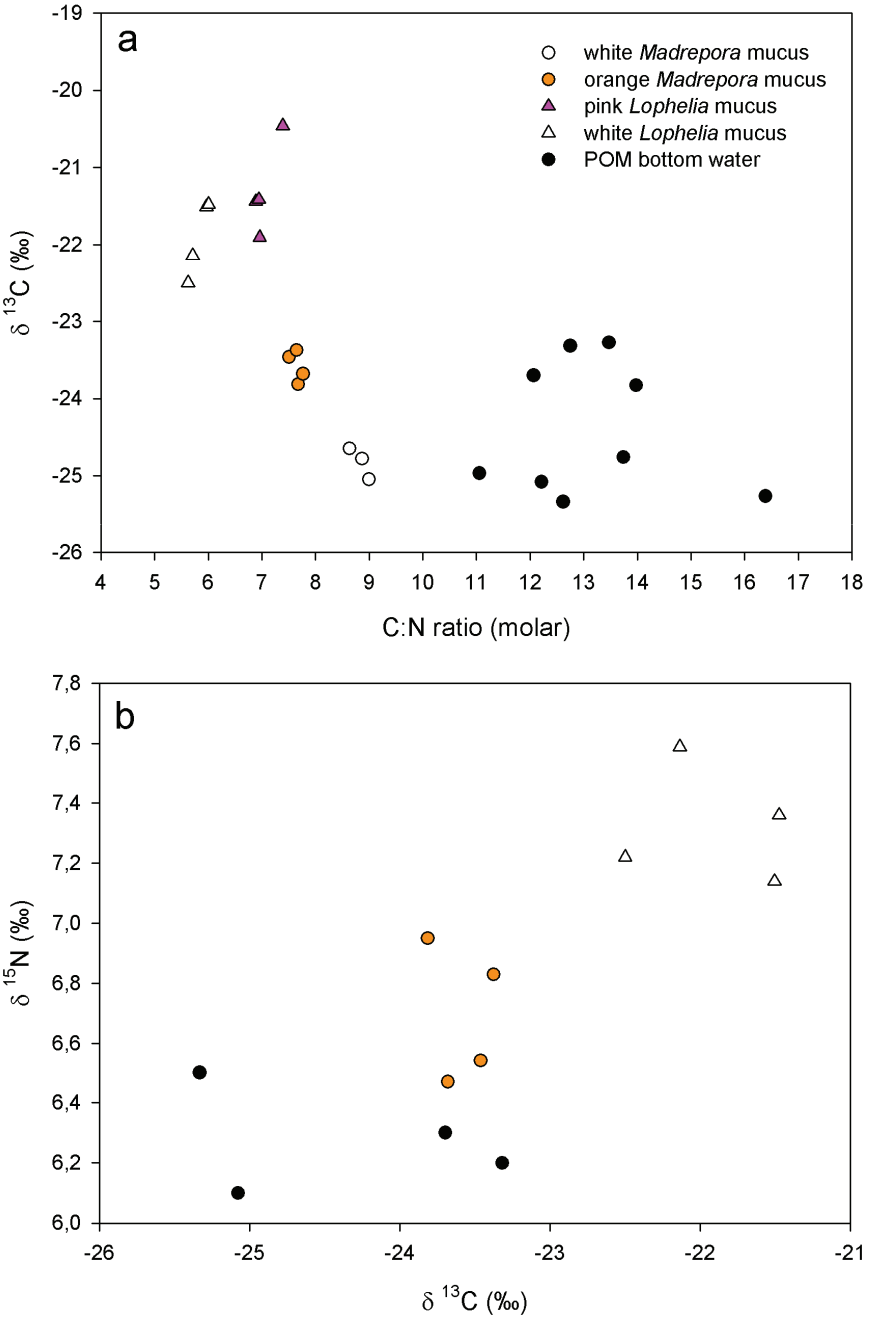

Fig. 2 *a) C:N ratios versus carbon stable isotope signatures and b) carbon versus nitrogen stable isotope signatures of mucus released by different colour morphotypes of the cold water corals Lophelia pertusa and Madrepora oculata in comparison with particulate organic matter (POM) suspended in bottom water samples close (less than 40 m distance) to Røst Reef, Norway.*

Mucus composition and microbial degradation

Cold water coral mucus collected from the four different investigated colour morphotypes showed clear compositional differences (Figure 2a). However, all coral mucus samples were significantly enriched in N compared to POM suspended in the water close to the cold water coral reef (two-sided U-test after Wilcoxon, Mann and Whitney, $\alpha = 0.05$). Mucus collected from *Lophelia* showed significantly lower C:N values (one-sided U-test after Wilcoxon, Mann and Whitney, $\alpha = 0.001$)

compared to all mucus samples collected from *Madrepora*, with minimum values of less than 6 for the white *Lophelia*, which is even lower than the Redfield ratio (Fig. 2a). Values of $\delta\ ^{13}C$ for all mucus samples from the *Lophelia* fragments were significantly heavier compared to the $\delta\ ^{13}C$ values of *Madrepora* mucus samples and POM suspended in bottom waters (one-sided U-test after Wilcoxon, Mann and Whitney, $\alpha < 0.05$), whereas no significant differences occurred between *Madrepora* mucus and POM. Coral mucus $\delta\ ^{15}N$ values showed similar trends with samples from white *Lophelia* fragments exhibiting significantly ($\alpha = 0.05$) heavier N isotope signatures (increase of ca. 1 ‰) compared to both orange *Madrepora* mucus samples and bottom water POM, whereas the latter two were not significantly different (Fig. 2b).

The mucus degradation experiment revealed that mucus released by cold water corals serves as an attractive substrate for reef-associated planktonic microorganisms. Oxygen was consumed 7 times faster in those incubation bottles filled with 1:10 diluted coral mucus compared to those only filled with seawater (Fig. 3). This corresponds to a microbial organic C degradation of circa 8 % of the added coral mucus per day assuming that 1 mol organic C is oxidized by 1 mol of O_2.

Microbial activity in the water column above Røst Reef

Microbial O_2 consumption rates were less than 10 µg l^{-1} d^{-1} in all samples, except the sample from the station closest to the reef, where values of more than 60 µg l^{-1} d^{-1} were recorded (Fig. 4). These maximum values were limited to the water column directly (less than 4 m distance) above the reef. By 10 m distance above the coral reef, O_2 consumption rates dropped down to the above mentioned low values (Fig. 4) In the remaining water column, oxygen consumption rates varied between 0 and 10 µg l^{-1} d^{-1}, reflecting the pattern of the POM concentrations, which varied between 40 and 60 µg l^{-1}.

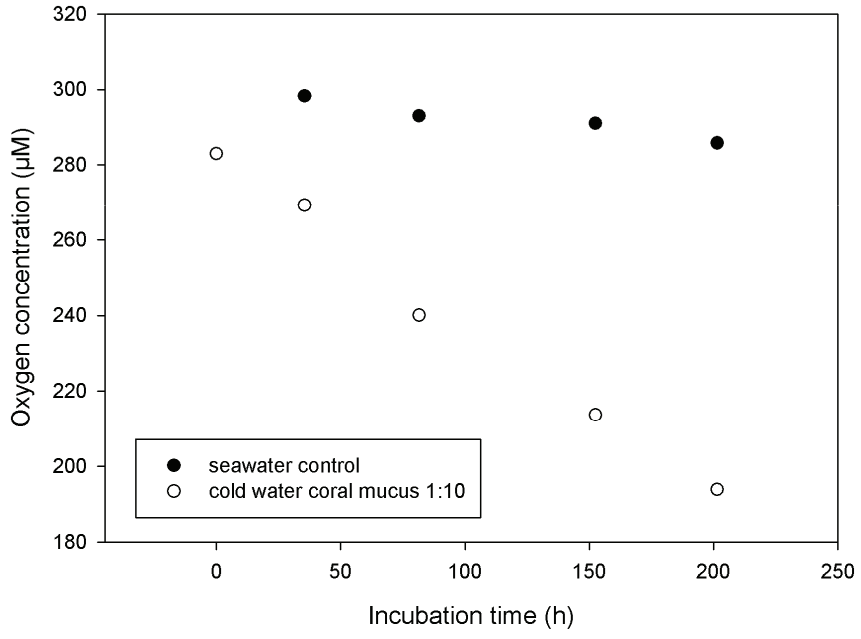

Fig. 3 *O_2 concentrations in glass incubation bottles filled with bottom water collected at Røst Reef and cold water coral mucus, 1:10 diluted with the bottom water.*

Discussion

Cold water coral-derived organic matter: quantity and quality

The results of this study show that cold water corals, similar to warm water corals, release organic matter in dissolved and particulate form. The investigated cold water coral *Lophelia pertusa* released clearly more DOC than POC, which contrasts with the recent study of Tanaka et al. (2008), who found higher POC than DOC release by the warm water corals *Acropora pulchra* and *Porites cylindrica* (Table 1). In general, POC release by the cold water coral tended to be lower, and DOC release rates tended to be higher than rates reported for warm water corals (see table 1 and references therein).

It is known from warm water corals that POM release may function as a protection mechanism against bio-fouling (Ducklow & Mitchell 1979), high particle loads in the water column (Rublee et al. 1980) and sedimentation (Schuhmacher 1977). While bio-fouling does seem to affect cold water corals as well (Freiwald et al. 2004, Hall-Spencer et al. 2007), the Norwegian cold water coral reefs apparently often experience low turbidity and sedimentation rates (Mortensen et al. 2001). Release of POM may thus have a general function against bio-fouling for all corals independent of the surrounding conditions including light and temperature. However, the question arises how azooxanthellate cold water corals are able to invest similar amounts of energy and nutrients in the production of released organic matter compared to zooxanthellate warm water corals, which derive large proportions of their energy demand from their endosymbiotic algae (Muscatine et al. 1981). 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 are directly transferred from the algae to the coral host (Meikle et al. 1988).

The low C:N ratio measured for mucus from different cold water corals indicate that N-containing components like glycoprotein, as found in warm water coral-derived organic matter (Meikle et al. 1987), are main components of this organic material. These components may be responsible for the high N-content, which was close to or even slightly below the Redfield ratio, thereby valorising cold-water coral mucus to an attractive and easily accessible food source for heterotrophic microorganisms.

Release of DOC could be measured for all investigated cold water corals, whereas quantification experiments for DOC release of warm water corals often showed net uptake of DOC through the corals (Ferrier 1991, Ferrier-Pages et al. 1998, Naumann et al. unpublished data). Warm water corals thus seem to behave more conservatively with DOC than cold water corals, probably due to the often more oligotrophic conditions of warm water coral reefs, which necessitates the retention of DOC- associated essential nutrients such as nitrogen and phosphorus. In cold water coral reefs, where usually more planktonic food is available for the corals (e.g. Freiwald et al. 2004), it may be energetically more favourable to release DOC into the surrounding water.

Carbon stable isotope signatures show clear differences between the mucus released by the two investigated cold water corals *Lophelia pertusa* and *Madrepora oculata*. Such differences were also observed for the nitrogen stable isotope signatures of the mucus from the white *Lophelia* and orange *Madrepora* morphotypes. Assuming that mucus reflects tissue signatures (which is supported by

very similar C stable isotope signatures of -22.0 ± 0.2 ‰ for *Lophelia* tissue samples from the fragments used in Bergen compared to -21.9 ± 0.5 ‰ for coral mucus samples from Røst Reef), these species-specific differences in carbon and nitrogen stable isotope signatures may point towards species-specific metabolic differences as suggested by Weisz et al. (2007) for sponges, or towards different feeding strategies, which is supported by higher $\delta^{15}N$ and monounsaturated fatty acid contents found in the tissue of *L. pertusa* compared to *M. oculata* (Kiriakoulakis et al. 2005). Carbon stable isotope signatures of all POM samples from the water column close to (Fig. 1) or above Røst Reef (Fig. 3) were in the range of values for mucus samples from *Madrepora*, but always more negative compared to the respective values for *Lophelia*. The $\delta^{15}N$ values of *Lophelia* mucus were enriched by 1 ‰ compared to POM, while those of *Madrepora* mucus were in the range of POM values. This may indicate that POM cannot act as the only food source for neither *Madrepora* nor *Lophelia*, which would result in a ~ 3 ‰ increase of $\delta^{15}N$ per trophic level (Peterson and Fry 1987). The low $\delta^{15}N$ values in both coral species may be explained by substantial inputs of N from autochthonous microbial sources (Weisz et al. 2007). However, seasonal variations of the $\delta^{15}N$ signature of POM (Duineveld et al. 2004) as well as direct feeding on zooplankton, as observed by Rapp & Sneli (1999) for *Lophelia*, may also contribute to the stable isotope composition of cold-water corals. Further investigation is needed to explain the observed differences in the isotopic signature of *Lophelia* and *Madrepora*, and to identify the main food sources of these species.

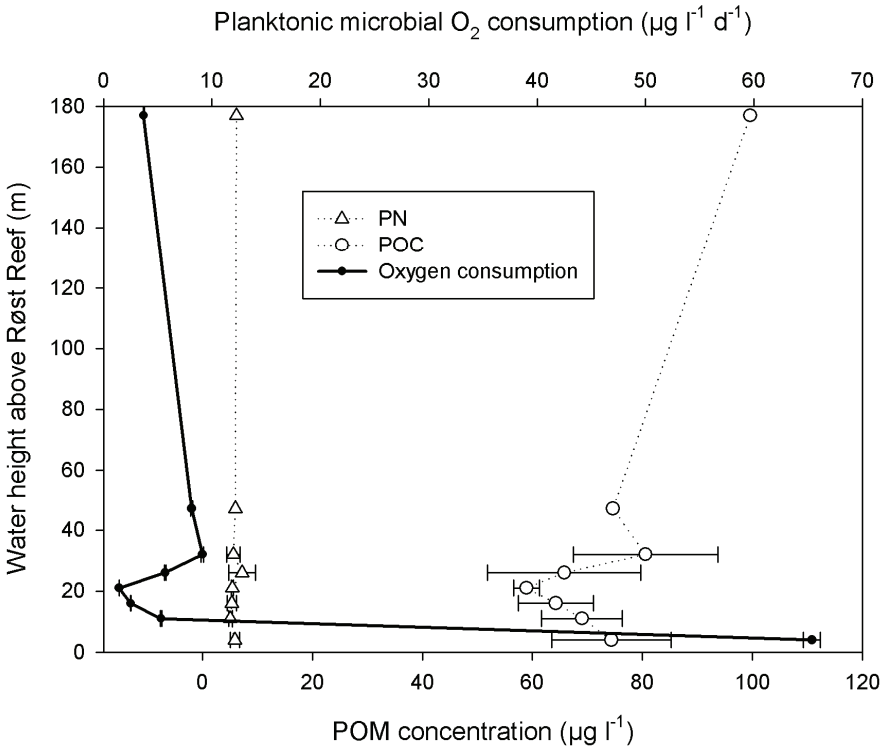

Fig. 4 *POM concentrations and microbial O_2 consumption measured in a vertical profile in the water column above Røst Reef. Values are means ± SD (n = 1-3). Error bars for microbial O_2 consumption indicate the methodological error of 1.2 % (n = 4).*

Organic matter degradability and fauna-microbe interaction

The enhanced respiration rates in mucus-enriched seawater showed that mucus released by cold water corals is an attractive substrate for planktonic microorganisms living in the water column. Thus, mucus can act as a vector for energy from the corals to these microbes. Approximately 8 % of the added coral mucus was mineralized by planktonic microbes per day at an *in-situ* temperature of 6 °C. It is difficult to compare these degradation rates with those of warm water coral mucus because of the apparent large temperature differences. However, assuming that after Van't Hoff's rule a 10 °C temperature increase doubles the velocity of biological processes (Harvey 1916), this would result in a temperature-corrected microbial organic C degradation rate of 1.4 % h^{-1} at 26 °C compared to a rate of 7.0 % h^{-1} measured for warm water coral mucus at identical temperature (Wild et al. 2004b, Wild et al. 2004c). This also illustrates the similarity of both coral-derived substrates with respect to attractiveness for microbial degradation, although cold water coral mucus seems to exhibit a more refractory character.

Intense fauna-microbe interaction via coral mucus is apparently established in both warm and cold water coral reef ecosystems. This may have strong effects on microbial diversity and activity as well as concomitant recycling of organic matter and ecosystem productivity. The present study shows that there may be a stimulation of the planktonic microbial activity in the water column directly above a proliferating reef, likely due to organic matter release by reef corals. The vertical measurements presented in Fig. 4 indicate that POM, which may fuel planktonic respiration in large parts of the water column, could not cause this stimulation of respiration rates directly above the reef. More likely, increased DOC concentrations in direct reef vicinity, with coral-released mucus as a possible DOC source, were responsible for the observed activity stimulation. This is supported by the high DOC : POC ratio of coral-derived organic matter as obtained in the laboratory study, and by the attractiveness of mucus to heterotrophic microbes, which is shown by its high N-content and by the results of the bottle incubations presented in this study. A study by Schöttner et al. (unpublished data) also confirms a potential parallel effect of coral derived organic matter on microbial diversity by showing that mucus from *Lophelia pertusa* supports a specifically associated microbial community, which is significantly different compared to that in the surrounding seawater and reef sediments.

Furthermore, the study of Wehrmann et al. (unpublished data), who investigated biogeochemical processes in sediments associated to the Norwegian cold water coral reefs, including Røst Reef, indicates that a large amount of the suspended organic carbon may already be turned over in the water column or the reef framework while microbial mediated carbon turnover rates including organoclastic sulphate reduction rates (SRR) in the subsurface sediments underlying these reefs were very low (< 3 nmol cm^{-3} d^{-1}). This is supported by the recent study of van Duyl et al. (2008), who showed that sponge-microbe consortia, which are often associated with cold water coral reefs (Roberts et al. 2003, Roberts et al. 2006, van Soest et al. 2007), can incorporate DOC at high rates. It remains speculation and a potential target issue of future studies if DOM excretion by cold water corals and subsequent stimulation of microbial growth near the reef may help the corals and associated organisms to prey on microbes.

Unlike warm water reefs, which mostly rely on sunlight as a direct energy source, cold water coral reefs entirely rely on the export of organic matter from primary production in surface waters to the seafloor (Duineveld et al. 2004, Thiem et al. 2006). The findings of this study suggest that organic matter not only functions as pre-requisite for cold water coral reef occurrence, but also acts as a trophic link for intense interactions between the different reef organisms.

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References

Allers E, Niesner C, Wild C, Pernthaler J (2008) Microbes enriched in seawater after the addition of coral mucus. Appl and Envir Microbiol 74(10): 3274-3278

Coplen TB (1995) Reporting of stable hydrogen, carbon, and oxygen isotopic abundances (Technical Report). Geothermics 24:707-712

Craig H (1957) Isotopic standards for carbon and oxygen and correction factors for mass-spectrometric analysis of carbon dioxide. Geochim Cosmochim Acta 12:133-149

Crossland C (1987) In situ release of mucus and DOC-lipid from the corals *Acropora variabilis* and *Stylophora pistillata* in different light regimes. Corals Reefs 6:35-42

Davies PS (1984) The role of zooxanthellae in the nutritional energy requirements of *Pocillopora eydouxi*. Coral Reefs: 181-186

Ducklow H, Mitchell R (1979) Bacterial populations and adaptations in the mucus layers on living corals. Limnol Oceanogr 24:715-725

Duineveld GCA, Lavaleye MSS, Berghuis EM (2004) Particle flux and food supply to a seamount cold-water coral community (Galicia Bank, NW Spain). Mar Ecol Prog Ser 277:13-23

Eyre BD, Glud RN, Patten N (2008) Mass coral spawning: A natural large-scale nutrient addition experiment. Limnol Oceanogr 53(3): 997-1013

Ferrier-Pages C, Gattuso JP, Cauwet G, Jaubert J, Allemand D (1998) Release of dissolved organic carbon and nitrogen by the zooxanthellate coral *Galaxea fascicularis*. Mar Ecol Prog Ser 172:265-274

Ferrier-Pages C, Leclercq N, Jaubert J, Pelegri SP (2000) Enhancement of pico- and nanoplankton growth by coral exudates. Aqu Micr Ecol 21:203-209

Ferrier MD (1991) Net uptake of dissolved free amino acids by 4 Scleractinian corals. Corals Reefs 10:183-187

Freiwald A, Fossa JH, Grehan A, Koslow T, Roberts JM (2004) Cold-water coral reefs, Vol 1. UNEP-WCMC, Cambridge, UK

Glud RN, Eyre BD, Patten N (2008) Biogeochemical consequences to mass coral spawning at the Great Barrier Reef. Limnol Oceanogr 53(3): 1014-1024

Hall-Spencer JM, Pike C, Munn CB (2007) Diseases affect cold water corals too: *Eunicella verrucosa* (Cnidaria: Gorgonacea) necrosis in SW England. Dis Aqu Org 76:87-97

Harrison PL, Babcock RC, Bull GD, Oliver JK, Wallace CC, Willis BL (1984) Mass spawning in tropical reef corals. Science 4641: 1186-1189

Harvey EN (1916) Temperatur und Lebensvorgänge. Science 43:466-467

Herndl GJ, Velimirov B (1986) Microheterotrophic utilization of mucus released by the Mediterranean coral *Cladocora cespitosa*. Mar Biol 90:363-369

Jensen A, Frederiksen R (1992) The fauna associated with the bank-forming deep-water coral *Lophelia pertusa* (Scleractinaria) on the Faroe Shelf. Sarsia 77(1): 53-69

Kiriakoulakis K, Fisher E, Wolff GA, Freiwald A, Grehan A, Roberts JM (2005) Lipids and nitrogen isotopes of two deep-water corals from the North-East Atlantic: initial results and implications for their nutrition. In: Freiwald A, Roberts JM (eds) Cold water corals and ecosystems. Springer, Berlin Heidelberg, p 715-729

Mariotti A (1984) Atmospheric nitrogen is a reliable standard for natural 15N abundance measurements. Nature 303:685-687

Meikle P, Richards GN, Yellowlees D (1987) Structural determination of the oligosaccharide side-chains from a glycoprotein isolated from the mucus of the coral *Acropora formosa*. J Biol Chem 262:16941-16947

Meikle P, Richards GN, Yellowlees D (1988) Structural investigations on the mucus from 6 species of coral. Mar Biol 99: 187-193

Mortensen PB, Hovland M, Brattegard T, Farestveit R (1995) Deep-water bioherms of the scleractinian coral *Lophelia pertusa* (L) at 64° N on the Norwegian Shelf - Structure and associated megafauna. Sarsia 80(2): 145-158

Mortensen PB, Hovland MT, Fossa JH, Furevik DM (2001) Distribution, abundance and size of *Lophelia pertusa* coral reefs in mid-Norway in relation to seabed characteristics. J Mar Biol Ass UK 81:581-597

Muscatine L, McCloskey LR, Marian RE (1981) Estimating the daily contribution of carbon from zooxanthellae to coral animal respiration. Limnol Oceanogr 26: 601-611

Naumann M, Niggl W, Laforsch C, Glaser C, Wild C (submitted) Coral surface area quantification – evaluation of established methods by comparison with computer tomography. Limnol Oceanogr Methods

Peterson BJ, Fry B (1987) Stable isotopes in ecosystem studies. Ann Rev Ecol Syst 18: 293-320

Rapp HT, Sneli JA (1999) *Lophelia pertusa* (L.) - Myths and reality. 2nd Nord Mar Sci Meet, Hirtshals

Reitner J (2005) Calcifying extracellular mucus substances (EMS) of *Madrepora oculata* - a first geobiological approach. In: Freiwald A, Roberts JM (eds) Cold water corals and ecosystems. Springer-Verlag, Berlin Heidelberg, p 731-744

Roberts JM, Long D, Wilson JB, Mortensen PB, Gage JD (2003) The cold-water coral *Lophelia pertusa* (Scleractinia) and enigmatic seabed mounds along the north-east Atlantic margin: are they related? Mar Poll Bull 46:7-20

Roberts JM, Wheeler AJ, Freiwald A (2006) Reefs of the deep: The biology and geology of cold-water coral ecosystems. Science 312:543-547

Rogers AD (1999) The biology of *Lophelia pertusa* (Linnaeus 1758). Int Rev Ges Hydrobiol 84:315-406

Rublee P, Lasker H, Gottfried M, Roman M (1980) Production and bacterial colonization of mucus from the soft coral *Briarium asbestinum*. Bull Mar Sci 30:888-893

Schuhmacher H (1977) Ability of fungiid corals to overcome sedimentation Proceedings of the Third Int Coral Reef Symp, Miami, Florida, p 503-509

Thiem O, Ravagnan E, Fossa JH, Berntsen J (2006) Food supply mechanisms for cold water corals along a continental shelf edge. J Mar Syst 60:207-219

Tanaka Y, Miyajima T, Koike I, Hayashibara T, Ogawa H (2008) Production of dissolved and particulate organic matter by the reef-building corals *Porites cylindrica* and *Acropora pulchra*. Mar Poll Bull 82(2): 237-245(9)

van Duyl FC, Hegeman J, A. H, Maier C (2008) Dissolved carbon fixation by sponge-microbe consortia of deep water coral mounds in the northeastern Atlantic Ocean. Mar Ecol Prog Ser 358:137-150

van Soest RWM, Cleary D, de Kluijver MJ, Lavaleye MSS, Maier C, van Duyl FC (2007) Sponge diversity and community composition in Irish bathyal coral reefs. Contrib Zool 76:121-142

Weisz JB, Hentschel U, Lindquist N, Martens CS (2007) Linking abundance and diversity of sponge-associated microbial communities to metabolic differences in host sponges. Mar Biol 152:475-483

Wild C, Tollrian R, Huettel M (2004). Rapid recycling of coral mass spawning products in permeable reef sediments. Mar Ecol Prog Ser 271: 159-166

Wild C, Huettel M, Klueter A, Kremb SG, Rasheed M, Jørgensen BB (2004b) Coral mucus functions as an energy carrier and particle trap in the reef ecosystem. Nature 428:66-70

Wild C, Rasheed M, Werner U, Franke U, Johnstone R, M. H (2004c) Degradation and mineralization of coral mucus in reef environments. Mar Ecol Prog Ser 267:159-171

Wild C, Woyt H, Huettel M (2005) Influence of coral mucus release on nutrient fluxes in carbonate sands. Mar Ecol Prog Ser 287:87-98

Wild C, Jantzen C, Struck U. Hoegh-Guldberg O, Huettel M (2008) Biogeochemical responses following coral mass spawning on the Great Barrier Reef: pelagic-benthic coupling. Coral Reefs 27(1): 123-132

Winkler LW (1888) The determination of dissolved oxygen in water. Berichte der Deutschen Chemischen Gesellschaft 21: 2843-2857

11

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

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Abstract

Coral reef associated benthic algae may control important metabolic processes in reef ecosystems via organic matter release. Yet little information is available about its quantity and chemical composition. Therefore, dissolved organic carbon (DOC) and particulate organic carbon and nitrogen (POC, PN) release by dominant reef associated benthic algae (*Caulerpa serrulata*, *Peyssonnelia capensis*, turf algae) were quantified during 4 seasonal expeditions to the Northern Red Sea. Additionally, 6 seasonal or patchy growing algae species were included in these investigations, and organic matter release by *Caulerpa* was studied under different simulated water depths (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. Turf algae displayed highest and seasonal algae lowest organic matter release rates. Results indicate that organic matter release rates by benthic reef algae were 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 related to temperature and light availability within light intensities of 0 to 300 µmol quanta m^{-2} s^{-1}, suggesting photoinhibition as limiting factor for productivity and subsequent organic matter release. These data provide essential information for understanding the contribution of benthic algae to coral reef organic matter dynamics and relevant environmental factors.

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 detected (Pandolfi et al. 2003, Hoegh-Guldberg et al. 2007), there is only 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 biocatalytical active reef sands (Wild et al. 2005) and particularly, by the release of organic matter, functioning 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 & 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 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 involvement of algae-derived organic matter in habitat competition between benthic algae and hermatypic corals in the reef ecosystem is therefore suggested, only a small number of quantitative studies have been conducted regarding the 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) described only minimal proportions (0 – 6 %) of the photosynthetic products to be released as organic matter. This could be an underestimation as the released photosynthetic products may not have reached the isotopic equilibrium due to short time-ranges of 1 to 24 h (Wada et al. 2007). Higher proportions of photosynthetic products released as dissolved organic matter (2 – 62 %) have been described by studies using closed systems to directly measure the increase of DOC (Khailov & Burlakova 1969, Abdullah & Fredriksen 2004). High variations in the proportion of photosynthetic products released as organic matter, found in these studies, can potentially be ascribed to methodical differences and pronounced seasonal variations in the release rates of organic matter by benthic algae (Wada et al. 2007).

The present study, based on investigations in the Northern Red Sea, aims to give an overview of the quantity of organic matter released as DOC, particulate organic carbon (POC) and particulate organic nitrogen (PN), by the dominating representatives of benthic macro and turf algae. Studies

were conducted in a seasonal resolution to regard the variations in the productivity of macroalgae (Hatcher et al. 1977, Wada et al. 2007) and present the algal organic matter release rates in relation to variances in environmental key factors (water temperature, light availability, inorganic nutrient availability), therefore establishing a comprehensive overview of organic matter release by benthic algae in a fringing reef ecosystem of the Northern Red Sea.

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 regard those changes in environmental parameters, the studies were carried out in a seasonal resolution (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).

Environmental parameters

In order to investigate the influence of environmental parameters on organic matter release rates of the studied benthic algae, 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 in 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. unpublished) were used in order to identify the dominant benthic macroalgae and turf algae which coved between 19 and 22 % of the available substratum at the study site. Those were: the green algae *Caulerpa serrulata*, the brown algae *Peyssonnelia capensis* and different consortia of turf algae predominately composed of green algae of the genus *Cladophora*, red algae of the genus *Gelidium* and associated cyanobacteria assemblages. *Caulerpa*, *Peyssonnelia* and turf algae were tested in incubation experiments for organic matter release in a seasonal resolution. *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 clathrata*, *Liagora turneri*, *Hydroclathrus clathratus*) were also used for incubation

experiments to establish a comprehensive overview of algae-derived organic matter release. Algae species, used in this study, accounting 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 detaching dead *Acropora* fragments, serving as substratum for the algae consortium, with side cutting pliers. 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 & 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. 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.

To 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 simulate similar light intensities as at the collection place. 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^{TM} 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 standard, 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 was 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) light availability at 5 m water depth of 400 µmol quanta m^{-2} s^{-1} during August 2007 (summer) and a minimum of 216 µmol quanta m^{-2} s^{-1} in February 2008 (winter). Dependent upon water depth, light 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, $p = 0.019$), although subjected to 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 one order of magnitude higher than those of the seasonally blooming algae *Enteromorpha* during winter (1.7 ± 0.3 mg m^{-2} h^{-1}) and *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 between systematic 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, $p < 0.001$). Congruent 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 between the systematic groups. Also congruent to 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: $p = 0.002$), whereby all algae displayed highest release rates in fall.

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.*

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

PN release rates were variable between different species (one way ANOVA, $p < 0.001$) and seasons (one way ANOVA, $p < 0.001$) even showing PN uptake of particular specimen (*Caulerpa* at 1 m water depth light in winter). 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 difference between the single systematic 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, $p > 0.001$). Within the algae tested in a seasonal resolution, only

Caulerpa exhibited significant seasonal differences (one way ANOVA, $p < 0.009$) in POC/PN ratios with the highest proportion of carbon in spring (13.9 ± 1.2) and lowest in winter (5.9 ± 1.3).

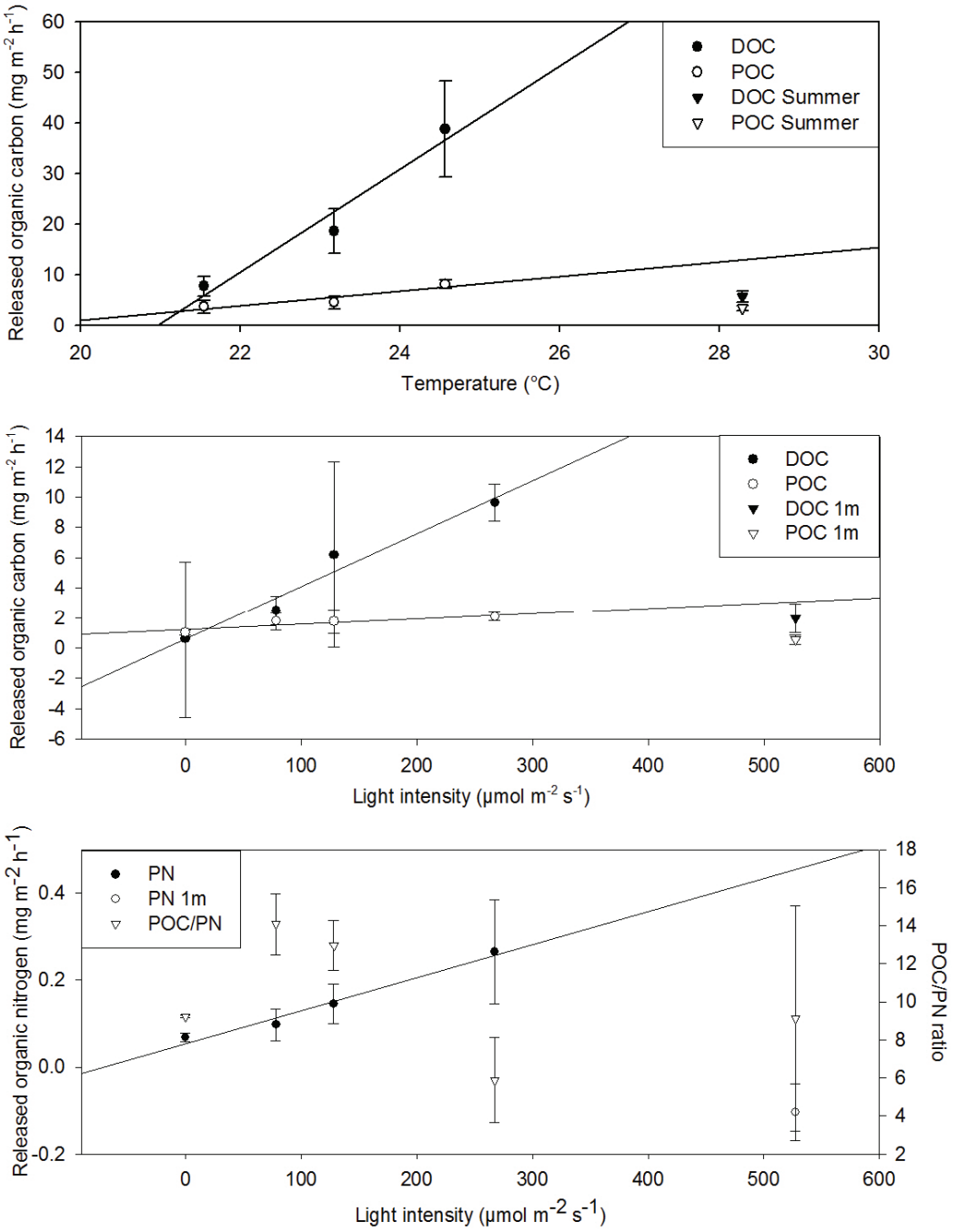

Figure 1 *a. Vertical resolution of organic carbon release (mean ± SE) for Caulerpa (1m, 5m, 10m, 20m), compared to the respective light intensity. b. Seasonal resolution of mean organic carbon release (mean ± SE) for the three most abundant algae in the study area (Caulerpa, Peyssonnelia, turf algae), compared to the respective temperature regime. 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.*

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 light 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 light 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.

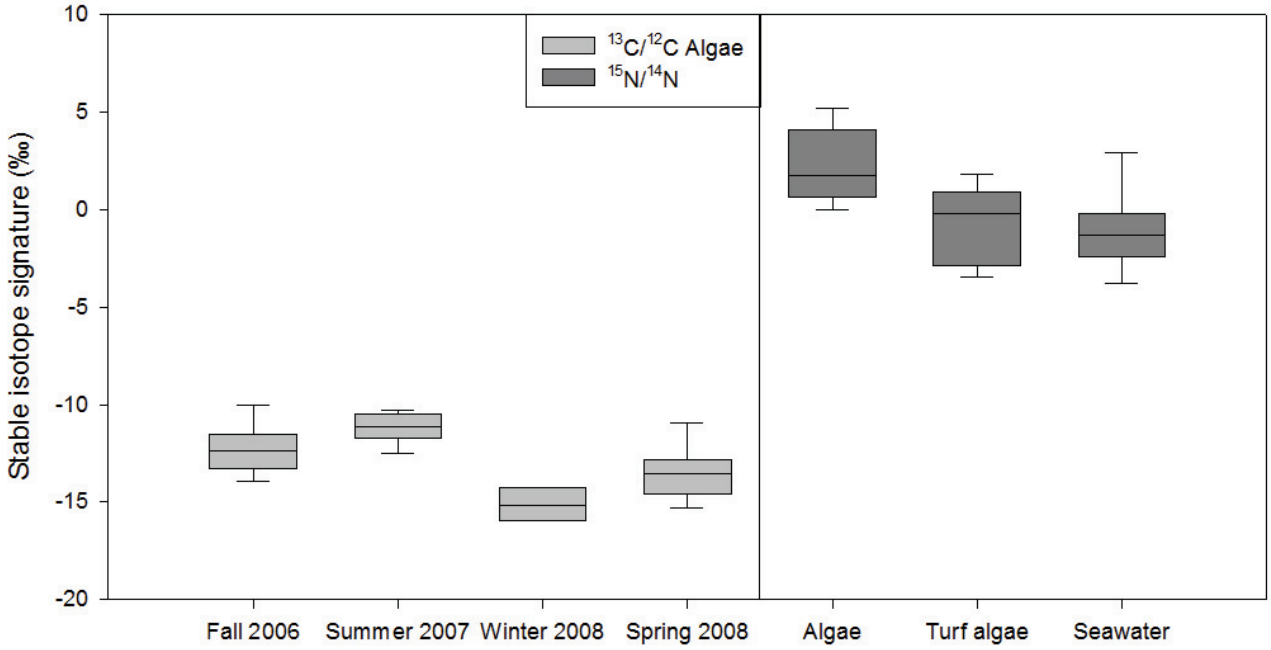

Figure 2 *Carbon stable isotope signature ($\delta^{13}C$ values) from algae-derived organic matter in a seasonal resolution and overall nitrogen stable isotope signature ($\delta^{15}N$ values) of algae, turf algae with associated cyanobacteria, and seawater controls.*

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).

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 dissolved organic carbon 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 as an important pathway of the photosynthetic products of algae (Wada et al. 2007). Although organic matter release by phytoplankton has been studied (Zlotnik & Dubinsky 1989, Baines & 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 & 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 chapter.

Species specific organic matter release by reef algae

The amount of all investigated algae-derived organic matter sources (DOC, POC, PN) varied between species regardless of environmental conditions. Although there were no differences found between the systematic groups of algae, the varying life strategies were reflected by the respective organic matter release rates. Whereas organic matter release by turf algae exceeded those of all other specimens, the comparatively 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 requires energy and resources which are then lost to 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 systematic groups of algae has already been described in studies by Littler & 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 & Arnold (1982) that the filamentous algae group displayed the highest productivity, are also reflected in algal organic matter release rates of the present study, as filamentous turf algae displayed the highest release rates of all measured parameters. 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 & 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 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 light 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 & Dubinsky (1989), who isolated the effects of light from other environmental factors in their experiments. Congruent to the present study, Zlotnik & 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 light 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 light 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 & 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 & 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 displayed in ^{13}C enriched $\delta^{13}C$ 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 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 & 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 & 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 turf algae associated cyanobacteria which use fixation of 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. submitted). 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).

Algae derived organic matter, predominately dissolving immediately in the ambient water, 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 & 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. As heterotrophic activity involves increased oxygen consumption, implications on oxygen dynamics and availability in the ecosystem may be a possible consequence. 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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References

Abdullah MI, Fredriksen S (2004) Production, respiration and exudation of dissolved organic matter by the kelp *Laminaria hyperborea* along the west coast of Norway. J Mar Biol Assoc UK 84: 887-894

Azam F, Smith DC, Steward GF, Hagström Å (1993) Bacteria-organic matter coupling and its significance for oceanic carbon cycling. Microb Ecol 28: 167-179

Baines SB, Pace ML (1991) The production of dissolved organic matter by phytoplankton and its importance to bacteria: Patterns across marine and freshwater systems. Limnol Oceanogr 36: 1078-1090

Belay A (1981) An experimental investigation of phytoplankton photosynthesis at lake surfaces. New Phyto 198: 61-74

Biddanda B, Benner R (1997) Carbon, nitrogen, and carbohydrate fluxes during the production of particulate and dissolved organic matter by marine phytoplankton. Limnol Oceanogr 42: 506-518

Biersmith A, Benner R (1998) Carbohydrates in phytoplankton and freshly produced dissolved organic matter. Mar Chem 63: 131-144
Brylinsky M (1977) Release of dissolved organic matter by some marine macrophytes. Mar Biol 39: 213-220
Critchley C (1981) Studies on the mechanisms of photoinhibition in higher plants. I. Effects of high light intensity on chloroplast activities in cucumber adapted to low light. Plant Physiol 67: 1161-1165
Dahl AL (1973) Surface area in ecological analysis: Quantification of benthic coral-reef algae. Mar Biol 23: 239-249
De-lin D, Pan F, Shuai L, Zhang J, Liu X, Fei X (1996) Identification of cytokinin in a green algae extract. Chin J Oceanol Limnol 14: 160-164
DeNiro MJ, Epstein S (1976) You are what you eat (plus a few ‰): The carbon isotope cycle in food chaines. Geol Soc Am Abstracts Program 8: 834-835
Done TJ (1992) Phase shifts in coral reef communities and their ecological significance. Hydrobiologia 247: 121-132
Dytham C (1999) Choosing and using statistics - a biologist´s guide, 2nd ed. Blackwell Publishing.
France LR (1995) Carbon-13 enrichment in benthic compared to planktonic algae: foodweb implications. Mar Ecol Prog Ser 124: 307-312
Fry B (2006) Stable isotope ecology. Springer New York.
Gillooly JF, Brown JH, West GB, Savage VM, Charnov EL (2001) Effects of size and temperature on metabolic rate. Science 293: 22-48
Grasshoff K, Ehrhardt M, Kremling K (1999) Methods of seawater analysis, 3rd ed. Verlag Chemie
Goericke R, Montoya JP, Fry B (1994) Physiology of isotope fractionation in algae and cyanobacteria. In: Lajtha K, Michener B (eds) Stable isotopes in ecology and environmental science. Blackwell
Häder DP, Porst M, Herrmann H, Schäfer J, Santas R (1997) Photosynthesis of mediterranean green alga *Caulerpa prolifera* measured in the field under solar irradiation. J Photoch Photobio B 37: 66-73
Hatcher BG, Chapman ARO, Mann KH (1977) An annual carbon budget for the kelp *Laminaria longicruris*. Mar Biol 44: 85-96
Hedges JI (2002) Why dissolved organic matter?. In: Hansell DA, Carlson CA (eds) Biogeochemistry of Marine Dissolved Organic Matter. Academic pr inc
Henley WJ, Lindley ST, Levavasseur G, Osmond CB, Ramus J (1992) Photosynthetic response of *Ulva rotundata* to light and temperature during emersion on an intertidal sand flat. Oecologia. 89: 516-523
Herndl GJ, Velimirov B (1986) Microheterotrophic utilization of mucus released by the Mediterranean coral *Cladocora cespitosa*. Mar Biol 90: 363-369
Hoegh-Guldberg O, Mumby PJ, Hooten AJ, Steneck RS, Greenfield P, Gomez E, Harvell CD, Sale PF, Edwards AJ, Caldeira K, Knowlton N, Eakin CM, Iglesias-Prieto R, Muthiga N, Bradbury RH, Dubi A, Hatziolos ME (2007) Coral Reefs Under Rapid Climate Change and Ocean Acidification. Science 318: 17-37
Jonas RB (1997) Bacteria, Dissolved Organics and Oxygen Consumption in Salinity Stratified Chesapeake Bay, an Anoxia Paradigm. Amer Zool 37: 612-620
Keeling CD (1958) The concentration and isotopic abundances of atmospheric carbon dioxide in rural areas. Geochim Cosmochim Ac 13: 322-334
Khailov KM, Burlakova ZP (1969) Release of dissolved organic matter by marine seaweeds and distribution of their total organic production to inshore communities. Limnol Oceanogr 14: 521-527
Kline DI, Kuntz NM, Breitbart M, Knowlton N, Rohwer F (2006) Role of elevated organic carbon levels and microbial activity in coral mortality. Mar Ecol Prog Ser 314: 119-125
Kok B, Gassner EB, Rurainski HJ (1965) Photoinhibition of chloroplast reactions. Photochem Photobiol 4: 215-227
Kübler JE, Davison IR (1993) High-temperature tolerance of photosynthesis in the red alga *Chondrus crispus*. Mar Biol 117: 327-335
Kuntz NM, Kline DI, Sandin SA, Rohwer F (2005) Pathologies and mortality rates caused by organic carbon and nutrient stressors in three Caribbean coral species. Mar Ecol Prog Ser 294: 173-180
Littler MM, Arnold KE (1982) Primary productivity of marine macroalgal functional-form groups from southwestern North America. J Phycol 18: 307-311
Littler MM, Littler DS (1992) Photosynthesis vs. irradiance curves for six species of macroalgae from the Seychelles Islands under four levels of nutrient enrichment. Atoll Res Bull 374: 1-14
Lotze HK, Schramm W, Schories D, Worm B (1999) Control of macroalgal blooms at early developmental stages: *Pilayella littoralis* versus *Enteromorpha spp*. Oecologia 119: 46-54
Lüning K (1990) Seaweeds: their environment, biogeography and ecophysiology. Wiley & Sons
Mann KH (1973) Seaweeds: Their productivity and strategy for growth. Science 182: 975-981
Mergner H, Svoboda A (1977) Productivity and seasonal changes in selected reef areas in the Gulf of Aqaba (Red Sea). Helgoländer wiss Meeresunters 30: 383-399
Muscatine L, Kaplan IR (1994) Resource partitioning by reef corals as determined from stable isotope composition. 2. $\delta^{15}N$ of zooxanthellae and animal tissue versus depth. Pac Sci 48: 304-312
Naumann MS, Niggl W, Laforsch C, Glaser C, Wild C (2009) Coral surface area quantification – evaluation of established methods by comparison with computer tomography. Coral Reefs 28: 109-117
Pandolfi JM, Bradbury RH, Sala E, Hughes TP, Bjorndal KA, Cooke RG, McArdle D, McClenachan L, Newman MJH,

Paredes G, Warner RR, Jackson JBC (2003) Global trajectories of the long-term decline of coral reef ecosystems. Science 301: 955-958

Pataki DE, Ehleringer JR, Flanagan LB, Yakir D, Bowling DR, Still CJ, Buchmann N, Kaplan JO, Berry JA (2003) The application and interpretation of Keeling plots in terrestrial carbon cycle research. Global Biogeochem Cy 17: 1022

Pregnall AM (1983) Release of dissolved organic carbon from the estuarine intertidal macroalga *Enteromorpha prolifera*. Mar Biol 73: 37-42

Rasheed M, Badran MI, Richter C, Huettel M (2002) Effect of reef framework and bottom sediment on nutrient enrichment in a coral reef of the Gulf of Aqaba, Red Sea. Mar Ecol Prog Ser 239: 277-285

Scheffers SR, de Goeij J, van Duyl FC, Bak RPM (2003) The cave-profiler: a simple tool to describe the 3-D structure of inaccessible coral reef cavities. Coral Reefs 22: 49-53

Schouten S, Klein Breteler WCM, Blokker P, Schogt N, Rupstra WIC, Grice K, Baas M, Sinninghe Damasté JS (1998) Biosynthetic effects on the stabile carbon isotopic compositions of algal lipids: Implications for deciphering the carbon isotopic biomarker record. Geochim Cosmochim Ac 62: 1397-1406

Sieburth JM (1969) Studies on algal substances in the sea. III. The production of extracellular organic matter by littoral marine algae. J Exp Mar Biol Ecol 3:290-309

Smith JE, Shaw M, Edwards RA, Obura D, Pantos O, Sala E, Sandin SA, Smriga S, Hatay M, Rohwer FL (2006) Indirect effects of algae on coral: algae-mediated, microbe-induced coral mortality. Ecol Lett 9: 835-845

Steinberg PD (1995) Seasonal variation in the relationship between growth rate and phlorotannin production in the kelp *Ecklonia radiata*. Oecologia 102: 169-173

Swart PK, Saied A, Lamb K (2005) Temporal and spatial variation in the $\delta^{15}N$ and $\delta^{13}C$ of coral tissue and zooxanthellae in *Montastrea faveolata* collected from the Florida reef tract. Limnol Oceangr 50: 1049-1058

Thomas JP (1971) Release of dissolved organic matter from natural populations of marine phytoplankton. Mar Biol 11: 311-323

Verity PG (1981) Effects of temperature, irradiance, and day length on the marine diatom *Leptocylindrus danicus* Qeve. II. Photosynthesis and cellular composition. J Exp Mar Biol Ecol 55: 79-91

Wada S, Aoki MN, Tsuchiya Y, Sato T, Shinagawa H, Hama T (2007) Quantitive and qualitative analyses of dissolved organic matter released from *Ecklonia cava* Kjellman, in Oura Bay, Shimoda, Izu Peninsula, Japan. J Exp Mar Biol Ecol 349: 344-358

Wild C, Huettel M, Klueter A, Kremb SG, Rasheed M, Jørgensen BB (2004) Coral mucus functions as an energy carrier and particle trap in the reef ecosystem. Nature 428: 66-70

Wild C, Rasheed M, Jantzen C, Cook P, Struck U, Boetius A (2005) Benthic metabolism and degradation of natural particulate organic matter in carbonate and silicate reef sands of the Northern Red Sea. Mar Ecol Prog Ser 298: 69-87

Wild C, Jantzen C, Struck U, Hoegh-Guldberg O, Huettel M (2008) Biogeochemical responses following coral mass spawning on the Great Barrier Reef: pelagic-benthic coupling. Coral Reefs 27: 123-132

Work TM, Aeby GS, Maragos JE (2008) Phase shift from a coral to a corallimorph-dominated reef associated with a shipwreck on Palmyra Atoll. PLoS ONE 3: e2989, doi:10.1371/journal.pone.0002989

Zlotnik I, Dubinsky Z (1989) The effect of light and temperature on DOC excretion by phytoplankton. Limnol Oceanogr 34: 831-839

12

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 (e.g. (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 flow-through 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 & 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 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, 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-dinitrophenylhydra-zone; Thermo Quest, Italy) and typically showed standard deviations < 3 %. Stable isotope ratios are given in the conventional delta notation ($\delta\ ^{13}C$, $\delta\ ^{15}N$) 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 & 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 algae-derived 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 ($\delta\ ^{13}C$: -10.1 ± 1.4 ‰) were significantly more positive ($p < 0.05$) than those of coral-derived POM ($\delta\ ^{13}C$: -18.3 ± 0.3 ‰). POM C stable isotope signatures were very similar to that of sterile coral mucus ($\delta\ ^{13}C$: -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.

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

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.

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.

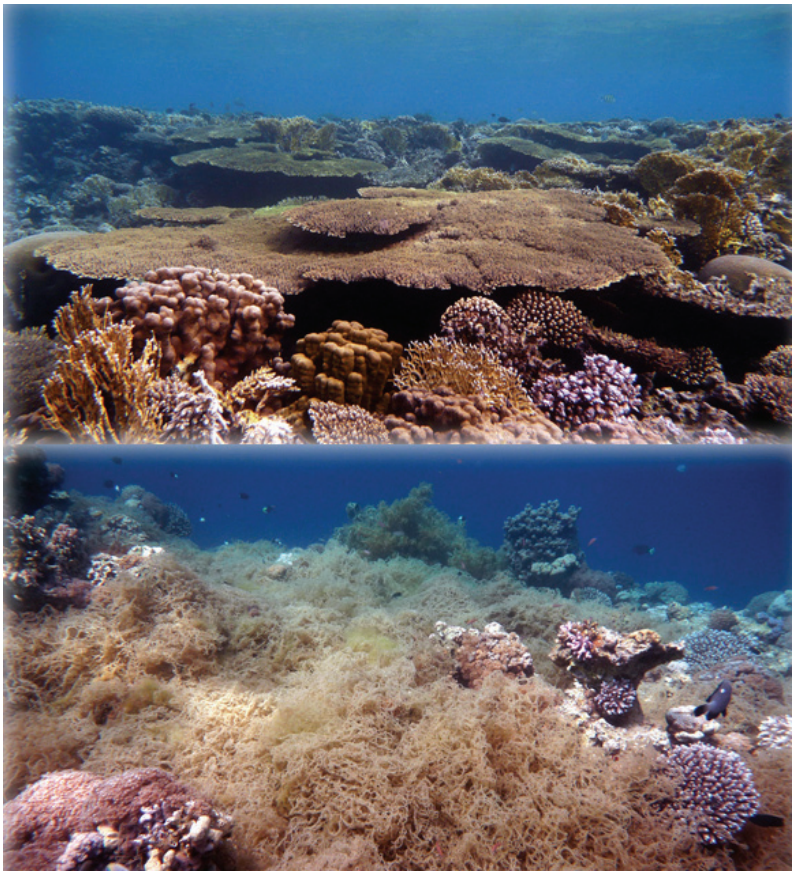

Fig. 1 *Coral (upper panel) versus benthic algae dominated (lower panel) fringing reef areas in front of MSS, Aqaba, Jordan, photographed during spring expedition 2008.*

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 (e.g. Rasheed et al. 2002). A higher availability of inorganic matter may have resulted in increased algae growth rates and associated high synthesis 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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References

Buchsbaum R, Valiela I, Swain T, Dzierzeski M, Allen S (1991) Available and refractory nitrogen in detritus of coastal vascular plants and macroalgae. Mar Ecol Prog Ser 73:131-143

Coplen TB (1995) Reporting of stable hydrogen, carbon, and oxygen isotopic abundances (Technical Report). Geothermics 24:707-712

Craig H (1957) Isotopic standards for carbon and oxygen and correction factors for mass-spectrometric analysis of carbon dioxide. Geochim et Cosmochim Acta 12:133-149

Dinsdale EA, Pantos O, Smriga S, Edwards RA, Angly F, Wegley L, Hatay M, Hall D, Brown E, Haynes M, Krause L, Sala E, Sandin SA, Vega Thurber R, Willis BL, Azam F, Knowlton N, Rohwer FL (2008) Microbial ecology of four coral atolls in the Northern Line Islands. PLoS ONE 3:1-17

Fry B (2006) Stable isotope ecology, Springer, New York

Herndl GJ, Velimirov B (1986) Microheterotrophic utilization of mucus released by the Mediterranean coral *Cladocora cespitosa*. Mar Biol 90:363-369

Hoegh-Guldberg O (1999) Climate change, coral bleaching and the future of the world's coral reefs. Mar Freshw Res 50:839-866

Hoegh-Guldberg O, Mumby PJ, Hooten AJ, Steneck RS, Greenfield P, Gomez E, Harvell CD, Sale PF, Edwards AJ, Caldeira K, Knowlton N, Eakin CM, Iglesias-Prieto R, Muthiga N, Bradbury RH, Dubi A, Hatziolos ME (2007) Coral reefs under rapid climate change and ocean acidification. Science 318:1737-1742

Huettel M, Gust G (1992) Solute release mechanisms from confined sediment cores in stirred benthic chambers and flume flows. Mar Ecol Prog Ser 82:187-197

Hughes T, Rodrigues M, Bellwood D, Ceccarelli D, Hoegh-Guldberg O, McCook L, Moltschaniwskyj N, Pratchett M, Steneck R, Willis B (2007) Phase shifts, herbivory, and the resilience of coral reefs to climate change Current Biology 17:360-365

Hughes TP, Baird AH, Bellwood DR, Card M, Connolly SR, Folke C, Hoegh-Guldberg O, Jackson JBC, Kleypas J, Lough JM, Marshall P, Nyström M, Palumbi SR, Pandolfi JM, Rosen B, Roughgarden J (2003) Climate change, human impacts, and the resilience of coral reefs. Science 301:929-933

Kline D, Kuntz NM, Breitbart M, Knowlton N, Rohwer FL (2006) Role of elevated organic carbon levels and microbial acitivity in coral mortality. Mar Ecol Prog Ser 314:119-125

Kristensen E (1994) Decomposition of macroalgae, vascular plants and sediment detritus in seawater: Use of stepwise thermogravimetry. Biogeochemistry 26:1-24

Kuntz NM, Kline DI, Sandin SA, Rohwer FL (2005) Pathologies and mortality rates caused by organic carbon and nutrient stressors in three Caribbean coral species. Mar Ecol Prog Ser 294:181-188

Mariotti A (1984) Atmospheric nitrogen is a reliable standard for natural 15N abundance measurements. Nature 303:685-687

Naumann MS, Niggl W, Laforsch C, Glaser C, Wild C (2009) Coral surface area quantification – evaluation of established methods by comparison with computer tomography. Corals Reefs 28:109-117

Pandolfi JM, Jackson JBC, Baron N, Bradbury RH, Guzman HM, Hughes TP, Kappel CV, Micheli F, Ogden JC, Possingham HP, Sala E (2005) Are U.S. coral reefs on the slippery slope to slime. Science 307:1725-1726

Rasheed M, Badran MI, Richter C, Huettel M (2002) Effect of reef framework and bottom sediment on nutrient enrichment in a coral reef of the Gulf of Aqaba, Red Sea. Mar Ecol Prog Ser 239:277-285

Smith JE, Shaw M, Edwards RA, Obura D, Pantos O, Sala E, Sandin SA, Smriga S, Hatay M, Rohwer FL (2006) Indirect effects of algae on coral: algae-mediated, microbe-induced coral mortality. Ecol Let 9:835-845

Wild C, Huettel M, Klueter A, Kremb SG, Rasheed M, Jørgensen BB (2004a) Coral mucus functions as an energy carrier and particle trap in the reef ecosystem. Nature 428:66-70

Wild C, Jantzen C, Struck U, Hoegh-Guldberg O, Huettel M (2008) Biogeochemical responses on coral mass spawning at the Great Barrier Reef: Pelagic-benthic coupling. Corals Reefs 27:123-132

Wild C, Laforsch C, Huettel M (2006) Detection and enumeration of microbial cells in highly porous carbonate reef sands. Mar Freshw Res 57:415-420

Wild C, Rasheed M, Jantzen C, Cook P, Struck U, Huettel M, Boetius A (2005a) Benthic metabolism and degradation of natural particulate organic matter in silicate and carbonate sands of the Northern Red Sea. Mar Ecol Prog Ser 298:69-78

Wild C, Rasheed M, Werner U, Franke U, Johnstone R, M. H (2004b) Degradation and mineralization of coral mucus in reef environments. Mar Ecol Prog Ser 267:159-171

Wild C, Woyt H, Huettel M (2005b) Influence of coral mucus release on nutrient fluxes in carbonate sands. Mar Ecol Prog Ser 287:87-98

Winkler LW (1888) The determination of dissolved oxygen in water. Ber Deutsch Chem Ges 21:2843-2857

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