# Organic matter release by Red Sea cnidarians and its function as energy carrier – effects of environmental variables


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**Wolfgang Niggl**
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# Thesis Abstract

Release of organic matter into the ambient seawater is a phenomenon described for a variety of marine plants, algae and animals and is particularly important for the oligotrophic coral reef ecosystems. Present coral reefs are affected by changing environmental conditions caused by both local and global anthropogenic impacts. However, investigations on potential influence of environmental factors on the release of organic matter by coral reef organisms are rare. In addition, the Red Sea represents a largely under-investigated area of the world ocean. The main objective of the present PhD thesis therefore was to investigate the impacts of environmental factors on organic matter release by dominant Northern Red Sea coral reef cnidarians and to trace the pathway of released organic matter as a trophic vector in a series of interconnected field and laboratory studies.

The reasons for extracellular release of organic matter are manifold, but include active disposal of excess carbon, passive permeation through the cell membrane, protection, feeding or locomotion. Once released to the water column, the organic matter can fulfil important roles for coral reef ecosystem functioning. The adhesive, insoluble organic compounds released by corals efficiently trap particles from the water column increasing their initial organic carbon and nitrogen content by orders of magnitude before they rapidly settle. Coral-derived particulate organic matter (POM) thereby supports benthic life and reduces loss of energy and nutrients from the reef ecosystem. Additionally, organic exudates are directly taken up by a variety of reef organisms thus serve as a source of food and thereby initiate metabolic communication. While the particulate fraction of released organic matter can be consumed directly by e.g. fishes, crabs or worms, the dissolved fraction is primarily accessible for microbes, but become accessible for higher trophic levels via the microbial loop.

Due to its importance for reef ecosystem functioning, organic matter release by scleractinian corals has been quantified recently by several studies. Most of these studies thereby chose the corals surface area as reference parameter. Hence, the accuracy of the applied surface area quantification is crucial to guarantee reliable results. The complex and delicate 3-D structures of many coral species pose a big challenge and require sophisticated approaches. Given, that coral tissue is only a thin layer covering the coral skeleton in scleractinian 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 which is based on surface area to mass correlation. An alternative approach for estimating the surface area is to coat corals by dipping them into liquids such as dye or melted paraffin wax and subsequently correlating the amount of the adhering liquid to the surface of the coral skeleton. However, techniques described above are all harmful or destructive, thus generally not recommendable and additionally not applicable for long-term studies requiring repeated measurements. Non-destructive techniques such as 2D planar projection of 3D structures or simplifying 3D

structures to geometric forms have also been applied but depending on the colonies growth form may rather represent inaccurate approximations.

For these reasons, as part of this thesis, a novel, non-invasive and completely harmless technique was developed to quantify the coral skeleton's surface area at a yet nonpareil accuracy (see **Chapter 1**). Conventional high-resolution medical computed tomography (CT) was used to scan living coral colonies. The resulting 'X-ray slices' were than processed using 3D modelling software, providing realistic 3D coral skeleton surface reconstructions enabling high accuracy surface area measurements. This technique however is restricted to the availability of CT, thus usually not applicable in the field.

Therefore, in a second study the surface area estimates obtained from the state-of-the-art CT method were compared to those obtained by application of four establish surface area quantification methods (see **Chapter 2**). Using CT as reference, approximation factors could be given in order to correct surface area estimates obtained from less accurate established techniques. This allows transformation of previous studies facilitating standardised comparison of surface related results.

On the one hand accurate determination of reference parameters is important to estimate in-situ organic matter release. On the other hand, investigating the abundance of organic matter releasing species is inevitable to evaluate their relevance for the ambient reef ecosystem. Therefore, two studies investigating the abundance and distribution patterns of dominant benthic reef-associated organisms were conducted in the Northern Red Sea (see **Chapter 3** and **Chapter 4**). In **Chapter 3**, the benthic community composition around the urbanized area of Dahab, Egypt, and potential impacts of relevant bottom-up and top-down controlling factors were examined. Besides the abundance and distribution of corals and benthic algae, the abundance of reef fish as well as the number of coral-algae interactions was assessed. Thereby, a comprehensive, highly resolved baseline dataset and methodological template was provided proofing scleractinian corals to be the dominant benthic organisms in investigated area and revealing a potentially human activity-induced bias of the benthic community composition.

In **Chapter 4,** distribution patterns and abundance of the upside-down jellyfish *Cassiopea* sp., a prominent invasive member of the phylum cnidaria usually associated with mangrove habitats, but also known to occur in coral reefs, was investigated. Observed distribution patterns in the Northern Red Sea close to the city of Aqaba represent the first results helping to illuminate the unexplored life cycle of *Cassiopea* sp. in coral reef environments. In the investigated study area, *Cassiopea* sp. represented one of the key organisms within the benthic community reaching high abundances of up to 31 animals $m^{-2}$ and a benthic cover of more than 20 %, thereby acting even as the dominant benthic organism at some locations. Although organic matter release by *Cassiopea* has been described before, no quantitative data was available.

Therefore, in **Chapter 5** the release of dissolved and particulate organic matter by *Cassiopea* sp. was quantified under in-situ conditions. The findings revealed substantial jellyfish POM

release exceeding release rates reported for scleractinian corals by factors of 2 to 15. As known from coral-derived organic matter, also jellyfish-derived organic matter could provide a source of energy for planktonic organisms. Particularly, the mysid *Idiomysis tsurnamali*, hovering in large swarms above the jellyfish and withdrawing between the jellyfish's tentacles in case of danger, could take up and degrade jellyfish-derived organic matter. Consequently, tracer experiments using stable isotopes were conducted and revealed uptake and fast mineralization of *Cassiopea*-derived organic matter by planktonic microbes and by the jellyfish commensal *Idiomysis tsurnamali*. These findings suggest that *Cassiopea*-derived organic matter may function as a newly discovered trophic pathway for organic matter from the benthic environment to pelagic food chains in coral reefs and other marine ecosystems.

The chemical composition, rather then the quantity, of released organic matter determines its nutritional value for consumers. However, related information is rare. In **Chapter 6** therefore the carbohydrate composition of organic matter released by dominant and cosmopolitan warm- and cold-water coral genera was analyzed and compared with each other. While compositional genus-specific differences occurred among the warm-water corals, no significant differences in the released organic matter carbohydrate composition between warm- and cold-water corals were detectable. The heterogeneous mixture of labile monosaccharides found in both warm- and cold-water coral-derived organic matter explains the observed stimulation of both planktonic and benthic microbial activity, thus supports previous studies. Thriving in complete darkness, cold-water corals lack the zooxanthellae-derived monosaccharide arabinose. This indicates the influence of environmental factors on the composition of organic matter released by corals. Given that up to 45 % of the photosynthetically fixed carbon is released to the ambient seawater by the coral host, factors influencing the photosynthetic performance of the zooxanthellae likely do also affect the quantity of released organic matter.

Temperature-induced coral bleaching, a phenomenon describing the extensive expulsion of zooxanthellae from the host, obviously affects the photosynthetic performance of the coral holobiont. A potential impact on organic matter release therefore seems likely and was investigated in **Chapter 7**. By exposing corals of the genus *Acropora* to elevated temperatures, a bleaching event was induced in the laboratory leading to elevated zooxanthellae exposure on the one hand, but on the other hand also to elevated coral-derived particulate organic carbon and nitrogen release, mainly originating from coral mucus. This study thus revealed an effect of temperature on organic matter release and indicates the release of coral mucoid exudates to be a general response to environmental stress, including coral bleaching.

Another key environmental factor, occurring during most times in coral reefs, is water current. While very strong water currents may also pose stress for corals, usually corals benefit from water currents. Generally, water current-induced mass exchange and transport processes proceed by orders of magnitude faster than the exchange via molecular diffusion. Consequently, water current is known to affect many coral physiological processes.

Additionally, water current impacts corals mechanically; an influence on organic matter release thus seems highly probable and has been discussed previously. However, all previous quantifications of coral organic matter release were carried out under still water conditions, neglecting potential impacts of water current. Therefore, in **Chapter 8** the effects of exposure to different water currents on coral organic matter release were investigated. While no effect of current velocity on dissolved organic carbon (DOC) release was detectable, POM release by all three investigated coral species was significantly increased compared to still water conditions. The detected immediate effect of ambient water currents suggests that POM release by corals was obviously mainly controlled by hydro-mechanical impacts, while DOC fluxes were influenced more by the coral's physiological condition. These findings indicate that previous POM release quantification results were rather conservative and likely underestimated actual in situ POM release. The ecological role of coral-derived POM may therefore be even more important than already assumed.

Both, the quantity and quality of released organic matter are influenced by environmental factors and vary between organisms. Hence, the organic matter released in different qualities and quantities by the various organisms likely is subject to varying microbial degradation rates. A comprehensive study comparing organic matter release by the dominant benthic reef-associated organisms from the Northern Red Sea and subsequent effects on microbial activity as well as in-situ $O_2$ availability is presented in **Chapter 9**. Results revealed that algae-derived organic matter, presumably its DOC fraction, stimulated microbial activity in the adjacent water significantly more than organic matter released by the investigated scleractinian and fire corals. Consequently, the daily mean and minimum in situ $O_2$ concentrations in the water directly above the reef (≤ 10 cm) were significantly higher at coral- dominated than at algae-dominated sites.

These findings were supported by the study presented in **Chapter 10** in which similar effects of the benthic community composition on in situ $O_2$ availability were found. Minimum $O_2$ concentrations were significantly negatively, while diurnal variability in $O_2$ concentration was significantly positively correlated with increasing benthic cover by algae. In contrast, no correlation with coral cover was found. These results indicate that shifts from corals to benthic algae, as reported from many reef locations world-wide, may likely affect both in situ $O_2$ availability and variability. Further, both studies of **Chapter 9** and **Chapter 10** confirm in-situ relevance of results of previous laboratory studies, which investigated the stimulation of microbial activity and subsequent $O_2$ depletion via the release of organic matter by various reef organisms.

The present PhD thesis links laboratory data on organic matter release by dominant cnidaria of the Northern Red Sea under varying environmental factors to actual in situ abundances and consequences to in situ $O_2$ dynamics. It thereby proves that the released organic matter acts as an important trophic vector in the oligotrophic coral reef ecosystem and that it affects in situ $O_2$ availability via the stimulation of microbial activity. The thesis provides another

component towards the understanding of the complex cycles of organic matter in coral reefs and how benthic coral reef organisms are able to influence their surrounding via the release of organic matter. It thus helps to better predict the effects of future environmental changes on the coral reef community.

# 1

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

**Christian Laforsch[1], Eva Christoph[2], Christian Glaser[2], Malik Naumann[3], Christian Wild[3] and Wolfgang Niggl[3]**

[1]Department Biology II and GeoBio-Center, Ludwig-Maximilians-Universität München, Großhadernerstraße 2, 82152 Martinsried, Germany

[2]Department for Clinical Radiology, University Hospital of Munich, Ludwig-Maximilians-Universität München, Marchioninistraße 15, 81377 München, Germany

[3]Coral Reef Ecology Work Group (CORE), GeoBio-Center and Department of Earth and Environmental Science, Ludwig-Maximilians-Universität München, Richard-Wagner-Str.10, 80333 München, Germany

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REPORT

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

**C. Laforsch · E. Christoph · C. Glaser · M. Naumann · C. Wild · W. Niggl**

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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 are 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 datasets 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 sample *t*-test: all n.s.). Data analysis of a single coral colony required approximately 15 to 30 min 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.

**Keywords** Scleractinian corals · Surface area determination · X-ray computed tomography · Surface rendering

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C. Laforsch (✉)
Department Biology II and GeoBio-Center, Ludwig-Maximilians-University Munich, Großhadernerstraße 2, 82152 Martinsried, Germany
e-mail: laforsch@bio.lmu.de

E. Christoph · C. Glaser
Department for Clinical Radiology, University Hospital of Munich, Ludwig-Maximilians-University Munich, Marchioninistraße 15, 81377 Munich, Germany

M. Naumann · C. Wild · W. Niggl
Coral Reef Ecology Work Group (CORE), GeoBio-Center and Department of Earth and Environmental Science, Ludwig-Maximilians-University Munich, Richard-Wagner-Str. 10, 80333 Munich, Germany

## 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 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, and thus are 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 two-dimensional planar projection derived from photographs to assess the surface area of coral colonies. However, planar projections of three-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 to calculate 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 dataset 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 the 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 three-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 artefacts caused by the container. Aquaria made of glass can cause "starburst" artefacts, which occur when scanning materials of 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 × 30.01 × 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

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

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 a virtually isotropic resolution of 0.400 × 0.400 × 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 × 512 pixels. Hounsfield Units (HU; standard computed tomography units), which correspond 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 datasets (DICOM format files) were transferred to a personal computer (Fujitsu Siemens Celsius, 3 GB 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 dataset was edited by the "Crop Editor" tool to reduce the entire dataset to a volume containing the voxels of a single object, being either a coral colony or a calibration body. Reduction of the dataset 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.

#### *"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 gray 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 artefacts in the reconstruction process due to increased background noise. Raising the threshold value subtracts voxels from the material of

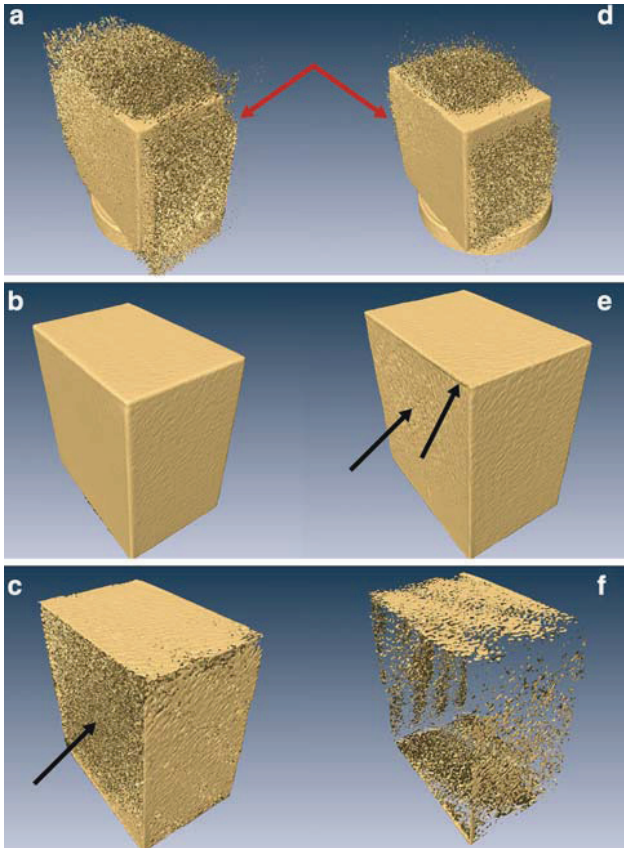


**Fig. 1** Isosurfaces of the calibration body made of Laas marble generated with six different threshold values corresponding to Hounsfield Units ((**a**) −800; (**b**) −900; (**c**) +350; (**d**) +2,000; (**e**) +2,500; (**f**) +2,700)). Setting a lower threshold value adds voxels to the object (red arrows). Raising the threshold value subtracts voxels from the material (black arrows) leading to visible degradation of the reconstructed calibration body

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 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. In order 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.

*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 dataset 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).

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 that would cause artefacts 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 gray 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. In order 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.

The "Segmentation editor" provided by Amira 4.1 was easily applicable to remove distracting artefacts. 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 regions (coral skeleton) were subtracted (exterior–interior). In the "options field" of the "LabelVoxel" module,

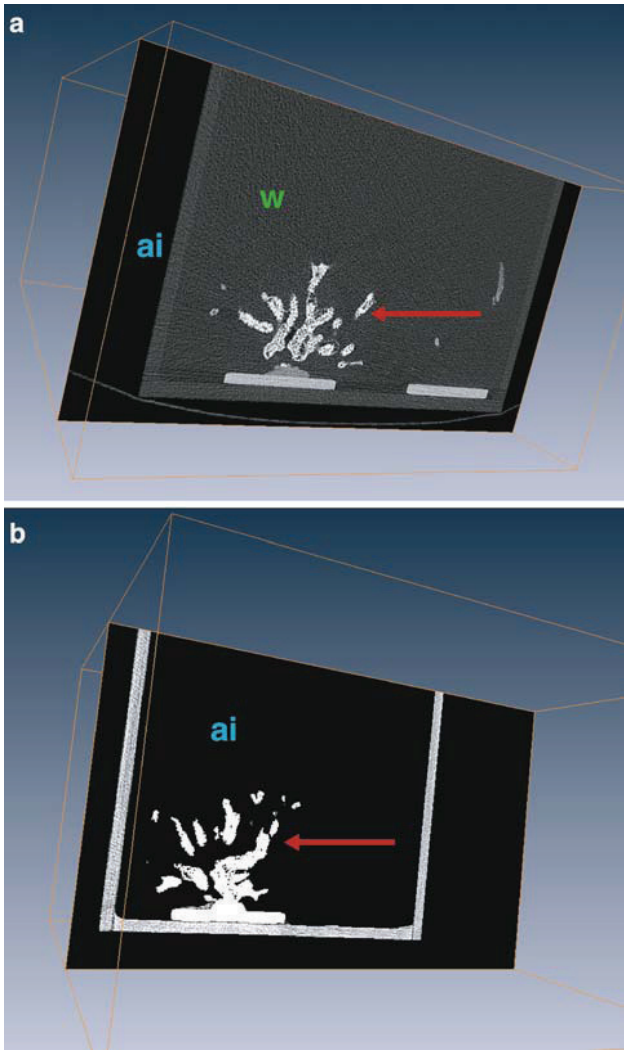


**Fig. 2** (**a**) Orthoslice of a coral colony (red arrow) scanned in sea water (w). (**b**) Orthoslice of a coral colony (red arrow) scanned in air (ai). The level of background noise is increased in the scan conducted in sea water

"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 strongly 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.

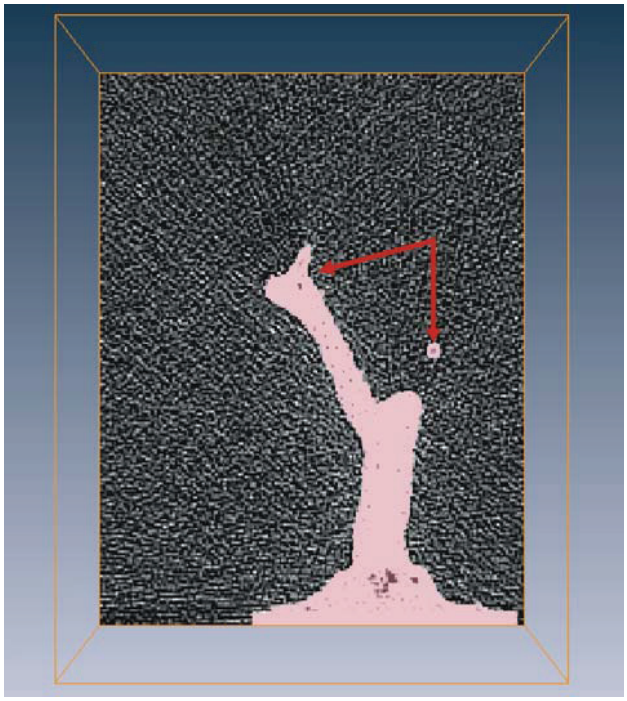

**Fig. 3** Image segmentation of a coral colony scanned in sea water. A single Orthoslice is divided into different sub-regions: coral colony (light red and red arrows) vs. background

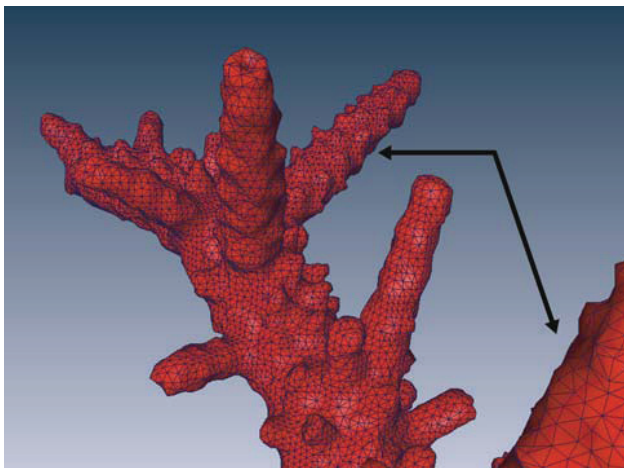

**Fig. 4** Triangulated surface (black arrows) of a coral colony created by the segmentation process

## 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 "Segmentation" methods. 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 sample *t*-tests ($P < 0.05$; SPSS for Windows). The results for the threshold value determination and for 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. 5a). 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. 5b). 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 grayscale 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).

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

Suface area of the 3D models of the same coral colony computed with both methods (−350 HU) 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).

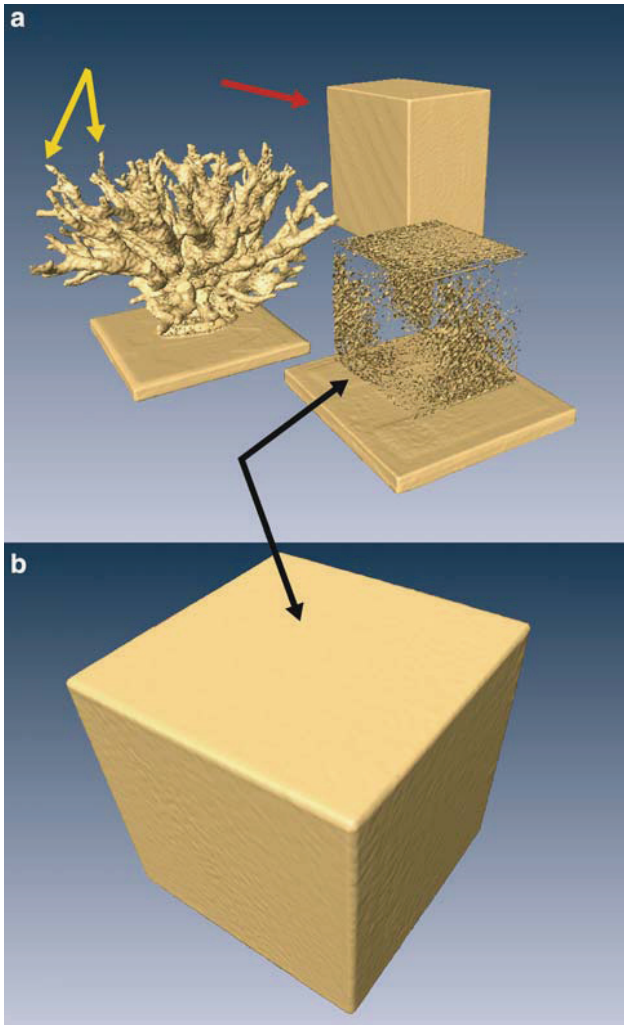


**Fig. 5** (**a**) Isosurface of both calibration bodies created at a threshold value of +1,000 HU. The silhouette of the calibration body made of Laas marble (red arrow) was distinctly visible, whereas surface area of the calibration body made of PVC (black arrow) showed a distinct degradation of the 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

**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 (bold) 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** |

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

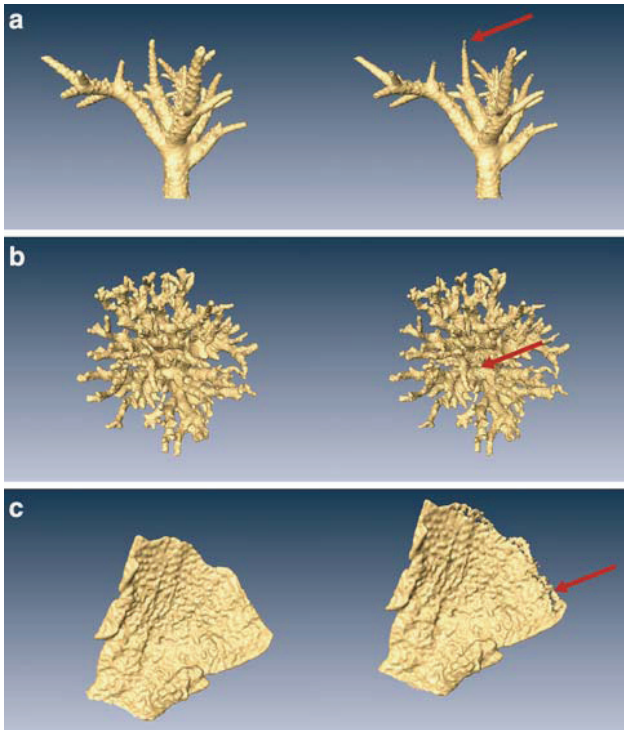


**Fig. 6** Comparison of isosurfaces of three coral genera ((**a**) *Acropora*; (**b**) *Pocillopora*, (**c**) *Montipora*)) created with both best-fit threshold values (left: −350 HU; right: +350 HU). Artefacts (red arrows) are clearly visible at a threshold value of +350 HU

**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"

| Threshold (HU)/method | Surface area ($cm^2$) | | |
|---|---|---|---|
| | *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 |

Deviation of both thresholds and methods are shown in percent

colony showed no significant difference between all test persons in all coral genera and in both methods (independent sample *t*-tests: Person 1 vs. Person 2; Person 2 vs. Person 3; Person 1 vs. Person 3, all n.s.; df = 4; Fig. 8).

## Discussion

This study introduces an accurate and novel approach to quantify surface areas of coral colonies using X-ray

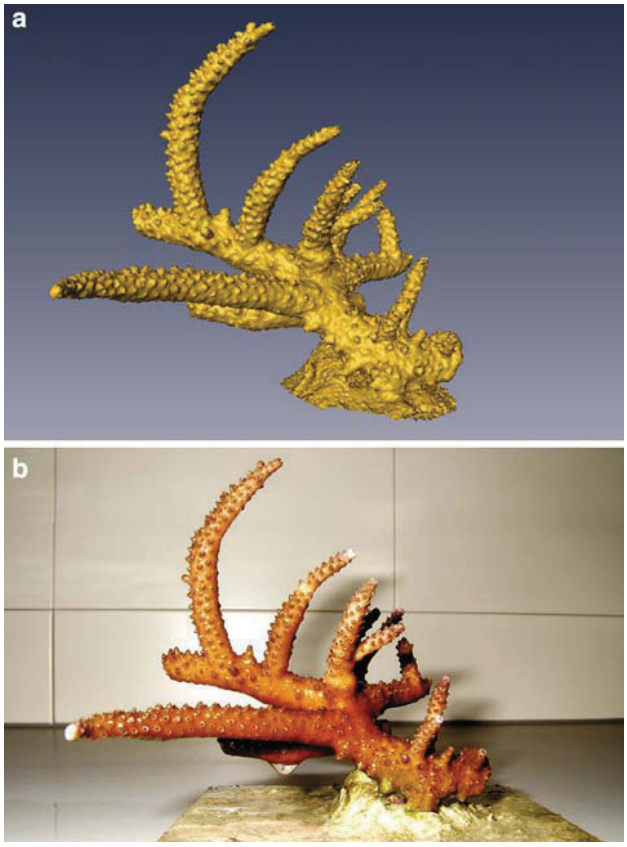


**Fig. 7** Visual comparison of the computed surface model using the segmentation method at a threshold value of −350 HU with the shape of the respective living colony shows distinctly the accuracy of the reconstructed surface

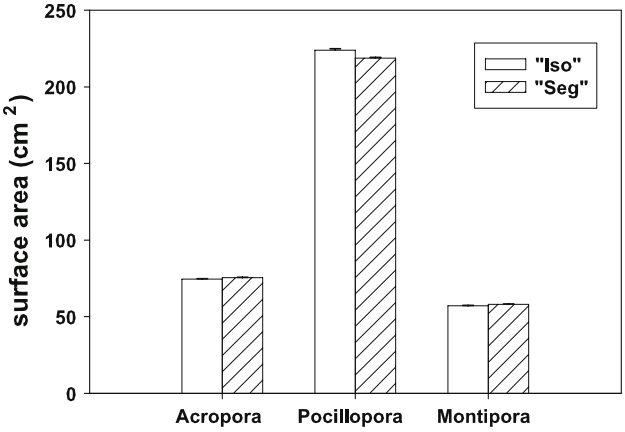


**Fig. 8** Bar chart of repeated surface area measurements conducted by three different persons. The bars indicate the mean surface area and the respective standard error of both surface rendering techniques ("Isosurface" white; "Segmentation" coarse lines) of the same coral colonies

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 min for a trained user applying the isosurface method, thus highlighting the rapid processing time as a further advantage of this method.

In studies primarily 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 that 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).

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.

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 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). 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 favored over scanning in water (Fig. 2). 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 artefacts 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 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 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 favorable 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 labeled 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 favored 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 (Figs. 4 and 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 modelling may be potentially utilized 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).

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# 2

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

**Malik S. Naumann[1], Wolfgang Niggl[1], Christian Laforsch[2], Christian Glaser[3], and Christian Wild[1]**

[1]Coral Reef Ecology Work Group (CORE), GeoBio-Center & Department of Earth and Environmental Science, Ludwig-Maximilians-Universität München, Richard-Wagner-Strasse 10, 80333 München, Germany

[2]Department Biology II and GeoBio-Center, Ludwig-Maximilians-Universität München, Großhadernerstrasse 2, 82152 Martinsried, Germany

[3]Department for Clinical Radiology, University Hospital of Munich, Ludwig-Maximilians-Universität München, Marchioninistrasse 15, 81377 München, Germany

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REPORT

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

**M. S. Naumann · W. Niggl · C. Laforsch · C. Glaser · C. Wild**

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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 with 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 genera-wide low accuracy (12–36%). Comparison of area values derived from established techniques and CT additionally yielded approximation factors (AFs) applicable as factors in the mathematical improvement of surface area estimates by established techniques in relation to CT reference accuracy.

**Keywords** Coral · Surface area · Methods · Evaluation · Computer tomography

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M. S. Naumann (✉) · W. Niggl · C. Wild
Coral Reef Ecology Work Group (CORE), GeoBio-Center and Department of Earth and Environmental Science, Ludwig-Maximilians-University Munich, Richard-Wagner-Strasse 10, 80333 Munich, Germany
e-mail: m.naumann@lrz.uni-muenchen.de
URL: http://www.palmuc.de/core

C. Laforsch
Department Biology II and GeoBio-Center, Ludwig-Maximilians-University Munich, Großhadernerstrasse 2, 82152 Martinsried, Germany

C. Glaser
Department for Clinical Radiology, University Hospital of Munich, Ludwig-Maximilians-University Munich, Marchioninistrasse 15, 81377 Munich, Germany

## 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 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 digitisation 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 after 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.

This study aimed to evaluate the accuracy of surface area estimates derived from the 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 (AFS) 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 six genera and four 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 with 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 the 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 Fig. 2.

## Geometric approximations

Geometric measurements of coral colonies were divided into two approach categories, Simple Geometry and

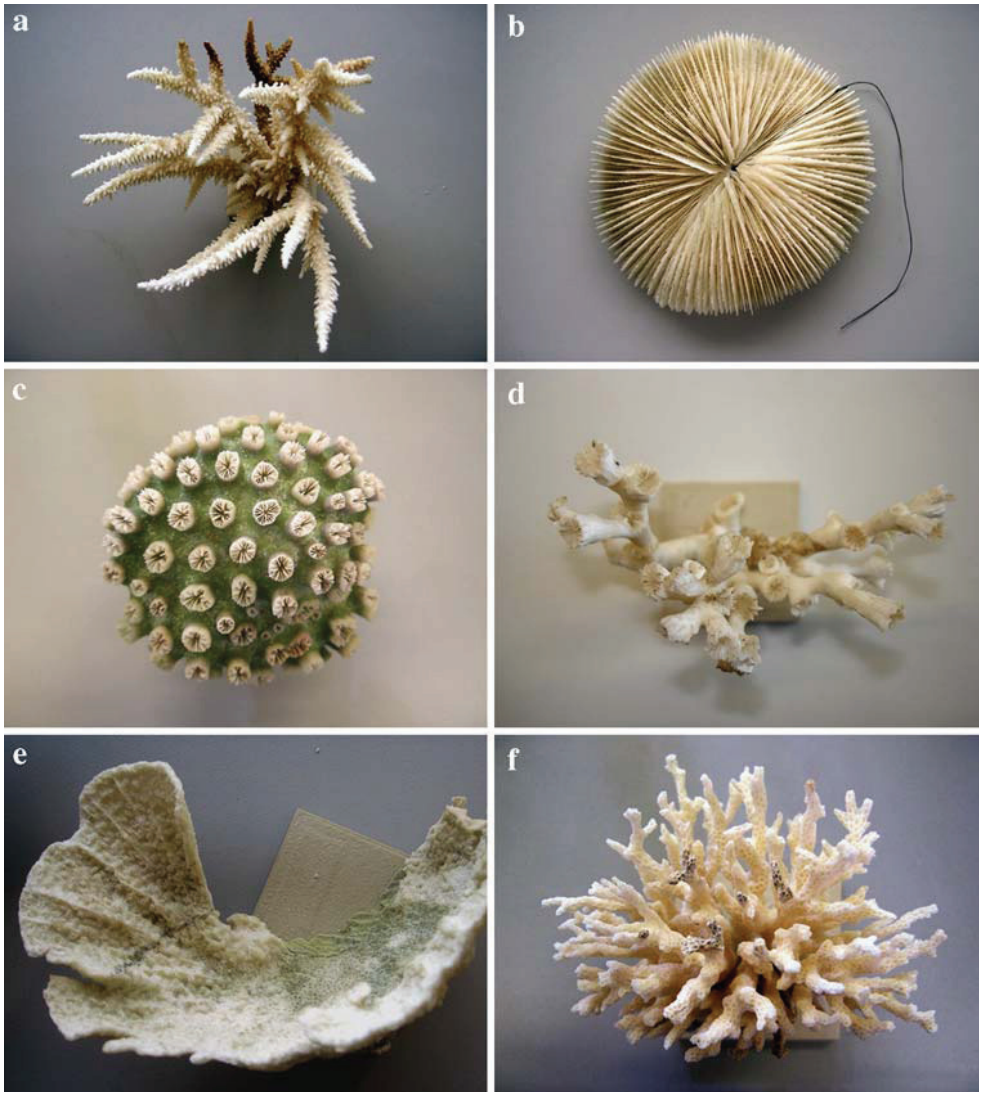


**Fig. 1** Top view of specimens assessed in coral surface area measurements. Panels **a**, *Acropora* spp.; **b**, *Fungia* spp. with attached thread used for Wax Coating; **c**, *Galaxea fascicularis*; **d**, *Lophelia pertusa*; **e**, *Montipora* sp. and **f**, *Pocillopora damicornis*

Advanced Geometry, defined as follows: Simple Geometry assesses the whole structure of a coral colony at once and assigns a geometric body that shows the 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 the approaches, measured parameters of all the 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 the 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.

### *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 four 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.

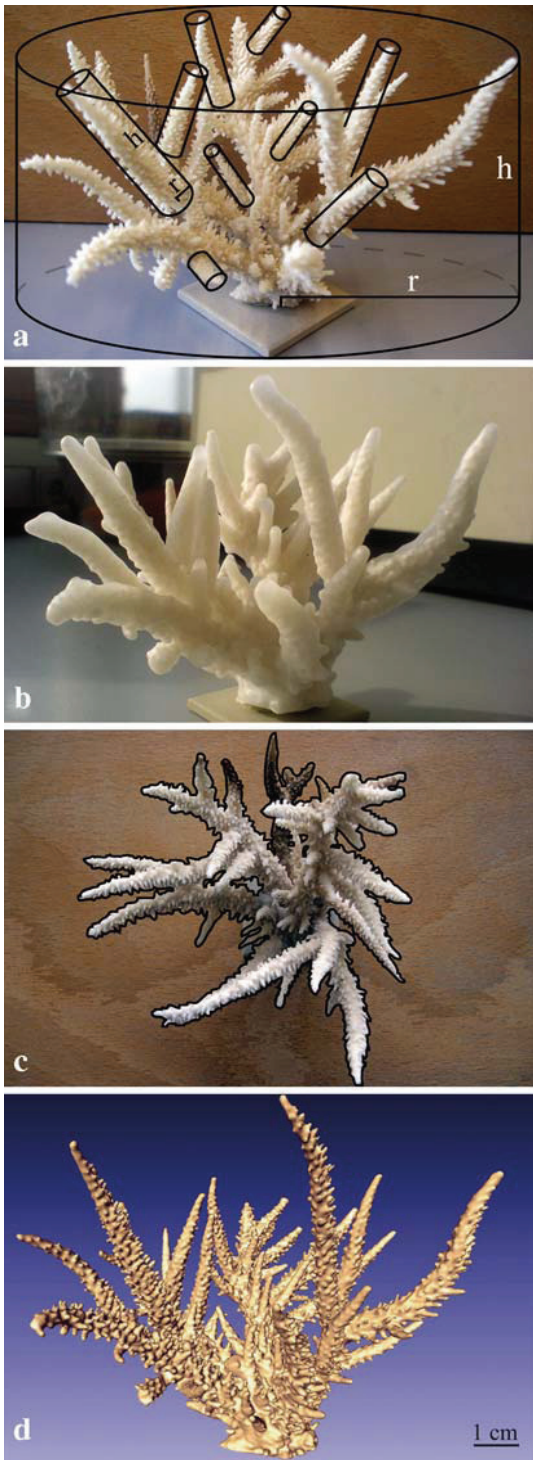


**Fig. 2** Techniques for coral surface area quantification applied in this study. (Panels **a–d**, *Acropora* coral specimen #10); Panel **a**, Simple Geometry and Advanced Geometry; **b**, Wax Coating; **c**, Planar Projection Photography; **d**, 3-D surface reconstruction of computer tomography-derived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

**Table 1** Area equations for geometric shapes and forms used in geometric approximation calculations (Simple and Advanced Geometry)

| 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 = Area, $\pi$ = pi, h = height, r = radius; [a] Where x and y are side lengths of a rectangle; [b] Where m and n are side lengths of a triangle

*Disc-like growth form*

A *Fungia* polyp resembles a disc, which is composed of two 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 four 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.

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 the 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 (four metal cubes and four 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. 1.37 m, 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 the three selected colonies representing the largest, smallest, and average size classes from three growth forms, resulted in an error of $0.3 \pm 0.3\%$. The methodological error determined by repeated digitising of one fragment was 0.4%, which results in a negligible total error of <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:

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

From these results, the genus-specific average percentage accuracy was computed for all the 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

Surface area values for all coral specimens derived from 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 AFs were subsequently calculated from ratios of all the 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 established techniques analysed 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 accurately with branching *P. damicornis* and foliose *Montipora* sp. corals, while Advanced Geometry showed the 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

**Table 2** Accuracy of established techniques in comparison to CT reference

| 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 | $n = 11$** |

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

**Table 3** Surface area values for *Acropora* specimen #10 obtained by different techniques

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

Abbreviations: SG = Simple Geometry; AG = Advanced Geometry; WA = Wax Coating; PP = Planar Projection Photography; CT = Computer Tomography and 3-D surface reconstruction

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%). AFs 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).

## 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 studies have used 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). CT 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 with 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

**Table 4** Approximation factors (AF) for conversion of surface area values derived by established techniques in relation to CT accuracy

| 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 |

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

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 advantages of non-invasive application with living coral specimens, which are 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, the 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) that were 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 study represents the first comparison between a variety of established techniques and the contemporarily the 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. Owing 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 this 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 in this study, 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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# 3

# Benthic community composition, coral-algal contact and fish distribution in coral reefs around the urbanized area of Dahab, Northern Red Sea

**Wolfgang Niggl[1], Andreas F. Haas[1], Christoph Haacke[1], Katrin Korczyk[1], Christopher Williamson[1], Annamarie Gabrenya[1], Vanessa N. Bednarz[1] and Chrisitan Wild[1]**

[1]Coral Reef Ecology Group (CORE), GeoBio-Center & Department of Earth and Environmental Science in the Faculty of Geosciences, Ludwig-Maximilians Universität, Richard Wagner Str. 10, 80333 München, Germany

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# Benthic community composition, coral-algal contact and fish distribution in coral reefs around the urbanized area of Dahab, Northern Red Sea

**W. Niggl • A. F. Haas • C. Haacke • K. Korczyk • C. Williamson • A. Gabrenya • V. Bednarz • C. Wild**

**Abstract.**
Shifts in benthic coral reef community composition from coral to algae dominance have frequently been associated with anthropogenic activities. In areas with strong coastal development, regular monitoring is an important tool to assess human-induced changes and enables to take appropriate measures for sustainable reef management. The South Sinai region in the Red Sea represents an under-investigated area with relatively intact corals reefs that are affected by intense coastal development and ensuing tourism activities. This emphasizes the need to establish baseline data and methodology for future monitoring. Therefore, in the present study, 8 different shallow-water reef locations around the rapidly developing urbanized area of Dahab, Egypt were surveyed in high spatial resolution targeting benthic community composition, coral-algal contact zones, abundance of reef fish, availability of inorganic nutrients and local waste water management strategy as key bottom-up and top-down factors. This revealed mean living coral cover of 44 ± 4 % (32 % scleractinian corals, 10 % soft corals and 2 % fire corals) and 8 ± 1 % cover by benthic macroalgae. About 11 % of all scleractinian corals were in direct contact with benthic algae (thereof 88 % with turf algae), wherefrom 26 % suffered algae overgrowth and 14 % showed signs of damage. The abundance of reef fish positively correlated with scleractinian coral cover, but not with algae cover and averaged at 88 ± 9 individuals 250 $m^{-2}$. Survey locations south of Dahab exhibited a significantly lower scleractinian coral and higher algae cover than the northern locations, indicating a potential impact by the urbanized area as the main current direction in the Gulf of Aqaba is from north to south. Future reef monitoring in the Dahab area is needed for which the present study provides a comprehensive baseline dataset and methodological template.

**Keywords:** Coral Reef, survey, coral cover, benthic community composition, reef fish, coral-algal contact, coastal development

**W. Niggl • A. F. Haas • C. Haacke • K. Korczyk • C. Williamson • A. Gabrenya • V. Bednarz • C. Wild**
Coral Reef Ecology Group (CORE), GeoBio-Center & Department of Earth and Environmental Science, Ludwig-Maximilians-Universität Munich, Richard Wagner Str. 10, 80333 München, Germany

## INTRODUCTION

Coral reefs undergo serious changes world-wide (see Wilkinson, 2008). Across the entire Caribbean basin, the average scleractinian coral cover decreased from about 50 % to 10 % cover within only three decades since the late 1970s (Côté *et al.*, 2005, Gardner *et al.*, 2003). In contrast, within the same time period from 1977 to 2001, absolute cover of macroalgae increased from 2 % to 10 % in Caribbean coral reefs (Côté *et al.*, 2005). For the Indo-Pacific region, the results of Bruno and Selig (2007) suggested an average coral cover decline from 43 % during the early 1980s to 22 % in 2003. Even the Great Barrier Reef, considered as the "best-managed reef in the world" (Pandolfi *et al.*, 2005), experienced a "system-wide decline" in living coral cover (Bellwood *et al.*, 2004).
Also in the Red Sea and the Gulf of Aden where reefs still appear generally healthy (Wilkinson, 2008), there is some evidence of local coral reef deterioration (Rinkevich, 2005, Wilkinson, 2008). Regular monitoring will be needed to assess such changes. The present study aims to contribute in this context.
The underlying causes of world-wide reef decline have frequently been questioned. Generally, they can be distinguished into global and regional causes. Global causes such as climate change are responsible for extensive damages in coral reefs (Hoegh-Guldberg

et al. 2007). Temperature-induced coral bleaching events have resulted in significant losses of live coral cover in many parts of the world (Berkelmans *et al.*, 2004, Brown, 1997, Hoegh-Guldberg, 1999). Ongoing ocean acidification poses another major risk for the world's coral reefs likely leading to structural changes and decline (Hoegh-Guldberg *et al.*, 2007). Besides human-induced impacts on a global scale, anthropogenic activity can negatively affect reef ecosystem functioning regionally by altering the general framework conditions or the top-down (e.g. the abundance of predators and herbivores) and bottom-up (e.g. the availability of inorganic nutrients) controlling factors. Increased sedimentation caused by dredging, logging, runoff, extensive tourism, reef exploitation by over-harvesting, nutrient enrichment and pollution thus can synergistically cause degradation of coral reefs (Davenport and Davenport, 2006, Hodgson, 1996, Hughes, 1994, Rogers, 1990, Sebens, 1994, Szmant, 2002). In this context, Mora (2008) found that human activities left a clear foot print in abundances of fishes, live coral and macroalgae benthic cover on Caribbean reef systems. For the Central Pacific area, the results of Sandin et al. (2008) indicate human-activity-induced changes in benthic reef community structure and a decline in live coral cover. But often, the ultimate causes for reef degradation are unresolved, and results from different studies are controversial.

In the Northern Red Sea, Tilot et al. (2008) found no general relationship between the level of tourist activity and coral cover. In contrast, Hasler and Ott (2008) reported a significant negative influence of diving tourism on coral cover within the same area. In another study reviewing published literature about a Northern Sinai coral reef of Eilat, Israel, Rinkevich (2005) pointed out that despite this reef being one of the most studied reefs world-wide, the knowledge of the causes of reef deterioration is sparse. However, he concluded that the tourist industry probably was the major cause for reef decline. In the Gulf of Aqaba, tourism is a rapidly expanding industry. Between 1989 and 2006, the number of hotels increased from 5 to 141, and the number of rooms from 565 to more than 48000 (Wilkinson, 2008). In the Dahab area (South Sinai, Egypt), the increasing number of tourists entails a rapid coastal development resulting in an estimated 3.5-fold increase of guest rooms within the time period from 2003 to 2017 (SEAM, 2004). This rapid increase in tourism will likely be accompanied by increasing impacts on adjacent reefs as in most cases the number of people will correlate with the intensity of their activities (Mora, 2008). Gradual decline of living coral cover, a key measure of reef habitat quality (Bruno and Selig, 2007), seems likely without appropriate counteracting measures such as effective waste water treatment and management of the local coral reef resources.

This emphasizes the need to assess the benthic community composition in order to detect changes, and also to assess the potentially relevant bottom-up and top-down factors in order to be able to take appropriate measures. However, comprehensive datasets are not available. Additionally, detailed comparison between previous studies is difficult due to the non-unified methodologies used.

Therefore, the present study aims to provide a comprehensive baseline dataset for future monitoring focusing on benthic community structure and key bottom-up and top-down factors as well as their impacts on the benthic community structure.

In the present study, 8 different reefs within and around the urbanized city-area of Dahab (South Sinai, Egypt) were therefore investigated focusing on live coral and macroalgal benthic cover as primary indicators for reef degradation. Competition between hard corals and algae as a key ecological process, especially during reef degradation (Jompa and McCook, 2003), was also recorded and assessed as the number of coral-algal contact zones and their character (occurrence of coral damage). Additionally, abundance of reef fishes, especially herbivorous species, as the key top-down factor and concentrations of inorganic nutrients as the key bottom-up factor were recorded. Statistical analysis was applied to investigate potential correlation between assessed parameters.

# METHODS

## Study Site

The study was conducted in the Gulf of Aqaba in the Northern Red Sea in March 2010 (Fig. 1). All survey sites were located within or near the city area of Dahab, Egypt between the coordinates 28° 26' 5" N / 34° 27' 26" E and 28° 34' 17" N / 34° 32' 12" (Table 1).

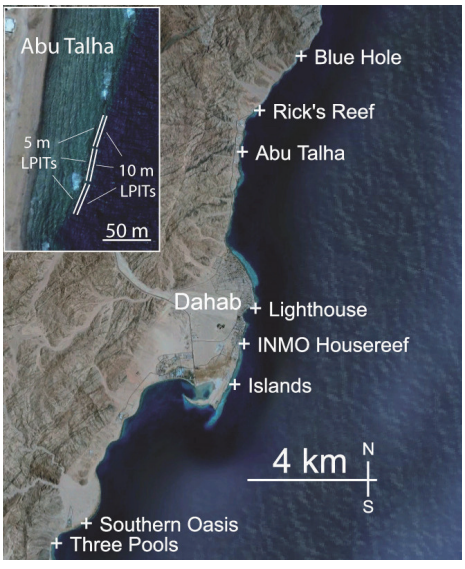


Fig. 1: Areal picture of the 8 survey sites located within or near the city area of Dahab (large picture) and schematic illustration of the arrangement of conducted line point intercept transects, exemplary for the site Abu Talha (small picture).

Table 1: Coordinates of the 8 survey sites, date of survey and distance to survey locations 'Blue Hole' and 'Lighthouse'. Abbreviations used are given in brackets.

| Location | Date of survey | Coordinates | Distance to BH (km) | Distance to LH (km) |
|---|---|---|---|---|
| Blue Hole (BH) | 16.03.2010 | 28° 34' 17" N / 34° 32' 12" E | 0 | 8.21 |
| Rick's Reef (RR) | 15.03.2010 | 28° 33' 19" N / 34° 31' 22" E | 2.25 | 6.32 |
| Abu Talha (AT) | 15.03.2010 | 28° 32' 37" N / 34° 31' 01" E | 3.74 | 4.99 |
| Lighthouse (LH) | 17.03.2010 | 28° 29' 55" N / 34° 31' 15" E | 9.02 | 0 |
| INMO House Reef (IN) | 12.03.2010 | 28° 29' 19" N / 34° 30' 59" E | 10.59 | 1.19 |
| Islands (IS) | 14.03.2010 | 28° 28' 38" N / 34° 30' 50" E | 11.88 | 2.46 |
| Southern Oasis (SO) | 13.03.2010 | 28° 26' 23" N / 34° 27' 58" E | 21.49 | 8.35 |
| Three Pools (TP) | 13.03.2010 | 28° 26' 5" N / 34° 27' 26" E | 22.57 | 9.34 |

### Benthic Surveys

The benthic community composition and substrate type was assessed at 8 different survey sites (Table 1) using the linear point intercept transect (LPIT) method after Nadon and Stirling (2006). Using SCUBA, at each survey site, triplicate 50 m LPITs were conducted at 5 and 10 m water depth (Fig. 1) resulting in a total number of 48 LPITs. For each transect, a 50 m measuring tape was unrolled on the seafloor at the respective water depth, and the substrate type was recorded every 50 cm directly beneath the transect line resulting in 101 data points per transect and 4848 data points in total. The living substrate was categorized as 'scleractinian coral', 'fire coral' (hydrozoan coral), 'soft coral' or 'fleshy alga' (macroalgae). Stable biogenic rock never occurred completely uncovered, but was either overgrown by turf algae or by crustaceous coralline red algae (CCA). These were thus recorded as either 'turf algae' or 'CCA'. The category 'turf algae' therefore also included stable substrate with only sparse overgrowth by small filamentous algae. Unstable biogenic substrate was subdivided into 'coral rubble' (unconsolidated dead coral fragments) and 'carbonate sands'. All other substrate types were summarized as 'others' and included e.g. other animals or seagrass. Whenever possible, scleractinian corals and fleshy algae were identified down to the genus level.
Three persons conducted the benthic surveys. Prior to surveys, trial transects and subsequent comparison as well as matching of data was performed in order to guarantee consistency of data collection between different surveyors.

### Coral-algal contacts

Every recorded scleractinian coral colony was investigated for potential contact with algae. Thereby, only contact zones visible from vertically above the colony were recorded. The type of algae involved (macroalgae, turf algae or CCA) and the type of contact (algal overgrowth, coral damage, equilibrium, bulge formation by the coral) were recorded. 'Algal overgrowth' described overgrowth with or without any visible responding changes in the coral colony. In case of changes in the coral pigmentation, tissue damages or a "dead zone" (white coral skeleton without overgrowth, very recently died) the interaction was categorized as 'coral damage' when no overgrowth of the coral was visible. 'Equilibrium' described a co-existence without visible signs of overgrowth or coral damage. 'Bulge formation' described the development of a bulge towards the adjoining algae, potentially functioning as a physical barrier to restrain the competitor (see also Haas *et al.*, 2010).

### Fish surveys

Fish abundance surveys were conducted using the 'coral reef fish visual census' methodology of English et al. (1997). Prior to the conduction of surveys, fish identification tests were performed in order to guarantee consistency of data. A team of 4 divers conducted transects, with 1 diver (the same diver) of each buddy pair recording fish abundance during each transect. All surveys took place between 10am and 3pm. At each location (n = 8) and water depth (5 and 10 m), 2 of the 3 benthic survey transects (see above) were used for fish abundance surveys. Before beginning the visual surveys, divers withdrew from the transect area for a minimum of 5 min to allow fishes to resume normal behaviour. For each transect, the observer swam slowly (approximately 1 min per 5 metres) along the transect, recording fish encountered within 2.5 metres on either side of the 50 m transect, therefore providing a total census area of 500 $m^2$ per water depth. In addition, fish were surveyed in the area 5 m directly above each transect. Categories of recorded fishes were based on the Reef Check Manuel 2004 indicator fish categories for the Red Sea, with a few alterations. The following fish categories were utilised by survey teams; Surgeon (Acanthuridae); Wrasse (Labridae) with individual categories for Broomtail wrasse (*Cheilinus lunulatus*) and Humphead wrasse (*Cheilinus undulates*); Parrot (Scaridae); Damsel (Pomacentridae), only including visible species, e.g. Sulphur damsel (*Pomacentrus sulfureus*) and White belly damsel (*Amblyglyphidodon leucogaster*); Butterfly (Chaetodontidae); Angel (Pomacanthidae); Grouper (Serranidae); Sweetlip (Plectorhinchinae), Snapper (Lutjanidae); Lion (Scorpaenidae); Rabbit

(Siganidae); and Moray eel (Muraenidae). These categories represent important herbivores, i.e. surgeon and parrot fish, on reefs and / or important indicators of reef degradation, e.g. overfishing. For statistical analysis, surgeon, parrot and rabbit fish were grouped together as 'Herbivores' whilst 'Total Fish' denoted all surveyed groups.

### Measurement of inorganic nutrient concentrations

The concentration of the inorganic nutrients ammonium and phosphate of surface waters was analysed at 5 different locations within the city area of Dahab starting at 'Lighthouse' and collecting with regular intervals, ending at the end of the Dahab promenade. An additional water sample was taken at the southern location 'Southern Oasis'. Triplicate water samples (20 ml) were collected from 50 cm beneath the water surface and 5 m away from the shoreline within a 2h time period on a calm day with minimal wind and no waves. Water samples were instantly stored in a cooler at 4 °C and then filtered through a syringe filter (VWR®, polyethersulfone membrane, 0.2 µm pore size) no longer than 60 min after sampling. Immediately after filtration, inorganic nutrient analysis was conducted using a Genesys 10 UV spectro-photometer and photometric ammonium and phosphate test kits (Spectroquant, Merck®). The lower detection limit of the photometric ammonium test as specified in the test kit's manual was 0.76 µM. For the phosphate test, linear regression revealed reliable measurement of phosphate concentrations to as low as 0.05 µM ($R^2 = 0.99$, n = 6).

### Effect of Dahab

The coastline distance of the survey site to the city centre of Dahab was used as an easy accessible proxy indicating potential anthropogenic influence due to the urbanized area, under the hypothesis that a reef located further from the city centre supposedly has fewer anthropogenic influences than reefs located closer to the city centre. The survey site 'Lighthouse' was located centrally in Dahab (Fig. 1) and therefore was set as the reference zero point for distance measurement of the other survey sites. Additionally, as the main current in the Gulf of Aqaba is from north to south (Berman *et al.*, 2000), effects induced by the city-area of Dahab could be north-south biased. In order to assess a proxy for potential North to South changes, the northern-most survey site 'Blue hole' was chosen as zero point for coastline distance measurements. For statistical analysis, all survey sites north of 'Lighthouse' were grouped together as 'Northern locations' (Abu Talha, Rick's Reef and Blue Hole), all sites south of 'Lighthouse' as 'Southern locations' (INMO house reef, Islands, Three Pools, Southern Oasis). Distance measurements were conducted using the geographic information system Google Earth (Google Inc.).

### Statistical analyses

Data recorded from 5 m and 10 m water depth were analysed separately. Differences between 5 and 10 m water depth were investigated applying paired t-tests using mean values from triplicate LPITs (duplicate transects for fish census). Where assumptions for a paired t-test were not met, the Wilcoxon signed rank test was used instead. Correlations between parameters were tested using the Pearson product-moment correlation. Where multiple comparisons were carried out, the p-value was adjusted respectively using the Bonferroni-correction. Differences in benthic coverage by the respective groups between the survey sites were analysed using analysis of variance (ANOVA) and the non-parametric Kruskal-Wallis test where assumptions of ANOVA were not met. Differences in scleractinian coral and macroalgae benthic coverage between 'Northern' and 'Southern locations' were investigated applying independent samples t-tests.
Data was tested for normal distribution using the Kolmogorov-Smirnov test and for homogeneity of variances using the Levene-test.

## RESULTS

### Benthic community composition

The benthic community in the surveyed area was dominated by scleractinian corals exhibiting a mean benthic coverage of 32 ± 2 % (mean ± SE, n = 48), followed by biogenic rock covered with turf algae (17 ± 1 %) or CCAs (11 ± 2 %). Soft corals (almost exclusively *Xeniidae*) and fire corals covered 10 ± 1 % and 2 ± 1 % of the seafloor, respectively. Fleshy macroalgae exhibited a mean benthic coverage of 8 ± 1 % (Table 2). There was a significant ($p < 0.05$, paired t-test) decrease in mean benthic coverage by scleractinian corals (- 19 %), fire corals (- 81 %), macroalgae (- 23 %) and CCA (- 60 %) from 5 to 10 m water depth. In contrast, mean benthic coverage by

Table 2: Benthic coverage by dominant groups of organisms (mean % ± SE, n = 48 LPITs). Asterisks indicate significant differences between 5 and 10 m water depth.

| Water depth | Scleractinian corals | Soft corals | Fire corals | Macroalgae | Turf | CCA |
|---|---|---|---|---|---|---|
| 5 m | 35 ± 3 | 8 ± 1 | 4 ± 1 | 9 ± 1 | 15 ± 2 | 16 ± 2 |
| 10 m | 29 ± 3 | 12 ± 2 | 1 ± 0 | 7 ± 1 | 19 ± 2 | 6 ± 1 |
| **mean** | 32 ± 2*[(a)] | 10 ± 1 | 2 ± 1*[(b)] | 8 ± 1*[(a)] | 17 ± 1 | 11 ± 2*[(b)] |

a. paired t-test
b. Wilcoxon signed ranks test

soft corals (+ 45 %, p = 0.080, paired t-test) and turf-covered biogenic rock (+ 24 %, p = 0.055, paired t-test) increased, albeit not significantly.

Among scleractinian corals, branching growth forms dominated and contributed to 52 ± 2 % with no significant change with increasing water depth (52 ± 3 % at 5 m water depth vs. 53 ± 2 % at 10 m water depth). Massive growth forms accounted for 38 ± 2 % (40 ± 3 % at 5 m vs. 36 ± 2 % at 10 m) of all scleractinian corals. *Acropora* was the most abundant coral genus accounting for 35 ± 3 % of all scleractinian corals (37 ± 3 % at 5 m water depth, 33 ± 2 % at 10 m water depth). Within the group of fleshy macroalgae, *Peyssonnelia* was by far the most abundant genus accounting for 94 ± 2 % of all macroalgae.

At 5 m water depth, the benthic cover by scleractinian, soft and fire corals differed significantly between the sites ($p < 0.05$, Kruskal-Wallis test), while there was no difference in cover by macroalgae, turf algae and CCA between the sites. The same applied for 10 m water depth, where scleractinian,and soft corals (both $p < 0.01$, one-way ANOVA) as well as fire corals ($p < 0.05$, Kruskal-Wallis test) significantly differed between sites, while cover by macroalgae, turf algae and CCA did not.

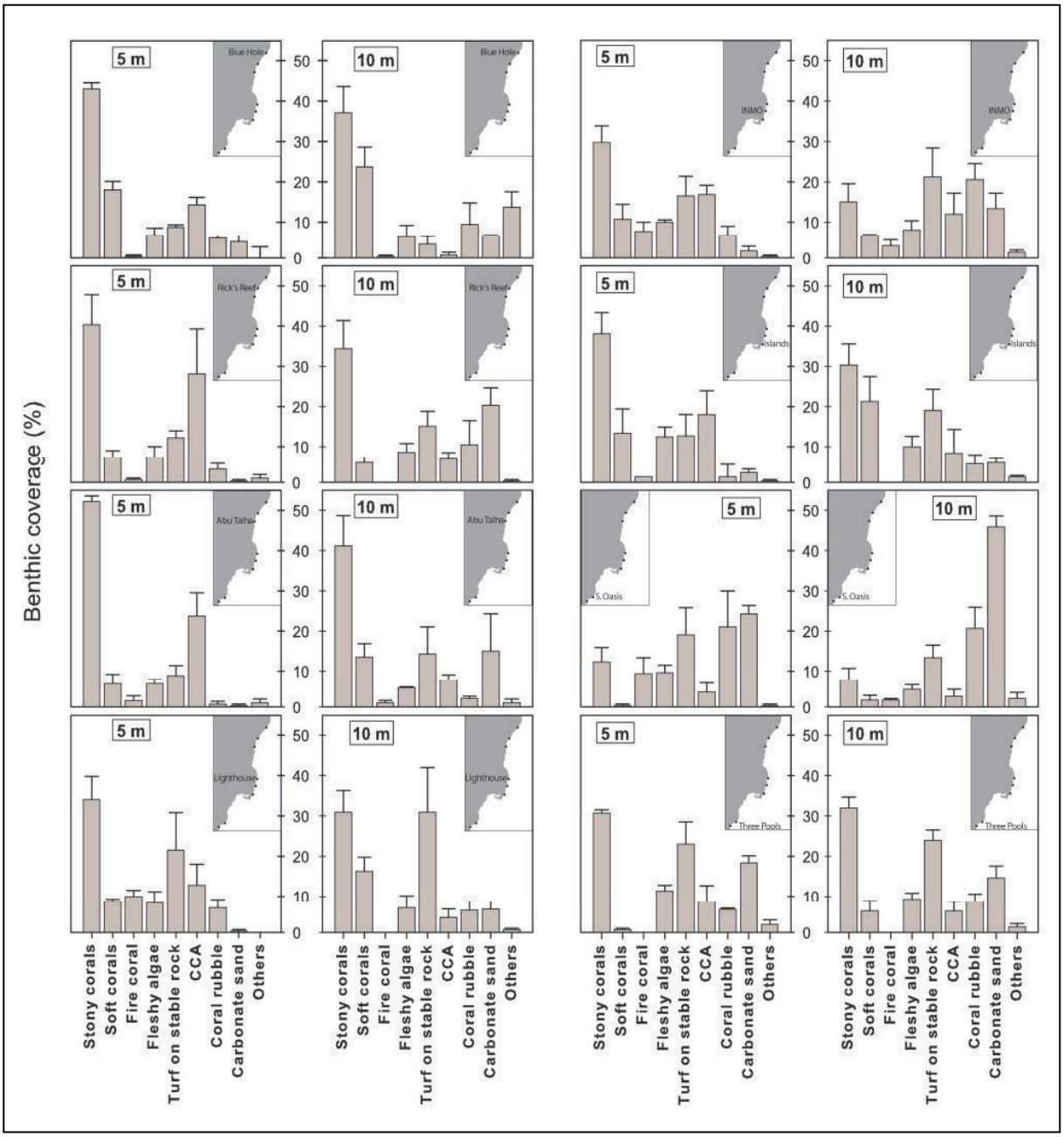


Fig. 2: Benthic coverage (columns are indicating mean values of triplicate transects with error bars indicating SE) by various living and dead substrate types at different locations at 5 and 10 m water depth.

## Coral-algal contacts

In total, 11 % of all scleractinian corals (18 % of branching species, 3 % of massively growing species) exhibited direct contact with algae, whereof 81 % interacted with turf, 16 % with macroalgae and 3 % with CCA (Fig. 3). In 86 % of all interactions, branching coral species were involved, while in only 11 % massive species were involved. Most susceptible for interactions with algae were *Pocillopora* corals of which 31 % exhibited interactions with reef algae. In contrast, only 2 % of *Porites* colonies were in direct contact with algae.

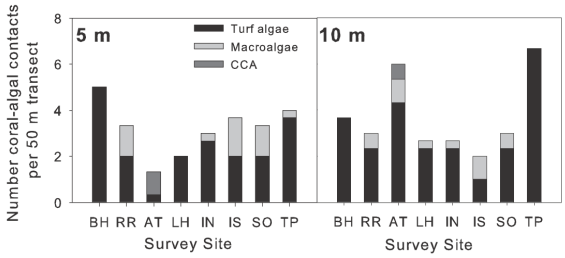


Fig. 3: Number of direct coral contacts with turf algae, macroalgae and CCA at 5 and 10 m water depth at respective locations.

Most coral-algal contacts appeared to be in equilibrium (58 %) without any signs of coral damage or algal overgrowth, but 26 % of all coral colonies in contact with algae showed signs of tissue damage or changes in pigmentation. In 14 % of all coral-algal contacts, algae overgrew the coral colony. Only 1 % of corals in contact with algae showed bulge generation. Whereas the absolute number of coral-algal contacts per transect was similar in 5 m (3.2 ± 0.3) and 10 m (3.7 ± 0.4) water depth, the percentage of coral colonies per transect in direct contact with algae increased from 12 ± 2 % in 5 m to 17 ± 2 % in 10m water depth, but not significantly ($p = 0.54$, paired t-test).

There was no significant correlation between the number of coral-algal contacts per transect, and the benthic cover by scleractinian corals, macroalgae, turf algae, CCA or total algae (macroalgae + turf algae + CCA). Similarly, no correlation between the number of coral-algal contacts per transect and the abundance of total or herbivorous fish was found.

## Fish abundance

Fish surveys revealed a mean abundance of 88 ± 9 individuals 250 $m^{-2}$ (mean ± SE, N = 36 transects), whereof 19 ± 2 individuals 250 $m^{-2}$ (22 % of all fish) were classified as herbivorous fish (Fig. 4). There was no significant change of total and herbivorous fish abundances with increasing water depth. On the contrary, total fish (88 ± 12 individuals 250 $m^{-2}$) and herbivorous fish (19 ± 4 individuals 250 $m^{-2}$) abundances at 5 m water depth were very similar to those at 10 m water depth (88 ± 13 total fish, 18 ± 3 herbivorous fish individuals 250 $m^{-2}$).

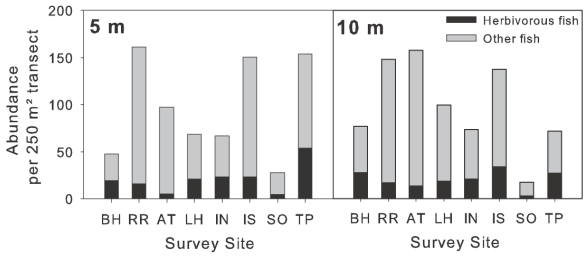


Fig. 4: Mean herbivorous and total fish abundance at 5 and 10 m water depths at respective survey sites.

The most abundant fish group (Table 3) was *Pomacentridae* (52 ± 3 % of total fish), followed by *Acanthuridae* (16 ± 2 %), *Labridae* (10 ± 1 %) and *Chaetodontidae* (9 ± 1 %). No correlation was found between the abundance of herbivorous fish and the benthic cover by scleractinian corals, macroalgae or turf algae. The abundance of total fish per 250 $m^2$ positively correlated to scleractinian coral cover at 10 m water depth ($p < 0.05$, $r = 0.750$, Pearson product-moment correlation), but remained unaffected by scleractinian coral cover at 5 m water depth ($p = 0.345$, Pearson product-moment correlation) and did not show any correlation to benthic cover by macroalgae or turf at any investigated water depth.

Table 3: Fish abundances per 250 m² transect (mean ± SE) at 5 m and 10 m water depth and mean of both water depths.

| Family or species | 5m | 10m | Mean |
|---|---|---|---|
| Pomacentridae | 48.6 ± 9.1 | 47.8 ± 9.7 | 48.2 ± 6.5 |
| Acanthuridae | 14.0 ± 3.2 | 12.0 ± 1.9 | 13.0 ± 1.8 |
| Labridae | 8.7 ± 2.1 | 8.0 ± 1.7 | 8.3 ± 1.3 |
| Chaetodontidae | 8.5 ± 1.6 | 6.7 ± 1.0 | 7.6 ± 0.9 |
| Scaridae | 3.7 ± 1.0 | 5.2 ± 1.3 | 4.4 ± 0.8 |
| Siganidae | 1.4 ± 0.8 | 1.2 ± 0.6 | 1.3 ± 0.5 |
| Serranidae | 0.8 ± 0.3 | 5.0 ± 3.0 | 2.9 ± 1.5 |
| Pomacanthidae | 0.7 ± 0.3 | 1.2 ± 0.4 | 1.0 ± 0.2 |
| Plectorhynchinae | 0.3 ± 0.2 | 0.3 ± 0.1 | 0.3 ± 0.1 |
| Scorpaenidae | 0.3 ± 0.2 | 0.3 ± 0.1 | 0.3 ± 0.1 |
| Lutjanidae | 0.3 ± 0.2 | 0.0 ± 0.0 | 0.2 ± 0.1 |
| *Cheilinus lunulatus* | 0.3 ± 0.2 | 0.2 ± 0.2 | 0.3 ± 0.1 |

## Inorganic nutrient concentrations

Photometric analysis of water samples from all 5 stations (measured in triplicates per station) within the city area of Dahab and from the location 'Southern Oasis' revealed ammonium and phosphate

concentrations below the detection limit at all stations. Correct absolute concentrations of phosphate and ammonia therefore cannot be assessed from the relative photometer values, but were below 0.76 µM for ammonium and 0.05 µM for phosphate.

### Effect of Dahab

The distance to the survey site Lighthouse was used as a primary proxy to estimate the influence of the urbanized area of Dahab on the benthic community. Neither for the benthic cover by scleractinian or soft corals nor for the benthic cover by macroalgae, turf algae or CCA, a correlation between the distance of the respective survey sites to Dahab could be found. Thus, the distance to the survey site 'Lighthouse' as proxy for potential human impact did not reveal any influence of the city area of Dahab on the benthic community.

While benthic cover did not correlate with the distance to the city centre of Dahab, the benthic composition displayed a strong north-south bias. At 5 m water depth, the benthic cover by scleractinian corals ($p = 0.001$, $r = -0.64$) and soft corals ($p = 0.003$, $r = -0.59$) significantly decreased from north to south. The benthic cover by CCA followed this pattern and decreased significantly ($p = 0.018$, $r = -0.48$) from north to south, while benthic cover by turf algae ($p = 0.022$, $r = 0.47$) increased significantly. Macroalgae cover also increased from north to south but did not show a significant correlation with the distance to 'Blue Hole' ($p = 0.037$, $r = 0.43$). At 10 m water depth, only scleractinian corals showed a significant negative correlation with the distance to 'Blue Hole' ($p = 0.013$, $r = -0.50$), thus decreased from north to south.

The mean scleractinian coral benthic cover at 'Northern locations' (45 ± 3 % at 5 m; 38 ± 4 % at 10 m water depth) was significantly elevated compared to the 'Southern locations' (28 ± 4 % at 5m; 21 ± 4 % at 10 m water depth) at both 5m and 10m water depth. In contrast, mean macroalgae benthic cover at 5 m water depth exhibited the reverse pattern being significantly ($p < 0.01$, independent samples t-test) lower at 'Northern locations' (7 ± 1 %) than at 'Southern locations' (11 ± 1 %). Total fish distribution followed the pattern of scleractinian coral cover and was significantly lower at the Southern locations (75 ± 24 individuals per 250 m² transect) compared to the Northern locations (127 ± 25 individuals per 250 m² transect), however only at 10m water depth ($p < 0.05$, independent samples t-test).

## DISCUSSION

Missing baseline datasets are a major problem in the evaluation of anthropogenic impacts on modern coral reefs (Knowlton and Jackson, 2008). Although earlier anthropogenic impacts have already left their footprints, assessing present status of coral reefs is an important tool to detect ongoing changes. In areas with rapid coastal development, such changes can be considered particularly strong, and regular comprehensive surveys can deliver important insights into the suitability of applied reef management strategies or the effects of non-managed impacts on the reef. The results of the present study deliver the largest comprehensive baseline dataset about the status of the benthic coral reef community as well as fish distribution, concentrations of inorganic nutrients and abundance of coral-algal contacts in the Dahab area so far, thereby providing a methodological template for future surveys and indicating potential impact of the urbanized area on the benthic community.

### Status of the coral reefs in the Dahab area

Generally, scleractinian corals, soft corals and benthic algae were the dominant benthic groups, a pattern previously described for the Gulf of Aqaba (Benayahu and Loya, 1981). The observed hard coral cover of 34 % (scleractinian + hydrozoan corals) is very similar to the average hard coral cover reported for Egyptian reefs for the time period from 2004 to 2008 (Wilkinson, 2008). Total living cover of 44 % is slightly lower due to lower soft coral cover of 10 % compared to 13 % average of Egyptian reefs (Wilkinson, 2008). The results of the present study revealed a higher hard coral cover of the Dahab area compared to the average cover of the South Sinai region (Table 4). This is supported by the studies of Wagler and Brümmer (2009) and Hasler and Ott (2008), who described similar hard coral cover for the Dahab area as the present study. Similar results within the Dahab area further indicate constant hard coral cover, thus, do not support significant decline in hard coral cover since 2006 (Table 4).

Mean macroalgae benthic cover in the Dahab area of 8 % was also found to be elevated compared to other areas in the Northern Red Sea. In a study from the Northern Gulf of Aqaba, Haas et al. (2010) reported a mean macroalgae cover of approximately 3 % (also mainly genus *Peyssonnelia*), while the macroalgae

Table 4: Mean benthic cover by scleractinian corals (SC), soft corals (SO), fire corals (HY) and macroalgae (MA) in comparison to previous studies. Scleractinian cover reported by Hasler and Ott (2008) included fire coral cover.

| Study area | Year | Water depth | SC | SO | HY | MA | Reference |
|---|---|---|---|---|---|---|---|
| South Sinai | 2002/2003 | 7 m | 18.3 | 11.4 | 1.7 | 0.8 | Tilot *et al.* (2008) |
| South Sinai | 2002/2003 | 16 m | 11.8 | 14.7 | 0.6 | 0.7 | Tilot *et al.* (2008) |
| Dahab area | 2006 | 12 m | 28.4 | | | | Hasler and Ott (2008) |
| Dahab area | 2007/2008 | mean 10m/16m | 30.2 | 16.6 | | 6.3 | Wagler and Brümmer (2009) |
| Dahab area | 2010 | 10 m | 28.6 | 11.5 | 0.8 | 6.8 | This study |

cover in the South Sinai region was even lower (Table 4). However, in comparison with the study from Wagler and Brümmer (2009), percent macroalgae benthic cover remained relatively constant from the years 2007/2008 to 2010 in the Dahab area. The marginal increase of 0.5 % could be attributed rather to the deeper water depth surveyed by Wagler and Brümmer (2009) than to an a actual increase over time as macroalgae cover was found to decrease with increasing water depth.

The mean soft coral cover of 10 % across all sites and water depths was slightly below the average of 13 % reported for Egyptian reefs (Wilkinson, 2008), but still was well within the range of 5.5 % (3 m water depth) to 14.7 % (16 m water depth) described by Tilot et al. (2008) for the South Sinai region.

Less data than on the benthic community is available on the fish distribution in the investigated area. As many studies focused on specific taxa according to their specific ecosystem functioning, comprehensive datasets including all non-cryptic species are rare. For the Dahab area, this study presents the first comprehensive dataset. Thereby, in accordance with previous studies from the Red Sea (Khalaf and Kochzius, 2002, Pilcher and Abou Zaid, 2000, Roberts and Ormond, 1987) Pomacentridae were found to be the most abundant family of reef fish. The abundances of Acanthuridae, Chaetodontidae and Serranidae in the Dahab area were similar or slightly elevated compared to those previously reported for the South Sinai and the Northern Gulf of Aqaba region, indicating no significant decline during last decades (Table 5). The high abundance of 35 Chaetodontidae per 500 m² transect reported by Hasler and Ott (2008) seems to be atypical and could not be confirmed here. In the Northern Gulf of Aqaba, Scaridae exhibited an abundance of 33.2 and 21.7 individuals per 500 m² in the years 1981 and 2000, respectively. In the Southern Sinai region including the Dahab area, Scaridae abundance was reported to range from 10.3 and 12.4 individuals per 500 m² within the time period from 2002 to 2010, thus either indicating a general decline in the Northern Red Sea and/or generally higher Scaridae abundance in the Northern Gulf of Aqaba compared to the Southern region. Within the South Sinai region, a consistent decrease from 11.1 to 2.3 individuals per 500 m² transect within the time period from 2002 to 2010 could only be observed for the Siganidae. For all other families, no consistent decrease was observed (Table 5).

For the South Sinai, the present study presents the first available dataset on the abundance and types of coral-benthic algae contacts. In the Northern Gulf of Aqaba, Haas et al. (2010) found 21 % of all branching and 15 % of all massive corals in interaction with algae, which exceeds the percentage of corals involved in direct contacts found in the present study (11 %). While the percentage of branching corals in contact with algae (18 %) was similar to the study of Haas et al. (2010), in the present study fewer massively growing coral colonies were found in contact with algae (3 %). An explanation for fewer contacts on massive growth forms may deliver the results of Bennett et al. (2010), who found lower rates of algal removal by herbivorous fish within areas of branching *Acropora* colonies, while planar surfaces exhibited the highest bite rates. Branching colonies were thus found to offer refuge for macroalgae by inhibiting predation by fishes. In another study from the Great Barrier Reef, Tanner (1995) found more than 90 % of coral colonies to be in direct contact with macroalgae, which may be explained by the 5-fold elevated macroalgae cover compared to the present study. According to the study of Haas et al. (2010), who found turf algae to be involved in most direct contacts (77 – 90 %), in the present study, turf algae accounted for 81 % of all contacts.

Data from the Northern Gulf of Aqaba revealed phosphate concentrations of approximately 0.09 µM in March/April (Rasheed *et al.*, 2002). Phosphate concentrations of < 0.05µM detected in the present study thus do not indicate atypically high phosphate concentrations for the Gulf of Aqaba. Due to the limitations of the applied test kits, actual ammonium concentrations could not be determined but were < 0.79µM at all stations. However, these concentration are in the same range as concentrations of approximately 0.3 - 0.5 µM reported by Rasheed et al. (2002). Low concentrations of inorganic nutrients and no differences between stations distributed along the Dahab coastline indicate the functioning of the Dahab

Table 5: Mean abundance per 500 $m^2$ transect of Acanthuridae (AC), Chaetodontidae (CH), Scaridae (SC), Pomacanthidae (PO), Siganidae (SG), Serranidae (SR), Lutjanidae (LU) in comparison to previous studies.

| Study area | Year | Water depth | AC | CH | SC | PO | SG | SR | LU | Reference |
|---|---|---|---|---|---|---|---|---|---|---|
| Aqaba, Jordan | ≤ 1981 | 10 m | 21.8 | | 33.2 | | 0.0 | | | Bouchon-Navaro and Harmelin-Vivien (1981) |
| Dahab area | 1996 | 10 m | | 11.9 | | | | | | Ormond (1996) |
| Northern Red Sea | 2000 | reef slope | | | 21.7 | | | | | Alwany *et al.* (2009) |
| Nabq, South Sinai | 2002 | 10 m | 33.3 | 10.3 | 11.2 | 0.4 | 11.1 | 2.7 | 0.3 | Ashworth and Ormond (2005) |
| South Sinai | 2002/2003 | 10 m | 21.8 | 11.8 | 12.4 | 5.1 | 2.5 | 6.3 | 0.3 | Tilot *et al.* (2008) |
| Dahab area | 2006 | 12 m | | ~ 35 | ~ 11 | | | | | Hasler and Ott (2008) |
| Dahab area | 2010 | 10 m | 24.0 | 13.4 | 10.3 | 2.4 | 2.3 | 10.0 | 0.0 | This study |

waste water treatment system. An interview with a waste water treatment facility employee of Dahab (Nasser Rifaat Kamel), conducted during the period of the present study, revealed that two waste water treatment facilities are in place, and all houses and hotels of Dahab are connected to this facility. These facilities consist of interconnected cement lined ponds in which the wastewater is pumped from the city and collected until it evaporates. Every three years, the ponds are dredged for solid waste, which is then burnt. As solely evaporation reduces the amount of waste water, new ponds need to be established regularly. For the future, a new three-stage wastewater treatment facility, designed to handle the wastewater of the city of Dahab, is under construction and is scheduled to be opened in September 2010. The cleaned water is planned to be used for irrigation purposes. To our knowledge, our study provides the first description of waste water management strategies and first data on inorganic nutrient concentrations for investigated area.

**Location specific differences - North to South bias**

The main surface water current direction in the Gulf of Aqaba is from north to south (Berman *et al.*, 2000). Therefore, potential Dahab-induced effects on the benthic community composition were expected to be more pronounced at the southern locations. Indeed, statistical comparison revealed significantly lower scleractinian coral and higher macroalgae benthic cover at the southern locations compared to the locations north of the Dahab city centre.

Distinguishing between possible causes is difficult. The benthic cover by the various reef organisms and resulting benthic community composition underlies biotic (e.g. abundance of predators or grazers) and abiotic factors such as the availability of inorganic nutrients, water movement and water sediment load or topographic conditions (Sheppard, 1982, Van Den Hoek *et al.*, 1978). The nutrient measurements conducted in the present study indicate a functioning wastewater management system for the Dahab area, but temporarily elevated nutrient levels may accumulate at southern locations due to the main current direction potentially leading to alterations in the benthic community composition (Smith *et al.*, 2001). Additionally, only the buildings in the Dahab area down south of the Dahab lagoon are connected to the central waste water treatment facility (pers. comment to Nasser Rifaat Kamel, waste water treatment facility employee of Dahab). Potential insufficient sewage treatment of the extensive tourist resorts South of Dahab could lead to increased concentrations of inorganic nutrients or generally decreasing water quality at the adjacent coral reefs, which in turn may lead to alterations in the benthic community composition (Smith *et al.*, 2001).

Also, abundance of fish may influence the benthic community. However, no effect of herbivorous fish on scleractinian or macroalgae benthic cover was found, thus solely predation by fish as the investigated top-down factor likely could not explain the observed north-south bias.

Although the present study could not ultimately determine the causes for the observed north-south bias, it identified key locations and fields for future surveys and delivered a baseline dataset to compare future surveys to.

**Lessons learned and recommendations**

The present study delivered the most comprehensive baseline dataset about the benthic community composition of the rapidly developing Dahab area. Thereby, in contrast to monitoring programs involving volunteers (e.g. Reef Check), high resolution triplicated data was recorded by trained scientists guaranteeing reliable accuracy. The focus of the present study was on living coral cover as a key measure of reef habitat quality (Bruno and Selig, 2007). Additionally, distribution of reef fish, concentrations of inorganic nutrients and abundance of coral-algae contacts were recorded. Certainly, as discussed by Sutton (1983), 'coral reefs and their fish fauna must be considered together, in terms of their influence on each other'. Fish may benefit from corals (shelter, nutrition) and corals from fish (removal of algae as competitors for substrate). Due to this mutual influence, we recommend recording the distribution of reef fish as an important top-down factor when describing the status of coral reefs in future surveys.

Human activities-induced pollution is often associated with elevated concentrations of inorganic nutrients. As an important bottom-up factor potentially impacting the benthic community composition (Smith *et al.*, 2001), we also recommend assessing concentrations of inorganic nutrients in future monitoring. As a new parameter extending previous studies (Hasler and Ott, 2008, Tilot *et al.*, 2008), the abundance of coral-algal contacts was assessed. Competition between hard corals and algae is a key ecological process on coral reefs, especially during reef degradation (Jompa and McCook, 2003). Algae overgrowth of initially healthy coral colonies has often been observed (e.g. Hughes, 1989, Jompa and McCook, 2003, Riegl and Velimirov, 1991). Additionally, recent studies indicated the potential of algae to trigger diseases in corals when being in direct physical contact (Nugues *et al.*, 2004) and to cause coral mortality by stimulating microbial activity with ensuing hypoxia likely via the release of dissolved organic compounds (Kline *et al.*, 2006, Smith *et al.*, 2006). Using microprobes, Barott et al. (2009) could demonstrate such algae-induced hypoxic areas and coral tissue disruption at the zones of direct coral-algal contact also in situ. This highlights the importance to assess the abundance of coral-algal contacts and to distinguish between different algae groups being involved as they differently affect corals. While CCA and filamentous turf algae are reported to have relatively minor effects on corals, fleshy turf and macroalgae in contrast can cause distinct hypoxic areas and coral tissue disruption

(Barott *et al.*, 2009, Jompa and McCook, 2003). We therefore recommend assessing the abundance and type of coral-algal contacts in future surveys, which is further supported by the high percentage (26 %) of coral colonies showing signs of damage when in direct contact with algae.

Although the present study could not ultimately determine the ultimate cause/causes for the observed north-south bias, it identified key areas and fields for future surveys. Testing for inorganic nutrient concentrations using sensitive test kits will be needed in order to assess location-specific differences and to be able to fully identify the influence of potential sewage discharge on the benthic community. Additionally, assessment of the numbers of fish taken from the different locations will be helpful in order to understand if low abundance of fish (due to high fishing rates) facilitates low coral cover or if rather low coral cover (for whatever reason) leads to a naturally low abundance of fish.

However, as the present study has not been temporarily resolved, future surveys will be needed in order to put the present results into context. As a standardized methodology is inevitable to guarantee maximum comparability between the surveys, the methodology used in the present study could function as a template. For instance, although the visual fish census technique is a frequently applied methodology to quantify coral reef fish, details of the applied procedure vary widely among different workers (Sale and Douglas, 1981), making it difficult to compare the respective results. Moreover, due to small-scale variations even at the same locations, at least triplicate surveys per location are recommended. Additionally, setting permanently installed transect starting points and marking coral-algal contacts could help to accurately monitor changes in the benthic community composition and outcomes of coral-algal contacts over time.

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# 4

# Spatial distribution of the upside-down jellyfish *Cassiopea* sp. within fringing coral reef environments of the Northern Red Sea: implications for its life cycle

**Wolfgang Niggl[1] and Christian Wild[1]**

[1]Coral Reef Ecology Group (CORE), GeoBio-Center & Department of Earth and Environmental Science in the Faculty of Geosciences, Ludwig-Maximilians Universität, Richard Wagner Str. 10, 80333 München, Germany

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ORIGINAL ARTICLE

# Spatial distribution of the upside-down jellyfish *Cassiopea* sp. within fringing coral reef environments of the Northern Red Sea: implications for its life cycle

**Wolfgang Niggl · Christian Wild**

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**Abstract** The zooxanthellate mangrove jellyfish *Cassiopea* sp. represents a prominent invasive species and a potential bioindicator for nutrient monitoring in coral reefs. However, information about its spatial distribution in combination with abundance, habitat specificity and life cycle elements is barely available. This study, therefore, presents the results of field surveys conducted within four different benthic habitat types (coral reef, seagrass meadow, reef-sand transition and sand flat) in the Northern Red Sea. *Cassiopea* sp. exhibited a highly patchy distribution within the entire study area with mean abundance of $1.6 \pm 0.3$ animals $m^{-2}$ and benthic coverage of 3.2%. Within coral reef habitats, maximum abundance of up to 31 animals $m^{-2}$ and benthic coverage of up to 20% were detected. Additionally, this study revealed that 65% of all observed *Cassiopea* specimens were associated with the commensalistic crustacean mysid *Idiomysis tsurnamali*. *Cassiopea* abundance and size as well as association patterns with mysids differed between most of the surveyed habitats. In summary, the findings of the present study (1) characterize *Cassiopea* as one of the key organisms in investigated benthic habitats, (2) indicate active habitat selection by the jellyfish and (3) may hint to an unexplored life cycle of *Cassiopea* with central role of seagrass meadows providing cues for larval settlement and metamorphosis in the absence of mangroves.

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W. Niggl (✉) · C. Wild
Coral Reef Ecology Group (CORE),
GeoBio-Center and Department of Earth and Environmental Science, Ludwig-Maximilians Universität,
Richard-Wagner-Str. 10, 80333 München, Germany
e-mail: w.niggl@lrz.uni-muenchen.de

**Keywords** *Cassiopea* · Abundance · Coral reef · Benthic habitat · *Idiomysis tsurnamali* · Life cycle

## Introduction

*Cassiopea* sp., commonly referred to as mangrove or upside-down jellyfish, represents a study organism within several scientific disciplines. Interorganismic interaction—typically an ecological topic—such as the jellyfish's symbiotic relationship with dinoflagellates or the commensalistic relationship with the crustacean mysid *Idiomysis tsunrnamali* (Bacescu 1973) has been addressed by marine scientists from various fields (e.g. Bacescu 1973; Hofmann and Kremer 1981; Thornhill et al. 2006). The lifecycle of the scyphozoan *Cassiopea* is classically metagenetic and, therefore, alters between a medusoid and polypal stage, with an intermediate larval stage (e.g. Bigelow 1900; Gohar and Eisawy 1960b; Smith 1936). Many studies have focused on factors influencing the development of *Cassiopea*, dealing with endogenous (Thieme and Hofmann 2003a, b) or exogenous (e.g. Bischoff et al. 1991; Curtis and Cowden 1971; Fleck and Fitt 1999; Fitt and Costley 1998; Hofmann et al. 1978) cues for larval settlement and metamorphosis, as well as factors influencing strobilation (e.g. Fitt 1984; Hofmann and Kremer 1981; Ludwig 1969; Rahat and Adar 1980). Additionally, the upside-down jellyfish has drawn attention as a possible bioindicator species for low-nutrient environments. In this context, Todd et al. (2006) demonstrated the potential of *Cassiopea* sp. as a susceptible indicator species for environmental phosphates.

However, information about its spatial distribution in combination with abundance is barely available. Holland et al. (2004) described *Cassiopea* as globally distributed, occurring in shallow, tropical inshore marine waters on

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sandy mudflats. Reports on remarkably high *Cassiopea* abundances, e.g. in the Caribbean and the Red Sea, were first summarized by Mayer (1910). However, quantitative data are only provided by Collado Vides et al. (1988) for the Nichupte lagoon system off the Mexican Caribbean coast and by Mergner and Schuhmacher (1981) for a 25 m² seafloor patch in the Northern Red Sea. Recently, a *Cassiopea* sp. was reported to increase its spatial distribution by invading into, for example, Hawaiian and Mediterranean waters (Çevik et al. 2006; Holland et al. 2004; Özgür and Öztürk 2008; Panucci-Papadopoulou et al. 2005). As invasive species are a principle threat to biodiversity and responsible for enormous economic losses (Bolton and Graham 2006), and especially jellyfish are known for their direct negative effect on human enterprises (Purcell et al. 2007), alterations in distribution and abundance of *Cassiopea* sp. require monitoring.

The present study, therefore, aims to provide data on abundance, habitat selection and life cycle elements like size classes and the commensalism with *Idiomysis tsurnamali* for *Cassiopea* in Northern Red Sea fringing reef environments. It thereby aims to contribute to understand the ecology of the jellyfish and to deliver a dataset for further monitoring. For this purpose, a study area in the Northern Gulf of Aqaba, Jordan, comprising several different benthic habitat types, was surveyed using two transect techniques and subsequent digital image as well as statistical analyses.

## Materials and methods

### Description of study site

The study was conducted in May 2008 in the Northern Gulf of Aqaba at the marine reserve of the Marine Science Station (MSS), Aqaba, Jordan (29° 27′ N, 34° 58′ E). All surveys took place at water depths of 5 to 20 m in a study area located directly north of the MSS jetty, ranging from 50 m north and 50 m south relative to the coordinates 29° 27′ 31″ N, 34° 58′ 34″ E (Fig. 1). Because surveys for the present study took place only during one season, and jellyfish blooms may occur seasonally (Mills 2001), supporting semi-quantitative data from other investigations during other seasons and years, but at identical study site, were collected. These data are summarized in Table 1 and confirm that the observations displayed in the present study are not atypical.

### Benthic composition

Benthic composition of the study area was determined using 50 m line point intercept (LPI) transects (modified after English et al. 1994) with recording intervals of 1 m. These transects were conducted at 5.0, 7.5, 10.0, 12.5, 15.0 and 20.0 m water depth each to the north and the south of the MSS jetty. Sediment type and seafloor coverage by benthic fauna and flora were recorded to get an overview of occurring benthic habitats in the study area. LPI transects revealed four distinct, main habitats: (a) the *reef* habitat, which describes the patchy reef northern of the MSS; (b) the *sand flat* consisting predominantly of silicate sands (Wild et al. 2005); (c) the *transition zone*, which is located between the reef habitat and the sand flat and (d) the *seagrass meadow*. These four habitats were examined for habitat-specific *Cassiopea* abundance and size dimensions as well as association with mysids (see below). Results of

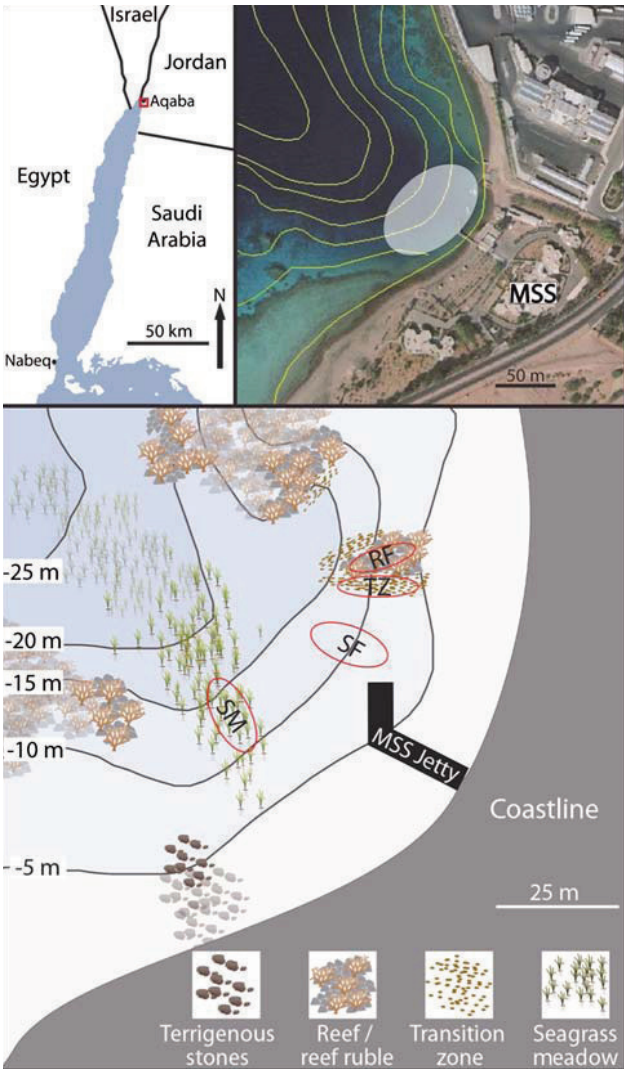


**Fig. 1** Map of the Gulf of Aqaba (*top left*). Aerial image (property of Aqaba Special Economic Zone Authority) of the Marine Science Station (MSS) in Aqaba with the study area indicated by transparent ellipses (*top right*). Benthic composition of the study area revealed by line point intercept transects (*bottom*). No transect-derived data were recorded in areas indicated by *transparent icons*. *Red ellipses* indicate distinguished main habitats (*RF* reef, *TZ* transition zone, *SF* sand flat, *SM* seagrass meadow)

**Table 1** Summary about previous and successive observations of high *Cassiopea* abundances (>5 animals $m^{-2}$) in benthic habitats identical to those investigated in present study during spring 2008

| Year | Season | Observer |
|---|---|---|
| 2002 | Spring | E.M. Zetsche |
| 2004 | Spring | C. Jantzen |
| | Summer | C. Jantzen/C. Wild |
| | Autumn | M. Naumann |
| 2005 | Winter | M. Naumann |
| 2006 | Autumn | C. Jantzen/C. Wild |
| 2007 | Summer | C. Wild |
| | Autumn | C. Jantzen |
| 2008 | Winter | C. Wild/W. Niggl/A. Haas |
| | Summer | L. Kamphausen |

Holland et al. (2004) indicate that the species investigated by the present study was likely *Cassiopea andromeda*. However, as no molecular analysis was performed and determination of species affiliation based on morphology is rather vague within the genus *Cassiopea* (Holland et al. 2004), in the following, the generic notation *Cassiopea* sp. was used.

### *Cassiopea* sp. abundance

In order to determine average abundance of *Cassiopea* sp. (specimen $m^{-2}$) within each benthic habitat type, multiple single quadrate surveys were conducted. Thereby, a quadrate of 1 m side length was randomly placed on the seafloor at 13 m water depth. Subsequently, the quadrate was flipped over towards a more shallow area, and again the number of abundant *Cassiopea* sp. was recorded. This was repeated until a water depth of 7 m was reached. With this preset methodology, subjective placement on obviously high abundance spots was avoided. This procedure was carried out on all four distinguished main habitats except the *seagrass meadow*, where due to its spatial extensions (upper limit at 10 m water depth), the survey was conducted between 16 and 10 m water depths. The abundance of *Cassiopea* sp. in all 14 to 23 resulting replicate quadrates was recorded, and a photograph taken from directly above using a Panasonic TZ5 (9.1 megapixel) digital camera with underwater housing. There was no correlation ($P = 0.44$; Spearman rank-order correlation) between *Cassiopea* abundance and water depth, so that the slightly deeper benthic habitat *seagrass meadow* could be compared to the other habitats.

*Cassiopea* sp. abundance was related to the *entire study area* by multiplication of habitat-specific mean abundance and proportional seafloor coverage of each habitat.

Maximum abundance of *Cassiopea* sp. in each habitat was quantified by placing quadrates specifically at spots with high *Cassiopea* sp. abundance. This procedure was repeated within each of the four habitat types at least 10 times, and maximum abundances were recorded from these subjective assessments.

### *Cassiopea* sp. benthic coverage

Benthic coverage of *Cassiopea* sp. as well as the proportion of unoccupied sand flats in the four habitats was analysed using the image processing software ImageJ on digital photographs of the single quadrate surveys (see above) using the quadrate side length of 1 m as a scale. Maximum benthic coverage at each habitat was analysed accordingly using the pictures from maximum abundance determination (see above). Benthic coverage of *Cassiopea* sp. in the *entire study area* was calculated from the data obtained by LPI transects.

### *Cassiopea* sp. size and association with *Idiomysis tsurnamali*

*Cassiopea* sp. specimens at all four habitats were also parallel surveyed concerning jellyfish diameter and association with *Idiomysis tsurnamali*. Specimens ($n = 22$ to 36 per habitat) were randomly selected, and their oral surface diameters were measured using a ruler (accuracy: ±0.1 cm). All specimens were also examined for the occurrence of associated *Idiomysis tsurnamali* (Fig. 2a), and the swarm size of the mysids was estimated using the categories described in Table 2.

### Statistical analysis

In order to investigate habitat-specific differences in jellyfish diameter, one-way ANOVAs followed by a LSD post hoc test were carried out. One-way ANOVA was chosen as the parameter "jellyfish diameter" was independent within and between samples. In contrast, due to the "end to end" placement, quadrates of the habitat surveys and resulting data for *Cassiopea* abundance and benthic coverage were not independent. Hence, these parameters were tested for habitat-specific differences using Mann–Whitney *U* tests. All categorical data (e.g. mysids size categories) were converted into discrete data and tested using Mann–Whitney *U* tests. Correlations were tested using the Spearman rank-order correlation.

## Results

### Benthic composition

Sand flats, consisting of predominantly silicates sands, dominated the study area, comprising 60.2%. However,

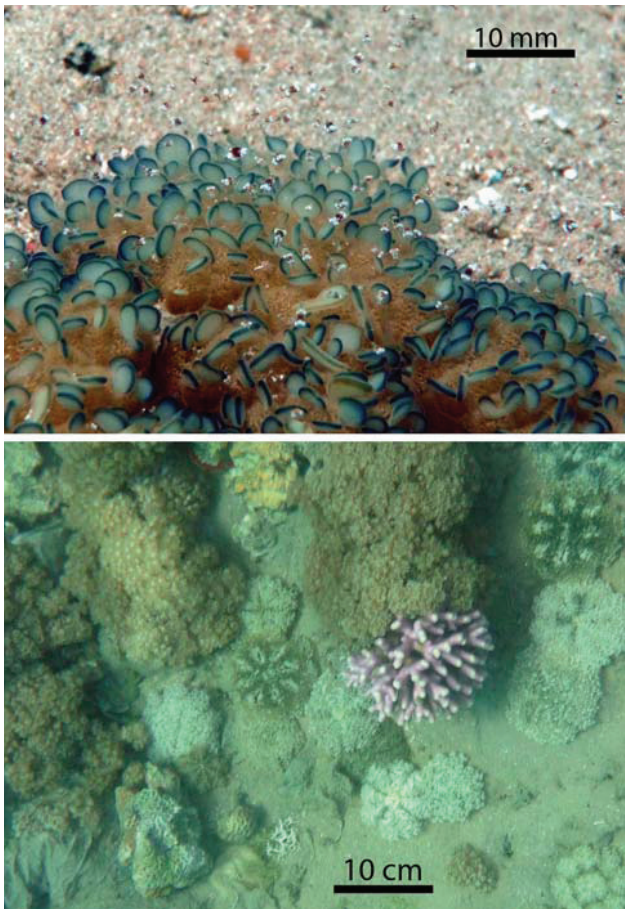


**Fig. 2** **a** *Cassiopea* sp. exhibiting a swarm of *Idiomysis tsurnamali*. **b** High abundance of *Cassiopea* sp. in the *reef* habitat

patchy reef structures (19.0%), areas with high seagrass cover (10.5%) and the transition zone (5.3%) between reef and sand flats also contributed to the benthic composition in the study area. Artificial constructions, terrigenous stones and carbonate sand flats each accounted for less then 3% of the study area.

*Cassiopea* sp. abundance

*Cassiopea* sp. exhibited a patchy distribution (Fig. 2b) between and within different benthic habitats, as indicated by the high standard deviations (Table 2). Nevertheless, there were significant (Table 3) differences concerning *Cassiopea* sp. abundances between the four habitats, ranging from less than one jellyfish $m^{-2}$ in the *seagrass meadow* to almost eight jellyfish $m^{-2}$ in the *transition zone* (Table 2). Maximum abundance occurred in the *reef*, exhibiting more than sixfold higher abundances than the *seagrass meadow*.

Within the *entire study area*, *Cassiopea* sp. exhibited a mean abundance of 1.6 ± 0.3 with a range from 0 to 22 animals $m^{-2}$ ($n = 71$).

*Cassiopea* sp. benthic coverage

Results obtained from image processing revealed significant differences (Table 3) in benthic coverage by *Cassiopea* sp. between the four main habitats, ranging from less than 1% in the *seagrass meadow* to more than 7% at the *transition zone* (Table 2). Maximum benthic coverage was reached in the *reef* habitat, with *Cassiopea* sp. covering up to 20.1% of the seafloor.

The *seagrass meadow* exhibited lowest proportion of unoccupied bare sand flats (Table 2).

*Cassiopea* sp. benthic coverage in the *entire study area* was 3.2% as revealed by line point intercept transect work. There was a strong bias between transects to the north (6%) and transects to the south (0.4%).

*Cassiopea* sp. size and association with *Idiomysis tsurnamali*

Additional to habitat-specific differences in abundance and benthic coverage, significant habitat-specific differences in jellyfish mean diameter were found. Mean diameter was lowest at the *seagrass meadow* and highest at the *sand flat* (Table 2). The percentage of *Cassiopea* sp. associated with *Idiomysis tsurnamali* also differed significantly (Table 3) between the habitats, with the highest percentage of associations at the *transition zone* and lowest jellyfish–mysids association at the *seagrass meadow* (Table 2).

Furthermore, a correlation between jellyfish diameter and association with mysids was found ($P < 0.001$), with

**Table 2** *Cassiopea* sp. abundance, benthic coverage, diameter and association with *Idiomysis tsurnamali* in the respective benthic habitat

| Site | Mean abundance (animals $m^{-2}$) | Maximum abundance (animals $m^{-2}$) | Mean diameter (cm) | Mean benthic coverage (%) | Unoccupied, bare sand flats (%) | *Cassiopea*–mysids association (%) | Mysids swarm size distribution (0:1:2:3) |
|---|---|---|---|---|---|---|---|
| Reef | 1.6 ± 2.2 | 31 | 10.3 ± 2.3 | 1.0 ± 1.1 | 24 ± 19 | 81.5 | 19:30:30:22 |
| Transition zone | 7.9 ± 6.7 | 27 | 13.1 ± 2.7 | 7.5 ± 5.8 | 84 ± 11 | 92.9 | 7:11:21:61 |
| Seagrass meadow | 0.4 ± 0.8 | 5 | 8.3 ± 3.2 | 0.1 ± 0.3 | 8 ± 9 | 18.2 | 81:5:0:14 |
| Sand flat | 1.3 ± 1.4 | 14 | 13.5 ± 2.4 | 1.6 ± 1.8 | 95 ± 4 | 61.1 | 39:14:8:39 |

Swarm size categories describe abundance of mysids per jellyfish (0 = no mysids, 1 = 0–4 mysids, 2 = 5–9 mysids, 3 = more than 10 mysids)

**Table 3** Statistical analyses of habitat-specific *Cassiopea* abundance, benthic coverage and association with mysids

| | Mean abundance | | | Benthic coverage | | | Mean diameter | | | *Cassiopea*–mysids association | | | Mysids swarm size | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | TZ | SM | SF | TZ | SM | SF | TZ | SM | SF | TZ | SM | SF | TZ | SM | SF |
| RF | *** | 0.08 | 0.96 | *** | * | 0.39 | *** | ** | *** | 0.21 | *** | 0.08 | ** | 0.23 | * |
| TZ | | *** | *** | | *** | *** | | *** | 0.56 | | *** | ** | | 0.93 | 0.71 |
| SM | | | 0.07 | | | * | | | *** | | | ** | | | 0.81 |

Given are *P* values for hypothesis for no differences between respective habitats (*TZ* transition zone, *SM* seagrass meadow, *SF* sand flat, *RF* reef) concerning listed parameters. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

smaller *Cassiopea* sp. specimens exhibiting associations to *Idiomysis tsurnamali* less frequently.

*Cassiopea* sp. located at the reef exhibited significantly different swarm sizes of *Idiomysis tsurnamali* compared to those at the transition zone and the sand flat (Tables 2 and 3). *Cassiopea* sp. swarm sizes were largest within the transition zone. *Idiomysis tsurnamali* swarm sizes were found to positively correlate with *Cassiopea* sp. diameter ($P < 0.01$). No habitat-specific differences could be found concerning size classes of *Idiomysis tsurnamali* specimens themselves.

## Discussion

The findings of the present study characterize *Cassiopea* to be one of the key organisms within the benthic community in investigated coral reef-associated environments of the Northern Red Sea, exhibiting abundance and benthic coverage comparable to soft corals. On smaller scales, *Cassiopea* can even act as the dominant benthic organism due to its patchy distribution that further results in habitat-specific differences concerning *Cassiopea* abundance, size and association patterns to *Idiomysis tsurnamali*.

These findings complement previous studies (e.g. Collado Vides et al. 1988; Holland et al. 2004; Mergner and Schuhmacher 1981) by describing high *Cassiopea* abundance in coral reef ecosystems for the first time and indicating active habitat selection potentially controlled by the availability of suitable substrates, inorganic nutrients and prey.

### *Cassiopea* sp. abundance and benthic coverage

There are only two previous studies in which mean *Cassiopea* abundance and benthic coverage was quantified. In a fringing reef adjacent to the area investigated in the present study, Mergner and Schuhmacher (1981) observed two *Cassiopea* sp. specimens in a 25 m² survey area resulting in an abundance of 0.08 animals $m^{-2}$ and a benthic coverage of 0.03%. This is much lower than the values observed in the present study. Although the study of Mergner and Schuhmacher (1981) was conducted during the winter half-year and jellyfish abundances are known to rapidly fluctuate (Pitt et al. 2005), differences to present study likely cannot be ascribed to seasonal changes. Observations during other investigations at the study site (Table 1) indicate a constantly high *Cassiopea* abundance throughout all seasons over the last 7 years. This is further confirmed by the study of Fitt and Costley (1998), in which no seasonal changes in *Cassiopea* medusae population were found.

The higher medusae abundance and benthic coverage described in the present study may, therefore, indicate an increase in *Cassiopea* abundance over the last decades. A second explanation for lower abundances reported by Mergner and Schuhmacher (1981) may be the patchy distribution *Cassiopea* exhibited (Fig. 2). Mergner and Schuhmacher only investigated a small section of 25 m², thereby potentially omitting patches with high jellyfish abundances.

The patchy distribution of *Cassiopea* is further reflected by the significant differences in jellyfish abundance and benthic coverage between the four investigated main habitats (*reef*, *transition zone*, *sand flat*, *seagrass meadow*). Limited availability of bare sediment likely caused the lowest *Cassiopea* abundances in the *seagrass meadow*, which is supported by the findings of Collado Vides et al. (1988). Availability of prey, e.g. copepods or crustacean larvae, which *Cassiopea* was shown to feed on (Gohar and Eisawy 1960a), may also influence *Cassiopea* abundance and likely differs between the four habitats. In addition, *Cassiopea* was shown to actively extract and take up inorganic nutrients from the sediment (Jantzen et al. unpublished). Sites with higher input of organic matter, which is rapidly recycled to regenerate nutrients in permeable reef sands (Wild et al. 2004a, b, 2005) may, therefore, be favoured by *Cassiopea* specimens. The direct vicinity to reef corals could lead to a high supply of organic matter to the adjacent sediments (Wild et al. 2005) and potentially explain the highest *Cassiopea* abundances within coral reef-associated habitats (Table 2).

Maximum observed *Cassiopea* abundance of up to 31 animals $m^{-2}$ is similar to that observed by Collado Vides et al. (1988), who reported a very high mean abundance of 42 medusae $m^{-2}$ in Bojórquez lagoon (Mexican Caribbean).

For coral reef ecosystems, the high abundances observed in present study are described for the first time.

The average benthic cover by *Cassiopea* sp. of 3.2% in the study area was lower than described for hermatypic corals (Bouchon-Navaro and Bouchon 1989; Khalaf and Kochzius 2002; Mergner and Schuhmacher 1974) and benthic algae (Haas et al. unpublished), but similar compared to soft corals (Khalaf and Kochzius 2002), thereby characterizing *Cassiopea* as one of the key representatives of the benthic community. On smaller scales, *Cassiopea* can even act as the dominant benthic organism in coral reef-associated habitats.

### Implications for *Cassiopea* life cycles

It is generally reported that *Cassiopea* is associated with mangrove-dominated habitats (Holland et al. 2004). In this context, Fleck and Fitt (1999) demonstrated in a laboratory study that degrading mangrove leaves provide a natural cue for *Cassiopea* sp. larval settlement and metamorphosis, whereas settlement on the seagrass *Thalassia testudinum* only marginally occurred. These findings were supported by in situ observations, which showed that *Cassiopea* sp. polyps frequently occur on mangrove leaves, but rarely on any other substratum (Fitt 1991; Fitt and Costley 1998). However, in the present study, high abundances of *Cassiopea* sp. were found more than 150 km north from the closest mangrove ecosystem (Nabeq, Egypt). As the main current in the Gulf of Aqaba runs from north to south (Berman et al. 2000), it is unlikely that observed high abundances of the almost stationary jellyfish *Cassiopea* are solely maintained by migration. Therefore, the *seagrass meadow* (*Thalassia testudinum*) is suggested to have provided natural cues, e.g. degrading organic material, for larval settlement and metamorphosis sufficient to sustain high abundances. This is further supported by the occurrence of very small medusae of about 1.5 cm in bell diameter and an estimated age of about 6 weeks (Kaiser, personal communication) and the lowest mean diameters of all four habitats in the *seagrass meadow*.

When medusae grow, bare substrate becomes limited in the *seagrass meadow*, which may force the jellyfish to leave. This may deliver an explanation for lowest numbers of medusae in the *seagrass meadow* despite the provision of natural cues. Highest mean *Cassiopea* diameter at the *sand flat*, the habitat with the highest percentage of unoccupied substrate, supports this assumption.

### *Cassiopea* association with *Idiomysis tsurnamali*

More than 60% of all jellyfish exhibited an association to mysids, whereby a positive correlation between jellyfish diameter, the presence of mysids and the mysids swarm size was found. An explanation may be that larger-sized *Cassiopea* offer more refuge to the mysids, which withdraw between the tentacles in case of danger (Niggl, personal observation). In addition, *Cassiopea* sp. was found to release organic matter (Ducklow and Mitchell 1979), which is consumed and mineralized by the mysids (Niggl et al. unpublished). As organic matter release by *Cassiopea* sp. positively correlates with surface area, larger *Cassiopea* specimens may be able to provide larger mysid swarms with food.

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**Wolfgang Niggl[1], Malik S. Naumann[1], Ulrich Struck[2], Riyad Manasrah[3] and Christian Wild[1]**

[1]Coral Reef Ecology Group (CORE), GeoBio-Center & Department of Earth and Environmental Science in the Faculty of Geosciences, Ludwig-Maximilians Universität, Richard Wagner Str. 10, 80333 München, Germany

[2]Museum for Natural History, Humboldt-University Berlin, 10099 Berlin, Germany

[3]Marine Science Station, University of Jordan, Aqaba 77110, Jordan

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

Wolfgang Niggl [a,*], Malik S. Naumann [a], Ulrich Struck [b], Riyad Manasrah [c], Christian Wild [a]

[a] *Coral Reef Ecology Group (CORE), GeoBio-Center and Department of Earth and Environmental Science, Ludwig-Maximilians University Munich, Richard-Wagner-Strasse 10, 80333 Munich, Germany*
[b] *Museum for Natural History, Humboldt-University Berlin, 10099 Berlin, Germany*
[c] *Marine Science Station, University of Jordan, Aqaba 77110, Jordan*

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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 mucoid particulate organic matter release was $21.2 \pm 9.4$ mg POC and $2.3 \pm 1.1$ 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 $O_2$ consumptions by microbes and zooplankton were 5.9- and 3.8-fold higher compared to seawater controls, respectively, when *Cassiopea*-derived organic matter was present. Total organic carbon (TOC) degradation rates increased 5.0-fold (0.27% $h^{-1}$ versus 1.38% $h^{-1}$), thereby indicating fast mineralization of *Cassiopea*-derived organic matter. These findings suggest that *Cassiopea*-derived organic matter may function as a newly discovered trophic pathway for organic matter from the benthic environment to pelagic food chains in coral reefs and other marine ecosystems.



## 1. 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. Besides 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,

* Corresponding author. Tel.: +49 89 2180 6633; fax: +49 89 2180 6601.
*E-mail addresses:* w.niggl@lrz.uni-muenchen.de (W. Niggl), m.naumann@lrz.uni-muenchen.de (M.S. Naumann), ulrich.struck@museum.hu-berlin.de (U. Struck), r.manasrah@ju.edu.jo (R. Manasrah), c.wild@lrz.uni-muenchen.de (C. Wild).

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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 dominate the benthic community reaching abundances of up to 31 animals $m^{-2}$ and a benthic coverage of up to 20% (Niggl and Wild, in press). *Cassiopea*, similar to most jellyfish, may release organic matter by several mechanisms such as sloppy feeding or excretion of fecal material or mucus (Pitt et al., 2009). 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 undisturbed 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 potentially function as a carrier of energy and nutrients to fuel pelagic food webs. The focus thereby lay on the two primary mechanism of organic matter release by jellyfish, excretion and mucus production (Pitt et al., 2009).

For this purpose, net release of particulate and dissolved organic matter was quantified by incubating *Cassiopea* sp. individuals in seawater filled beakers under in-situ light and temperature conditions at the Northern Red Sea. Released mucoid organic matter was labelled with stable isotopes and provided to the common jellyfish commensal zooplanktonic mysid *Idiomysis tsurnamali* that hover in swarms directly above the jellyfish and withdraw between its tentacles in case of danger (Bacescu, 1973). This was carried out in order to investigate potential uptake of *Cassiopea*-derived organic matter by the mysids. In addition, $O_2$ consumption by mysids and planktonic microbes was quantified to determine mineralization of *Cassiopea*-derived organic matter by these organisms.

## 2. Material and methods

### 2.1. 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 *I. 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.

Cassiopea-associated specimens of the zooplanktonic mysid *I. tsurnamali* 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 of the genera *Fungia* ($n=9$) and *Ctenactis* ($n=6$) were collected as reference organisms from the fore reef directly in front of the MSS, at water depths ranging 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).

### 2.2. Quantification of organic matter release by Cassiopea sp.

Organic matter was subdivided by size classes into particulate and dissolved organic matter. Particulate was defined as material being retained by a Whatman GF/F filter (0.6 µm–0.8 µm particle retention) and dissolved as material passing a 0.2 µm pore size filter (VWR®, polyethersulfone membrane). 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 and followed the established beaker incubation method (Herndl and Velimirov, 1986). In total, $n=18$ *Cassiopea* specimens were incubated, whereby each specimen was incubated only once.

All glass beakers prior to incubation experiments were first washed with acetone and then with distilled water to ensure complete removal of acetone. Immediately before the experiment, the beakers were thoroughly flushed with water from the maintenance tank and then submerged in the maintenance tank. Six *Cassiopea* sp. specimens (5–8 cm in diameter) were transferred into one beaker each by carefully lifting them from the bottom and then letting them descend into the beakers placed underneath the jellyfish. When removing the beakers from the maintenance tank by slowly lifting them, most of the initial water in the beakers, possibly contaminated with organic matter released by the jellyfish due to the transferring procedure, was replaced by fresh, non-contaminated water from the maintenance tank. Macroscopic investigation of the jellyfish ensured no occurrence of epibiotic animals or adhesive external material associated with the jellyfish. Five additional beakers were treated accordingly just without transferring jellyfish specimens 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. During the incubation, the jellyfish were not fed in order to avoid organic matter release due to sloppy feeding.

Frequent observation of the jellyfish during incubation confirmed that no differences (e.g. extensive pumping) compared to in-situ behaviour occurred. 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 used to clean the filter and 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.

After DOC sampling, the incubation water in all beakers was inspected for fecal pellets which, when occurring, were removed using plastic pipettes. Approximately 200 ml of the incubation water of each beaker was then 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 graduated glass cylinder (accuracy ± 10 ml) and filtered onto pre-combusted GF/F filter (Whatman®, 25 mm diameter, 0.6 µm–0.8 µm particle retention). The filters were dried for at least 48 h 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

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

Jellyfish surface area was used as non-invasive reference unit for organic matter release rate calculations in order to ensure comparability to previous studies (e.g. Herndl and Velimirov, 1986; Crossland, 1987; Wild et al., 2004a; Wild et al., 2005a; Niggl et al., 2009).

### 2.3. Mineralization of Cassiopea-derived organic matter by I. tsurnamali and planktonic microbes

In order to assess mineralization of *Cassiopea*-derived organic matter, $O_2$ consumption rates of microbes and mysids in control seawater and in seawater with previous *Cassiopea* incubation were determined. In the first approach, $O_2$ consumption by the natural microbial assemblage, likely including bacteria, archaea, viruses and small protozoa (visual examination ensured the absence of larger plankton) was investigated in both control seawater and in water with previous *Cassiopea* incubation. Therefore, aliquot samples were taken from each of the 11 incubation waters of the organic matter quantification experiment – 5 controls and 6 with previous *Cassiopea* incubation – and filled into 60 ml Winkler bottles. The initial $O_2$ concentration in each of the 11 Winkler glass bottles was determined using an $O_2$ optode (HACH, HQ 10). Afterwards, the Winkler bottles were closed and stored in the dark at in-situ temperature for 19.0–20.2 h. At the end of this period, $O_2$ concentrations were measured. By subtracting end from initial $O_2$ concentrations the microbial $O_2$ consumption was determined in both, the control water as well as in water with previous *Cassiopea* incubation. Microbial $O_2$ consumption rates were expressed in mg $O_2$ $L^{-1}$ $d^{-1}$.

In the second approach, combined $O_2$ consumption rates of mysids plus microbes were assessed in both control seawater and in water with previous *Cassiopea* incubation. Therefore, again aliquots were taken from each of the eleven incubation waters of the organic matter quantification experiment – 5 controls and 6 with previous *Cassiopea* incubation – and filled into 120 ml Winkler bottles. The initial $O_2$ concentration in each of the eleven Winkler bottles was determined. Before closing the bottles, 5 medium sized (body length: approximately 3–4 mm) *I. tsurnamali* specimens were added to each of the eleven 120 ml Winkler bottles. After 4.0–4.5 h of dark incubation at in-situ temperature, $O_2$ concentrations were determined and subtracted from initial $O_2$ concentrations. The changes in $O_2$ concentrations in that approach displayed the combined $O_2$ consumption rates of mysids plus the microbial assemblage in control seawater and in seawater with previous *Cassiopea* incubation. $O_2$ consumption rates of solely mysids were calculated by subtracting microbial $O_2$ consumption rates from combined $O_2$ consumption rates (microbes plus mysids) for both control seawater and seawater with previous *Cassiopea* incubation, assuming that predation of mysids on microbes had no significant effect on $O_2$ consumption rates of the microbial assemblage. The high volume (120 ml) to animal (only 5 mysids) ratio may have justified this assumption. $O_2$ consumption rates were expressed as mg $O_2$ $mysid^{-1}$ $d^{-1}$.

Organic carbon degradation was calculated assuming that 1 mol of added organic C was mineralized by 1 mol of consumed $O_2$. TOC degradation rates were calculated dividing microbial carbon degradation rates by the respective amounts of available TOC.

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 experiments. 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 (Wild et al., 2009).

### 2.4. 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 added to the tank water. Immediately before addition of $Na^{15}NO_3$, the water flow was stopped. 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. 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 daily addition of $Na^{15}NO_3$. 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).

### 2.5. Sampling of labelled Cassiopea- and coral-derived organic matter

For triggering mucus release 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. In order to prevent contamination of released mucus with draining seawater, the mucus of the first 60 s was discarded and only subsequently released mucus

was collected in a 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 was carried out according to a modified technique described in Wild et al. (2005b). Briefly, *Fungia* and *Ctenactis* polyps were washed in fresh running seawater for 1 min and subsequently exposed to air which immediately triggered mucus release. The initial 30 s of mucus release was 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 analyses, 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 h and kept dry until further analysis, as described below.

### 2.6. Incubation experiments with labelled organic matter and I. tsurnamali

*Cassiopea*- and coral-derived mucus enriched with the heavy isotope $^{15}N$ were provided to *I. 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 *I. 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 ($\delta\ ^{15}N = 177 \pm 2.0$‰), *Cassiopea*-derived mucus (jellyfish mucus = JM) 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 ($\delta\ ^{15}N = 925.2 \pm 67.0$‰), coral-derived mucus (coral mucus = CM) as reference. The remaining six glass dishes without any supplement served as controls. Experiments were conducted in sterile seawater and 0.2 µ filtered JM and CM 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 were 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 JM treatment samples), 4.0 and 25.0 h (JM treatment, CM treatment and control samples). All sampled mysids were washed and filtered on pre-combusted GF/F filter 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 JM samples (5.97–6.17 mg $O_2$ $L^{-1}$) followed by the CM samples (6.58–6.61 mg $O_2$ $L^{-1}$) and the controls (6.92–6.96 mg $O_2$ $L^{-1}$). Hence, all $O_2$ concentrations were well above critical values.

### 2.7. Analyses of samples

DOC concentrations were determined by high-temperature catalytic oxidation (HTCO) using a Rosemount Dohrmann DC-190 total organic carbon analyser and potassium hydrogenphtalat as standard (standard deviation <3%). Each sample was acidified by adding 100 µl of 20% phosphoric acid to pH <2 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 ($n \geq 3$) were 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.

Total organic carbon (TOC) concentrations in the incubation waters were calculated by adding DOC and POC concentrations.

## 3. Results

### 3.1. Release of organic matter by Cassiopea sp.

Tables 1 and 2 give an overview on POM and DOC release rates as well as on POC and DOC concentrations 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 surface area $h^{-1}$ and from 1.1 to 5.0 mg PN $m^{-2}$ jellyfish surface area $h^{-1}$. In contrast, DOC release was highly variable with 44% of all incubated jellyfish exhibiting net DOC uptake. The POC/PN ratio of the released organic matter of $11.0 \pm 0.3$ (mean ± standard error; $n=18$) was significantly ($p<0.01$, Mann–Whitney *U* test) elevated compared to the controls ($10.2 \pm 0.1$). 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.

### 3.2. 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

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

| Experiment | 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 |

**Table 2**
POC, DOC and TOC concentrations, microbial $O_2$ consumption and total organic carbon degradation rates for incubation water samples with previous *Cassiopea* incubation and controls for respective experiments (Exp.). Values are given as mean ± SD. * indicates significant differences of *Cassiopea*-incubation water to the controls.

| Exp. | Controls | | | | | *Cassiopea*-incubation water | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | POC conc. (µg C $ml^{-1}$) | DOC conc. (µg C $ml^{-1}$) | TOC conc. (µg C $ml^{-1}$) | $O_2$ consump. (mg $L^{-1}$ $d^{-1}$) | Carbon degradat. (% TOC $h^{-1}$) | POC conc. (µg C $ml^{-1}$) | DOC conc. (µg C $ml^{-1}$) | TOC conc. (µg C $ml^{-1}$) | $O_2$ consump. (mg $L^{-1}$ $d^{-1}$) | Carbon degradat. (% TOC $h^{-1}$) |
| 1 | 0.12 ± 0.01 | 1.4 ± 0.4 | 1.5 ± 0.3 | 0.1 ± 0.1 | 0.14 ± 0.09 | 0.58 ± 0.14 | 1.5 ± 0.3 | 2.1 ± 0.3 | 1.8 ± 0.6 | 1.3 ± 0.4 |
| 2 | 0.10 ± 0.01 | 1.5 ± 0.2 | 1.6 ± 0.2 | 0.2 ± 0.1 | 0.17 ± 0.10 | 1.06 ± 0.54 | 1.8 ± 0.2 | 2.8 ± 0.8 | 2.3 ± 0.5 | 1.4 ± 0.6 |
| 3 | 0.09 ± 0.01 | 1.6 ± 0.3 | 1.6 ± 0.2 | 0.6 ± 0.1 | 0.63 ± 0.17 | 0.38 ± 0.11 | 1.4 ± 0.5 | 1.8 ± 0.6 | 1.4 ± 0.6 | 1.4 ± 0.6 |
| Mean | 0.10 ± 0.01 | 1.5 ± 0.3 | 1.6 ± 0.3 | 0.3 ± 0.3 | 0.27 ± 0.24 | 0.67 ± 0.42* | 1.6 ± 0.4 | 2.2 ± 0.7* | 1.8 ± 0.7* | 1.4 ± 0.5* |

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

Natural $\delta^{15}N$ of freshly collected mysids was 3.9 ± 0.1 ‰ ($n = 15$). Mysids removed from JM 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 (Fig. 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 *I. tsurnamali*.

### 3.3. Microbial degradation of Cassiopea-derived organic matter

While DOC concentrations were similar between *Cassiopea*-incubation waters (0.9–2.1 µg C $ml^{-1}$) and controls (1.0–2.1 µg C $ml^{-1}$), POC and TOC concentrations in all *Cassiopea*-incubation waters were significantly ($p < 0.01$, Mann–Whitney *U* test) higher than those in the controls (Table 2). Microbial $O_2$ consumption rates (Table 2) in *Cassiopea*-incubation water samples ranged from 0.91 to 3.03 mg $O_2$ $L^{-1}$ $d^{-1}$ ($n = 18$) and were significantly elevated ($p < 0.001$, Mann–Whitney *U* test) compared to microbial $O_2$ consumption rates in the controls ranging from 0.05 to 0.70 mg $O_2$ $L^{-1}$ $d^{-1}$ ($n = 15$). TOC degradation rates in the *Cassiopea*-incubation waters ranged from 0.27 to 2.14% $h^{-1}$ thus were significantly ($p < 0.001$, Mann–Whitney *U* test) higher than TOC degradation rates in the controls (0.05–0.80% $h^{-1}$). Statistical analysis revealed no correlation between DOC concentration and microbial $O_2$ consumption ($p = 0.24$, 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).

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. However, POC and DOC concentrations as well as the POC/PN ratio remained similar compared to experiments 1 and 2.

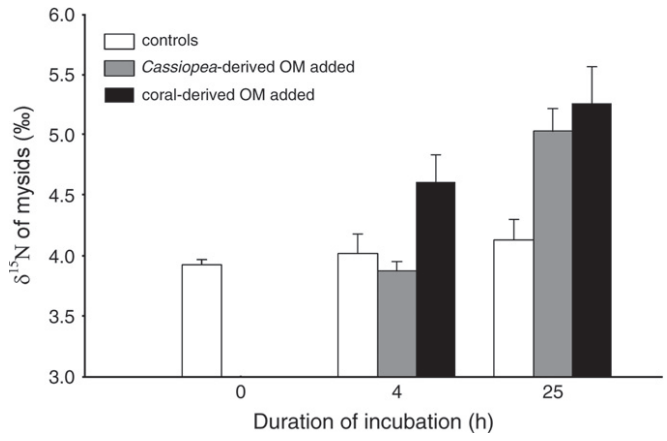


**Fig. 1.** Stable nitrogen isotopic signature of mysids provided with $^{15}N$-labelled *Cassiopea*- and coral-derived organic matter.

### 3.4. Degradation of Cassiopea-derived organic material by zooplankton

During the second incubation experiment, all *I. 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 5.9 to 24.8 µg $O_2$ $mysid^{-1}$ $d^{-1}$ ($n = 12$) whereas $O_2$ consumption of mysids in the control incubation water samples ranged from 1.0 to 5.1 µg $O_2$ $mysid^{-1}$ $d^{-1}$ ($n = 10$). 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 *I. 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 $h^{-1}$ $mysid-^{1}$ ($n = 10$).

## 4. Discussion

### 4.1. Release of organic matter by Cassiopea sp.

Copious amounts of mucus released by jellyfish are colloidal in nature (Wells, 2002). Colloidal material is defined as particles with a linear dimension between the size range from 0.001 to 1 µm (Hiemenz and Rajagopalan, 1997). A large fraction of colloids (0.001–0.2 µm) therefore was included by our DOC measurements, while another part (0.6–1 µm) was covered by the POM measurements of this study. However, some colloidal material within the size range of 0.2–0.6 µm released by the jellyfish may not have been covered by either DOM or POM measurements. Organic matter release rates presented here therefore can be considered as rather conservative. Microbial activity may have resulted in degradation of organic matter during the incubation, which may have led to an underestimation of release rates. Estimations revealed *Cassiopea*-derived POM to be degraded at circa 5% $h^{-1}$ (see below) which could lead to a microbial utilization of up to 30% of the released POC within the incubation time of 6 h. As DOC concentrations were similar in the controls and in *Cassiopea*-incubation waters, underestimations of release rates due to microbial consumption can be neglected.

**Table 3**
$O_2$ consumption rates of mysids in incubation waters with previous *Cassiopea* incubation (input of *Cassiopea*-derived organic matter) and controls (d.e. = data excluded) of the respective experiments (Exp.). Values are given as mean ± SD.

| Exp. | Controls | | Organic matter addition | |
|---|---|---|---|---|
| | N | $O_2$ consumption (µg $O_2$ mysid$^{-1}$ d$^{-1}$) | N | $O_2$ consumption (µg $O_2$ mysid$^{-1}$ d$^{-1}$) |
| 1 | 5 | 3.8 ± 1.6 | 6 | 13.0 ± 4.2 |
| 2 | 5 | d.e. | 6 | d.e. |
| 3 | 5 | 3.7 ± 0.4 | 6 | 15.2 ± 5.9 |

All release rates were correlated to the surface area of the jellyfish. A geometric approach was chosen in order to approximate the relatively complex surface area of *Cassiopea*. However, such a geometric approach does not include surface enlargement due to appendages. The absolute release rates related to the 3D surface area of the animal should therefore be regarded with caution, but the presented 2D release rates are highly suitable for calculations using data on benthic coverage as conducted in this study.

Nevertheless, surface area determination is subject to similar problems within most cnidarian taxa (e.g. corals, hydroids), which are characterised by a very similar blueprint and ensuing morphological complexity. Established methods such as wax coating or aluminium foil wrapping techniques, computerized tomography or simple geometric approaches usually neglect surface enlarging parts, e.g. extracted tentacles. Our approach therefore allows maximal comparability of *Cassiopea* organic matter release rates data with data generated with similar methodology in mentioned reference studies.

All release rates present net release rates, as the jellyfish holobiont including the animal, zooxanthellae and associated microbes may have taken up organic matter, particularly DOM.

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 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, which likely consisted of mainly mucoid organic matter as fecal pellets were largely removed and precautions were made to prevent contamination via sloppy feeding. This POM release was higher than reported for other warm water coral reef cnidaria under submersed conditions (Table 4). Only Herndl and 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, in press), 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 and Velimirov (1986) calculated a release of 32 mg mucus-derived C m$^{-2}$ seafloor area d$^{-1}$ by the Mediterranean coral *C. 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 of POC and PN in the Northern Gulf of Aqaba related to the seafloor area may be comparable to that described for other cnidarians.

### 4.2. 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 *I. tsurnamali* was demonstrated. Owing to the experimental setup (e.g. conducting the experiments in 0.2 µm filtered seawater, providing 0.2 µm filtered mucus) 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. As the labelled mucus was 0.2 µm filtered and thus provided in the dissolved form, the question remains concerning possible uptake mechanisms of DOM by mysids. To date, there is no study demonstrating DOM uptake by mysidacae, but there is some evidence for another Arthropode, *Neocalanus plumchrus* (Copepoda) to take up glucose via dermal glands and midgut (Chapman, 1981). The same may possibly apply for *I. tsurnamali*. Further, Chin et al. (1998) demonstrated that DOM polymers can spontaneously assemble to form polymer gels which could have happened and would have led to the availability of labelled particulate organic matter.

Regardless of the mechanisms, the present study could demonstrate uptake of jellyfish organic matter by mysids. The results of this study thereby append the scyphozoan *Cassiopea* sp. to the list of coral reef cnidaria providing organic matter to other coral reef organisms via the release of organic matter, particularly mucus. This study also demonstrates uptake of coral-derived nitrogen by *I. tsurnamali*, and

**Table 4**
Release of particulate organic matter by various cnidarian taxa (n.m. = not measured).

| 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.m. | n.m. | Perspex chambers | Crossland (1987) |
| Gulf of Aqaba/Eilat | *Stylophora pistillata* | Anthozoa | 2.7–4.0 | n.m. | n.m. | Perspex chambers | Crossland (1987) |
| Laboratory | *Acropora* sp. | Anthozoa | 7.8 ± 2.1 | 1.1 ± 0.3 | 8.7 ± 1.0 | Beaker | Niggl et al. (2009) |
| Bight of Piran | *Cladocora cespitosa* | Anthozoa | 58 | n.m. | n.m. | Beaker | Herndl and Velimirov (1986) |
| Gulf of Aqaba/Eilat | *Cassiopea* sp. | Scyphozoa | 21 ± 9 | 2.3 ± 1.1 | 11.0 ± 1.1 | Beaker | Present study |

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 can likely use these energy-rich components for their metabolic demands. This may be reflected by 3.8-fold elevated $O_2$ consumption rates of mysids transferred to *Cassiopea*-incubation waters compared to mysids in control waters. Likely, mysids mineralize both present microbes as well as *Cassiopea*-derived organic matter. However, assuming similar microbe-mineralization rates by the mysids in control seawater and in water with previous *Cassiopea* incubation, elevated $O_2$ consumption rates can only be attributed to the presence of *Cassiopea*-derived organic matter, thus indicate mineralization of *Cassiopea*-derived organic matter by *I. tsurnamali*.

The present study further demonstrated increased microbial $O_2$ consumption rates in *Cassiopea*-incubation waters compared to controls (Table 2). As DOC concentrations in the *Cassiopea*-incubation waters were not increased compared to the controls, the increases in microbial $O_2$ consumption may largely be attributed to released POC. This may explain why POC concentrations correlated to microbial $O_2$ consumption rates, while no correlation could be found between DOC concentrations and microbial $O_2$ consumption rates.

Attributing increases in microbial $O_2$ consumption largely to increases in POC concentrations allows an estimation of degradation rates of *Cassiopea*-derived POC of $5.1 \pm 2.7\%\ h^{-1}$.

A comparison to microbial degradation of coral mucus, which can dominate suspended POM in coral reef waters (Johannes, 1967; Marshall, 1968), helps to put the results of the present study in context. Wild et al. (2004b) reported a microbial respiration of 10.7 mg $O_2$ $L^{-1}$ $d^{-1}$ 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 (1.5 mg $O_2$ $L^{-1}$ $d^{-1}$) 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 of the present study 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.

### 4.3. Energy and nutrient pathways

There are two possible pathways explaining how *Cassiopea* could have utilized inorganic $Na^{15}NO_3$. Likely both pathways described in the following may have occurred. On the one hand, the jellyfish may have taken up provided $^{15}NO_3$ indirectly via the microbial loop (Azam et al., 1983); mysids may feed on bacterivorous protozoa which took up $^{15}NO_3$-assimilating bacteria (Caron, 1994). A second possible pathway is the direct uptake of $^{15}NO_3$ by the jellyfish. 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 supported by the study of Wilkerson and Kremer (1992), who reported assimilation of dissolved inorganic N and P from the water column by the zooxanthellae of *Linuche ungiculata*.

This assimilated nitrogen is likely transferred from the algal endosymbionts to their jellyfish host. 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 like in anthozoans do also likely occur in their scyphozoan relatives (Pitt et al., 2009), which is supported by the results of the present study.

DOC and dissolved free amino acids released by jellyfish have been described as easily 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 $O_2$ consumption rates of the microbial assemblage could be found, but POC concentrations significantly correlated with $O_2$ consumption of the natural microbial assemblage. 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.

### 4.4. Ecological implications

The commensalistic relationship between *Cassiopea* sp. and *I. 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 *I. 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).

Besides *Cassiopea* sp., 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 *I. 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 to 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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Contributors: W. Niggl was responsible for the implementation of the experiments. W. Niggl and C. Wild designed the experiments and prepared the manuscript. M. Naumann, U. Struck and R. Manasrah assisted in the experiments and sample analyses. **[SS]**

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# 6

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

**Christian Wild[1], Malik S. Naumann[1], Wolfgang Niggl[1] and Andreas F. Haas[1]**

[1]Coral Reef Ecology Group (CORE), GeoBio-Center & Department of Earth and Environmental Science in the Faculty of Geosciences, Ludwig-Maximilians Universität, Richard Wagner Str. 10, 80333 München, Germany

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NOTE

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**Christian Wild*, Malik Naumann, Wolfgang Niggl, Andreas Haas**

**Coral Reef Ecology Group (CORE), GeoBio-Center & Department of Earth and Environmental Science, Ludwig-Maximilians-Universität, Richard Wagner Str. 10, 80333 München, Germany**

ABSTRACT: Mucus, a complex composed primarily of carbohydrates, is released in similar quantities by scleractinian warm- and cold-water reef corals, and can function as an important carrier of organic material from corals to a range of consumers, microbes in particular. 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 coral genera. Arabinose was the major mucus carbohydrate component for the genus *Acropora*, but was not found in cold-water coral mucus. Mucus derived from corals of the genus *Fungia* contained significantly more fucose than the mucus of all other coral genera. However, comparison of mucus carbohydrate composition for the warm- and cold-water corals in the present study and in the literature revealed no significant differences. This indicates use of similar carbohydrate components (with the exception of arabinose) during mucus synthesis by scleractinian corals, largely irrespective of zooxanthellate or azooxanthellate carbon supply mechanisms.

KEY WORDS: Warm-water coral · Cold-water coral · Mucus · Chemical composition · Carbohydrate · Microbes · Degradability



## INTRODUCTION

Both scleractinian warm- and cold-water corals (termed WC and CC hereafter) continuously release mucus into their surroundings in similar quantities (Wild et al. 2005a, 2008) for various purposes (reviewed in Brown & Bythell 2005). This mucus is released in such quantities that it can dominate the suspended matter around WC reefs (Johannes 1967, Marshall 1968) and may also control the carbon cycle in the water column above CC reefs by stimulating microbial growth that contributes to fast conversion of coral-derived dissolved organic carbon (DOC) into particulate organic carbon (POC) (Wild et al. 2008, 2009).

Previous studies confirmed the important function of WC derived mucus as an energy carrier and particle trap in the reef ecosystem (Wild et al. 2004a, Huettel et al. 2006, Naumann et al. 2009). Coral mucus is 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 CC derived mucus, fauna–microbe interactions via this material and its fast recycling by planktonic microbes have been observed (Wild et al. 2008). Supplementary research revealed similar planktonic microbial degradation of mucus released by CC coral *Lophelia pertusa*, compared to degradation of the carbohydrates starch and glucose (Wild et al. 2009).

However, information about the chemical composition of WC derived mucus is very limited and is non-existent for CC derived mucus. WC derived mucus has been described as a primarily carbohydrate complex (Coffroth 1990), but more detailed chemical analyses

*Email: c.wild@lrz.uni-muenchen.de

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 6 different coral species within the genus *Acropora* and found arabinose, mannose, galactose, glucose and N-acetyl glucosamine present in all samples, whereas rhamnose, fucose and xylose were detected only in some samples. Such differences in mucus composition may control microbial community composition in WC (Allers et al. 2008) and CC reef habitats (Schöttner et al. 2009), with ensuing effects on microbial activity.

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

Substrate specificity in marine polysaccharide complexes is, however, critical for microbial degradation and concomitant organic matter recycling (Arnosti 2000). The present study therefore presents carbohydrate compositions of mucus released from dominant WC genera (*Acropora*, *Stylophora*, *Pocillopora*, *Fungia* and *Ctenactis*) in comparison to the 2 cosmopolitan CC 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 here for the first time.

## MATERIALS AND METHODS

**Collection of mucus samples.** Warm-water scleractinian corals were collected by SCUBA from water depths of ca. 5 m within a fringing reef close to the Marine Science Station in Aqaba, Jordan (29° 27′ N, 34° 58′ E), during 3 seasonal expeditions (Aug–Sep 2007, Feb–Mar 2008 and May 2008). For each mucus sampling, 4 to 6 different *Acropora*, *Stylophora* or *Pocillopora* colonies (diameter: 21 to 45 cm) or polyps of *Fungia* or *Ctenactis* (diameter: 21 to 43 cm) were used. All coral colonies or polyps were kept in flow-through aquaria at *in situ* temperature and light availability for 24 to 48 h prior to mucus sampling in order to avoid mucus contamination because of lesion leakage. Mucus was then collected from each coral genus by using the methodology described in Wild et al. (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 was 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 the RV 'Polarstern' expedition ARK-XXII/1a, or by a remotely operated vehicle (ROV) of type Sperre SUBfighter 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 Reefs, 4 to 8 fragments (length: 10 to 25 cm) from different colonies of the genera *Lophelia* (both reefs) and *Madrepora* (only Røst Reef) were collected and kept in seawater at *in situ* temperature for at least 5 d prior to mucus sampling in order to avoid mucus contamination because of lesion leakage. This maintenance water was collected from either the water depth of coral sampling (Røst Reef) or pumped from the field (water depth: ca. 50 m) and prefiltered over coarse sand (Tisler Reef). Water was exchanged at a rate of 50% at least every second day. Corals were not externally fed during maintenance in the aquaria, but could feed on dissolved and particulate natural organic matter suspended in the incubation water. Maintenance conditions of corals were therefore very close to *in situ* conditions. Mucus was then collected from both cold-water coral genera during the 2 expeditions as described above in volumes of 2 to 10 ml. Coral mucus samples were kept frozen at –20°C until further analysis.

**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 Da. A length of membrane sufficient to hold 2 ml of liquid was cut off from the 10 m strip and washed using deionized, sterile water. The membrane was then filled with approximately 2 ml of sample and placed in a 4 l bucket that was continuously filled with new deionized, sterile water from the bottom and emptied from the top. A stir bar was employed to aid mixing at 4°C. After 3 d, the samples were removed, frozen and lyophilized. Glycosyl composition analysis was performed by combined gas chromatography-mass spectrometry (GC-MS) of the per-*O*-trimethylsilyl (TMS) derivatives of the monosaccharide methyl glycosides produced from the sample by acidic methanolysis. An aliquot was taken from each sample and added to separate tubes with 40 µg of inositol as the internal stan-

dard. Methyl glycosides were then prepared from the dry sample following a mild acid treatment by methanolysis in 1 M HCl in methanol at 80°C for 16 h, followed by re-*N*-acetylation with pyridine and acetic anhydride in methanol (for detection of amino sugars). The sample was then per-*O*-trimethylsilylated by treatment with Tri-Sil (Pierce) at 80°C for 0.5 h, as described in York et al. (1986) and Merkle & Poppe (1994). GC-MS analysis of the TMS methyl glycosides was performed on an AT 6890N GC interfaced to a 5975B mass selective detector (Agilent Technologies), using a Supelco EC-1 fused silica capillary column (30 m × 0.25 mm ID).

**Statistical analysis.** *U*-rank sum tests after Wilcoxon, Mann and Whitney were carried out for all statistical evaluations as this test does not require homogeneity of variances or a normal distribution.

## RESULTS AND DISCUSSION

C6 sugars (glucose, mannose and galactose) occurred most often, followed by deoxysugars (fucose and rhamnose), amino sugars (N-acetyl glucosamine) and C5 sugars (arabinose and xylose) (Table 1). The monosaccharide arabinose, often detected as a compound of biopolymers such as hemicellulose and pectin, was only found in mucus released by warm-water corals of the genus *Acropora*, where it was the major carbohydrate component. Analysis of all available similar data sets on the carbohydrate composition of WC mucus from the literature (Richards et al. 1983, Meikle et al. 1988, Wild et al. 2005a) confirmed that *Acropora* mucus (n = 8 samples from different species including *A. aspera*, *A. digitera*, *A. formosa*, *A. millepora*, *A. nobilis* and *A. pulchra*) contained significantly

Table 1. Carbohydrate composition (in mole percentage of all detected carbohydrates) of mucus released from different scleractinian warm- and cold-water coral genera investigated in the present study in comparison with all available data from the literature. Ara: arabinose; Rha: rhamnose; Fuc: fucose: Xyl: xylose; Man: mannose; Gal: galactose; Glc: glucose; GlcNAc: N-acetyl glucosamine; nd: not detected; n/a: not analyzed. Glucuronic acid, galacturonic acid, N-acetyl galactosamine and N-acetyl mannosamine could not be detected in any of the samples

| Origin (season) | Ara | Rha | Fuc | Xyl | Man | Gal | Glc | GlcNAc | Source |
|---|---|---|---|---|---|---|---|---|---|
| *Acropora* | | | | | | | | | |
| Aqaba, Jordan (summer) | 76.4 | nd | 6.5 | nd | 5.7 | 3.7 | 1.2 | 6.6 | Present study |
| Heron Island, Australia | 50.8 | nd | 5.5 | nd | 10.6 | 6.2 | 13.2 | 13.7 | Wild et al. (2005a) |
| | 13.9 | 2.8 | 5.0 | 4.0 | 12.0 | 5.3 | 40.5 | 16.4 | |
| | 36.7 | nd | 5.6 | nd | 12.8 | 5.4 | 22.2 | 17.2 | |
| | 63.2 | nd | nd | nd | 11.1 | 5.3 | 12.5 | 7.9 | |
| | 24.6 | 8.0 | 6.6 | 4.7 | 13.4 | 5.9 | 22.1 | 10.6 | |
| | 25.4 | nd | 7.8 | 9.8 | 11.1 | 2.9 | 32.2 | 10.7 | |
| Magnetic Island, Australia | 47.0 | n/a | 2.0 | nd | 18 | 2.0 | 1.0 | 29.0 | Meikle et al. (1988) |
| *Ctenactis* | | | | | | | | | |
| Aqaba, Jordan (winter) | nd | nd | 5.2 | nd | 22.1 | 6.0 | 5.9 | 60.8 | Present study |
| *Fungia* | | | | | | | | | |
| Aqaba, Jordan (spring) | nd | nd | 68.4 | nd | 31.6 | nd | nd | nd | Present study |
| Aqaba, Jordan (summer) | nd | nd | 78.7 | nd | 15.0 | 0.7 | 0.9 | 4.7 | |
| Aqaba, Jordan (winter) | nd | nd | 85.8 | nd | 14.2 | nd | nd | nd | |
| Magnetic Island, Australia | 2.0 | n/a | 41.0 | 2.0 | 19.0 | 4.0 | 3.0 | 22.0 | Meikle et al. (1988) |
| *Pocillopora* | | | | | | | | | |
| Aqaba, Jordan (winter) | nd | nd | 25.3 | nd | 49.5 | nd | 25.2 | nd | Present study |
| *Stylophora* | | | | | | | | | |
| Aqaba, Jordan (winter) | nd | nd | nd | nd | nd | nd | 100.0 | nd | Present study |
| *Pachyseris* | | | | | | | | | |
| Magnetic Island, Australia | 16.0 | n/a | 14.0 | nd | 12.0 | 46.0 | nd | 10.0 | Meikle et al. (1988) |
| *Madrepora* | | | | | | | | | |
| Røst Reef, Norway | nd | 31.4 | nd | nd | 42.6 | nd | 26.0 | nd | Present study |
| *Lophelia* | | | | | | | | | |
| Røst Reef, Norway | nd | nd | 8.0 | 1.5 | 18.8 | 4.7 | 9.8 | 57.2 | Present study |
| Tisler Reef, Sweden | nd | nd | nd | nd | 40.4 | nd | 59.6 | nd | |
| All warm-water corals (mean ± SE) | 22.3 ± 6.4 | 0.8 ± 0.6 | 22.3 ± 7.4 | 1.3 ± 0.7 | 16.1 ± 2.8 | 5.8 ± 2.7 | 12.0 ± 3.5 | 13.1 ± 3.0 | |
| All cold-water corals (mean ± SE) | nd | 10.5 ± 10.5 | 2.7 ± 2.7 | 0.5 ± 0.5 | 33.9 ± 7.6 | 1.6 ± 1.6 | 31.8 ± 14.7 | 19.1 ± 19.1 | |

($p < 0.001$) more arabinose than all samples (n = 8) from 5 other WC genera in the present study. Similarly, mucus derived from different corals of the genus *Fungia* (n = 4 samples in the present study) contained significantly ($p < 0.001$) more fucose than the mucus of all other 7 WC genera in the present study. This indicates a similar carbohydrate composition at the genus level for warm-water corals (Table 1).

The carbohydrate composition difference between the 2 *Lophelia* mucus samples (Table 1) may be explained by the different CC reef sampling locations with different environmental conditions, but not by differences in handling, as identical methodologies were used. This is supported by studies on WC indicating that quantity (Naumann et al. in press) and composition (Drollet et al. 1997) of released mucus can change when corals are exposed to different environmental parameters, e.g. light availability, UV radiation, water temperature or inorganic nutrient concentrations.

In the present study, the only carbohydrate component found in the mucus of all coral genera was glucose (Table 1), which represents a universal energy source for most organisms. Glucose contents in mucus from both WC and CC may explain its excellent microbial degradability described by several previous studies (Ducklow 1990, Wild et al. 2004a,b, 2005b). Besides glucose, the neutral monosaccharides arabinose, galactose, xylose and mannose, as well as the amino sugar N-acetyl-glucosamine, have been identified as important substrates supporting bacterial growth and contributing to the flux of labile dissolved organic matter (DOM) in marine waters (Rich et al. 1996, Riemann & Azam 2002). The concentration of labile monosaccharides in marine waters is usually low (Benner et al. 1992), as hydrolysable neutral sugars are subject to rapid microbial decomposition (Ogawa et al. 2001). Thus the finding that the carbohydrate fraction of coral mucus includes a heterogeneous mixture of labile monosaccharides explains the stimulating influence of both warm- and cold-water coral mucus on planktonic or benthic microbial metabolism (Wild et al. 2005b, 2008). The remaining monosaccharide constituents of coral mucus, fucose and rhamnose, likely contribute to the large pool of refractory marine DOM, as previous studies attested a low bacterial degradability of these deoxysugars (Amon et al. 2001, Ogawa et al. 2001).

Arabinose was not detected in any of the azooxanthellate CC, likely because this monosaccharide is usually not a constituent of animal cells, but a characteristic monosaccharide for photosynthetic organisms (Meikle et al. 1988). But rhamnose, fucose, xylose, mannose, galactose, glucose and N-acetyl glucosamine were found in both the mucus of zooxanthellate warm-water and azooxanthellate CC (Table 1), 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; see Table 1) revealed no significant differences in glycosyl composition ($p > 0.05$ for single glycosyls except arabinose) between WC (n = 16 samples) and CC genera (n = 3 samples). This indicates use of similar carbohydrate components during mucus synthesis by corals, largely irrespective of different energy supply mechanisms in zooxanthellate or azooxanthellate corals.

The importance of WC derived mucus as a trophic link was suggested in previous studies (Benson & Muscatine 1974, Ducklow & Mitchell 1979, Wild et al. 2004b). Cold-water coral-derived mucus, with its high content of the C6 sugars glucose and mannose, which have been shown to primarily fuel microbial production in aquatic ecosystems (Rich et al. 1996), may likely function as a key energy carrier from corals to microbes in CC reef ecosystems.

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# 7

# First evidence of coral bleaching stimulating organic matter release by reef corals

**Wolfgang Niggl[1], Martin Glas[1], Christian Laforsch[2], Christoph Mayr[3] and Christian Wild[1]**

[1]Coral Reef Ecology Group (CORE), GeoBio-Center & Department of Earth and Environmental Science in the Faculty of Geosciences, Ludwig-Maximilians Universität, Richard Wagner Str. 10, 80333 München, Germany

[2]Department Biology II and GeoBio-Center, Ludwig-Maximilians-Universität München, Großhadernerstrasse 2, 82152 Martinsried, Germany

[3]GeoBio-Center & Department of Earth and Environmental Sciences, Ludwig-Maximilians-Universität München, Richard Wagner Str. 10, 80333 München, Germany

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# First evidence of coral bleaching stimulating organic matter release by reef corals

**W. Niggl[1,2], M. Glas[1,2], C. Laforsch[2,3], C. Mayr[2], and C. Wild[1,2]**

1) Coral Reef Ecology Work Group (CORE), LMU, 80333 München, Germany
2) GeoBio-Center, LMU München, Richard-Wagner-Str. 10, 80333 München, Germany
3) Department Biology II, LMU München, Großhadenerstr. 2, 82152 Martinsried, Germany

**Abstract.** Corals continuously release mucoid organic exudates in order to clean their surfaces. Additionally, recent research highlighted the fact that this coral-derived organic matter acts as energy carrier and particle trap in the oligotrophic coral reef ecosystem, thus playing an important ecological role for recycling of matter and conservation of nutrients. Environmental stressors such as air exposure, high sediment loads and turbidity are known to increase the release of coral-derived organic matter. However although it is a common statement in the literature, scientific data verifying increased coral-derived organic matter release rates during temperature-induced bleaching events is lacking. This is critical as coral bleaching is the most extensive coral disease world-wide, and bleaching-induced changes in organic matter release potentially have far reaching consequences for reef functioning. In this study, a bleaching event was induced in the laboratory and release of dissolved and particulate organic carbon (DOC and POC) and nitrogen (PN) by the hermatypic coral *Acropora* spec. was quantified. Results show that during a bleaching event coral derived POC and PN release almost doubled compared to unstressed controls. This is the first experimental evidence that coral bleaching affects coral-derived organic matter release and potentially ensuing element cycles in tropical reef ecosystems.

**Key Words:** organic, matter, release, bleaching, stimulating

## Introduction

Mucoid organic exudates continuously released by corals (Meikle et al. 1988) play an important role in heterotrophic feeding (Duerden 1906, Yonge 1930, Lewis and Price 1975, Lewis 1977, Sleigh et al. 1988, Goldberg 2002) as a defence against smothering by sediment (Schumacher 1977), desiccation (Daumas and Thomassin 1977, Krupp 1984), physical (Brown and Bythell 2005) and UVR related (Drollet et al. 1997) damage, pathogens (Ducklow and Mitchell 1979, Rublee et al. 1980, Cooney et al. 2002) or pollutants (Mitchell and Chet 1975, Neff and Anderson 1981, Bastidas and Garcia 2004). However, as mucoid organic exudates can dominate the suspended particulate matter (Johannes 1967, Marshall 1968) in reef waters, they are obviously also major components of the coral reef ecosystem's nutrient cycles. Wild et al. (2004a) suggested that these coral-derived mucoid exudates may function as energy carrier and particle trap, thereby helping to conserve essential nutrients in oligotrophic tropical coral reefs. Astonishingly, although the importance of coral-derived organic matter for reef ecosystem functioning is well documented, studies quantifying release rates in correlation to variations in the key environmental factors are rare.

During the last decades, the phenomenon of coral bleaching, i.e. the whitening of corals due to the loss of their symbiotic algae and/or pigments (Brown 1996), has become more and more evident all over the world. Mass coral bleaching events, triggered mainly by increases in water temperature, have affected the world's coral reefs with increasing frequency and intensity since the late 1970s (Hoegh-Guldberg 2004). It is predicted that due to a continued increase in seawater surface temperature (Bijlsma et al. 1995) from the year 2030 large scale bleaching events will occur annually (Coles and Brown 2003), leaving only a very short recovery period for the affected corals.

Despite the apparent actual threat of coral bleaching for the survival of coral reefs, no data is available concerning the associated release rates of coral-derived organic matter. However, such data is indispensable in order to allow any prediction concerning nutrient and energy budgets for future environmental scenarios in coral reefs. In this laboratory study, release rates of particulate organic carbon (POC), particulate nitrogen (PN) and dissolved organic carbon (DOC) by hermatypic corals of the genus *Acropora* during a temperature induced bleaching event were investigated.

In contrast to the methods of previous studies that have investigated release rates of organic matter in

relation to varying environmental factors excluding coral bleaching (Crossland 1987, Riegl and Branch 1995, Wild et al. 2005a), this study distinguished released coral-derived organic matter (mucoid exudates and host cells) from algal (zooxanthellae)-derived organic matter.

**Material and Methods**

*Experimental description*

All experiments were conducted in August and September 2007 in the aquarium facilities of the Department Biology II of LMU München, Germany. One coral colony of the genus *Acropora* was fragmented three weeks prior to the subsequent experiment in order to allow healing and regeneration. After the fragmentation, 10 coral fragments (surface Area: 72.4 - 126.2 $cm^2$) were fixed on ceramic tiles (4.6 x 4.6 cm) using conventional coral glue. The experimental set-up consisted of two aquaria, the resident aquarium (215 L control aquarium), in which the fragments were maintained at non-heat-stress conditions, and a 30 L aquarium (bleaching aquarium), in which the temperature could be adjusted using a thermostat (HAAKE E52, Germany). The temperature in the resident aquarium was monitored by an ONSET underwater temperature logger revealing a temperature range between 25.6 °C and 29.3 °C in diurnal cycles. At the beginning of the incubation experiments, the coral fragments were placed in ten 1000 ml beakers filled with ca. 900 ml of filtered seawater (0.2 µm pore size) from the control aquarium. Manual transference into the beakers resulted in an expose to air of less than two seconds. Five beakers, each with one submersed colony (C1-C5), were placed in the control aquarium, thereby being exposed to the same temperature conditions as prior to the start of the experiment. The submersed fragments in the remaining five beakers were placed in the bleaching aquarium and acted as bleaching samples (B1-B5).

Initial water temperature for the bleaching samples was adjusted to 27 °C and kept at that temperature for 24 h, which complied with two incubation periods (one incubation period = 12 h). Introduction of compressed air ensured sufficient air supply and water circulation. After 12 h incubation, all coral colonies (B1-B5, C1-C5) were transferred to additional 1000 ml beakers filled with ca. 900 ml of freshly filtered seawater (0.2 µm). The incubation water of the precedent incubation period (IP) was kept for further processing as described below. This procedure was repeated every 12 h. After 24 h at 27 °C (IP 1 and 2), the temperature of the bleaching aquarium was raised every 12 h to a maximum of 32 °C at IP 7. Temperature was decreased to 29 °C and 27 °C for IP 8 and IP 9, respectively.

The occurrence of bleaching was defined as the point in time when zooxanthellae release rates of the bleaching samples were significantly higher than the release rates of the control samples. The surface areas of all coral fragments were measured as a reference parameter using the advanced geometry method described in Naumann et al. (submitted) and based on computer tomography reference as described by Laforsch et al. (2008).

*Incubation water processing*

The exact volume of the incubation water from all beakers was determined using a graduate 1000 ml glass cylinder with an accuracy of ± 20 ml. The incubation water was then stirred using a glass pipette and sub-samples (n = 1 for each parameter) were taken in order to determine the following parameters.

For subsequent **DOC measurements,** 5 ml of the incubation water were filtered through 0.2 µm syringe filters (FP 30/0.2 CA, Schleicher and Schell). The first 2 ml of the filtrate were discarded, but the following 3 ml were collected in precombusted brown glass bottles, which were instantly shock-frozen at -80 °C and kept frozen until analysis. For **POM quantification** (particulate organic matter), 50 ml of the incubation water were extracted and filtered by a vacuum filtration unit onto precombusted GF/F filters (Whatman, 25 mm diameter). Filters were dried for at least 48 h at 40 °C and kept dry until analysis. Another 50 ml were fixed with 2-3 drops of Lugol's solution and stored at room temperature for subsequent **enumeration of zooxanthellae** using counting towers and backlight microscopy at 400-times magnification (Axioplan, Zeiss Germany).

The remaining incubation water was fixed with formaldehyde (1 % formaldehyde end concentration) and stored in the dark at 4 °C until further treatment.

*Organic matter analysis*

POM analyses were conducted using an Elemental Analyzer NC 2500 for C- and N determinations (Carlo Erba, Italy). For calibration of the elemental content of the samples, two standards, Atropine ($C_{17}H_{23}NO_3$) and Cyclohexanone-2,4-dinitrophenylhydrazone ($C_{12}H_{14}N_4O_4$) were used. Obtained POC and PN values equal the total amount of released particulate organic matter (POMt). In order to obtain the released amounts of coral-derived particulate organic matter (POMc) the amount of released algal-derived organic matter (POMa) was subtracted from POMt**.** Consequently coral-derived organic matter is defined as any organic matter (mucoid exudates, host cells) released by the corals except algal cells.

For calculating the amount of released POMa, the POC and PN contents of a distinct number of

zooxanthellae was determined. Therefore a zooxanthellae suspension was produced by centrifugation (6000 g) of 400 ml incubation water from B2 after IP8. The pellet was resuspended in filtered seawater and the zooxanthellae concentration (9330 cells $ml^{-1}$) was determined (methodology see above). Dilution series of 0.1, 1.0, 10.0 and 50.0 ml of this solution were subsequently filtered in triplicates onto precombusted GF/F filters (Whatman). The filters were dried at 40 °C for at least 48 h before POM analysis as described above.

The released amounts of algal derived particulate organic matter (POMa) was determined by multiplying released numbers of zooxanthellae by the respective calculated carbon and nitrogen contents of a single *Symbiodinium* cell.

Bacteria abundances for 2 bleaching and 2 control samples were determined using standard DAPI coloration and fluorescence microscopy. Assuming a carbon content of 20 fg C per cell (Lee and Fuhrmann 1987), bacteria in the bleaching samples would account for 3.1 to 5.2 % of the total recorded C content. In the control samples, bacteria would account for 4.0 to 6.0 % respectively. In the light of these calculations microbial contribution was considered minor.

Unfortunately, bleaching samples B1, B4 and B5 showed necrosis after IP 7 (12 h at 32 °C). The incubation water of these fragments was therefore excluded from all further analyses unless otherwise stated.

DOC concentrations were determined by high temperature catalytic combustion (HTCO) using a Rosemount Dohrmann DC-190 total organic carbon (TOC) analyser and Potassium hydrogenphtalat as standard solution. Each sample was acidified by adding 100 µl of 20 % phosphoric acid and purged for 5 min in order to remove inorganic carbon. The DOC concentrations of each sample were measured five times. An outlier test was conducted, and the DOC concentrations of the remaining sub-samples were averaged.

## Results

*Induction of a bleaching event*

A bleaching event was induced in the laboratory by exposing the investigated coral fragments to temperatures increased by 3 to 5 °C (Fig. 1a). Zooxanthellae enumeration revealed that from the IP 5 (30 °C) to the IP 9 (27 °C) significantly more (Table 1) zooxanthellae were released by the coral fragments incubated under elevated temperature compared to the controls (Fig. 1a).

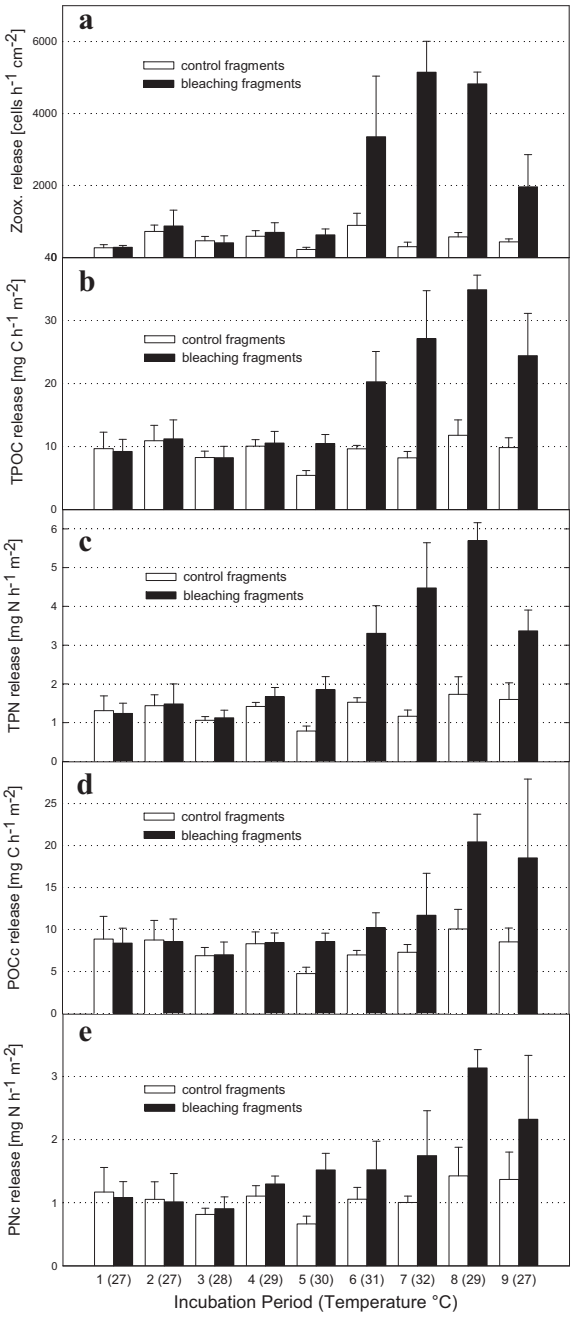


Figure 1: Summary of organic matter release rates during artificial bleaching experiment a) zooxanthellae release b) total POC release c) total PN release d) coral-derived POC release e) coral derived PN release.

*Release of POCt and PNt*

Throughout non-bleaching conditions there was no significant difference between the controls and the bleaching samples (Fig. 1b,c). Under bleaching conditions, from IP 5 (30 °C) until IP 9 (27 °C),

bleaching samples showed significantly (Table 1) higher POCt and PNt release rates. During IP 8 (29 °C), bleaching samples exhibited highest POCt and PNt release rates.

Regarding all bleaching samples (B1-B5), including those partially necrotic, POCt release rates were also highest during IP 8 (29 ° C) with release rates of 114 ± 75 mg C $h^{-1}$ $m^{-2}$ (mean ± SD, n = 5) and 14.0 ± 7.6 mg N $h^{-1}$ $m^{-2}$ (mean ± SD, n = 5).

Table 1: Summary of statistical analysis (independent samples t-test): given are p values for hypothesis for no differences between control fragments and bleaching fragments. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

| Temp | Zoox. | TPOC | TPN | POCa | PNa | POCc | PNc |
|---|---|---|---|---|---|---|---|
| **30 °C** | *** | *** | *** | *** | *** | *** | *** |
| **31 °C** | * | ** | *** | * | * | ** | 0.065 |
| **32 °C** | *** | ** | *** | *** | *** | 0.079 | * |
| **29 °C** | *** | *** | *** | *** | *** | ** | ** |
| **27 °C** | ** | ** | ** | ** | ** | * | 0.115 |

*Release of POMa and POMc*

The released amounts of algal derived particulate organic matter (POMa) directly correlated with the zooxanthellae release rates, as POMa release was calculated by multiplying the released numbers of zooxanthellae with obtained POC and PN content of 0.3 and 0.05 ng $cell^{-1}$, respectively (linear regression of zooxanthellae numbers against respective POC content, $R^2 = 0.999$). Consequently, POCa and PNa release of the bleaching samples was significantly higher than that of the control samples whenever bleaching, as defined above, occurred (Table 1).

Algal-derived POC and PN release of the bleaching samples was lowest at IP 1 (27 °C) and highest during IP 7 (32 °C). The highest increase of algae-derived POC/PN release was found during IP 8 (32 °C), when bleaching samples released 11 to 35 times more algae-derived POC and PN than the control samples.

POMc was calculated subtracting POMa from POMt. Throughout non-bleaching conditions there was no significant difference between the treatments concerning POCc and PNc release (Fig. 1d,e). Coral-derived POC release accounted for 76 to 91 % of the total released POC and coral-derived PN for 68 to 88 % of total released PN under non-bleached conditions. Bleaching samples during IP 5 (30 °C) released significantly (Table 1) more POCc and PNc compared to the controls. During bleaching, coral derived POC release accounted for 42 to 82 % of released POCt and coral-derived PN accounted for 38 to 82 % of total released PN. Maximum POCc and PNc release could be detected during IP 8 (29 °C).

*Release of DOC*

No significant differences between bleaching and control samples concerning DOC concentrations could be found after any incubation period and treatment.

## Discussion

*Coral bleaching and organic matter release*

Coral bleaching was induced at elevated temperatures (30 °C – 32 °C), but also occurred when temperature was decreased to 29 °C and 27 °C at the end of the experiment. This temporal delay may be explained by the effect of heat stress, which can lead to the breakdown of enzymatic pathways in plants and animals, resulting in metabolic or biochemical dysfunction (Cossins and Bowler 1987). Reinstalling these enzymatic pathways may take a few hours to days, depending on the damage evoked by heat stress. Thus, although temperatures were adjusted to non-bleaching conditions, the release rates were still elevated in the bleaching samples.

Besides the total release of POM, the exclusive release of particulate coral-derived organic matter was increased. This may be attributed to either increased release of mucoid exudates or increased release of coral cellular material as a consequence of the bleaching mechanism (e.g. host cell detachment). If the mechanism of bleaching, i.e. the release of zooxanthellae, was solely responsible for increased coral-derived organic matter release, the release of coral-derived organic matter should be highest when zooxanthellae release during bleaching was highest. However, coral-derived organic matter release was highest at 29 °C when zooxanthellae release had already decreased (Fig. 1a,d,e). Consequently, the mechanism of bleaching was very likely not the only factor responsible for increased coral-derived organic matter release. Therefore, increased release of mucoid exudates apparently co-occurred during bleaching.

The measured total organic matter release rates of the non-stressed control fragments are in the same range as release rates described in previous field studies (Table 2). Including coral fragments with partial necrosis, release rates were similar to those measured during air exposure (Table 2). As necrosis is one of five possible mechanisms resulting in expulsion of zooxanthellae (Gates et al. 1992), and commonly occurs during bleaching (Glynn et al. 1985), these findings underline the relevance of bleaching events for energy and nutrient cycles in the reef ecosystem. This is confirmed by the study of Wild et al. (2004b), who found that coral-derived organic matter is rapidly degraded by reef microbes, in contrast to zooxanthellae-derived organic matter, which may rather represent a loss of energy and nutrients for the reef ecosystem (Wild et al. 2005b).

Table 2: Summary of studies examining organic matter release rates by corals of the genus *Acropora*. In the present study 45 replicates are displayed, because 5 coral fragments were incubated at 9 different periods. (Note: Previous studies used old definition of mucus and did not distinguish between coral- and algal-derived organic matter).

| Study site | Stress | Mucus C release ($mg\ h^{-1}\ m^{-2}$) | | Mucus N release ($mg\ h^{-1}\ m^{-2}$) | | N | Method | Reference |
|---|---|---|---|---|---|---|---|---|
| Heron Island | Air expos. | 117 ± 79 | | 13 ± 8 | | 8 | Container | Wild et al. 2005 |
| Heron Island | No | 10 ± 5 | | 1.3 ± 0.8 | | 8 | Beaker | Wild et al. 2005 |
| Heron Island | No | 7 ± 3 | | 0.8 ± 0.4 | | 8 | Beaker | Wild et al. 2005 |
| Eilat | No | 1.4 - 4.2 | | | | | Perspex Chamber | Crossland 1987 |
| Aqaba | No | 1.0 - 3.0 | | 0.1 - 0.4 | | 5 | Beaker | Naumann unpubl. |
| | | coral-derived | algal-derived | coral-derived | algal-derived | | | |
| Laboratory | No | 7.8 ± 2.1 | 1.5 ± 0.8 | 1.1 ± 0.3 | 0.3 ± 0.1 | *45* | Beaker | This study |
| Laboratory | Bleach. 30° | 8.6 ± 1.0 | 1.9 ± 0.5 | 1.5 ± 0.3 | 0.3 ± 0.1 | 5 | Beaker | This study |
| Laboratory | Bleach. 31° | 10.2 ± 1.8 | 10.1 ± 5.0 | 1.5 ± 0.5 | 1.8 ± 0.9 | 5 | Beaker | This study |
| Laboratory | Bleach. 32° | 11.7 ± 5.0 | 15.4 ± 2.6 | 1.7 ± 0.7 | 2.7 ± 0.5 | 2 | Beaker | This study |
| Laboratory | Bleach. 29° | 20.4 ± 3.3 | 14.5 ± 1.0 | 3.1 ± 0.3 | 2.6 ± 0.2 | 2 | Beaker | This study |
| Laboratory | Bleach. 27° | 18.5 ± 9.4 | 5.9 ± 2.7 | 2.3 ± 1.0 | 1.0 ± 0.5 | 2 | Beaker | This study |

This study also showed that DOC release was not influenced by coral bleaching. This is surprising as Wild et al. (2004a) demonstrated that between 56 and 80 % of coral mucus can dissolve in the surrounding seawater. However, it is very likely that a high proportion of the released DOC was re-consumed by the coral and associated bacteria (Sorokin 1973, Al-Moghrabi et al. 1993). This explanation is supported by the studies of Ferrier-Pages et al. (1998) and Naumann et al. (unpublished data), who found that it is generally difficult to detect any DOC release by corals in a closed system such as a beaker.

Furthermore, DOM polymers can spontaneously assemble to form polymer gels, thus entering the POM pool (Chin et al. 1998), which could have lead to a removal of surplus DOC from the incubation water.

*Ecological implications*

Increased coral-derived POM release during bleaching can probably be attributed to increased release of cellular matter and/or to increased release of mucoid exudates. Increased release of cellular matter during bleaching can be explained by the mechanism of bleaching, which may lead to loss of parts of or entire coral cells. However, the reason for increased release of mucoid exudates is harder to surmise. Up to 45 % of carbon fixed daily by the zooxanthellae can be released as organic matter by the host coral (Davies 1984, Crossland 1987, Bythell 1988, Edmunds and Davies 1989). A bleached coral is in a state of energy shortage as the algal symbionts, which are capable of providing the coral host with up to 100 % of its daily metabolic energy requirements (Muscatine et al. 1981), are lost. Thus, it is not surprising that coral bleaching can affect the release rates of organic matter.

However, there are some ecological advantages and disadvantages of up-regulation of mucoid organic matter release during coral bleaching. On one hand, energy loss via mucoid organic matter release may further reduce the ability of corals to cope with bleaching, whereas on the other hand mucoid exudates release may function for heterotrophic feeding (reviewed by Brown and Bythell 2005), which could partly compensate the missing autotrophic contribution to the coral's energy demand during bleaching. Further, increased mucoid exudates release during bleaching may also help to protect the coral against high UV radiation often associated with coral bleaching (e. g. Jokiel 1980, Fisk and Done 1985, Gleason and Wellington 1993) as UV-absorbing substances such as mycosporine-like amino acids (MAAs) have been detected in coral mucus (reviewed by Dunlap and Shick 1998).

Coral mucus may play an important role in providing various defence capabilities against pathogenic organisms (reviewed by Brown and Bythell 2005). During bleaching, corals are in a state of stress owing to energy shortage and damaged epithelia, and thus are more vulnerable to pathogens which may occur with increased abundances at elevated temperatures. Increased mucus release may be a response to decrease vulnerability and support defence against pathogens.

Recent research revealed that azoxanthellate cold water corals release POM in comparable quantities to zooxanthellate warm water corals (Wild et al. in press). This indicates that the release of mucoid exudates by corals is largely decoupled from the presence of zooxanthellae and thus represents a general response to any kind of environmental stress, including bleaching.

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# 8

# Effect of water currents on organic matter release by three scleractinian corals

**Wolfgang Niggl[1], Christian Laforsch[2], Miriam Schutter[3], Roland Fuß[4], Christoph Mayr[5], Holger Saar[1] and Christian Wild[1]**

[1]Coral Reef Ecology Group (CORE), GeoBio-Center & Department of Earth and Environmental Science in the Faculty of Geosciences, Ludwig-Maximilians Universität, Richard Wagner Str. 10, 80333 München, Germany

[2]Department Biology II and GeoBio-Center, Ludwig-Maximilians-Universität München, Großhadernerstrasse 2, 82152 Martinsried, Germany

[3]Aquaculture and Fisheries Group, Wageningen University, P.O. Box 338, 6700 AH Wageningen, The Netherlands

[4]Helmholtz Zentrum München - Institute of Soil Ecology, Ingolstädter Landstrasse 1, 85764 Neuherberg, Germany

[5]Institute of Geography, Friedrich-Alexander-Universität Erlangen-Nürnberg, Kochstr.4/4, 91054 Erlangen

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# Effect of water currents on organic matter release by three scleractinian corals

**W. Niggl • C. Laforsch • M. Schutter • R. Fuß • C. Mayr • H. Saar • C. Wild**

**Abstract**
Organic matter released by corals fulfils an important role as a carrier of energy and particle trap in reef ecosystems. For this reason, organic matter release by corals has recently been quantified in several studies using incubation experiments under still water conditions. However, the impact of water currents, an essential abiotic factor in coral reefs, has not been investigated yet. This study therefore describes the findings of a series of experiments with three species of scleractinian corals (*Euphyllia* sp., *Montipora digitata* and *Galaxea fascicularis*) using two different types of closed-system flow-through chambers under different water current velocities ranging from 4 to 18 cm $s^{-1}$. After 4 - 5 h of incubation, release rates of particulate organic carbon (POC), particulate organic nitrogen (PON) and dissolved organic carbon (DOC) were measured. While no effect of current velocity on DOC release was detectable, POM (particulate organic matter) release by all investigated coral species was significantly increased by factors of 2 – 7 for POC and 2 - 3 for PON compared to still water conditions. After 3 weeks of water current exposure, no adaption of initially increased POM release was observed. The detected immediate effect of ambient water currents suggests that POM release by corals is mainly controlled by hydro-mechanical impacts, while DOC fluxes are rather influenced by the coral's physiological condition. These findings indicate that previous POM release quantification results are conservative estimates and may have underestimated actual in situ POM release. The ecological role of coral-derived POM may therefore be even more important than already assumed.

**C. Mayr • H. Saar • C. Wild**
Coral Reef Ecology Group (CORE), GeoBio-Center & Department of Earth and Environmental Science, Ludwig-Maximilians-Universität Munich, Richard Wagner Str. 10, 80333 München, Germany

**C. Laforsch**
Department Biology II and GeoBio-Center, Ludwig-Maximilians-Universität Munich, Großhadernerstraße 2, 82152 Martinsried, Germany

**M. Schutter**
Aquaculture and Fisheries Group, Wageningen University, P.O. Box 338, 6700 AH Wageningen, The Netherlands

**R. Fuß**
Helmholtz Zentrum München - Institute of Soil Ecology, Ingolstädter Landstrasse 1, 85764 Neuherberg, Germany

**C. Mayr**
Institute of Geography, Friedrich-Alexander-Universität Erlangen-Nürnberg, Kochstr.4/4, 91054 Erlangen

## INTRODUCTION

Extracellular release of dissolved and/or particulate organic matter is known from various marine taxonomic groups such as phytoplankton, macrophytes, gastropods, bivalves, zooplankton and several cnidarian species (e.g. Khailov and Burlakova 1969; Thomas 1971; Ducklow and Mitchell 1979; Kappner et al. 2000; Morton 2000; Steinberg et al. 2004). The reasons for extracellular release are manifold and diverse, but include active disposal of excess carbon (Fogg 1983), passive permeation through the cell membrane (Bjornsen 1988), protection, feeding or locomotion (summarized by Wotton 2004). Once released into the water column, the organic matter can fulfil important roles for aquatic ecosystem functioning in various ways. Dissolved organic carbon (DOM) is an important source of energy for heterotrophic bacteria (Cole et al. 1982; Baines and Pace 1991). After being processed by bacteria, DOM-derived energy enters the pelagic food chain via the microbial loop (Azam et al. 1983), thus becomes available for higher trophic levels. The importance of particulate organic matter (POM) release is particularly well documented for oligotrophic coral reef ecosystems. Released as mucus by hard corals, its adhesive particulate fraction efficiently traps e.g. planktonic algae, grains, foraminifers, detritus and also very small particles such as picoplanktonic cyanobacteria from the water column and enhances sedimentation velocity (Wild et al. 2004; Naumann et al. 2009a). It thus functions as both carrier of energy and trap of particles, thereby supporting benthic life and reducing loss of energy and nutrients from the reef ecosystem (Wild et al. 2004). Additionally, mucus exudates of e.g. corals or jellyfish may function as trophic vectors to microbes or many reef animals such as fish, crabs or

zooplankton (e.g. Knudsen 1967; Benson and Muscatine 1974; Herndl and Velimirov 1986; Niggl et al. 2010).

There have been several efforts to quantify organic matter release rates by coral reef organisms. In an early study, Ducklow and Mitchel (1979) investigated organic matter release rates by various benthic reef organisms including corals under artificial stress, exposing specimens to air thus found release rates not applicable to in situ conditions. In a more recent study, Wild et al. (2004) quantified coral organic matter release at the 6.9 km² reef rim of Heron Island (Great Barrier Reef, Australia) and calculated total daily release rates of at least 1.8 t carbohydrate polymers, 1.0 t of proteins and 0.2 t of lipids, solely by corals of the genus *Acropora*. Some studies also addressed the influence of environmental factors on organic matter release by corals. Thereby, the corals' trophic status, the ambient light and temperature conditions or different water sediment loads positively affect coral organic matter release (Crossland 1987; Riegl and Branch 1995; Ferrier-Pages et al. 1998; Niggl et al. 2009; Naumann et al. 2010). However, as previous incubation experiments were conducted under still water conditions, the influence of water flow on organic matter release by corals has not been assessed, although water flow is known to strongly impact corals' physiology and morphology (e.g. Dennison and Barnes 1988; Kaandorp et al. 1996; Sebens et al. 2003) and occurs during most times in the natural reef environment. Generally, water current-induced mass exchange and transport processes proceed by orders of magnitude faster than the exchange via molecular diffusion in still water (Huettel et al. 2003; Precht and Huettel 2003). In zero current incubation experiments organic matter fluxes are mainly driven by diffusive processes. Application of water current will enhance mass exchange processes, but may also facilitate mechanical exchange, thereby enhancing the release rates of organic matter. Main working hypothesis of this study therefore is that organic matter release by corals is stimulated by water flow. For this purpose, three different species of scleractinian corals were exposed to different water current velocities in an aquarium system and both dissolved and particulate organic matter fluxes were quantified using two different types of closed-system flow through chambers. The present study hereby complements previous studies implementing the first quantifications of organic matter release under in situ current conditions representing a further step towards precise quantifications under natural conditions.

# METHODS

This study consists of two separate experiments conducted in two different aquarium facilities and variations in the experimental procedure. Hence, the two experiments, comprising *Galaxea fascicularis* on the one hand and *Euphyllia* sp. and *Montipora digitata* on the other hand were described and analyzed separately. Organic matter release by *Euphyllia* sp. and *Montipora digitata* was quantified three times; before, after 1 and 3 weeks of continuous exposure to different current velocities. In contrast, organic matter release by *Galaxea fascicularis* was quantified only once without previous continuous exposure to different current velocities. Prior to the experiment, all colonies were kept under a non-laminar current with a current velocity < 2 cm $sec^{-1}$.

### Experiments comprising *Euphyllia* sp. and *Montipora digitata*

*Setup and experimental design:*

The experiments involving *Euphyllia* sp. and *Montipora digitata* were conducted in March 2010 in the aquarium facilities of the Coral Reef Ecology Group (CORE) at GeoBio-Center, LMU Munich, Germany. The closed-circuit coral culture system consisted of approximately 2000 L in three interconnected tanks hosting several hard and soft coral species. Three weeks prior to the experiment, coral fragments of *Montipora digitata* (19.6 – 56.0 cm² surface area) and *Euphyllia* spec. (5.5 – 33.1 cm² surface area) from the Indopacific (maintained in the aquarium system for at least 1 year prior to the experiments) were glued on ceramic tiles (4.6 x 4.6 cm) using Reef Construct (Aqua Medic). Fragments were kept in a 200 L flow-through tank illuminated by an Ocean Light 150 Plus (Aqua Medic) providing 12 h of light $day^{-1}$ with an intensity of 2000 – 3500 Lux

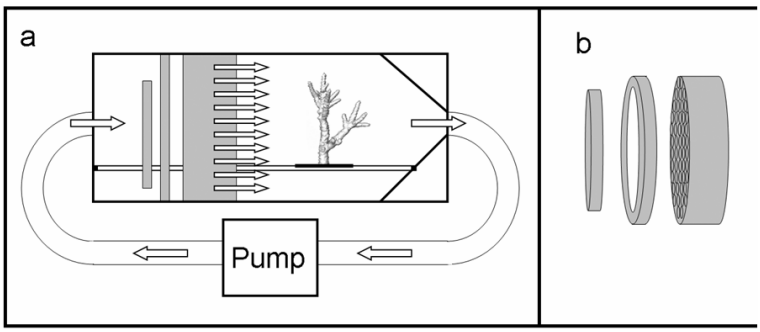


Fig. 1: Fig. 1: Schematic illustration of custom-made flow Plexiglas chamber with PVC tubes and submersible pump used for the experiments with *Euphyllia* sp. and *Montipora digitata* (panel a) and detailed view of the flow straightener with its 2 discs and the terminal perforated plate (panel b). Arrows indicate the water flow.

as measured with a Onset HOBO Pendant UA-002-64 light logger. During the experiment, the temperature ranged from 24.0 – 27.1 °C, the salinity from 38 – 40 as measured with a Onset HOBO Pendant UA-002-64 data logger and a refractometer (Aqua Medic) respectively.

*Setup for continuous exposure to different current velocities:*
For a total period of 3 weeks, 4 colonies of each species were exposed to low current (3.5 – 6.2 cm $sec^{-1}$) and 4 colonies of each species to high current (14.2 – 15.8 cm $sec^{-1}$). Therefore, the 200 L tank was divided into a high current and a low current section. A stream of unidirectional current was generated in the high current section by connecting a submersible pump (EHEIM) with a perforated PVC pipe functioning as water outlet. The low current section was placed downstream of the high current section with an interposed plastic mesh (mesh size 7.5 x 2.5 mm) reducing flow velocity accordingly. Coral colonies were randomly placed in the respective sections in a way that no coral was in the slipstream of another. Current velocity and direction was checked and readjusted weekly by tracking neutrally buoyant objects along a known distance using ruler and stopwatch (n > 45 measurements per section and week).

*Quantification of organic matter release:*
Quantification of organic matter release by *Montipora digitata* and *Euphyllia* sp. was conducted by incubating coral colonies in closed-system Plexiglas flow chambers (Fig. 1a). All incubations were conducted at the same daytime between 9 am and 3 pm. Water flow was created with submersible pumps (EHEIM) connected to the flow chambers via PVC tubes. The cylindrical flow chambers had a diameter of 12 cm and a total volume of 2.58 L. At the corals' position in the flow chamber a unidirectional current was ensured by the utilization of a flow straightener (Fig. 1b). Unidirectional current and appropriate current velocity were checked prior to the experiment by visually tracking neutrally buoyant objects along a known distance using ruler and stopwatch (n = 45 measurements per chamber).
After filling the flow chambers, the pumps and the PVC tubes with filtered (0.2 µm pore size) seawater from the culture system (see above), one coral colony was transferred to each chamber wearing non-powdered gloves and exposing the coral colony to air for ≤ 1 s. Subsequently, the chambers were closed, air was removed via a valve in the PVC tubes and the incubation was started at the respective current velocity (zero, low or high current). After 4 to 5 h of incubation, the coral colonies were quickly removed wearing non-powdered gloves, and the incubation water was instantly processed. Corals were handled touching only the ceramic tiles in order to avoid artificially increased organic matter release evoked by mechanical stress.
For later DOC (dissolved organic carbon) analysis, the incubation water was well mixed and 10 ml of incubation water was extracted using a 10 ml sterile syringe and filtered through a sterile syringe filter (VWR®, polyethersulfone membrane, 0.2 µm pore size). The first 4 ml of the filtrate was used to clean the filter and discarded, the following 6 ml collected in pre-combusted (450 °C; 6 h) 10 ml glass vials, which were instantly frozen at -20° C until further analysis. Prior to analysis defrosted water samples were acidified to a pH < 2 by adding 60 µl of 2M HCl and then purged with $O_2$ for 1 minute in order to remove inorganic carbon. Concentrations of DOC were determined using a Dimatoc 100 (Dimatech) and potassium hydrogenphthalate as a standard. Defrosted water samples were acidified to a pH < 2 by adding 60 µl of 2M HCl and then purged with $O_2$ for 1 minute in order to remove inorganic carbon. Multiple measurements of each sample (n = 3, standard deviation < 2%) were averaged.
For POM analysis, 1 L of the well mixed remaining incubation water was filtered onto pre-combusted GF/F filter (Whatman®, 25 mm diameter, 0.6 µm – 0.8 µm particle retention). The filters were dried for at least 48 h at 40 °C in a cabinet dryer and kept dry until further processing. POC (particulate organic carbon) and PON (particulate organic nitrogen) contents of the filters were analysed using an Elemental Analyzer NC 2500 for C- and N determinations (Carlo Erba, Italy) and atropine and cyclohexanone-2,4-dinitrophenylhydrazone (Thermo Quest, Italy) as elemental standards.
Concentrations of POC, PON and DOC in the well mixed filtered seawater before coral incubations were analysed accordingly (n = 1 for each parameter) and subtracted from the concentrations in the coral incubation waters in order to calculate amounts of coral-derived organic matter. Organic matter release rates were related to the surface area of the coral fragments and given as mg $m^{-2}$ $h^{-1}$. The surface area of all coral fragments was determined using the advanced geometry method described in Naumann et al. (2009b). Thereby, the highly variable and current depending surface areas of the tentacles were not included.
Release rates of total organic carbon (TOC) were calculated adding up POC and DOC release rates.
Quantification of organic matter release was conducted prior (Inc0), after one (Inc1) and three (Inc3) weeks of continuous exposure to either high or low water currents. At Inc0, non-conditioned fragments of each species were incubated under high (each species n = 8), low (each species n = 8) and zero current (*Euphyllia*, n = 20; *Montipora digitata*, n = 4). At Inc1 and Inc3, preconditioned fragments (n = 4 per species), i.e. fragments exposed to either high or low current for 1 or 3 weeks respectively and already incubated at Inc0 (see above), were incubated again. Thereby, each fragment was incubated in the current conditions they were preconditioned in.

### Experiments with *Galaxea fascicularis*

All experiments comprising *Galaxea fascicularis* were conducted in October 2008 in the aquarium facilities of Wageningen University in a second aquarium system comparable to that in Munich. Four colonies of *Galaxea fascicularis* (16.2 – 26.1 cm² surface area), grown from a single mother colony (clones) glued on 7 x 7 cm PVC plates using Reef Construct (Aquamedic), were kept in a 600 litre closed-circuit coral culture system. During the experiment, the temperature was maintained at 26.1 ± 0.4 °C and salinity at 34.4 ± 1.5. POM release quantifications of non-conditioned fragments were conducted under approx. 11, 19 and 27 cm $sec^{-1}$ (n = 4 for each velocity). POM sampling and analysis as well as incubation and transfer of corals were conducted as described above. In contrast to the studies in Munich, respirometric flow cells as described by Schutter et al. (2010) were used for incubations allowing additional measurements of coral net photosynthetic $O_2$ production. Net photosynthetic $O_2$ production was assessed subtracting initial $O_2$ concentration in the incubation water from $O_2$ concentration after the first 1.5 hours of incubation. Regression analysis revealed linear $O_2$ production over time so that potentially disturbing hyperoxia effects could be excluded. Net $O_2$ evolution rates were converted to net carbon fixation rates using a 1:1 molar ratio and relating fixation rates to the corals' surface areas.

### Statistical analyses

Data were tested for normal distribution using the Kolmogorov-Smirnov test and homogeneity of variances using the Levene test. Changes of organic matter release rates over time were investigated using repeated measures ANOVA or the non-parametric equivalent Friedman test when the assumptions of an ANOVA were not met.

Potential influence of the three applied current velocities (zero, low and high at Inc0 for *Euphyllia* sp. and *Montipora digitata*; 11, 19 and 27 cm $s^{-1}$ for *Galaxea fascicularis*) on organic matter release (DOC, POC, PON) was investigated applying one-way ANOVA or the Kruskal-Wallis test in cases when the preconditions for an ANOVA were not met.

Differences in DOC, POC and PON release rates by *Euphyllia* sp. and *Montipora digitata* between zero current conditions, low and high current conditions were investigated applying Mann-Whitney U tests and a Bonferroni correction for multiple pairwise comparisons (three comparisons resulting in a significance level of $p < 0.017$).

Influence of water current on photosynthesis by *Galaxea fascicularis* was tested using a one-way ANOVA.

## RESULTS

### Flow-dependent organic matter release by *Euphyllia* sp. and *Montipora digitata*

Incubation experiments revealed significant influence of water current velocities on the release of POC (Kruskal-Wallis test, $p = 0.019$) and PON (Kruskal-Wallis test, $p = 0.001$) by *Euphyllia* sp. and on POC release by *Montipora digitata* (Kruskal Wallis test, $p = 0.022$). In the next step, differences between release rates under zero, low and high current velocity were investigated conducting multiple pairwise comparisons using Mann-Whitney U tests and adjusting the significance level by application of the Bonferroni correction. This did not reveal any significant differences between zero, low and high current POC release by *Euphyllia* sp. although the Kruskal-Wallis test indicated a significant impact of current velocity (Table 1). This may be explained by the current-induced high variance of obtained release rates one the one hand and by the relatively small sample size, inevitable due to logistic reasons, on the other hand. However, all results supported the trend of lower POC and PON release rates under zero current conditions compared to when water current was applied (Table 1). Under zero current condition, non-preconditioned (Inc0) fragments of *Euphyllia* sp. on average released 2.1 – 3.8 times less POC and 2.4 – 2.8 times less PON than under low or high current conditions (Fig. 2, Table 1). Similarly, *Montipora digitata* released 3.8 – 6.8 times less POC and 2.3 – 2.5 times less PON under zero current conditions compared to conditions when current was applied. Also, there were no significant differences in the POC and PON release rates of *Euphyllia* sp. and *Montipora digitata* between high and low water currents (Mann-Whitney U test, n.s.). However, non-preconditioned fragments (Inc0) on average released almost twice as much POC under low compared to high water current conditions (*Euphyllia* sp.: 76.5 ± 27.4 mg $m^{-2}$ $h^{-1}$ vs. 42.0 ± 10.3 mg $m^{-2}$ $h^{-1}$; *Montipora digitata*: 28.0 ± 14.7 mg $m^{-2}$ $h^{-1}$ vs. 15.9 ± 3.4 mg $m^{-2}$ $h^{-1}$). After 3 weeks of water current exposure, these differences converged resulting in highly similar POC release rates under high and low water current conditions for both, *Euphyllia* sp. and *Montipora digitata* (Mann-Whitney U, n.s.). Release rates of PON under high and low water current conditions were similar before preconditioning the fragments (Inc0) and remained similar over the course of the experiment (Mann-Whitney U, n.s.). The patterns of POM release subjected to current velocities were similar for both coral species (Fig. 2).

Contrasting the release of particulate organic matter, no effect of water currents on the release of dissolved organic carbon was (Kruskal-Wallis test, n.s. for both species) found. Across all treatments and species both uptake and release of DOC occurred. At Inc0, mean DOC release rates by non-conditioned fragments were similar across all current velocities and ranged from

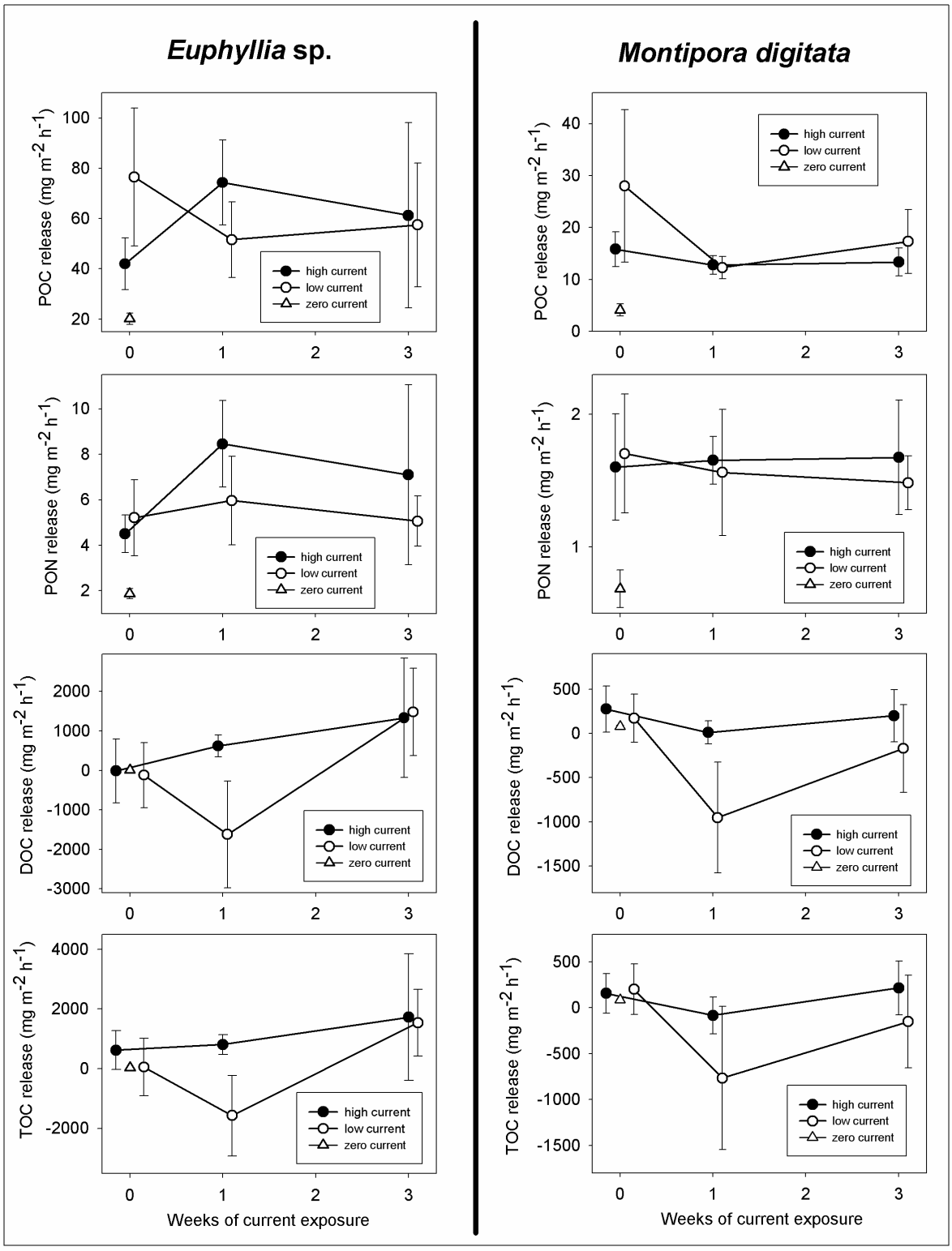


Fig. 2: TOC, DOC, POC and PON release rates by *Euphyllia* sp. and *Montipora digitata* subjected to different water current (mean ± SD) velocities and durations of current exposure prior to incubations.

-119 ± 826 mg $h^{-1}$ $m^{-2}$ (mean ± SE) at low current to 10 ± 5 mg $h^{-1}$ $m^{-2}$ at zero current for *Euphyllia* sp. and from 81 ± 11 mg $h^{-1}$ $m^{-2}$ at zero current to 276 ± 262 mg $h^{-1}$ $m^{-2}$ at high current for *Montipora digitata*. After 1 week of exposure to either high or low current, both species exhibited a similar pattern in which low current fragments tended to an average DOC uptake (-1625 ± 1353 mg $h^{-1}$ $m^{-2}$ by *Euphyllia* sp.; -952 ± 626 mg $h^{-1}$ $m^{-2}$ by *Montipora digitata*), while fragments exposed to high current on average took up DOC (619 ± 274 mg $h^{-1}$ $m^{-2}$ by *Euphyllia* sp.; 12 ± 129 mg $h^{-1}$ $m^{-2}$ by *Montipora digitata*). However, no significant differences between high or low current (Mann-Whitney U test, n.s.) or changes over time occurred (Repeated measures ANOVA, n.s.). After 3 weeks of current exposure,

Table 1: DOC, POC and PON release rates under zero current conditions compared to low or high current conditions at Inc0. Asterisks indicate significant differences to zero current, p-values in bold indicate trends. Please note the

| | *Euphyllia* sp. | | | *Montipora digitata* | | |
|---|---|---|---|---|---|---|
| **Current (cm sec$^{-1}$)** | **DOC release (mg m$^{-2}$ h$^{-1}$)** | **POC release (mg m-2 h-1)** | **PON release (mg m-2 h-1)** | **DOC release (mg m$^{-2}$ h$^{-1}$)** | **POC release (mg m-2 h-1)** | **PON release (mg m-2 h-1)** |
| **0** | 10 ± 5 | 20.2 ± 2.3 | 1.9 ± 0.2 | 81 ± 11 | 4.2 ± 1.2 | 0.7 ± 0.1 |
| **Low (3.5 – 6.2)** | -119 ± 826 p=0.344 | 76.5 ± 27.4 **p=0.023** | 5.2 ± 1.7 **p=0.004*** | 172 ± 271 p=0.788 | 28.0 ± 14.7 **p=0.012*** | 1.7 ± 0.4 **p=0.024** |
| **High (14.2 - 15.8)** | -10 ± 808 p=0.349 | 42.0 ± 10.3 **p=0.031** | 4.5 ± 0.8 **p=0.002*** | 276 ± 262 P=0.570 | 15.9 ± 3.4 **p=0.012*** | 1.6 ± 0.4 p=0.109 |

these differences converged resulting in highly similar release rates of *Euphyllia* sp. fragments exposed to high current (1330 ± 1511 mg DOC h$^{-1}$ m$^{-2}$) and low current (1481 ± 1106 mg DOC h$^{-1}$ m$^{-2}$). The DOC release rates of *Montipora digitata* also converged but not as pronounced, resulting in release of 200 ± 293 mg DOC h$^{-1}$ m$^{-2}$ by fragments exposed to high current and uptake of -169 ± 497 mg DOC h$^{-1}$ m$^{-2}$ by fragments exposed to low current. No significant difference between the DOC release patterns of the two investigated species (Fig. 2, Table 1) occurred.
Maximum DOC release/uptake rates exceeded POC release rates by one order of magnitude. TOC release was therefore mostly determined by DOC release/uptake and thus followed the patterns described above. Similarly, no significant effect of water currents on the release of total organic carbon was found. Across all treatments and species, both, uptake and release of TOC occurred. Likewise, there was no significant difference between the TOC release patterns of the two investigated species (Fig. 2, Table 1).

### POM release and net photosynthesis by *Galaxea fascicularis*

Water current significantly affected POC (Oneway ANOVA, p = 0.014) and PON (Oneway ANOVA, p = 0.016) release rates by *Galaxea fascicularis*. Despite the ANOVA indicated significant current effects on POC release, the subsequent Tamhane post-hoc test did not reveal any significant differences between POC release by fragments exposed to 11, 19 or 27cm s$^{-1}$. As described above, this is likely due to the logistically unavoidable small sample size. However, similar to current-depending release rates by *Euphyllia* sp. and
*Montipora digitata*, fragments of *Galaxea fascicularis* exposed to the here lowest current velocity of 11 cm s$^{-1}$ released 2.0 times more POC (Tamhane post-hoc test, p = 0.081) and 1.7 times more PON (LSD post-hoc test, p = 0.043) than fragments exposed to medium velocity of 19 cm s$^{-1}$. Further elevation of the current to the here highest velocity of 27 cm sec$^{-1}$ did not lead to further decrease, but to 1.9 times higher POC (Tamhane post-hoc test, p = 0.070) and 2.0 times higher PON (LSD post-hoc test, p = 0.006) release.

Flow velocity significantly affected net photosynthetic $O_2$ production rates and resulting net photosynthetic carbon fixation rates (one-way ANOVA, p = 0.004).

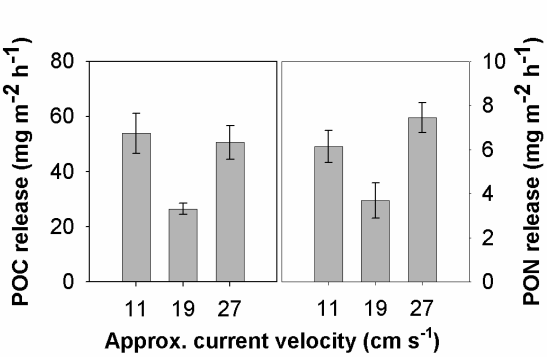


Fig. 3: Fig. 3: POC and PON release by *Galaxea fascicularis* subjected to different water current velocities (mean ± SD).

While there was no significant difference between colonies exposed to current velocities of 11 cm s$^{-1}$ and 19 cm s$^{-1}$ fixing on average 128 ± 20 mg C m$^{-2}$ h-1 and 158 ± 22 mg C m$^{-2}$ h$^{-1}$, respectively (Fig. 4), colonies exposed to the highest current velocity of 27 cm s$^{-1}$ exhibited significantly reduced net photosynthetic carbon fixation (42 ± 11 mg C m$^{-2}$ h$^{-1}$) compared to current velocities of 11 (Bonferroni post-hoc test, p = 0.028) and 19 cm s$^{-1}$ (Bonferroni post-hoc test, p = 0.005).

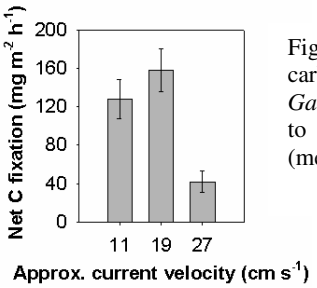


Fig. 4: Net photosynthetic carbon fixation rates by *Galaxea fascicularis* subject to different current velocities (mean ± SD).

## DISCUSSION

In previous studies a variety of methods has been applied to quantify organic matter release by scleractinian corals. Several studies used the beaker

incubation method introduced by Herndl and Velimirov (1986), in which coral colonies are incubated in open glass beakers under simulated in situ conditions for several hours (e.g. Ferrier-Pages et al. 1998; Wild et al. 2004; Niggl et al. 2009; Naumann et al. 2010). In another approach, organic matter release was quantified in situ by enclosing coral colonies in plastic bags (Richman et al. 1975) or in clear perspex chambers (Crossland 1987). A common task of all studies was to resemble in situ conditions as accurate as possible. Yet, water current, an important environmental factor substantially characterizing in situ conditions, has not been taken into account likely due to technical difficulties. Although the potential impact of water current on organic matter release had been discussed before (Richman et al. 1975), most studies were conducted under zero current conditions. Only the perspex chambers used by Crossland (1987) created non static incubation water conditions. However, the applied water exchange rate of 160 ml $min^{-1}$ at a total chamber volume of 1750 ml likely was too low to create water currents comparable to in situ conditions. The present study therefore is the first to quantify organic matter release under in situ-relevant water current conditions.

Water current is known to affect many coral physiological processes (Dennison and Barnes 1988; Patterson et al. 1991; Schutter et al. 2010). Additionally, water current impacts corals mechanically thereby washing away organic matter (mucus) from the coral's surface (Wild et al. 2004); an influence on organic matter release thus seems highly probable. Indeed, the present study revealed significant impact of current velocity on the release rates of organic matter by three scleractinian coral species. The bulk of organic matter released by scleractinian corals derives from coral mucus (Crossland 1987). Current-induced hydro-mechanical impact on the coral surface mucus layer, mainly composed of insoluble carbohydrate and/or protein polymers (Meikle et al. 1988), likely explains the 2.1 to 6.8-fold increased release of particulate organic carbon and nitrogen when water current is applied. The water current velocities applied in the present study represent a range often occurring in coral reefs worldwide; in areas with strong tides, current velocities usually even exceed those applied here (e.g. Roberts and Suhayda 1983; Gattuso et al. 1993; Huettel et al. 2006; Manasrah et al. 2006; Reidenbach et al. 2006). This emphasizes that previous quantifications carried out under zero current conditions (e.g. Richman et al. 1975; Wild et al. 2004; Naumann et al. 2010) may have underestimated actual in-situ POM release rates substantially. Results obtained from the additional experiment incubating *Galaxea fascicularis* indicate that underestimations may be especially pronounced in reef systems with strong tidal currents, as with increasing current velocity from 19 cm $s^{-1}$ to 27 cm $s^{-1}$ and resulting increasing –hydro-mechanical impact, POM release also increased. However, this hypothesized pattern of increasing POM release with increasing current velocity is only applicable when the current velocity exceeds a certain threshold value. Below this threshold velocity, the current-induced retraction of tentacles (Hubbard 1974) and resulting reduction of surface area likely leads to reduced POM release rates. Above the threshold velocity, the hydro-mechanical impact on POM release exceeds the effects of current-induced surface reduction. This may explain why *Galaxea fascicularis* released significantly more POM at a current velocity of 27 cm $s^{-1}$ compared to 19 cm $s^{-1}$ indicating a threshold value between 19 and 27 cm $s^{-1}$. This threshold value is supposed to be species-specific depending amongst others on the colony's growth form and the size of its polyps.

During the course of the experiment, no adaption of the long term-exposed corals *Montipora digitata* and *Euphyllia* sp. to applied current resulting in a reduction of initially increased POM release was observed. The increase of POM release rates under current exposure compared to zero current conditions observed for *Montipora digitata* and *Euphyllia* sp. thus does not display a temporarily limited artefact, but rather represents valid release rates that likely approach in-situ POM release by scleractinian corals. This is supported by the fact that all investigated species were affected similarly, although embodying different growth forms (branching, massive) and differently sized polyps.

In contrast to POM release, application of water current did not result in significantly altered release rates of DOC compared to zero current conditions. This indicates that the release of DOM is impacted to a lesser extent by hydro-mechanical impacts. The mechanisms of DOC release however still remain unknown. A part of the released DOC originates from released mucus. Up to 80 % of mucus has been shown to dissolve immediately when released in the ambient seawater (Wild et al. 2004). With current-induced increasing mucus release, mucus-derived DOC release should also increase. This could not be observed in the present study, which indicates that either the composition of released mucus changed towards a higher proportion of particulate compounds, or the DOC uptake/release kinetics depend on mucus release only to a limited extend. The composition of mucus released from scleractinian corals varies temporarily, between species or depending on environmental factors (Crossland et al. 1980; Muscatine et al. 1984; Meikle et al. 1988; Coffroth 1990; Wild et al. 2010). In the present study, compositional alterations towards a higher proportion of particulate compounds would have had to happen within less than 5 h as no time for current adaption was provided before incubation. This is considered unlikely and leads to the assumption that mucus-derived DOC contributes only to a limited extent to total DOC uptake/release kinetics. Thus, in contrast to POM, DOC uptake/release kinetics is considered to be mainly affected by physiological processes rather than by external hydro-mechanical impacts.

Ferrier-Pages et al. (1998) found DOC release rates to depend on the trophic status of the coral colonies with 'artemia-fed' colonies releasing more DOC than unfed colonies. As the corals were not fed during the experiment in the present study, the trophic status was likely determined by the photosynthetic performance and the transfer of photosynthetic products from the symbionts to the host. Data on the effects of water flow on the photosynthetic performance is controversial. While Sebens et al. (2003) did not find a significant influence of flow speed between 1-10 cm $s^{-1}$ on net photosynthesis by *Agaricia tenuifolia*, Lesser et al. (1994) found significantly stimulating effects of increasing flow (mean velocities of 0.2, 3.8 and 7.2 cm $s^{-1}$) on net photosynthesis by *Pocillopora damicornis*. Schutter et al. (2010) found similar net photosynthetic $O_2$ production rates by *Galaxea fascicularis* at water current velocities of 10 cm $s^{-1}$ and 20 cm $sec^{-1}$, but decreasing rates when the velocity was adjusted to 25 cm $s^{-1}$. This is in accordance with the present study where similar net photosynthetic $O_2$ production rates by *Galaxea fascicularis* at 11 and 19 cm $s^{-1}$, but significantly decreased rates at 27 cm $s^{-1}$ were found. Assuming similar patterns for *Euphyllia* sp. and *Montipora digitata*, the applied current velocities of approximately 5 and 15 cm $s^{-1}$ likely did not result in different photosynthetic performances thus implies similar nutritional conditions. This may explain why no significant effects of exposure to different currents velocities on the DOC uptake/release kinetics were found.

**In situ applicability and conclusions**

Results presented here where obtained by exposing relatively small coral colonies to an unidirectional flow. However, actual in situ flow dynamics in coral reefs are highly sophisticated (reviewed by Monismith 2007). The current conditions and mass transfer within a coral colony may be completely different from the outside conditions depending on e.g. the coral morphology or the prevalent flow, oscillatory or unidirectional (Reidenbach et al. 2006). Nevertheless, the present study revealed significant influence of water motion on POM release by scleractinian corals thus proved water current to be an important parameter for future quantifications. By conducting the first organic matter quantifications under simulated in situ current conditions, the present study complements previous studies indicating even higher release rates of particulate organic matter than previously estimated. The ecological role of the insoluble particulate part of released organic matter as a trap of particles and carrier of energy for coral reef ecosystems functioning (Wild et al. 2004) may therefore be even more important than already assumed. In contrast, the uptake/release of dissolved organic carbon was not immediately affected by water currents. Therefore, DOC uptake/release kinetics is likely rather influenced by physiological conditions than by immediate hydro-mechanical impacts.

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# 9

# Organic matter release by Red Sea coral reef Organisms - potential effects on microbial activity and *in situ* $O_2$ availability

**Christian Wild[1], Wolfgang Niggl[1], Malik S. Naumann[1], Andreas F. Haas[1]**

[1]Coral Reef Ecology Group (CORE), GeoBio-Center & Department of Earth and Environmental Science in the Faculty of Geosciences, Ludwig-Maximilians Universität, Richard Wagner Str. 10, 80333 München, Germany

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# Organic matter release by Red Sea coral reef organisms — potential effects on microbial activity and *in situ* $O_2$ availability

**Christian Wild*, Wolfgang Niggl, Malik S. Naumann, Andreas F. Haas**

**Coral Reef Ecology Group (CORE), GeoBio-Center & Department of Earth and Environmental Science in the Faculty of Geosciences, Ludwig-Maximilians Universität, Richard Wagner Str. 10, 80333 Munich, Germany**

ABSTRACT: This study presents a comprehensive dataset (223 reef organisms that were separately incubated during 44 independent experiments during 4 seasonal expeditions) of dissolved and particulate organic matter (DOM and POM) release by dominant benthic organisms from the Northern Red Sea. Reef organisms studied were scleractinian and fire corals, the upside-down jellyfish and reef-associated algae. Subsequently, the effect of this organic matter (OM) release on microbial activity was determined. These studies were complemented by high resolution, *in situ* $O_2$ concentration measurements within reef environments that were dominated by corals or algae. Dissolved organic carbon (DOC) release was 14.5 ± 2.3 mg $m^{-2}$ surface area $h^{-1}$ for all 9 investigated reef algae, which was significantly higher than DOC release by scleractinian corals during all seasons except winter. POM release (particulate organic carbon and nitrogen, POC and PON, respectively) was observed for all investigated reef organisms. Benthic reef algae released 5.1 ± 0.5 mg POC $m^{-2}$ $h^{-1}$ and 0.35 ± 0.03 mg PON $m^{-2}$ $h^{-1}$, which are significantly higher than POM release rates by scleractinian corals in spring and autumn. Algae-derived OM, presumably the DOC fraction, stimulated microbial activity in the adjacent water more significantly than OM released by the investigated scleractinian and fire corals. Consequently, the daily mean and minimum *in situ* $O_2$ concentrations in the water directly above the reef (≤10 cm) were significantly higher in coral dominated than in algae dominated sites, confirming the *in situ* relevance of results of previous laboratory studies. Findings also suggest that benthic reef algae decrease $O_2$ availability in waters close to reef environments via the release of labile OM and its subsequent fast microbial degradation.

KEY WORDS: Red Sea · Coral reefs · Benthic organisms · Organic matter release · Coral–algae–microbe interaction · *In situ* $O_2$ availability



## INTRODUCTION

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

Smith et al. (2006) indicated that coral reef-associated benthic algae might affect processes such as microbial activity in their surroundings via a hypothetical release of OM. This could reduce $O_2$ availability in coral reef environments and may, thus, have severe consequences for coral metabolism and ecosystem functioning. In coral reefs, hypoxia (i.e. dissolved $O_2$ concentrations that are below saturation) is a common phenomenon (Nilsson & Östlund-Nilsson 2004). During the night when no $O_2$ is produced by coral zooxanthellae, severe hypoxic conditions (down to 0.7 mg $O_2$ $l^{-1}$) can occur between the

*Email: c.wild@lrz.uni-muenchen.de

branches of coral colonies (Shashar et al. 1993, Kühl et al. 1995). Although corals may overcome periods of low $O_2$ concentrations by extending their tentacles or decreasing their respiration (Shashar et al. 1993), severe hypoxia or anoxia can cause widespread coral mortality (Simpson et al. 1993). A recent study also described the occurrence of hypoxia in interactions between corals and some turf or fleshy macroalgae in coral reef ecosystems (Barott et al. 2009).

In this context, it is surprising that only few data are available on $O_2$ availability in coral reef ecosystems. Low $O_2$ concentrations caused by the decomposition of organic materials may constitute a significant stress factor for corals (Johannes 1975), and the release of labile OM by reef organisms may stimulate microbial activity that could result in such $O_2$ deficiency (Simpson et al. 1993).

This study, therefore, presents comparative quantitative data of OM release by dominant benthic coral reef organisms that were investigated during 4 seasonal expeditions to a typical fringing reef in the northern Red Sea. These studies were supplemented by investigations on (1) the effects of this release on the planktonic microbial activity measured as $O_2$ consumption in the adjacent water, and (2) the potential implication for *in situ* $O_2$ availability within benthic communities dominated by different reef organisms.

## MATERIALS AND METHODS

**Transect surveys.** The study was conducted during 4 seasonal expeditions (autumn: Nov/Dec 2006, summer: Aug/Sep 2007, winter: Feb/Mar 2008, spring: May 2008) to a fringing reef close to the Marine Science Station (MSS), Aqaba, Jordan (29° 27′ N, 34° 58′ E). The target dominant benthic organisms were identified at the beginning of the first expedition using the line point intercept (LPI) transect survey technique modified from Loya (1978) and Nadon & Stirling (2006). At water depths of 0.5, 1.0, 5.0, 10.0 and 20.0 m, duplicate 50 m transect surveys at 0.5 m point intervals were carried out parallel to the reef crest at a northern and a southern reef location using SCUBA. LPI transect data were analysed to derive the percent coverage of the dominant benthic organisms in the study area. In total, 44 transect surveys were carried out during the 4 seasonal expeditions.

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

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

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

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

**Quantification of OM release**. OM release by all collected reef organisms was carried out as described by Herndl & Velimirov (1986). Animals and algae were separately transferred into 1000 ml glass beakers (previously rinsed with acetone followed by a thorough seawater rinse) that were filled with 800 to l000 ml of untreated, freshly pumped seawater from the field. Identical beakers filled with seawater served as controls. Beakers were kept in a flow-through aquarium during the day at *in situ* temperatures. Nylon gauze was clamped above the beakers to simulate light intensities at 5 m water depth as verified by light loggers (Onset Pendant). After a 6 h incubation, organisms were removed from the beakers and subsamples were

taken from the incubation water for determination of the following parameters.

***Dissolved organic carbon:*** Approximately 10 ml of the incubation water collected from both control and treatment beakers at the end of incubations were filtered through 0.2 µm sterile syringe filters (polyethersulfone [PES] membrane, VWR International). DOC leakage from PES filter membranes as noted by Khan & Subramania-Pillai (2007) was insignificant, as assured by repeated analyses of different lots of untreated filters according to the following sampling procedure: the first 4 ml of the filtrate were discarded and the remaining 6 ml were collected in pre-combusted brown glass bottles or ampoules, which were instantly frozen at –20°C and kept frozen until analysis. Potential contamination of samples was also prevented by using powder-free gloves during all manual handlings.

DOC concentrations were determined by high temperature catalytic oxidation (HTCO) using a total organic carbon (TOC) analyser (Rosemount Dohrmann DC-190) (e.g. Sharp et al. 1993). A certified TOC standard (ULTRA Scientific) was used for instrument calibration (10-point) and as a regular quality control after every 4th sample. Analytical precision was <3% of the certified value.

After defrosting, each sample was treated by adding 100 µl of 20% phosphoric acid and purged for 5 min using pure $O_2$ to remove dissolved inorganic carbon. The DOC concentration of each sample was measured using 5 single injections of 100 µl sample volumes. An outlier test of the resulting DOC concentrations was conducted, and the remaining values were averaged.

***Particulate organic carbon and nitrogen:*** Between 400 and 800 ml of the incubation water were filtered on pre-combusted GF/F (Whatman, 25 mm diameter), which were then dried for at least 48 h at 40°C and kept dry until analysis. During this sampling, inclusion of larger, visible particles such as faecal pellets (in the case of jellyfish) or tissue fragments (in the case of algae) was avoided. Particulate organic carbon (POC) and nitrogen (PON) concentration measurements and respective stable isotope analyses were performed with an elemental analyzer (Carlo Erba NC 2500). Elemental concentrations were calculated from certified elemental standards (atropine, cyclohexanone-2,4-dinitrophenylhydra-zone; Thermo Quest) and typically showed SDs that were <3%.

For the calculation of OM release rates (DOC as well as POC and PON), values from control beakers were subtracted from those measured in the incubation water of the beakers containing the organisms, followed by normalisation to individual organism surface area, incubation time and incubation water volume. Respective surface areas were measured as a reference parameter using geometric approximation for all corals and turf algae growing on dead coral fragments (see Naumann et al. 2009a for detailed methodology) or the image analysis software ImageJ to analyze digital photographs of the other organisms.

**Effects on planktonic microbial $O_2$ consumption**. Approximately 140 ml of the incubation water collected from each beaker at the end of the incubation experiments were used to fill two 60 ml glass bottles. $O_2$ concentration was measured immediately in one of the bottles, and after incubation of the enclosed water for at least 16 h in the dark at *in situ* temperature in the second bottle. $O_2$ concentration was measured using the modified Winkler titration technique described by Carpenter (1965) during the autumn expedition or a sensor (Hach HQ 10) during the spring, summer and winter expeditions. The sensor was calibrated using Winkler titration. $O_2$ consumption by microbes (including Bacteria, Archaea, small Protozoa) in the incubation water was determined by subtracting the final $O_2$ concentration from that at the start of the incubation. Final $O_2$ concentration values were always >10% lower than initial values, rendering differences clearly detectable with both methodologies used. Resulting values were then related to the surface area of the incubated organism.

**Supplementary *in situ* studies**. The effect of differences in benthic reef community composition on *in situ* $O_2$ availability in the overlying water column was investigated during both spring and winter expeditions by deploying dissolved $O_2$ loggers (Eureka Midge). During 3 (spring) or 6 (winter) occasions, 2 loggers were simultaneously deployed for 24 h at water depths of 4 to 7 m within different small (<5 $m^2$) reef sections dominated by scleractinian corals (coral cover: 20 to 95%, algal cover: 0 to 10%) or benthic reef algae (algal cover: 35 to 100%, coral cover: 0 to 15%). $O_2$ concentration and water temperatures were measured and logged every 5 min over the entire deployment period (288 data points for each deployment). Water currents were measured in triplicates during each deployment via tracking of natural suspended particles along known distances using a ruler and a watch.

**Data analysis**. Differences in mean DOC and POM concentrations in the incubation waters between the controls and the treatments (see Table 1) were investigated using paired *t*-tests. Where the requirements for paired *t*-tests were not met, Wilcoxon signed rank tests were used.

Net OM release (DOC, POC, PON) by the investigated reef organisms and subsequent effects on microbial $O_2$ consumption rates were calculated by subtracting mean control values of the respective parameters from those of each treatment. Statistical

analyses for these data were carried out using Mann-Whitney *U*-tests, as homogeneity of variances was proven (Levene test), but the data were not normally distributed (Kolmogorov-Smirnov test).

For comparison of data obtained from simultaneously deployed *in situ* $O_2$ loggers, paired *t*-tests were used. These data showed homogeneity of variances and were normally distributed.

## RESULTS

Fig. 1 gives an overview of the benthic coverage by the different groups of investigated organisms in the MSS fringing reef. Among the study organisms, hard corals exhibited the highest benthic coverage followed by reef algae, fire corals and jellyfish. Seasonal algae only appeared during the winter and spring expeditions and could account for up to 26% of the seafloor area of the investigated coral reef. Water currents reversed or changed direction regularly and exhibited velocities of 3.2 to 7.1 cm $s^{-1}$ during all logger deployments, with identical current velocities at the scleractinian coral dominated and the reef algae dominated sites during each deployment.

### Comparative OM release

In total, 223 separate incubations of the different coral reef organisms were conducted in 44 independent experiments using identical methodologies during the 4 different expeditions. Detailed information about the temporal, spatial and species-specific resolution of these investigations are presented in the studies of Naumann et al. (2010) for scleractinian and fire corals, Haas et al. (2010) for benthic reef algae, and Niggl et al. (2010b) for jellyfish.

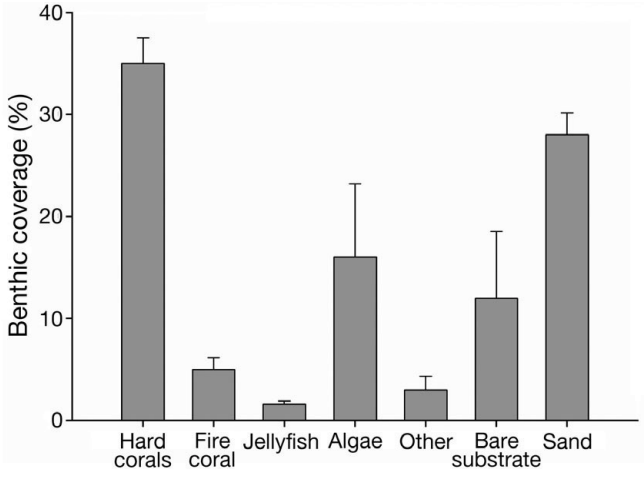


Fig. 1. Benthic coverage (mean + SD) by the different reef organisms and substrates at the Marine Science Station fringing reef as determined by Line Point Intercept surveys. 'Other' = other organisms

Table 1 shows the OM concentrations in all water samples from the incubation experiments. The difference in DOC concentrations between control and organism incubations was clearly above the analytical precision (<3%) for all experiments. OM release rates were calculated by subtracting OM concentrations of the control beakers from those measured in the incubation water of the beakers containing the organisms. Whereas POC concentrations in the beakers were consistent over all 4 seasons, DOC concentrations were up to one order of magnitude elevated in autumn (Table 1). However, there is no justification to remove DOC values for autumn from the dataset since identical methodologies were used throughout all seasonal samplings, contamination of samples was avoided, and similar values have been reported in the water column at the study area during autumn (Wild et al. 2009b).

DOC concentrations in waters of scleractinian coral incubations (3698 ± 984 µg $l^{-1}$) during all the 4 seasons (n = 17) were not significantly different ($p < 0.05$) from those of controls (5167 ± 1775 µg $l^{-1}$). In contrast, POC concentrations in waters with coral incubation (293 ± 35 µg $l^{-1}$) were significantly ($p < 0.001$) higher when compared to the controls (134 ± 12 µg $l^{-1}$), clearly indicating POC release. DOC concentrations (n = 22) in waters with algal incubation (6974 ± 2181 µg $l^{-1}$) were significantly ($p < 0.001$) higher compared to the controls (5026 ± 1638 µg $l^{-1}$). POC concentrations (n = 18) were also significantly ($p < 0.001$) higher in waters with algal incubation (566 ± 83 µg $l^{-1}$) when compared to the controls (129 ± 15 µg $l^{-1}$). This emphasizes the feasibility of net OM release measurement with the applied methodology.

Fig. 2 summarizes the mean net OM release by the different groups of reef organisms during the 4 seasons as related to the surface area of the incubated organism. Average net DOC release for all 9 reef algae species during all seasons was 14.5 ± 2.3 mg $m^{-2}$ $h^{-1}$, with turf algae releasing the highest DOC (33.6 ± 6.9 mg $m^{-2}$ $h^{-1}$). While scleractinian corals (–20.7 ± 21.2 mg $m^{-2}$ $h^{-1}$) and jellyfish (–1.2 ± 4.4 mg $m^{-2}$ $h^{-1}$) rather took up DOC, fire corals exhibited a net DOC release of 9.2 ± 12.8 mg $m^{-2}$ $h^{-1}$. Benthic algae released significantly more DOC compared to the scleractinian corals during all 4 seasons except in winter and significantly more DOC than jellyfish in spring (Fig. 2a).

POC and PON release was observed for all investigated reef organisms (Fig. 2b,c). The jellyfish *Cassiopea* released about one order of magnitude more POM compared to all other organisms. Benthic reef algae on average released 5.1 ± 0.5 mg POC $m^{-2}$ $h^{-1}$ and 0.35 ± 0.03 mg PON $m^{-2}$ $h^{-1}$, thereby exhibiting significantly

Table 1. DOC and POC (dissolved and particulate organic carbon) concentrations (µg $l^{-1}$) in the incubation waters of the various organisms and the respective controls during the 4 seasonal samplings. Values are mean ± SE, with numbers of replicates in parentheses; na = not assessed

| Season | Date | Organism | Control | | Treatment | |
|---|---|---|---|---|---|---|
| | | | DOC | POC | DOC | POC |
| Spring 2008 | May 17 | *Acropora* | 1501 ± 18 (2) | 85 ± 3 (5) | 1385 (1) | 164 ± 26 (5) |
| | 12 | *Fungia* | 1615 ± 86 (3) | 95 ± 8 (5) | 1328 ± 66 (4) | 184 ± 25 (5) |
| | 16 | *Stylophora* | 1778 ± 696 (2) | 93 ± 6 (5) | 1357 ± 121 (3) | 402 ± 68 (4) |
| | 10 | *Liagora* | 1292 ± 175 (3) | na | 1710 ± 87 (5) | na |
| | 11 | *Hydroclathrus* | 1435 ± 97 (4) | na | 1834 ± 114 (4) | na |
| | 12 | *Caulerpa* | 1495 ± 33 (2) | 136 ± 36 (5) | 2326 ± 211 (5) | 255 ± 62 (5) |
| | 16 | *Peyssonnelia* | 1080 (1) | 133 ± 46 (5) | 1316 ± 82 (4) | 449 ± 104 (5) |
| | 17 | Turf algae | 1499 ± 18 (2) | 84 ± 4 (5) | 3096 ± 257 (4) | 504 ± 102 (5) |
| | 10 | *Cassiopea* | 1399 ± 163 (4) | 117 ± 4 (5) | 1533 ± 132 (6) | 578 ± 59 (6) |
| | 11 | *Cassiopea* | 1507 ± 103 (5) | 102 ± 4 (5) | 1752 ± 89 (5) | 1058 ± 220 (6) |
| | 20 | *Cassiopea* | 1670 ± 137 (4) | 89 ± 6 (3) | 1425 ± 206 (5) | 384 ± 46 (6) |
| Summer 2007 | Aug 26 | *Acropora* | 1512 ± 185 (4) | 104 ± 8 (3) | 1832 ± 107 (5) | 398 ± 127 (5) |
| | 23 | *Fungia* | 2042 ± 515 (2) | 115 ± 5 (3) | 1646 ± 125 (5) | 346 ± 98 (5) |
| | Sep 04 | *Goniastrea* | 1715 ± 232 (3) | 86 ± 1 (4) | 2207 ± 124 (4) | 178 ± 21 (5) |
| | Aug 29 | *Pocillopora* | 1888 ± 100 (4) | 107 ± 5 (5) | 1651 (1) | 286 ± 48 (5) |
| | Sep 02 | *Stylophora* | 1617 ± 72 (3) | 75 ± 4 (5) | 1522 ± 224 (2) | 258 ± 28 (5) |
| | 07 | *Millepora* | 2938 (1) | 72 ± 8 (3) | 1469 (1) | 87 ± 4 (5) |
| | Aug 20 | Turf algae | 2424 ± 496 (3) | na | 2991 ± 226 (3) | na |
| | 23 | Turf algae | 2177 ± 328 (3) | 109 ± 7 (4) | 2182 ± 42 (2) | 688 ± 74 (5) |
| | 26 | *Caulerpa* | 1510 ± 185 (4) | 96 ± 8 (4) | 3331 ± 423 (4) | 500 ± 85 (4) |
| | 29 | Turf algae | 1918 ± 184 (4) | 170 ± 60 (5) | 2727 ± 380 (4) | 695 ± 82 (4) |
| | Sep 02 | *Peyssonnelia* | 1579 ± 55 (3) | 80 ± 5 (4) | 3057 ± 1055 (4) | 424 ± 89 (4) |
| Autumn 2006 | Nov 25 | *Acropora* | 6446 ± 372 (3) | 244 ± 14 (2) | 14213 ± 7377 (4) | 240 ± 37 (5) |
| | 19 | *Fungia* | 7129 ± 1263 (2) | 169 ± 1 (3) | 2545 ± 426 (5) | 361 ± 115 (5) |
| | 20 | *Goniastrea* | 11252 ± 8541 (3) | 201 ± 16 (2) | 10774 ± 3983 (5) | 267 ± 12 (5) |
| | 22 | *Pocillopora* | 28329 ± 1236 (3) | 140 ± 1 (3) | 5997 ± 1429 (4) | 476 ± 70 (5) |
| | 23 | *Stylophora* | 16103 ± 1960 (3) | 204 ± 18 (2) | 10192 ± 1176 (3) | 693 ± 65 (5) |
| | Dec 01 | *Millepora* | 1963 ± 151 (3) | 244 ± 19 (3) | 2796 ± 537 (5) | 285 ± 16 (5) |
| | Nov 15 | *Caulerpa* | 5887 ± 987 (3) | 164 ± 22 (3) | 10930 ± 3566 (5) | 1306 ± 283 (3) |
| | 21 | *Peyssonnelia* | 25294 ± 3725 (3) | 245 ± 148 (3) | 31626 ± 2684 (5) | 1100 ± 155 (5) |
| | 26 | Turf algae | 23517 ± 3594 (3) | 93 ± 8 (3) | 31748 ± 2837 (4) | 684 ± 130 (5) |
| | 28 | Turf algae | 19885 ± 2129 (3) | 95 ± 6 (3) | 29732 ± 4502 (5) | 1420 ± 220 (4) |
| | Dec 02 | *Caulerpa* | 11574 ± 3848 (2) | 280 ± 183 (3) | 13084 ± 2435 (5) | 699 ± 113 (4) |
| Winter 2008 | Feb 28 | *Acropora* | 1140 ± 67 (5) | 71 ± 10 (3) | 1535 ± 78 (5) | 137 ± 26 (3) |
| | 17 | *Fungia* | 1299 ± 45 (3) | 140 ± 10 (4) | 1743 ± 150 (4) | 191 ± 9 (5) |
| | 24 | *Goniastrea* | 1279 ± 80 (4) | 118 ± 53 (4) | 1529 ± 108 (4) | 170 ± 34 (5) |
| | Mar 01 | *Stylophora* | 1194 ± 36 (5) | 136 ± 9 (3) | 1416 ± 84 (5) | 229 ± 77 (4) |
| | Feb 17 | *Enteromorpha* | 1250 ± 54 (3) | 123 ± 19 (4) | 1969 ± 128 (5) | 264 ± 95 (4) |
| | 24 | Turf algae | 1283 ± 63 (4) | 230 ± 119 (4) | 1815 ± 273 (3) | 458 ± 196 (4) |
| | 28 | *Ulva* | 1139 ± 67 (4) | 61 ± 12 (3) | 1717 ± 271 (5) | 112 ± 11 (4) |
| | 29 | *Peyssonnelia* | 1201 ± 65 (3) | 45 ± 7 (4) | 1443 ± 119 (4) | 307 ± 43 (3) |
| | Mar 01 | *Caulerpa* | 1192 ± 43 (5) | 126 ± 11 (3) | 1935 ± 108 (5) | 174 ± 26 (3) |
| | 06 | *Lobophora* | 974 ± 117 (3) | 93 ± 12 (2) | 1485 ± 70 (5) | 313 ± 60 (4) |
| | 06 | *Sargassum* | 974 ± 117 (3) | 93 ± 12 (2) | 1368 ± 76 (5) | 405 ± 53 (2) |

higher POC release rates than scleractinian corals in spring and autumn and significantly higher PON release rates in autumn. Fire coral POC (0.34 ± 0.14 mg $m^{-2}$ $h^{-1}$) and PON (0.04 ± 0.01 mg $m^{-2}$ $h^{-1}$) release was always significantly lower when compared to benthic reef algae and scleractinian corals (POC: 2.8 ± 0.3 mg $m^{-2}$ $h^{-1}$; PON: 0.29 ± 0.03 mg $m^{-2}$ $h^{-1}$ for scleractinian corals).

### Effects on microbial activity and *in situ* $O_2$ availability

Induction of microbial activity (Fig. 2d), which was measured as $O_2$ consumption, was highest for *Cassiopea*-derived OM (15.2 ± 1.6 mg $O_2$ $l^{-1}$ $h^{-1}$ normalized $m^{-2}$ of surface area), significantly exceeding those of scleractinian corals and benthic algae. Corrected planktonic microbial $O_2$ consumption was 3.7 ± 0.2 mg

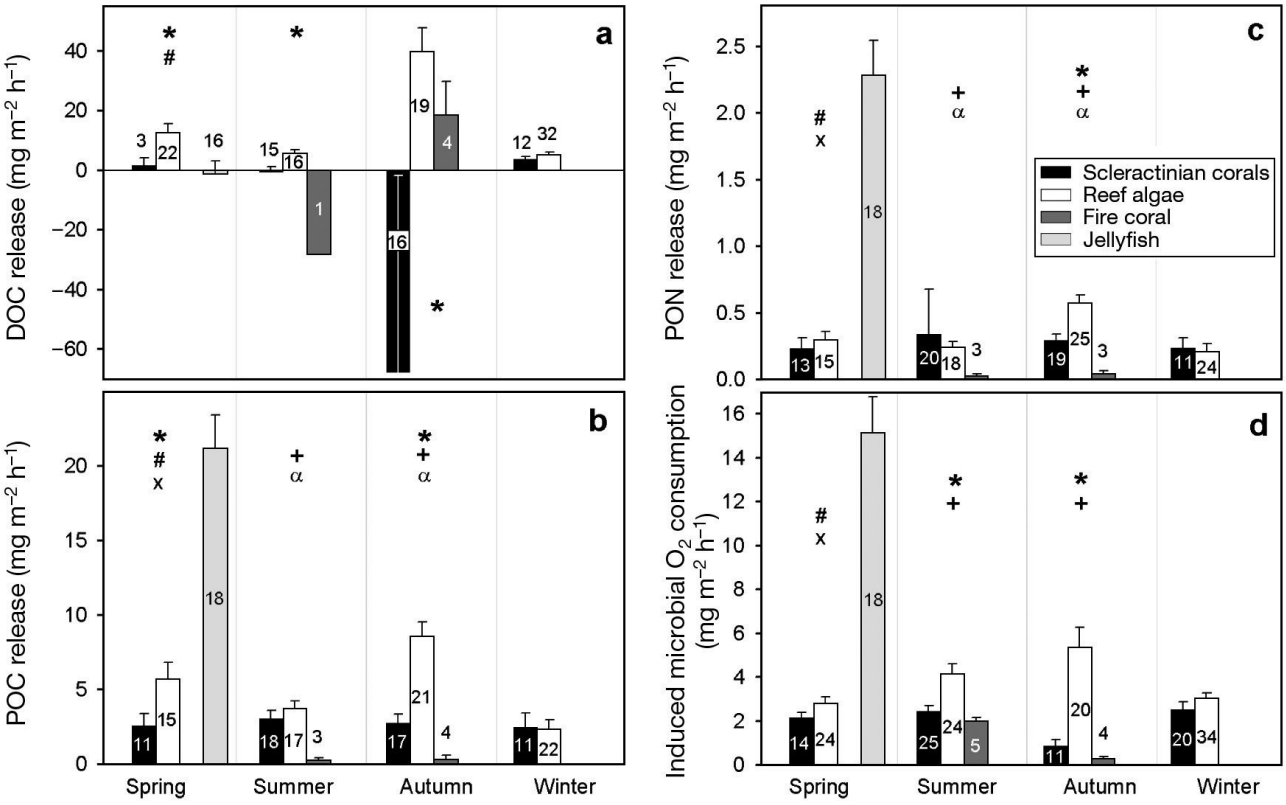


Fig. 2. Comparative organic matter (a) DOC, (b) POC, and (c) PON release by the investigated coral reef organisms and its effects on microbial $O_2$ consumption (d) during the 4 seasonal expeditions. Values for microbial activity in (d) are normalized to 1 l of incubation water. Values are mean + SE, numbers within or above columns: replication. Symbols above columns indicate statistically significant differences ($p < 0.05$) between scleractinian corals and reef algae (*), fire coral (α), or jellyfish (x), as well as between reef algae and fire coral (+), or jellyfish (#)

$O_2$ l$^{-1}$ h$^{-1}$ for algal incubations, and 2.2 ± 0.2 and 1.2 ± 0.3 mg $O_2$ l$^{-1}$ h$^{-1}$ for scleractinian and fire coral incubations respectively. Stimulation of microbial activity by reef algae-derived OM significantly exceeded those by scleractinian and fire coral-derived OM in summer and autumn.

Algae dominated sites showed strong diurnal variation in $O_2$ concentrations ranging from 5.1 to 9.3 mg l$^{-1}$ in spring and 5.1 to 10.3 mg l$^{-1}$ in winter, with lowest values being observed after dusk and before dawn, and highest values around midday (Fig. 3). In contrast, $O_2$ values at coral dominated sites ranged from 6.6 to 8.5 mg l$^{-1}$ in spring and 7.0 to 9.5 mg l$^{-1}$ in winter.

During all 9 parallel deployments of $O_2$ loggers at coral or algae dominated reef sites, daily mean $O_2$ concentrations in the water directly above the reef were significantly higher (2-sided, paired *t*-tests; $p < 0.05$) at the scleractinian coral dominated compared to the benthic reef algae dominated sites. Diurnal variations in $O_2$ concentrations at algae dominated sites were significantly higher than at coral dominated sites and displayed strong positive correlation with benthic algal cover (Pearson product moment correlation, $r = 0.90$, $p = 0.001$).

## DISCUSSION

### OM release by different groups of reef organisms

Net OM release by coral reef organisms has been investigated in the 1970s (Richman et al. 1975, Ducklow & Mitchell 1979). However, in contrast to these earlier studies, which either focused on a single group of organisms (Richman et al. 1975) or investigated OM release under manipulative stress conditions (Ducklow & Mitchell 1979), the present study delivers a comprehensive dataset on net OM release by the dominant benthic reef organisms in the Northern Red Sea under 'low stress' conditions.

Results of the present study showed that all investigated benthic reef organisms released POM (POC and PON) into their surroundings in significant quantities.

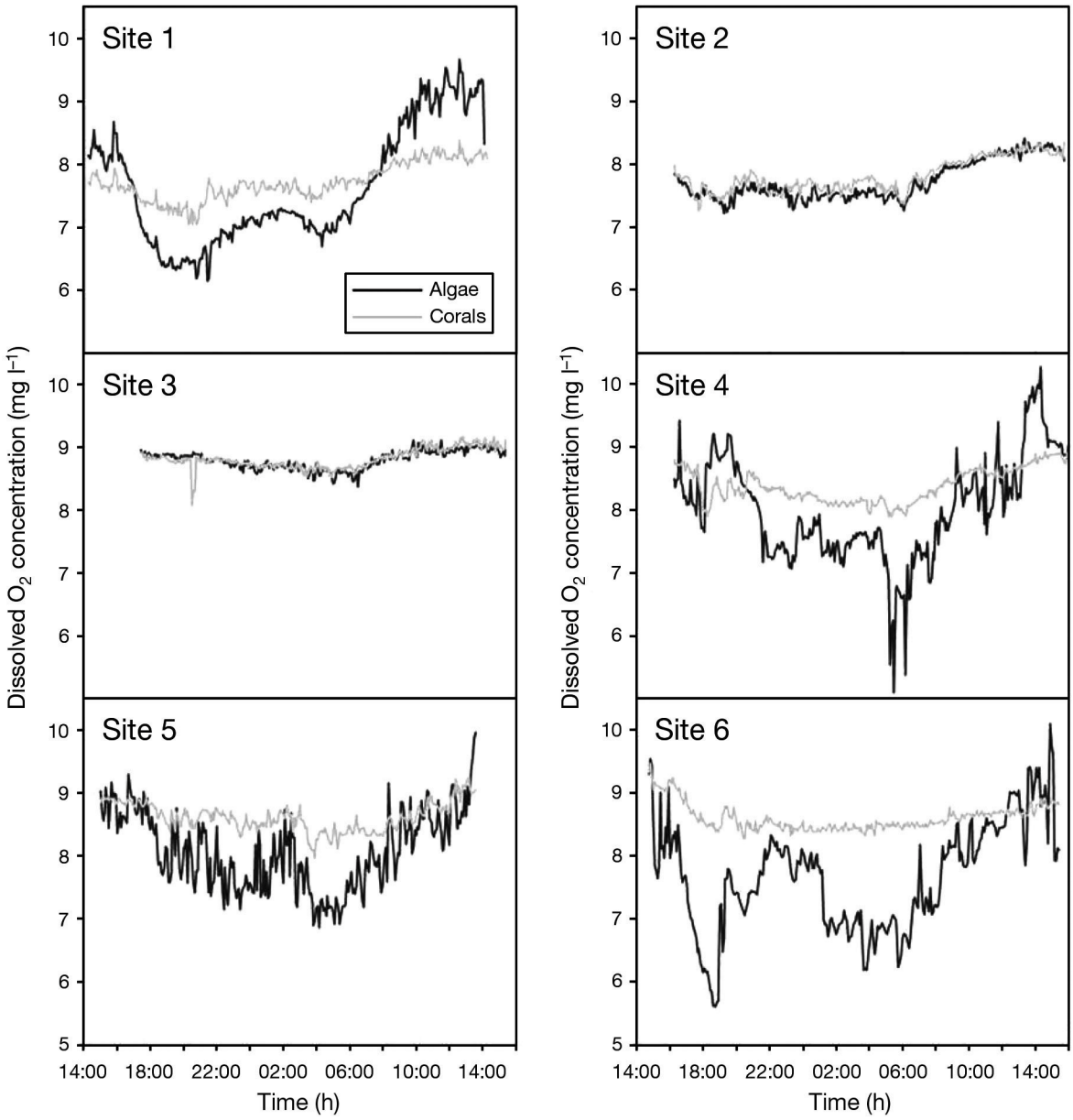


Fig. 3. Diurnal *in situ* dissolved $O_2$ concentrations in the water column directly above (<10 cm) reef sites with benthic communities that are dominated by scleractinian corals or reef algae as measured with $O_2$ loggers (Midge). Water temperatures were 21.5 to 21.8°C during winter and 22.3 to 22.7°C during spring measurements

For corals, this release can account for up to half of the carbon assimilated by their zooxanthellae (Crossland et al. 1980, Davies 1984, Muscatine et al. 1984). Coral-derived POM release rates were similar to those described elsewhere (Crossland 1987, Wild et al. 2005b), while hydrozoan, scyphozoan and macroalgal POM release rates were quantified here for the first time. As release of OM has been attributed to surplus carbon fixation during intense photosynthesis (Fogg 1983, Davies 1984), similarities in POM release between algae and corals may be explained by similar photosynthetic overproduction as well as concurrent limited nutrient availability since very low nitrate (0.12 to 0.90 µM) and phosphate (0.03 to 0.07 µM) concentrations were observed at the study site during the 4 expeditions (Wild et al. 2009b).

Net DOM release by corals has been demonstrated by Ferrier-Pages et al. (1998). In the present study, however, net release of DOC was only observed for 3 of the investigated coral genera. This finding is most likely due to the variety of feeding mechanisms of zooxanthellate corals and jellyfish. Besides using

photosynthetic products from zooxanthellae (Muscatine 1990), corals are able to change to other feeding modes, such as the capture of zooplankton by polyps and the uptake of dissolved organic compounds from the surrounding seawater (Sorokin 1973, Ferrier 1991, Muller-Parker & D'Elia 1996). This DOM uptake may have exceeded release for most of the investigated corals. The same explanation likely applies to the jellyfish *Cassiopea* sp. In contrast, all algal species, particularly turf algae, released DOC, which is in agreement with previous studies (Khailov & Burlakova 1969, Brylinsky 1977). In contrast to zooxanthellate corals and jellyfish, benthic algae are strictly photoautotrophic in terms of their energy and carbon requirement (Tuchman 1996); thus, reabsorption of DOM is unlikely, but may still occur.

The exceedingly high OM release rates found for turf algae can potentially be attributed to an underestimation of surface areas due to the exclusion of fine filaments. As larger proportions of structural tissue are needed for more complex morphologies, Littler & Littler (1984) suggested a higher performance of primary production in algae with filamentous morphology. This also creates an increased surface area, thereby providing a large interface with the surrounding that may lead to a faster exchange of metabolic products with the ambient environment. Another reason may be the N fixing ability of turf algae associated cyanobacteria (Williams & Carpenter 1998), which can affect net OM production by overcoming N limitation (Smith 1982).

### Effects on microbial activity and potential implications for *in situ* $O_2$ availability

The comparably high DOC release by benthic reef algae in combination with the observed high stimulation of planktonic microbial activity confirms previously postulated statements (Kline et al. 2006, Smith et al. 2006, Dinsdale et al. 2008), suggesting that DOM released by benthic algae may stimulate planktonic microbial $O_2$ consumption. High microbial respiration of the biologically labile algae-derived OM may reduce $O_2$ availability in the surrounding seawater (Nguyen et al. 2005). The results of several laboratory studies suggest that this $O_2$ deficiency can lead to damage or death of scleractinian corals (Mitchell & Chet 1975, Kuntz et al. 2005, Kline et al. 2006, Smith et al. 2006). The mesocosm study of Haas et al. (2009) in this context also indicated that DOC addition might negatively influence corals in interaction with algae via decreased water $O_2$ concentrations.

The present study supplements these laboratory studies by demonstrating that there could be similar effects *in situ* via a strong influence of benthic algae on $O_2$ availability in the reef, likely via the release of labile DOM. Various factors can influence the occurrence of hypoxia around corals *in situ*. The morphology of coral colonies can promote hypoxic conditions by creating a region of weak water exchange between the inner branches (Chamberlain & Graus 1975), and hypoxia on corals has also been found along natural interactions between corals and algae *in situ* (Barott et al. 2009). In contrast, the mutualistic relationship between branching corals and sleep-swimming fish helps to aerate the colony, thus preventing hypoxia (Goldshmid et al. 2004).

There are few studies using $O_2$ sensors to investigate water $O_2$ concentrations in coral reefs (Barnes 1983, Barnes & Devereux 1984). To our knowledge, the present study is the first comparison of adjacent sites with different benthic communities, revealing significantly lower water $O_2$ concentrations at algae dominated sites compared to adjacent coral dominated sites.

In the present study, the $O_2$ sensors did not measure a particular parcel of water since water from coral dominated sites likely exchanged with water from the algae dominated sites and vice versa because of alternating tidal currents. In addition, the pronounced reef topography and high sedimentary permeability at the study site (Wild et al. 2005a, Wild et al. 2009b) likely facilitated advective water exchange (Huettel & Gust 1992, Ziebis et al. 1996), which counteracted the establishment of $O_2$ gradients as described by Shashar et al. (1993). Nevertheless, significant differences in $O_2$ concentration between neighbouring sites were observed during all comparative logger deployments, which indicate *in situ* establishment of spatially limited $O_2$ gradients despite such counteracting factors as described in detail by Niggl et al. (2010a). Another reason may be that benthic algae grow preferentially in areas where high advective upwelling of nutrient rich and $O_2$ depleted pore water takes place. However, the laboratory findings of Smith et al. (2006) under exclusion of sediments and advection emphasized that there are indeed negative effects of benthic reef algae on $O_2$ concentrations; hence, the above mentioned explanation seems to be unlikely. Therefore, the recorded $O_2$ concentrations are obviously controlled by the benthic community composition of the respective site.

Physical factors such as water flow, or topographic characteristics as well as biological factors such as respiration and photosynthetic activity may influence water $O_2$ concentrations *in situ* (Kraines et al. 1996). The deployed $O_2$ loggers in the present study were placed at comparable sites in close proximity to each other where differences in topography and flow speeds between the algae and coral locations are unlikely to occur. In addition, higher respiration by benthic algae is also unlikely to explain the findings of the

present study since coral respiration is usually higher than benthic reef algal respiration (C. Jantzen unpubl. data). Lower $O_2$ concentrations at algae dominated sites may therefore be most likely due to strong stimulation of microbial activity by algae-derived OM. At algae dominated sites, $O_2$ concentrations in the dark dropped well below 100% saturation (~7 mg $l^{-1}$), but not at coral dominated sites (Fig. 3) or in the water column (Wild et al. unpubl. data). This may indicate that algae dominated sites are $O_2$ sinks in the coral reef. However, upstream–downstream measurements are required in order to confirm this assumption.

## Ecological implications

All investigated groups of organisms can obviously control reef processes, in particular interaction with microbes via OM release. However, corals likely contribute differently to reef functioning than benthic algae. The OM released by corals stimulates microbial activity generally less than algae-derived OM. Further, corals mainly release POM in the form of coral mucus (Crossland 1987), which is a transparent exopolymer (Krupp 1985, Meikle et al. 1987) that is able to trap particles, thereby fulfilling an important role as an energy carrier and nutrient trap in coral reef ecosystems (Wild et al. 2004a, Huettel et al. 2006, Naumann et al. 2009b). In contrast, algae release OM that is predominantly in dissolved form, and the particulate fraction of algae-derived organic material mainly consists of detritus and dead algal cells (Duarte & Cebrian 1996, Mannino & Harvey 2000). This is unlikely to substitute for the important role of coral mucus as particle traps as already hypothesized by Wild et al. (2009c). Apart from lacking the function of particle trapping, algae-derived OM potentially supports a different microbial community. Coral-derived OM can be degraded to some extent by microbes on the coral surface, but this material is mainly (>90%) degraded by the microbial community associated with the reef sands after detachment (Wild et al. 2004b). In contrast, algae-derived OM is likely utilized predominantly by the planktonic microbial community in the surrounding water column. This assumption is further supported by the *in situ* $O_2$ logger measurements that showed significantly lower water $O_2$ concentrations at algae dominated compared to coral dominated reef areas.

Thus, this study supports assumptions of negative consequences of OM release on $O_2$ availability in reefs subjected to a phase shift from coral to algae dominated ecosystems, owing to labile OM released by benthic algae (Kuntz et al. 2005, Kline et al. 2006, Smith et al. 2006). It further indicates that these 2 key groups of primary producers may contribute differently to coral reef ecosystem functioning, owing to the differential rates and locations of microbial utilization of their released OM.

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# 10

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

**Wolfgang Niggl[1], Andreas F. Haas[1] and Christian Wild[1]**

[1]Coral Reef Ecology Group (CORE), GeoBio-Center & Department of Earth and Environmental Science in the Faculty of Geosciences, Ludwig-Maximilians Universität, Richard Wagner Str. 10, 80333 München, Germany

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SHORT RESEARCH NOTE

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

**Wolfgang Niggl · Andreas F. Haas · Christian Wild**

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**Abstract** Many coral reef ecosystems experience shifts in benthic community composition from scleractinian corals to algae. However, consequences of such phase shifts on $O_2$ availability, important for many reef organisms, are unresolved. This study therefore comparatively investigated potential in situ effects of different benthic cover by reef macroalgae and scleractinian corals on water column $O_2$ concentrations in a Northern Red Sea fringing reef. Findings revealed that mean daily $O_2$ concentrations at algae-dominated sites were significantly lower compared to coral-dominated sites. Minimum $O_2$ concentrations were significantly negatively correlated, while diurnal variability in $O_2$ concentration was significantly positively correlated, with increasing benthic cover by algae. In contrast, no correlation with coral cover was found. These results indicate that shifts from corals to benthic algae may likely affect both in situ $O_2$ availability and variability. This may be particularly pronounced in reef systems with low water exchange (e.g. closed lagoons) or under calm weather conditions and suggests potential $O_2$-mediated effects on reef organisms.

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W. Niggl (✉) · A. F. Haas · C. Wild
Coral Reef Ecology Group (CORE), GeoBio-Center and Department of Earth and Environmental Science in the Faculty of Geosciences, Ludwig-Maximilians Universität, Richard Wagner Str. 10, 80333 Munich, Germany
e-mail: w.niggl@lrz.uni-muenchen.de

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Whilst 'pristine' coral reefs usually exhibit low fleshy macroalgal standing crop (Smith et al., 2001), degrading reefs often undergo a phase shift in which fleshy macroalgae become dominant over scleractinian corals (Done, 1992; Hughes, 1994; McCook, 1999). In this context, many studies investigated phase shift promoting factors such as overfishing of herbivores and nutrient enrichment (e.g. McManus et al., 2000; Smith et al., 2001; Belliveau & Paul, 2002; Ledlie et al., 2007) or focussed directly on coral–algae interactions (McCook et al., 2001; Jompa & McCook, 2003; Nugues & Roberts, 2003; Haas et al., 2009, 2010). Recent laboratory studies demonstrated the deleterious effect of coral reef macroalgae on scleractinian corals via the hypothetical release of biologically labile dissolved organic compounds (Nguyen et al., 2005) and a subsequent decrease in $O_2$ availability via stimulation of microbial activity (Kline et al., 2006; Smith et al., 2006). However, in situ relevance of these observations could not be demonstrated yet.

This study aims to contribute in this context by the comparative investigation of in situ $O_2$ availability and variability at reef sites dominated by algae or corals in the Northern Red Sea. Coral reefs in this

area usually display high benthic coverage by living scleractinian corals (Wilkinson, 2008). However, we observed extensive overgrowth of corals by benthic macroalgae during two field expeditions to the Gulf of Aqaba in winter and spring 2008 although the living hermatypic coral cover in investigated area was 36–41% (Haas et al., 2010) thus exceeded typical living hard coral cover (30%) of the Jordan coral reefs (Wilkinson, 2008). The high macroalgal abundance was likely caused by high availability of inorganic nutrients owing to seasonal water mixing in winter (Wolf-Vecht et al., 1992; Badran et al., 2005) and subsequent stimulation of algal growth, presumably at first on bare substrate. The resulting simultaneous occurrence of adjacent algae- and coral-dominated reef sites offered a good opportunity to study potential effects of benthic community composition on $O_2$ concentrations in the overlying water column in situ. Therefore, during the two expeditions, two *Eureka* Midge Dissolved $O_2$ Loggers were simultaneously deployed for 24 h within adjacent algae- (35–100% benthic cover by genera *Hydroclathrus*, *Enteromorpha* or turf algae) and coral- (20–95% benthic cover; ca. 60% of total coral cover by genera *Acropora*, *Lobophyllia*, *Porites*) dominated reef sites at nine different deployment occasions (6 in winter, 3 in spring) in water depths of 4–7 m (Fig. 1). Coral benthic coverage was less than 10% on algae-dominated sites and vice versa. The remaining seafloor was covered with sand or dead coral rock. During each of these deployment occasions, the two $O_2$ loggers were centrally placed, one on the algae- and the other one on the respective adjacent coral-dominated site resulting in a distance between the loggers of 3–10 m. All 18 different sites were spread over a total distance of approximately 400 m. $O_2$ concentrations and water temperatures were measured and logged every 5 min over the entire deployment period of 24 h (Fig. 2), whereby the sensors of the loggers were installed ca. 10 cm above the seafloor. Before deployment, the $O_2$ loggers were calibrated at in situ temperature and salinity using $O_2$ concentrations obtained from the dissolved $O_2$ determination method described by Winkler (1888) as reference. All deployment sites were exposed to similar water current velocities of 3.2–7.1 cm $s^{-1}$ (measured via in situ tracking of suspended particles along known distances using ruler and watch). The orientation of algal- to coral-dominated sites relative to the main current direction varied between the nine deployment occasions. Temperature ranged from 20.7 to 21.4°C during winter and from 22.0 to 22.7°C during spring expedition.

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

Analysis of data revealed that mean daily $O_2$ water concentrations were significantly lower at algae-dominated ($7.69 \pm 0.91$ mg $O_2$ $l^{-1}$; mean ± SD of all nine deployments, $n = 2{,}512$) compared to adjacent coral-dominated ($8.07 \pm 0.61$ mg $O_2$ $l^{-1}$; mean ± SD of all nine deployments, $n = 2{,}512$) sites (two-sided, paired *t*-tests; $P < 0.05$). The minimum daily $O_2$ concentrations exhibited a strong negative correlation with benthic algae cover (Pearson product-moment correlation, $r = -0.842$, $P = 0.004$) (Fig. 3a). In addition, diurnal variations in $O_2$ concentrations at algae-dominated sites were significantly higher than at coral-dominated sites and displayed strong positive correlation with benthic algae cover (Pearson product-moment correlation, $r = 0.90$, $P = 0.001$) (Fig. 3b). Coral cover in contrast did neither correlate to diurnal variation nor to minimum daily $O_2$ concentrations.

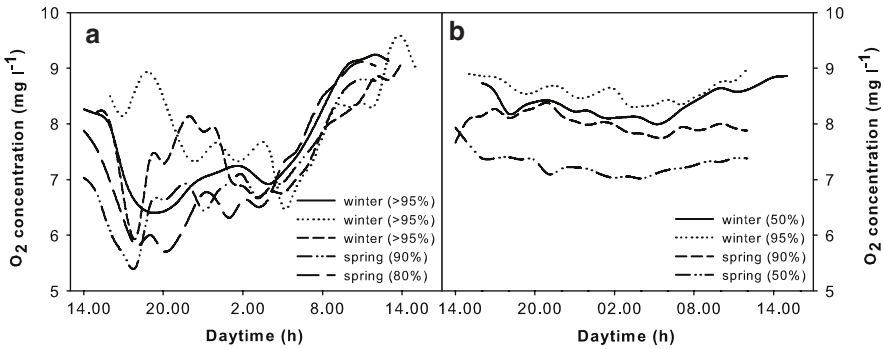


**Fig. 2** Hourly averaged $O_2$ concentrations above (**a**) algae-dominated sites (algae cover ≥80%) and (**b**) coral-dominated sites (coral cover ≥50%). The numbers in brackets describe the benthic cover on respective sites

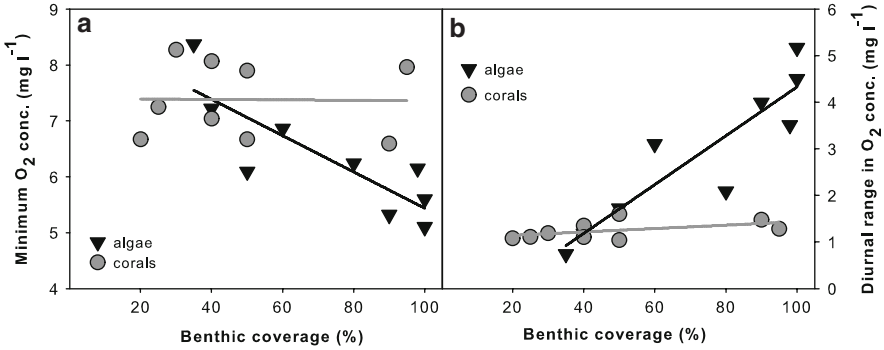


**Fig. 3** Minimum $O_2$ concentrations (**a**) and diurnal variation in $O_2$ concentrations (**b**) plotted against per cent benthic coverage of corals or algae in the surveyed locations

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

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

The results of this study therefore suggest more pronounced, longer-lasting hypoxia events under calm weather or other advection-inhibiting environmental conditions (e.g. small sediment grain size and resulting low permeability, low surface topography, weak bottom currents). This may particularly apply for closed reef lagoons with low water exchange and high cover by benthic macroalgae.

Occurring severe hypoxia could cause altered distribution and behaviour of fishes (Breitburg, 2002; Östlund-Nilsson & Nilsson, 2004) or even lead to mass mortality of reef organism, particularly if microbial activity is stimulated by natural (Simpson

et al., 1993) or anthropogenic organic matter and nutrient input. But already low $O_2$ concentrations in the range of 4.5–6.0 mg $O_2$ $l^{-1}$ which are similar to those of this study (Fig. 3a) may negatively affect animal growth, behaviour, and metabolism (Harris et al., 1999; Gray et al., 2002). Whilst physiological processes may only be marginally affected by a transitory $O_2$ decrease, benthic fauna composition may change under these conditions (Montagne & Ritter, 2006). Mobile dwellers can avoid low $O_2$ availability by migrating to sites with higher $O_2$ concentrations (Wu et al., 2002). But very active specimens which are unable to tolerate low oxygen levels (Nilsson et al., 2007) and some of the sessile benthic specimens could be particularly affected. Hypoxia-tolerant reef species (e.g. inactive organisms) could be favoured against those hypoxia-sensitive organisms, which may lead to changes in community composition in a particular habitat. Such changes in the benthic community composition due to periodically low dissolved $O_2$ concentrations have already been observed (Platon et al., 2005; Lim et al., 2006; Montagne & Ritter, 2006).

Recent studies indicated negative effects of benthic algae on scleractinian corals by reducing $O_2$ availability likely via the release of DOC (Kline et al., 2006; Smith et al., 2006), as algae-derived DOC mostly consists of glucose and positively correlates with microbial $O_2$ consumption (Haas & Wild, unpublished). In contrast, reef algae-derived POM does not correlate with microbial $O_2$ consumption, thus exhibits much lower microbial degradability (Haas et al., unpublished). The findings of this study therefore supplement previous laboratory studies (Kline et al., 2006; Smith et al., 2006) by demonstrating that there may be similar in situ effects of algae-derived DOC. Besides released organic matter, intense coral or algae respiration could also lead to decreased local $O_2$ concentrations. The observations of this study do therefore likely reflect a combination of high DOC release and respiration by algae. The study of Haas et al. (2009) in this context indicated that DOC addition may negatively influence corals in interaction with algae via decreased $O_2$ water concentrations. This could also imply positive feedback loops promoting reef algae in the competition with scleractinian corals. In contrast to the investigated seasonal phase shifts, which likely provide sufficient time for the reestablishment of original benthic community composition, permanent phase shifts may cause non-reversible subsequent changes.

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## Curriculum Vitae — Wolfgang Niggl

**PERSONAL DATA:**

| | |
|---|---|
| 04/04/1980 | born in Traunstein |

**EDUCATION:**

| | |
|---|---|
| 05/2007-09/2010 | Ludwig-Maximilans-Universität München<br>PhD Thesis: ‚Organic matter release by Red Sea cnidarians and its function as energy carrier – effects of environmental variables' |
| 10/2000-09/2006 | Ludwig-Maximilans-Universität München<br>Diploma Thesis: ‚Die zeitliche Komponente und die Rolle des Korallenmucus bei Coral Bleaching' |
| 09/1990-06/1999 | Chiemgau-Gymnasium Traunstein |
| 09/1986-08/1990 | Grundschule Waging |

**AWARDS:**

| | |
|---|---|
| 05/2007-10/2010 | PhD stipend of University of Bavaria/Bavarian Elite Advancement |