Proceedings of the 12th International Coral Reef Symposium, Cairns, Australia, 9-13 July 2012
4C Coral reef response to multiple stresses: organisms to ecosystems

Environmental factors affect soft coral-derived organic matter fluxes

Vanessa N. Bednarz, Malik S. Naumann, Christian Wild

Coral Reef Ecology Group (CORE), University of Bremen & Leibniz Center for Tropical Marine Ecology (ZMT), Fahrenheitstr. 6, 28359 Bremen, Germany
Corresponding author: vanessa.bednarz@zmt-bremen.de

Abstract. Coral-derived organic matter (OM) release importantly contributes to reef ecosystem functioning, but may be affected by several environmental factors. Although soft corals represent a dominant organism group in many reef habitats, their OM flux rates under variable environmental conditions remain unexplored. Therefore, the present study quantified OM fluxes induced by the common soft coral genus *Xenia* in response to low (LL) and high (HL) light availabilities (35 and 57 µmol photons m^{-2} s^{-1}, respectively) as well as to elevated ammonium (N, 20 µM), phosphate (P, 2 µM) and combined elevated (NP) concentrations. In addition, subsequent planktonic microbial activity induced by *Xenia*-derived OM was investigated. Findings revealed uptake of OM under HL (-0.32 ± 0.16 mg particulate organic carbon (POC), -0.05 ± 0.02 mg particulate nitrogen (PN), -4.82 ± 2.13 mg dissolved organic carbon (DOC) m^{-2} coral surface area h^{-1}) and release under LL (0.24 ± 0.14 mg POC, 0.03 ± 0.02 mg PN, 1.04 ± 1.15 mg DOC m^{-2} coral surface area h^{-1}), but only PN fluxes were statistically different. DOC uptake significantly increased in response to N and NP addition, while POC fluxes were not affected by inorganic nutrient enrichment. PN release significantly increased in response to P enrichment (under HL only) and consequently caused a significant stimulation of microbial O_2 consumption compared to low nutrient conditions. This study provides first information on the effect of environmental key factors on soft coral-derived OM fluxes with further implications for soft coral contribution to reef ecosystem functioning *via* OM release.

Key words: Organic matter, Release, Uptake, Inorganic nutrients, Light.

Introduction

Corals can release large amounts of particulate (POM) and dissolved (DOM) organic matter (OM) into surrounding waters (Crossland et al. 1980; Meikle et al. 1988; Naumann et al. 2010). This coral-derived OM fulfills important ecological functions *via* particle retention, establishing of fauna-microbe interactions and recycling of essential nutrients within the reef ecosystem (Wild et al. 2004a; Wild et al. 2008; Naumann et al. 2009).

Various environmental factors such as aerial exposure, depth-mediated light availability, water temperature or inorganic nutrient concentrations can significantly affect OM release by scleractinian corals (Crossland 1987; Naumann et al. 2010; Tanaka et al. 2010). Decreasing light availability strongly reduces OM release of corals most likely due to light-mediated changes in phototrophic carbon assimilation (Crossland 1987; Naumann et al. 2010). In addition, elevated inorganic nutrient concentrations can reduce OM release by scleractinian corals previously demonstrated under *in situ* and laboratory conditions (Naumann et al. 2010; Tanaka et al. 2010). Such changes of coral-derived OM fluxes may subsequently influence coral reef functioning, in particular OM degradation and recycling processes by planktonic and benthic microbes (Wild et al. 2010). Although environmental factors can affect OM fluxes of several hard coral reef species, to date no study investigated the effect on soft coral-derived OM.

Therefore, the objective of this study was to examine the effect of elevated inorganic nutrient concentrations and different light availabilities on particulate organic carbon (POC), particulate nitrogen (PN) and dissolved organic carbon (DOC) fluxes induced by the soft coral *Xenia*, a common species in several reef locations worldwide. In addition, subsequent effects induced by *Xenia*-derived OM on planktonic microbial activity were investigated by determining planktonic microbial O_2 consumption rates.

Material and Methods

Experimental design

A total of 48 soft coral colonies of the genus *Xenia umbellata* c.f. were used for the experiments. Two separate nutrient-enrichment experiments were carried out under controlled laboratory conditions. The first experiment took place under high light (HL) conditions (57.2 ± 0.6 µmol photons m^{-2} s^{-1}, mean ±

SE) representative of ambient light availability measured in a Red Sea fringing reef at ca. 5 m water depth (a typical *Xenia* environment) during March 2010 (Bednarz unpublished data). For the follow-up low light (LL) experiment (35.2 ± 0.6 µmol photons m^{-2} s^{-1}), light availability was reduced by ca. 50 % in order to obtain a potential light treatment effect on coral-derived OM fluxes between the HL and LL experiment. For each experiment, half of the colonies were randomly assigned to 4 different treatments (n = 6 per treatment): 1. phosphate enrichment (P, 2 µM PO_4^{3-}), 2. ammonium enrichment (N, 20 µM NH_4^+), 3. ammonium and phosphate enrichment (NP, 20 µM NH_4^+ and 2 µM PO_4^{3-}) and 4. non-enriched local aquarium water (control, < 0.8 µM NH_4^+ and < 0.1 µM PO_4^{3-}). Each coral colony was placed individually into a 1 l glass beaker filled with nutrient-enriched or non-enriched aquarium water to avoid pseudo-replication for each treatment. The respective water in all beakers was renewed daily over a period of 4 weeks for each experiment and light intensity as well as water temperature were continuously recorded with data loggers (Onset HOBO Pendant UA-002-64; spectral detection range: 150 – 1200 nm; temperature accuracy: ± 0.53°C).

Quantification of organic matter fluxes

After the experimental period of 4 weeks, the established beaker incubation technique described by Herndl & Velimirov (1986) was carried out to quantify OM fluxes by *Xenia*. For this purpose, each coral fragment was incubated individually in a pre-cleaned glass beaker (500 ml). Non-nutrient-enriched aquarium seawater was used as incubation medium for all treatments to ensure comparable water conditions, in particular equal inorganic nutrient availability during incubation procedure for all treatments. Beakers only filled with aquarium seawater (without coral) served as seawater controls (n = 5). During incubation, light availability and water temperature were the same as during the experimental period. After 5 – 6 h, the incubation was terminated by removing the corals from the beakers. Immediately after incubations (≤ 5 min), subsamples were drawn from the homogenized incubation water, and 80 – 90 ml were set aside for subsequent microbial O_2 consumption measurements carried out as described below.

For DOC, 10 ml subsamples were drawn from each beaker using clean syringes and passed through sterile polyethersulfone membrane filters (VWR; 0.2 µm pore-sized, pre-washed with 6 ml sample). The filtrate was collected in pre-combusted (450 °C; 6 h) glass vials that were kept frozen at -20 °C until further processing. DOC samples were defrosted, acidified by adding 50 µl of 2 M HCl to a pH < 2, purged with O_2 for 2 min in order to remove inorganic carbon and subsequently analyzed using a DIMA-TOC 100 total organic carbon analyzer (Dimatec, Germany) with potassium hydrogen phthalate as elemental standard.

The remaining incubation water (350 – 450 ml) was vacuum filtered onto pre-combusted (450 °C; 6 h) GF/F filters (Whatman, 0.6 - 0.8 µm pore size, diameter: 25 mm) in order to quantify POC and PN contents. The filters were immediately dried for at least 48 h at 40 °C and kept dry until further processing. For POM analysis, filters were wrapped in silver cups and measured using a THERMO™ NA 2500 elemental analyzer (standard deviation < 3 %) with atropine and cyclohexanone-2, 4-dinitrophenylhydrazone as elemental standards.

After beaker incubations, the number of polyps from each colony was counted and the mean surface area of a *Xenia* polyp was determined by measuring the polyp's diameter as well as the diameter and length of the tentacle foot on 100 completely protruded polyps randomly distributed over all 48 colonies. The mean surface area for a polyp was subsequently multiplied with the number of polyps to generate the total surface area of each incubated colony.

POC, PN and DOC concentrations (µg l^{-1}) of seawater controls were subtracted from the concentrations found in *Xenia*-incubated water and related to volume of incubation medium. The values were subsequently normalized to incubation time and colony surface area to calculate OM flux rates (mg m^{-2} h^{-1}). POC:PN ratios were calculated from molar contents of POC and PN concentrations in the incubation water.

Microbial O_2 consumption

After incubation, the initial O_2 concentration in the water of each beaker was measured using an O_2 optode sensor (Hach Lange HQ 10, accuracy ± 0.05 %). Thereafter, from each beaker one water subsample was incubated in an airtight 60 ml Winkler glass bottle in the dark for 18 – 23 h. Subsequently, the final O_2 concentration in each Winkler bottle was measured as described above, subtracted from initial O_2 concentration values and normalized to dark incubation duration. To calculate microbial O_2 consumption induced by *Xenia*-derived OM, the mean O_2 consumption of the seawater controls was subtracted from O_2 draw down in *Xenia*-incubated waters. The resulting concentration differences were finally related to the volume of coral incubation medium and normalized to coral surface area and coral incubation period (mg m^{-2} h^{-1}).

Data analysis

All data entering statistical comparisons were tested for homogeneity of variance and normal distribution using Levene and Shapiro-Wilk tests. If assumptions were fulfilled, one-way ANOVA analyses with following Tukey Post-hoc test were carried out. Otherwise non-parametric Kruskal-Wallis tests were used instead, followed by Mann-Whitney as Post-hoc test. The effect of light was analyzed by comparing data obtained from control corals under HL and LL condition using single Mann-Whitney tests.

Results

Effect of light availability

POC, PN and DOC fluxes of control corals (Fig. 1 a-c) were usually net negative (uptake) during HL and net positive (release) under LL condition. Statistical analysis (Mann-Whitney tests) revealed only significantly higher release rates for PN under LL compared to HL conditions ($p = 0.017$), while no light-mediated differences were found for POC ($p = 0.052$) or DOC ($p = 0.093$) fluxes.

Effect of inorganic nutrient concentration

Inorganic nutrient addition caused no effect on POC fluxes, irrespective of light condition (Fig. 1a). PN fluxes were affected under HL condition, only (Kruskal-Wallis test, $H = 10.01$, $df = 3$, $p = 0.019$; Fig. 1b). This revealed significantly stimulated release rates of P treated compared to control corals (multiple comparison of means, $p = 0.015$), while no effect was found in N and NP treated corals. Additionally, the POC:PN ratio in the incubation water of P (Post-Hoc: Tukey, $p < 0.001$) and NP ($p = 0.040$) treated corals was significantly reduced compared to controls under HL (One-way ANOVA, $df = 3$, $F = 13.47$, $p < 0.001$; Tab. 1), while no differences were found under LL.

Inorganic nutrient addition caused significant effects on DOC fluxes under HL (Kruskal-Wallis-Test, $H = 8.28$, $df = 3$, $p = 0.041$) and LL conditions (One-way ANOVA, $df = 3$, $F = 7.80$, $p = 0.002$; Fig. 1c). Increased uptake rates were found for N and NP treated corals being statistically significant for N compared to P treated corals under HL (multiple comparison of means, $p = 0.042$) and for NP compared to P treated (Post-hoc: Tukey, $p = 0.003$) and control corals (Post-hoc: Tukey, $p = 0.007$) under LL.

Microbial activity

Microbial O_2 consumption rates in the incubation water induced by OM fluxes of control corals revealed no differences between HL and LL conditions (Mann-Whitney test, $p = 0.132$; Fig. 1d). However, nutrient treated corals significantly affected

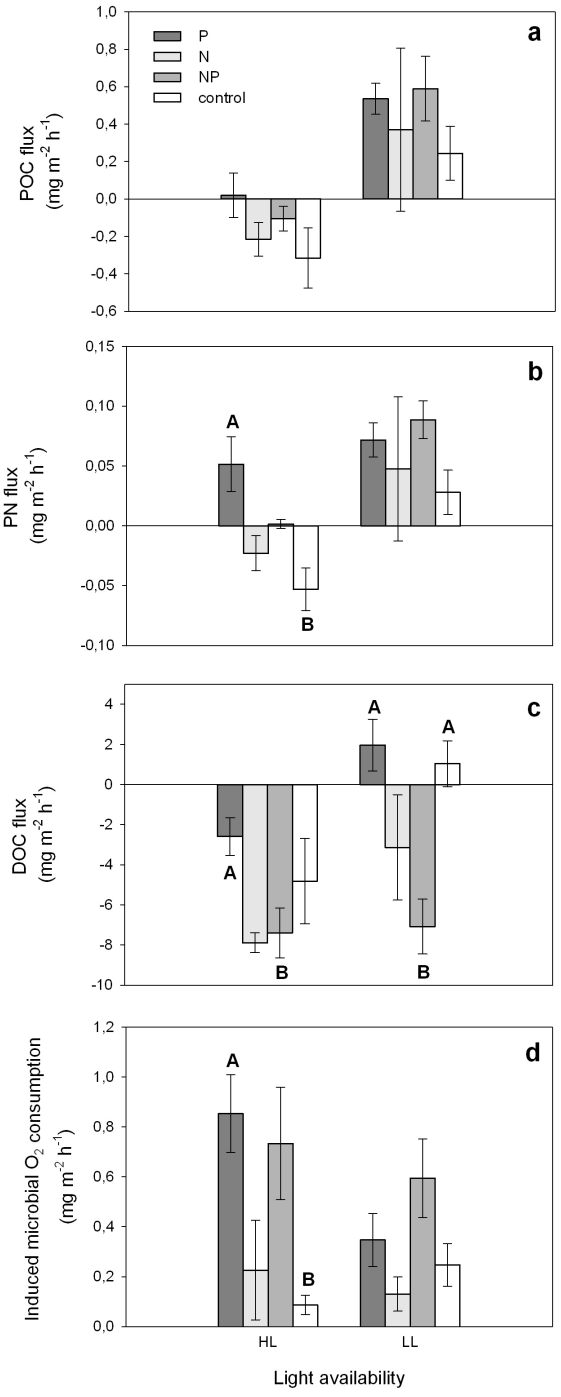

Figure 1: Particulate organic carbon (POC; a), particulate nitrogen (PN; b) and dissolved organic carbon (DOC; c) fluxes (mean ± SE, n = 3 – 6) by *Xenia* corals in response to treatments P (phosphate addition), N (ammonium addition), NP (combined ammonium and phosphate addition) and control (no nutrient addition) under ambient high (HL) and low (LL) light levels (positive values indicate net release and negative values net uptake) and its effect on microbial O_2 consumption (d). Columns marked with A are significantly different from columns marked with B within HL and LL, respectively.

microbial activity under HL conditions, only (One-way ANOVA, df = 3, F = 4.85, p = 0.011). There, OM release by P treated corals significantly stimulated microbial O_2 consumption rates in comparison to control corals (Post-hoc: Tukey, p = 0.023), while N (p = 0.937) and NP (p = 0.063) treated corals had no significant effect.

Table 1: POC:PN ratios (mean ± SE, n = 3 – 6) in *Xenia* incubation water in response to treatments P (phosphate addition), N (ammonium addition), NP (combined ammonium and phosphate addition) and control (no nutrient addition) under ambient high (HL) and low (LL) light levels. Values marked with A are significantly different from values marked with B within HL and LL, respectively.

Treatment	HL	LL
P	7.3 ± 0.4 ^{A}	9.5 ± 0.4
N	9.0 ± 0.3	9.1 ± 0.8
NP	8.7 ± 0.3 ^{A}	8.7 ± 0.6
control	10.0 ± 0.2 ^{B}	9.8 ± 0.2

Discussion

Effect of light availability

Light availability represents an environmental key factor potentially influencing soft coral-derived OM release. Previous studies on several scleractinian coral species observed elevated OM uptake or reduced OM release rates in response to decreasing ambient irradiance levels (Crossland 1987; Naumann et al. 2010). Decreasing light availability predominantly influences photosynthetic carbon assimilation by the symbiotic zooxanthellae, which is directly supported by our findings from parallel studies revealing reduced gross photosynthesis for *Xenia* corals under LL (13 ± 1 mg O_2 m^{-2} h^{-1}) compared to HL (22 ± 2 mg O_2 m^{-2} h^{-1}) conditions (Bednarz unpublished data). This likely reduces the amount of carbon translocated to the coral host (Muscatine et al. 1984) and consequently the coral may release less OM into the surrounding water. However, the present findings contrast to the above mentioned studies, as *Xenia*-derived PN release was significantly enhanced under decreasing light availability, and no light-mediated change was found for POC and DOC fluxes. The observed minor effect of light on *Xenia*-derived OM fluxes compared to scleractinian corals may result from differences regarding the coral's nutrition, as soft corals have been identified to rely less on autotrophy and to a greater extend on heterotrophy than scleractinian corals (Fabricius & Klumpp 1995).

Effect of inorganic nutrient concentration

Besides the minor effect of light, elevated ambient inorganic nutrient concentrations significantly affected OM fluxes by the soft coral *Xenia*.

While POC fluxes remained constant among all treatments, PN release significantly increased in P treated corals. A previous study found no effect of seasonally elevated ammonium or phosphate concentrations on POM fluxes induced by several scleractinian corals (Naumann et al. 2010). Autotrophic carbon acquisition by zooxanthellae plays an important role for the composition and release of coral-derived OM (Brown & Bythell 2005). As gross photosynthesis of P treated *Xenia* corals significantly increased compared to control corals (Bednarz et al. submitted), the amount of products translocated from zooxanthellae to the host may have likewise increased. This in turn could have stimulated POM release. Supporting this, scleractinian C:N ratios of translocated compounds have been shown to decrease in response to elevated ambient inorganic nutrient concentrations (Tanaka et al. 2006), thereby potentially influencing the composition of POM released by the coral. This is in line with the observed stimulated PN release and the consequently reduced POC:PN ratio in the incubation water of P treated compared to control corals.

DOC uptake from the surrounding water was significantly stimulated in N and NP treated *Xenia* corals, while no effect was found in response to phosphate addition. This indicates that elevated ambient ammonium concentrations predominantly influenced DOC flux in *Xenia*. Previous studies on several scleractinian corals found no change of DOC fluxes in response to elevated inorganic nutrient concentrations (Naumann et al. 2010; Tanaka et al. 2010). Increased DOC uptake rates of N and NP treated corals observed in the present study may be explained by a reduced translocation of photosynthates from the algal symbionts to the coral host. Although photosynthesis and respiration rates remained comparable between N, NP and control *Xenia* corals (Bednarz et al. submitted), elevated ambient ammonium concentrations may enable the zooxanthellae to retain more photosynthetically fixed carbon for their own metabolism, and thus less carbon is translocated to the host (Dubinsky & Jokiel 1994). Consequently, the coral may balance this carbon deficit by increasing organic carbon uptake from the surrounding water. As POC fluxes were not affected, we conclude that *Xenia* may predominantly rely on DOM as its heterotrophic food source in high nutrient waters.

Microbial activity and ecological implications

Coral-derived OM represents an energy-rich substrate supporting rapid microbial growth and activity in the direct reef vicinity (Ducklow & Mitchell 1979; Wild et al. 2004b).

In the present study, highest stimulation of microbial activity was observed in incubation water showing the lowest POC:PN ratio. Previously, it has

been shown that not only the quantity, but also the quality of coral-derived OM represents an important criterion for influencing planktonic microbial activity and planktonic community metabolism (Wild et al. 2008). Therefore, our findings indicate that elevated ambient inorganic nutrient concentrations may influence the composition and quality of *Xenia*-derived OM, thereby representing an attractive substrate for planktonic microbes.

Nevertheless, rates of planktonic microbial activity attributable to *Xenia*-derived OM were below values previously reported for several scleractinian corals (2.2 ± 0.2 mg O_2 m^{-2} h^{-1}; Wild et al. 2010). This may result from the small amount of OM released by *Xenia* compared to scleractinian corals (2.8 ± 0.3 mg POC m^{-2} h^{-1}, 0.29 ± 0.03 mg PN m^{-2} h^{-1}; Naumann et al. 2010). OM released by scleractinian corals acts as a trophic vector and is mainly (> 90 %) degraded by microbes associated with the reef benthos, thereby establishing fast recycling processes of essential nutrients within the reef ecosystem (Wild et al. 2004b). The present study provides first indications that soft corals may not sustain this important ecosystem engineering function *via* OM release. However, *in situ* experiments will be required in order to verify the present laboratory-based findings with data obtained under natural conditions, and to investigate the degradation of soft coral-derived OM by microbes associated with the reef benthos. These findings will be of special interest as soft corals represent a major sessile group of the reef benthos (Dinesen et al. 1983), but their specific contribution to biogeochemical nutrient cycles and reef ecosystem functioning *via* OM release still remains largely unexplored.

In summary of soft and scleractinian reef coral susceptibility to changes in some environmental variables, our findings reveal a differential response of both groups with regard to OM fluxes. While ambient light availability strongly influences OM fluxes of scleractinian corals, we show here that soft corals (i.e. *Xenia*) are rather affected by ambient inorganic nutrient concentrations than ambient light levels. In this context, comparative studies involving soft and scleractinian coral species would enable us to investigate group-specific physiological responses, and to eventually evaluate the potential impact of variable environmental parameters on biogeochemical OM cycles in scleractinian and soft coral dominated reef environments.

Acknowledgement

We would like to thank W. Niggl, A. Gabrenya, J. Müller, S. Becker and H-J. Chen for assistance during experimentation and U. Struck and R. Fuss for their help with sample analyses. This study was funded by grant Wi 2677/6-1 of the German Research Foundation (DFG) to C.Wild and a PhD stipend of Evangelisches Studienwerk e.V. Villigst to V. Bednarz.

References

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

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

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

Dinesen ZD (1983) Patterns in the distribution of soft corals across the central Great Barrier Reef. Coral Reefs 1:229-236

Dubinsky Z, Jokiel PL (1994) Ratio of energy and nutrient fluxes regulates symbiosis between zooxanthellae and corals. Pac Sci 48:313-324

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

Fabricius KE, Klumpp DW (1995) Widespread mixotrophy in reef-inhabiting soft corals: the influence of depth, and colony expansion and contraction on photosynthesis. Mar Ecol Prog Ser 125:195-204

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

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

Muscatine L, Falkowski PG, Porter JW, Dubinsky Z (1984) Fate of photosynthetic fixed carbon in light-and shade-adapted colonies of the symbiotic coral *Stylophora pistillata*. Proc R Soc Lond Ser B 222:181-202

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

Naumann MS, Haas A, Struck U, Mayr C, El-Zibdah M, Wild C (2010) Organic matter release by dominant hermatypic corals of the Northern Red Sea. Coral Reefs 29:649-659

Tanaka Y, Miyajima T, Koike I, Hayashibara T, Ogawa H (2006) Translocation and conservation of organic nitrogen within the coral-zooxanthella symbiotic system of *Acropora pulchra*, as demonstrated by dual isotope-labeling techniques. J Exp Mar Biol Ecol 336:110-119

Tanaka Y, Ogawa H, Miyajima T (2010) Effects of nutrient enrichment on the release of dissolved organic carbon and nitrogen by the scleractinian coral *Montipora digitata*. Coral Reefs 29:675-682

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

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

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

Wild C, Niggl W, Naumann MS, Haas AF (2010) Organic matter release by Red Sea coral reef organisms: potential effects on microbial activity and *in situ* O_2 availability. Mar Ecol Prog Ser 411:61-71