



Microbial degradation of cold-water coral-derived organic matter: potential implication for organic C cycling in the water column above Tisler Reef

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ABSTRACT: Cold-water corals release organic matter, in particular mucus, but its role in the ecological functioning of reef ecosystems is still poorly understood. The present study investigates the planktonic microbial degradation of mucus released by *Lophelia pertusa* colonies from Tisler Reef, Skagerrak. Results are compared to the degradation of dissolved and particulate organic substrates, including the carbohydrates glucose and starch, as well as gum xanthan and the cyanobacterium *Spirulina* spp. as the model organism for phytoplankton. Resulting microbial organic C degradation rates for the dissolved fraction of *L. pertusa*-derived mucus showed nearly linear progression over time and revealed similar degradation rates compared to glucose and starch. Degradation of the particulate mucus fraction, in contrast, displayed exponential progression and was much faster than degradation of the dissolved fraction. In addition, particulate mucus degradation showed a 4-fold increase compared to that of the added *Spirulina* spp. suspension. Mucus-associated microbial communities apparently play a key role in organic matter recycling, as degradation rates more than doubled in untreated compared to sterile coral-derived mucus over 3 d of incubation. Quantification of O₂ consumption in the water column above Tisler Reef showed significantly increased values in the direct vicinity of the reef. C-stable isotope signatures of suspended particulate organic matter close to Tisler were close to those of *L. pertusa*-derived mucus, and high dissolved organic carbon (DOC) concentrations were detected above Tisler Reef. These findings demonstrate the stimulating effect of cold-water coral reefs on microbial activity in the adjacent water column and may indicate some control over organic C cycling.

KEY WORDS: Cold-water coral reefs · *Lophelia pertusa* · Coral-derived mucus · Microbial degradation · O₂ consumption · DOC dynamics · Carbon cycle

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INTRODUCTION

Cold-water coral ecosystems are hotspots of biodiversity, hosting a benthic community of >1300 reef-associated species (Jensen & Frederiksen 1992, Mortensen et al. 1995, Freiwald et al. 2004, Roberts & Hirshfield 2004). The main framework-building, scleractinian corals *Lophelia pertusa* and *Madrepora*

oculata rely on food supply transported either down from productive surface waters or by redistribution of suspended particulate organic matter (POM) in the bottom boundary layer, as well as on zooplankton prey in the vicinity of corals (Freiwald 2002, Duineveld et al. 2004, Kiriakoulakis et al. 2004, 2005, White et al. 2005, Roberts et al. 2006). This filter-feeding lifestyle contrasts with the main lifestyle of

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hermatypic warm-water corals, which host zooxanthellae—endosymbiotic algae transferring photosynthetically fixed carbon to their host (Muscatine et al. 1981). Carbon and nutrient cycling, as well as ecosystem functioning (Goreau et al. 1979, Romaine et al. 1997, Wild et al. 2004a), are well studied in tropical coral reef systems. These ecological and biochemical processes are however far less understood in cold-water coral reefs, and studies investigating the interrelations between corals and reef-associated organisms, particularly planktonic microbial assemblages, are scarce.

Recent research at Norwegian cold-water coral reefs showed that cold-water corals release high quantities of particulate and dissolved organic matter, in particular mucus (Wild et al. 2008). The cold-water coral-derived mucus represents an attractive organic substrate for planktonic microbial assemblages and stimulates microbial activity in the adjacent water column, as evident by high microbial organic matter turnover rates (Wild et al. 2008). The establishment of fauna–microbe interactions may play an important role in ecosystem functioning, e.g. as a vector for carbon and nutrient cycling in the reef system. It also facilitates the generation of specific microbial habitats showing distinct patterns of microbial diversity (Schöttner et al. 2009). Furthermore, Lavaleye et al. (2009) described a 'reef effect' leading to the preferential removal of labile N-rich compounds and, thus, altering the quality of POM passing over a cold-water coral reef and suggest that cold-water coral reef ecosystems do play an important role in C mineralization. High aerobic microbial mineralization of organic matter in the overlying water column and coral framework (Wild et al. 2008) may, in addition, limit the amount of labile organic material reaching the reef-associated sediments, where extremely low rates of anaerobic C mineralization have been detected (Wehrmann et al. 2009).

The present pilot study aims to extend our understanding of the interplay between cold-water corals and associated planktonic microbial assemblages. It targets the microbial degradability of cold-water coral-derived organic material in comparison to other dissolved and particulate organic substrates. Supplementary studies investigating O₂ consumption rates as a proxy for microbial activity and dissolved organic carbon (DOC), as well as POM concentrations and C isotope signatures in the water column above Tisler Reef, Skagerrak, are presented. Studies were conducted in May 2008 through the combined deployment of line-attached Niskin bottles and a remotely operated vehicle (ROV), thereby allowing exact tracking of sampling locations in close proximity to the cold-water coral reef.

MATERIALS AND METHODS

Study site. Experiments presented in the current study were conducted between 20 and 27 May 2008 at Sven Lovén Centre for Marine Sciences, Skagerrak, Sweden. *In situ* sampling was carried out at 3 neighbouring stations above the central part of the nearby Tisler Reef, Skagerrak (Stn 1: 58° 59.902' N, 10° 57.736' E, Stn 2: 58° 59.806' N, 10° 57.979' E, Stn 3: 58° 59.796' N, 10° 58.001' E; water depth at all stations ca. 108 to 114 m), close to the border between Sweden and Norway. The Tisler Reef is a relatively large inshore reef (ca. 1200 × 200 m live reef over a depth range of from 70 to 160 m), dominated by the hermatypic scleractinian *Lophelia pertusa*.

Microbial degradation of *Lophelia pertusa*-derived mucus and reference substrates. To study the microbial degradation of *L. pertusa*-derived mucus in comparison with reference substrates, 2 independent experiments were conducted: a dissolved substrate and a particulate substrate experiment. In these experiments microbial dissolved and particulate organic carbon (DOC and POC) degradation of *L. pertusa*-derived mucus were separately investigated in comparison with different organic substrates, because coral-derived mucus comprises both a dissolved and a particulate fraction (Wild et al. 2004a, 2008). Both fractions were separated by filtration through sterile 0.2 µm syringe filters (Millipore). An overview of both experiments with the respective substrates added is given in Table 1. Immediately before each experiment, mucus was freshly collected from 10 to 20 small (lengths: 3 to 10 cm) *L. pertusa* fragments. The fragments were originally sampled from Tisler Reef and kept in 40 l glass aquaria with deep water flow-through (*in situ* water pumped from ca. 50 m water depth and pre-filtered over coarse sand) for between 2 and 60 d prior to the mucus sampling. Mucus was collected by exposing the coral fragments to air for 3 to 5 min. Mucus released during the first minute was discarded, and the subsequent production was collected in glass Petri dishes. When necessary, sterile-filtered seawater (0.2 µm, Millipore) was used to detach mucus from the coral fragments. In total, 200 to 400 ml of diluted mucus (approximately 1:5, mucus:seawater) was retrieved from all coral fragments during the 2 samplings. *L. pertusa*-derived mucus was added to 30 ml Winkler glass bottles in volumes of 5 ml (particulate substrate experiment) and 10 ml (dissolved substrate experiment), with n = 4 replicate volumes for each experiment. Glucose (monohydrate; 2 g l⁻¹ deep water) and starch (2 g l⁻¹ deep water) suspensions were used for the dissolved substrate experiment. For the particulate substrate experiment, in addition to the untreated mucus, 5 ml of sterile *L. pertusa*-derived mucus (heated to

Table 1. Overview of substrate incubation experiments and respective organic matter additions. All incubation experiments with *Lophelia pertusa*-derived mucus, glucose and starch additions took place in 30 ml; gum xanthan incubations in 60 ml; and *Spirulina* spp. as well as control incubations in 120 ml glass bottles. DOC: dissolved organic carbon; POC: particulate organic carbon; ND: not determined

Experiment	Start date	Incubation duration (h)	Substrate solutions used	DOC concentration (mM)	POC concentration (mM)	Added volumes (ml)	Added DOC (μmol)	Added POC (μmol)
Dissolved substrate	20 May 2008	95	<i>L. pertusa</i> -derived mucus (untreated)	60	ND	10	603	
			Glucose (monohydrate; 2 g l ⁻¹ deep water)	60	ND	10	600	
			Starch (2 g l ⁻¹ deep water)	74	ND	5	370	
			Deep-water control	3	ND			
Particulate substrate	24 May 2008	69	<i>L. pertusa</i> -derived mucus (untreated)	ND	2	5		11
			<i>L. pertusa</i> -derived mucus (sterile)	ND	2	5		11
			Gum xanthan (FLUKA; 1 g l ⁻¹ deep water)	ND	12	10		123
			<i>Spirulina</i> spp. (SIGMA; 1 g l ⁻¹ deep water)	ND	26	10		259
			Deep-water control	ND	0.003			

ca. 100°C in a glass vial) was also used. As reference substrates, gum xanthan (model mucus without any N components, FLUKA; 1 g l⁻¹ deep water) and *Spirulina* spp. (phytoplankton model organism) suspensions (Sigma; 1 g l⁻¹ deep water) were used. For each experiment, the respective reference substrate suspensions (Table 1) were added to Winkler glass bottles (volume: 30 to 120 ml) in replicates of 4. All bottles were subsequently filled with the deep water containing natural microbial assemblages and incubated at *in situ* temperature (5 to 6°C) in a cool room in the dark for an incubation period of 95 h (dissolved substrate experiment) or 69 h (particulate substrate experiment). Deep water without the addition of further substrates was also incubated in replicates of 4 for each experiment as controls. O₂ water concentrations in all treatments were measured in time series using Winkler titration (Winkler 1888), and O₂ consumption rates were calculated by linear regression of all 4 data points. Microbial organic carbon degradation was calculated by relating the resulting O₂ consumption rates to the respective total organic C content of each type of organic matter (measured as described is 'DOC, POM and isotope analyses', in replicates of n = 3 to 4, sample volumes: 5 to 10 ml) assuming that 1 mol of organic C is mineralized by 1 mol of O₂.

Microbial planktonic activity and organic matter concentrations above Tisler Reef. For this sampling, three 3 l Niskin bottles mounted on a steel cable were deployed from the RV 'Lophelia' to collect water samples from various water depths (108 to 114 m) above the central part of Tisler Reef. The lowest Niskin bottle was descended from the research vessel down to the reef. Exact positioning of this bottle was visually veri-

fied using simultaneous video survey by the contemporaneously deployed ROV of Type Sperre SUB-fighter 7500 DC. With this method it was possible to collect water samples from directly within or shortly above Tisler Reef. During 3 independent deployments, water samples were retrieved from ≤ 1.0 , 5.0 and 50.0 m above the reef on each occasion. *In situ* samplings took place during calm days. Parallel ADCP (Acoustic Doppler Current Profiler) measurements revealed that the NW components (the main current direction at Tisler) of the water currents above Tisler Reef on the days of our sampling (21 and 22 May) were similar and always $< 10 \text{ cm s}^{-1}$ at all stations at the 3 respective water depths (T. Lundalv unpubl. data).

Within 30 min after retrieval of each water sample, 10 ml aliquots were taken for later measurement of DOC as described below. Within 12 h after sample retrieval (samples were meanwhile kept at *in situ* temperatures of 4 to 6°C), 500 ml aliquots were taken for later measurements of POM as described below. In addition, 2 aliquots from each water sample were filled into two ~60 ml Winkler glass bottles for determination of O₂ concentration at the present and after incubation for 106 to 108 h in the dark at *in situ* temperature in a cold-room using Winkler titration. After this period, the O₂ concentration of the incubated water samples was measured as described in the previous section and subtracted from the initial values to calculate planktonic microbial O₂ consumption as a proxy for microbial activity.

DOC, POM and isotope analyses. For measurement of DOC concentrations, ~10 ml of the sample solutions was filtered through 0.2 μm sterile syringe filters (polyethersulfone membrane, VWR Collection). The first

4 ml of the filtrate was discarded, and the following 6 ml was collected in new, pre-combusted glass ampoules, which were instantly frozen at -20°C and kept frozen until analysis. Samples for DOC measurements retrieved from the water column above Tisler Reef were shock-frozen on the vessel using liquid nitrogen stored in a dry shipper. DOC concentrations were determined by high-temperature catalytic oxidation (HTCO) using a Rosemount Dohrmann DC-190 total organic carbon (TOC) analyser. After defrosting, each sample was treated by adding 100 μl of 20% phosphoric acid and purged with O_2 (using an injection needle) for 5 min to remove dissolved inorganic carbon. The DOC concentration of each sample was measured 5 times. An outlier test was conducted, and the DOC concentrations of the remaining samples were averaged. Potassium hydrogenphthalate was used as standard for calibrating the TOC analyser.

POC and PON (particulate organic nitrogen) concentrations were measured by filtering particulate substrate suspensions and collected water samples onto pre-combusted GF/F filters (Whatman, 25 mm diameter), which were dried for at least 48 h at 40°C and kept dry until analysis. Respective concentration and isotope measurements were performed with a Carlo Erba NC 2500 elemental analyser, coupled via a THERMO/Finnigan Conflo II-interface with a THERMO/Finnigan MAT Delta plus isotope ratio mass spectrometer. Elemental concentrations were calculated from certified elemental standards (atropine, cyclohexanone-2,4-dinitrophenylhydrazine; Thermo Quest) and typically showed standard deviations of $<5\%$. Carbon stable isotope ratios ($R = {}^{13}\text{C}/{}^{12}\text{C}$) are given in the conventional delta notation [$\delta^{13}\text{C} = (R_{\text{sample}}/R_{\text{VPDB}} - 1) \times 1000$] relative to the Vienna PeeDee Belemnite (VPDB) standard (Craig 1957, Coplen 1995) and atmospheric nitrogen (Mariotti 1983), respectively. Standard deviations for repeated stable isotope measurements of the laboratory standard (Peptone) were better than 0.15%.

Table 2. Summary of microbial carbon degradation rates for the substrates used in both experiments. O_2 consumption values are corrected for the respective background respiration in the controls and the volumes in the incubation bottles. DOC: dissolved organic carbon; POC: particulate organic carbon

Experiment	Substrate suspensions used	Added DOC (μmol)	Added POC (μmol)	O_2 consumption ($\mu\text{mol d}^{-1}$)	Organic matter degradation ($\% \text{d}^{-1}$)
Dissolved substrate	<i>Lophelia pertusa</i> -derived mucus	603		0.33	0.05
	Glucose	600		0.42	0.07
	Starch	370		0.29	0.08
Particulate substrate	<i>Lophelia pertusa</i> -derived mucus		11	1.58	15.0
	Gum xanthan		123	0.66	0.5
	<i>Spirulina</i> spp.		259	9.61	3.7

RESULTS

Microbial degradation of *Lophelia pertusa*-derived mucus and reference substrates

The dissolved substrate experiment showed that in all incubations with substrate addition, the O_2 concentration decreased significantly linearly (ANOVA, $p < 0.02$), whereas this was not the case in the seawater control ($p = 0.257$). *L. pertusa*-derived mucus was degraded by the microbial assemblage with an O_2 consumption rate of $12.7 \mu\text{M O}_2 \text{d}^{-1}$, which was similar to rates measured for the glucose ($15.4 \mu\text{M O}_2 \text{d}^{-1}$) and starch ($11.3 \mu\text{M O}_2 \text{d}^{-1}$) incubations (Fig. 1). All 3 kinds of substrate were degraded 15- to 20-fold faster than the deep-water controls ($0.8 \mu\text{M O}_2 \text{d}^{-1}$). Resulting microbial DOC degradation revealed similar rates for all incubations with added substrates (Table 2).

Statistical analyses of results from the particulate substrate experiment showed that only in the incuba-

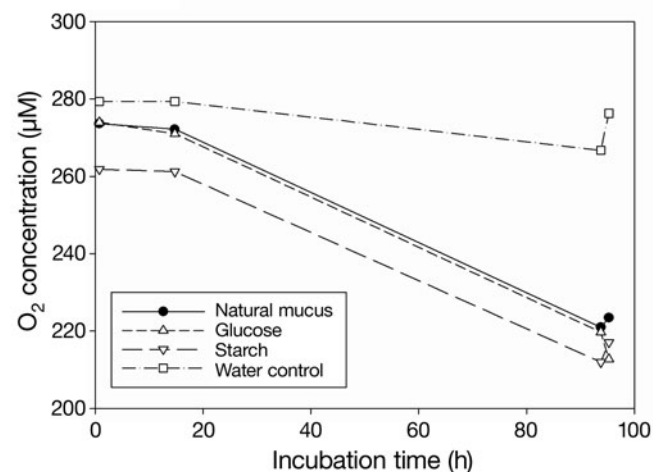


Fig. 1. O_2 concentration decrease during dissolved substrate incubation experiments. Mucus was collected from *Lophelia pertusa* fragments

tion with gum xanthan, did the O_2 concentrations show a significant linear decrease (ANOVA, $p < 0.05$). Both the addition of *Spirulina* spp. cells and untreated coral-derived mucus resulted in high microbial O_2 consumption rates of 81.9 and 60.1 $\mu M O_2 d^{-1}$ if calculated over the total duration of this experiment (Fig. 2). O_2 consumption in the sterile mucus incubation (13.1 $\mu M O_2 d^{-1}$) was >4-fold lower than that in the untreated mucus incubation and very similar to that in the gum xanthan solution (14.7 $\mu M O_2 d^{-1}$). All substrate-amended incubations showed higher microbial O_2 consumption than the controls with natural suspended organic matter (1.9 $\mu M O_2 d^{-1}$). The decrease in O_2 concentration was, however, not linear over the entire incubation period (ANOVA, $p = 0.075$), but initially (first day) showed comparably low microbial O_2 consumption for both, untreated and filtered mucus, with similar rates of 6.4 and 4.3 $\mu M O_2 d^{-1}$, respectively. The *Spirulina* spp. suspension was degraded very slowly during the first day (rate: 0.4 $\mu M O_2 d^{-1}$), which was an even lower rate than that of the seawater control (1.0 $\mu M O_2 d^{-1}$). After the first day, microbial O_2 consumption rates showed an approximately exponential progression ($R^2 > 0.65$ for both treatments), with maximum values of 120.3 and 225.9 $\mu M O_2 d^{-1}$ for the third day in the untreated mucus and *Spirulina* spp. incubation, respectively. O_2 concentrations in the other 3 treatments decreased almost linearly ($R^2 > 0.89$ for sterile mucus, gum xanthan and seawater control). Overall, resulting planktonic microbial organic matter degradation rates were much higher for the particulate compared to the dissolved substrates. Highest rates were determined for the untreated *Lophelia pertusa*-derived mucus with an ~4-fold higher value compared to the *Spirulina* spp. so-

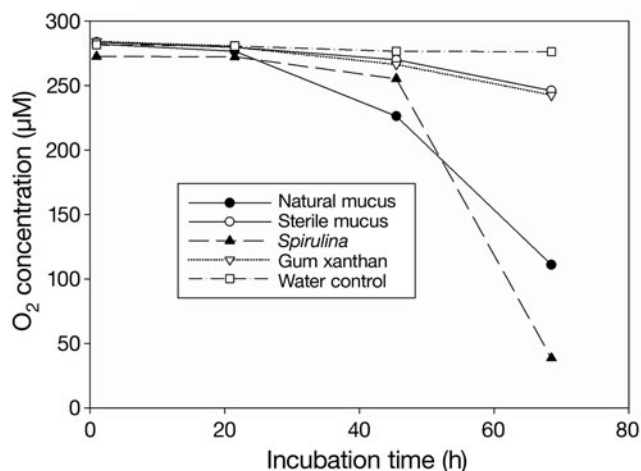


Fig. 2. O_2 concentration decrease during particulate substrate incubation experiments. Both kinds of mucus were collected from *Lophelia pertusa* fragments. For the phytoplankton treatment a suspension containing the cyanobacterium *Spirulina* spp. was used

lution. Gum xanthan represents an unfavourable organic substrate for microbial respiration, as indicated by the low degradation rates (Table 2).

Microbial planktonic activity above Tisler Reef

Analysis of microbial activity in water samples collected from 3 different stations at 3 depths above Tisler Reef revealed that microbial O_2 consumption was very similar and not significantly different between ≤ 1 m ($11.9 \pm 2.0 \mu M O_2 d^{-1}$) and 5 m above the reef ($12.1 \pm 2.8 \mu M d^{-1}$), but significantly higher ($p < 0.02$, 2-tailed *U*-test after Wilcoxon, Mann and Whitney) compared to 50 m above the reef ($8.2 \pm 0.4 \mu M d^{-1}$), i.e. in the middle of the water column (Fig. 3a).

POM, DOC and $\delta^{13}C$ distribution above Tisler Reef

POC and PON concentrations in the water column above Tisler Reef showed high variability and no significant differences ($p > 0.05$, 2-tailed *U*-test after Wilcoxon, Mann and Whitney) between the 3 investigated water depths (Fig. 3b). DOC water concentrations above Tisler Reef were very high, with maximum values of 55 ± 32 mM in the direct vicinity of the reef. However, similar to POM, DOC concentrations were highly variable and therefore were not significantly different between the 2 stations directly above Tisler Reef and the mid-water column station at 50 m water depth, which showed a DOC concentration of 15 ± 12 mM (Fig. 3c).

The C:N ratio of POM suspended in the water column above Tisler Reef was highly variable at the deepest station (Table 3) and not significantly different from the values at the other stations.

$\delta^{13}C$ signatures of suspended POM revealed significantly more positive values at the 2 stations close to Tisler Reef than that at the mid-water column station ($p < 0.02$, 2-tailed *U*-test after Wilcoxon, Mann and Whitney). The $\delta^{13}C$ values of suspended POM at the deep stations were close to those measured in the *Lophelia pertusa*-derived mucus suspensions used in the laboratory incubation experiments (Table 3).

DISCUSSION

Degradability of *Lophelia pertusa*-derived mucus and associated microbial activity

The present study broadens our understanding of the microbial degradability of cold-water coral-derived organic material, particularly mucus. The dissolved sub-

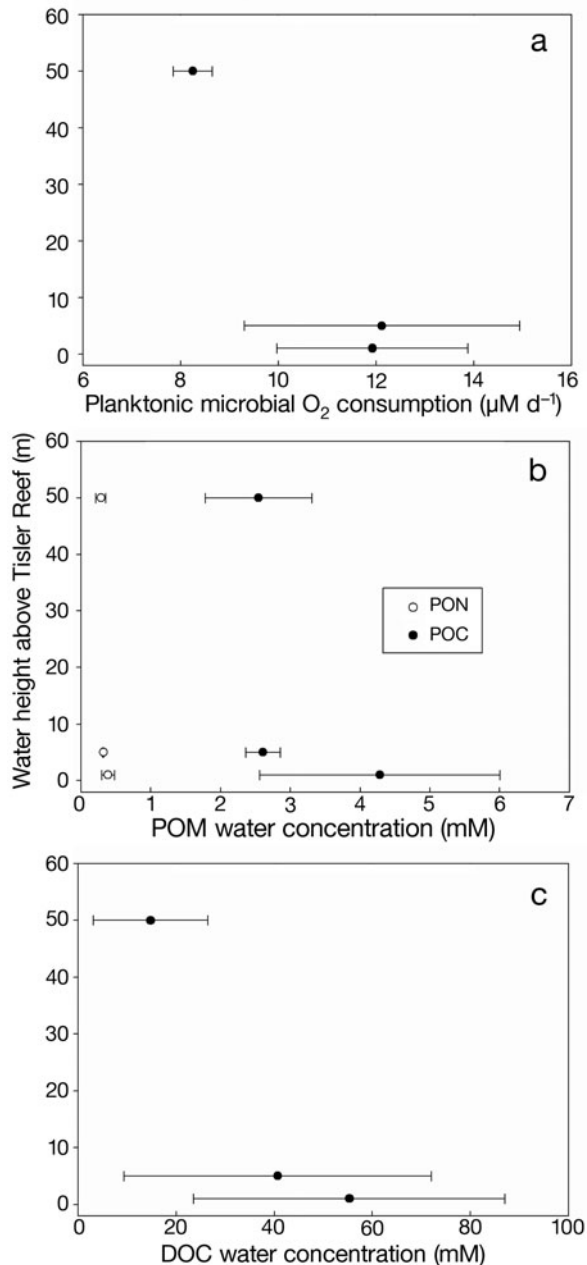


Fig. 3. (a) Microbial O₂ consumption, and (b) the concentrations of particulate organic matter (nitrogen and carbon; PON and POC) and (c) dissolved organic carbon (DOC) above Tisler Reef. Values are means (\pm SD) of 3 replicate *in situ* samplings at 3 different Tisler Reef locations over a total period of ca. 5 h on 22 May 2008

strate experiment showed that *Lophelia pertusa*-derived mucus was degraded at rates similar to that in the standard carbohydrate components glucose and starch, which are commonly used as the main carbon sources in culture media in the field of microbiology. Furthermore, the particulate substrate experiment showed that *L. pertusa*-derived mucus was degraded at

Table 3. C:N ratios and C stable isotope signature of suspended particulate organic matter in the water column above Tisler Reef in comparison to particulate substrates used in the laboratory incubation experiments. Values are averages (\pm SD) of n = 3 to 4 replicate measurements each. NA: not applicable

	C:N ratio	$\delta^{13}\text{C}$
In situ water sampling		
≤ 1 m above reef	12.0 ± 3.7	-23.7 ± 0.3
5 m above reef	9.0 ± 1.0	-23.8 ± 0.3
50 m above reef	8.6 ± 0.6	-25.2 ± 0.5
Laboratory substrates		
<i>Lophelia pertusa</i> -derived mucus	5.7 ± 0.3	-23.1 ± 1.0
<i>Spirulina</i> spp.	4.4 ± 0.1	-20.9 ± 0.1
Gum xanthan	NA	-24.8 ± 0.5

a rate similar to that in the *Spirulina* spp. suspension, which represents a model organism for phytoplankton. These findings emphasize the attractiveness of *L. pertusa*-derived mucus as a substrate for marine bacteria and potentially other microbes, including archaea, fungi and protozoans, which are described as primary consumers of warm- and temperate-water coral-derived organic matter (Ducklow & Mitchell 1979, Ducklow 1990, Wild et al. 2004a,b, 2005). The microbial community inhabiting *L. pertusa*-derived mucus apparently plays a key role in the degradation of this organic material, as revealed by the pronounced differences in microbial degradation rates between the untreated and sterile mucus incubations. This can be explained by mucus-specific microbial communities as described for warm-water corals (Ritchie & Smith 2004, Ritchie 2006, Kooperman et al. 2007, Allers et al. 2008). Such an explanation is also supported for cold-water corals by the studies of Neulinger et al. (2008) and Kellogg et al. (2009), which indicate that *L. pertusa*-specific microbial communities exist. Furthermore, the study of Schöttner et al. (2009), showed that different *L. pertusa*- and *Madrepora oculata*-generated habitats, including the coral-derived mucus surface layer, offer niches for specific microbial communities.

The finding that *Lophelia pertusa*-derived mucus is much more quickly degraded by microbes than gum xanthan, the model substrate for mucus, may be explained by the missing N-components in gum xanthan. Bacterial production in the degradation of fresh marine POM was reported to correlate best with PON (Grossart & Ploug 2001), and the low C:N ratios observed in cold-water coral-derived mucus indicate high PON contents (Wild et al. 2008).

Microbial activity measured as O₂ consumption was very similar in the mucus incubation of the dissolved substrate experiment ($12.7 \mu\text{M O}_2 \text{ d}^{-1}$) compared to all water samples collected from within and <5 m above Tisler Reef (11.9 to $12.1 \mu\text{M O}_2 \text{ d}^{-1}$). This may indicate

that *Lophelia pertusa*-derived mucus was a main component of the organic matter suspended in waters neighbouring Tisler Reef and that is controlled microbial activity. Such an assumption is further supported by the $\delta^{13}\text{C}$ values of POM suspended in the water column close to the reef showing similar values to the *L. pertusa*-derived mucus used in the laboratory incubation experiments during the present study. In addition, Wild et al. (2008) demonstrated that both dissolved ($47 \pm 19 \text{ mg DOC m}^{-2} \text{ d}^{-1}$) and particulate ($1.4 \pm 1.2 \text{ mg POC m}^{-2} \text{ d}^{-1}$) organic carbon can be released in high quantities into the surrounding water column by *L. pertusa*.

Microbial O_2 consumption in the water column above Tisler Reef revealed a similar gradient to that described by Wild et al. (2008) for Røst Reef, but with more than double the maximum values in the direct vicinity of Tisler Reef (12.5 to $14.3 \mu\text{M O}_2 \text{ d}^{-1}$) compared to near Røst Reef ($5.4 \mu\text{M O}_2 \text{ d}^{-1}$). This indicates that planktonic microbial activity is generally stimulated by the influence of cold-water coral reefs and suggests that microbial assemblages in the immediate reef vicinity contribute to the turnover of organic carbon in the reef ecosystem. The higher microbial activity directly above the reef observed in the present study by video-assisted sampling cannot be explained by the closer proximity of the origin of the water sample relative to the respective coral reef (<1 m above Tisler Reef compared to 4–5 m above Røst Reef), because the microbial activity at 5 m above Tisler Reef was also clearly higher than that at the same height above Røst Reef. Differences in bottom water temperature (5 to 6°C at both reef locations), which could also potentially have an effect on microbial activity, are obviously not the reason for the observed differences. Thus, organic matter availability may be the key factor in controlling microbial activity close to cold-water coral reefs.

The present study on Tisler Reef thus confirms some of the main findings of the study conducted by Wild et al. (2008) on reefs off northern Norway, in particular that: (1) cold-water coral-derived mucus represents an attractive substrate for the associated microbial assemblages and (2) that microbial activity in the water column in close proximity to a cold-water coral reef is elevated. The results of the present study thus extend the spatial validity of the previous findings and further support the hypothesis that cold-water coral-derived organic matter may have an important ecological function as a carrier of energy from corals to a range of (primarily microbial) consumers. Although the stimulation of microbial activity above Tisler Reef was less pronounced than that described above Røst Reef (Wild et al. 2008), it was detectable despite the more eutrophic character of the Skagerrak (especially during the study

period in spring with phytoplankton blooms) compared to the more oligotrophic Norwegian Sea (Freiwald et al. 2004).

Carbon cycling in the reef and the potential role of coral-derived mucus in ecosystem functioning

The present study showed that both POM and DOM concentrations are high in the direct vicinity of the reef. Concentrations of POC (22 to $80 \mu\text{g l}^{-1}$) and PON (3 to $6 \mu\text{g l}^{-1}$) in the water column above Tisler Reef were in the range of values reported from the water column above Røst Reef in the Norwegian Sea (Wild et al. 2008). DOC concentrations in the water column above Tisler Reef were very high, with values of $55 \pm 32 \text{ mM}$. These values exceed reported DOC concentrations in the deep ocean by 3 orders of magnitude (Hansell & Carlson 1998) and concentrations reported from estuaries (Guo & Santschi 1997, 2000, Klinkhamer et al. 2000, Lobbes et al. 2000) or warm-water coral reefs (van Duyl & Gast 2001, De Goeij & Van Duyl 2007) by >1 order of magnitude. The data of the present study are further supported by the high DOC concentrations (0.5 to 1.8 mM) measured in water samples collected at the high-latitude Røst Reef during RV 'Polarstern' Cruise ARKXXII/1a in June 2007 (Wild et al. unpubl. data). Contamination of samples can be largely excluded, as new pre-combusted ampoules were used for sample collection, and samples were immediately frozen onboard after collection.

The reported DOC values indicate the presence of a large pool of easily degradable, labile DOC available to the associated planktonic microbial community. DOC concentrations in the upper part of the water column can be attributed to exudates of the declining spring phytoplankton bloom (Dafner & Wangersky 2002). The increase of DOC concentrations close to the reef surface, however, indicates that further sources contribute to the DOC pool at the reef sites. The most likely source is organic matter production and release by the reef biota, particularly corals, which occur in great amounts at Tisler Reef (Roberts & Hirshfield 2004, Fossaa et al. 2005).

Ferrier-Pages et al. (1998, 2000) showed that the uptake of heterotrophic nutrition serves warm-water corals as a source for nutrients, e.g. nitrogen and phosphorous, while the surplus of carbon taken up is subsequently released again as DOC. Lavaleye et al. (2009) suggested that cold-water coral reefs, in contrast, serve as a filter for high-quality POM, as evident by the preferential removal of labile N-rich components during the passage over Tisler Reef and also indicated by the particulate substrate experiment in the present study. Contemporaneously, Wild et al. (2008) showed that

cold-water coral-derived mucus is characterized by C:N ratios close to or even below the Redfield ratio (i.e. ca. 6.6), which describes the typical molar C:N:P ratio for phytoplankton as 106:16:1. These findings suggest a transfer of N from the particulate to the dissolved fraction and argue against a comparable mechanism of nitrogen retention in cold-water coral ecosystems as previously described for warm-water coral reefs. Instead, the findings of the present study may indicate that cold-water corals actively release labile organic matter and stimulate heterotrophic bacteria in the adjacent water column. This is also supported by the fast degradation of coral-derived mucus in comparison to other labile compounds, e.g. glucose and starch, during the dissolved substrate experiment presented here.

Reef-associated fauna, particularly corals, consume POC that is transported down from the productive surface layer (Kiriakoulakis et al. 2005) and, in turn, can release organic carbon with a high DOC:POC ratio (Wild et al. 2008). While POC concentrations do not seem to change greatly between the beginning and end of the passage over Tisler Reef (Lavaleye et al. 2009), large amounts of DOC are obviously released, which accumulate in waters adjacent to the reef. This may indicate that C transferred down from primary production in the surface layers may undergo several cycles in the DOC–microbial food chain before storage or loss from the system by deposition, current transport, or transformation into refractory DOC that cannot be mineralised on short time scales. Such a theoretical concept termed ‘network virtual amplification’ (Higashi et al. 1993) has previously been suggested in relation to DOC–food web dynamics. It implies that no new energy is created, but that the amount of available energy is increased due to recycling. Via the release of labile components, such as mucus, corals may act as the main drivers of this recycling mechanism and ensure fast re-entry into the food web via recycling by heterotrophic bacteria, which, in turn, are consumed by e.g. protozoa, so that loss into the overlying water column is prevented. The chemical composition of cold-water coral-derived organic matter with its low C:N ratio (Wild et al. 2008) may facilitate this process. It results in longer C retention in the coral reef system, thus effectively sustaining C availability during times of low external input, e.g. during winter when phytoplankton activity and thus the supply of ‘fresh material’ is limited (Roberts et al. 2006). Scleractinian corals themselves are known to feed on pico- and nanoplankton, including bacteria and protozoa (Houlbrèque et al. 2004, 2006). Low $\delta^{15}\text{N}$ values of *Lophelia pertusa*- and *Madrepora oculata*-derived mucus (Wild et al. 2008) are in agreement with these explanations, as they indicate the input of N from autochthonous microbial sources.

The retention of essential nutrients such as N is likely of less importance in cold- compared to warm-water corals reefs, while the supply of C is of greater importance due to the high need for heterotrophic feeding in cold-water corals. The present study may, therefore, indicate that cold-water corals assure their long-term C supply by the actual release of C, which may represent a new mechanism of C storage in the reef system. Planktonic heterotrophic bacteria fulfil an important function in trophodynamics, because they can convert coral-derived DOC to POC, thereby making it available again for higher trophic levels. But also sponges, which occur in great abundances in cold-water coral reef ecosystems, may benefit from the release of large amounts of DOC, because it is known that sponge–microbe consortia can fix DOC in cold-water coral reef ecosystems (van Duyl et al. 2008).

The release of organic matter by cold-water corals and its subsequent microbial consumption, therefore, potentially depict a newly described combined assimilatory–dissimilatory process (Jones et al. 2006), while the trapping of suspended particles and formation of aggregates induced by coral-derived organic material in warm-water coral reef ecosystems (Wild et al. 2004a) represents true ecosystem engineering (Jones et al. 1997), because it involves several modifications on the physical properties of particles (e.g. aggregation, sedimentation rate) suspended in reef waters.

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