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Dynamics of nutrients, total organic carbon, prokaryotes and viruses in onboard incubations of cold-water corals

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Abstract

The potential influence of the cold-water corals (CWCs) *Lophelia pertusa* and *Madrepora oculata* on the dynamics of inorganic nutrient and total organic carbon (TOC) concentrations and the abundances of prokaryotes and viruses in bottom water was assessed in onboard incubation experiments. Ammonium, nitrite, dissolved inorganic nitrogen (DIN), dissolved inorganic phosphorus (DIP) and TOC concentrations and N:P ratios were typically higher in incubation water with corals than in controls, whereas nitrate concentrations did not reveal a clear trend. Mucus release (normalized to coral surface) was estimated by the net increase rate of TOC concentrations and averaged $23 \pm 6\,\mathrm{mg\,C\,m^{-2}\,h^{-1}}$ for *L. pertusa* and $21 \pm 8\,\mathrm{mg\,C\,m^{-2}\,h^{-1}}$ for *M. oculata*. Prokaryotic and viral abundance and turnover rates were typically stimulated in incubation water with corals. This prokaryotic stimulation averaged $6.0 \pm 3.0 \times 10^9$ cells $\mathrm{m^{-2}\,h^{-1}}$ for *L. pertusa* and $8.4 \pm 2.9 \times 10^9$ cells $\mathrm{m^{-2}\,h^{-1}}$ for *M. oculata*, whereas the viral stimulation averaged $15.6 \pm 12.7 \times 10^9$ particles $\mathrm{m^{-2}\,h^{-1}}$ for *L. pertusa* and $4.3 \pm 0.4 \times 10^9$ particles $\mathrm{m^{-2}\,h^{-1}}$ *M. oculata*. Our data suggest that prokaryotes and viruses are released from corals and that nutrient and mucus release enhanced prokaryotic and viral production. The result of this stimulation could be a fuelling of bottom water in CWC reefs with nutrients and organic matter and consequently an enhancement of microbe-mediated processes.

1 Introduction

The distribution of corals ranges from shallow waters to the deep abyssal plains. Coral reefs or bioherms do not only occur in tropical surface waters but also in deep and cold waters (Roberts et al., 2006). *Lophelia pertusa* and *Madrepora oculata* are important species as main frame-builders of these cold-water coral (CWC) ecosystems, which sustain a large biodiversity and biomass (Rogers, 1999). CWC reefs seem to thrive in areas of elevated hard substrata and enhanced water flow, which could prevent sedimentation and provide the necessary food sources (Rogers, 1999; Roberts et al., 2006; Mortensen et al., 2001; Davies et al., 2009).

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There is evidence that CWC reefs preferentially remove nitrogen and are hotspots of remineralization activity in the ocean (Lavaleye et al., 2009). Moreover, it has been demonstrated that dissolved carbon fixation by sponge-microbe consortia is high in this habitat (van Duyl et al., 2008). Corals (including CWC species such as *L. pertusa)* can also release significant amounts of mucus (e.g. Ducklow and Mitchell, 1979b; Herndl and Velimirov, 1986; Wild et al., 2008), which has been shown to function as an energy carrier and particle trap in tropical reef systems (Wild et al., 2004). This recycling loop supports the growth of benthic organisms and consequently reduces losses of energy and matter from the ecosystem (Wild et al., 2004). It has also been shown that *L. pertusa* and *M. oculata* release mucus, which stimulates oxygen consumption rates of microorganisms (Wild et al., 2008; Wild et al., 2009). It is known from studies in tropical areas that mucus and nutrient release into the interstitial space of corals can enhance microbial abundance (Schiller and Herndl, 1989; Ferrier-Pagès et al., 2000). Coral mucus harbours a high density of prokaryotes (e.g. Ducklow and Mitchell, 1979; Koren and Rosenberg, 2006). A first insight into the bacterial community structure is available for *L. pertusa* (Yakimov et al., 2006; Kellogg et al., 2009; Neulinger et al., 2008; Schöttner et al., 2009), *M. oculata* (Hansson et al., 2009) and CWC sediments (Jensen et al., 2008a; Jensen et al., 2008b).

Viral abundances have not been often quantified in coral reef systems (Paul et al., 1993; Dinsdale et al., 2008; Patten et al., 2008; Weinbauer et al., 2010). It is known that reef corals and zooxanthellae can be infected by viruses (e.g. Wilson et al., 2005; Danovaro et al., 2008) and lytic phages of coral pathogens have been isolated (Efrony et al., 2007). The morphological diversity of viruses in coral mucus and in the holobiont is high (Davy and Patten, 2007; Patten et al., 2008) and a high diversity was also shown by viral metagenomics of the holobiont (Marhaver et al., 2008). There is also some in situ evidence that viral abundance increases close to corals (Patten et al., 2006; Seymour et al., 2005). To the best of our knowledge, nothing has been published on viruses in CWC ecosystems.

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The aim of this study was to assess potential interactions of cold-water corals with some central components of the microbial food web in the dark ocean, i.e. nutrients, organic carbon, prokaryotes and viruses. The potential role of the cold-water corals *L. pertusa* and *M. oculata* for the dynamics of inorganic nutrients and total organic carbon (TOC) was addressed in on-board incubations. Moreover, it was investigated, whether prokaryotes and viruses are released from the corals. Finally, an attempt was made to quantify mucus release and the stimulation of prokaryotic and viral production in incubations water with cold-water corals.

2 Material and methods

2.1 Study site and sampling

The sampling site was at the CWC reef located on the southeastern Rockall Bank at the Clan mounds (55.444° N to 55.445° N, −16.072° E to −16.975° E) and Haas mounds (55.491° N to 55.501° N, −15.788° E to −15.801° E). The two mounds are ca. 20 km apart. Sampling was performed during the BIOSYS/HERMES cruise of the RV *Pelagia* between 21 June and 21 July 2005 (http://www.nioz.nl/public/dmg/rpt/crs/64pe263.pdf). Specimen of *L. pertusa* and *M. oculata* were collected from 560–780 m using boxcore samplers (for more details of the study site and sampling see van Duyl et al., 2008). Corals used in experiments originated from 12 casts. This increases the probability that the corals differed in clonal structure and physiological status thus, making them more representative for the coral ecosystem.

2.2 Keeping of corals

Onboard, small branches of *L. pertusa* (9 ± 4 polyps) and *M. oculata* (32 ± 16 polyps) without epibionts, were glued onto Petri dishes using underwater Epoxy®. Corals and micro-colonies were kept in acid-cleaned and seawater rinsed 20 L plastic tanks in

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bottom seawater. Tanks were kept in the dark at ca. in situ temperature (9 °C). Bottom water (580–770 m) for keeping corals was sampled with a 1000 l water box. One third of the seawater was replaced every third day with freshly collected bottom water without exposing corals to air. A water flow was generated by small submerged aquarium pumps with a capacity of 250 l^{-1} h^{-1}. The micro-colonies were kept for at least 2 days for acclimation before they were used in experiments.

2.3 Incubations to assess the dynamics of nutrients, organic carbon and microorganisms

Per replicate, three micro-colonies of *L. pertusa* or *M. oculata* were placed into an acid cleaned hardplastic jar filled with 3 l of sea water. A summary of the experimental approach is shown in Fig. 1. For coral treatments, experiments were performed in triplicates (3 coral branches x 3 jars, in total 9 coral branches per species and experiment). *L. pertusa* and *M. oculata* colonies were incubated in different jars. Incubations were kept at in situ temperature and under water flow (see above, section: Keeping of corals). Corals appeared healthy with extended polyps and tentacles during the experiment.

Three types of controls were run in duplicates each: (1) seawater without corals, (2) seawater with dead micro-colonies (containing biofilms) and (3) seawater with dead micro-colonies which were bleached (containing no biofilm). To remove the biofilm of dead corals, the skeletons were soaked in house-hold bleach, thoroughly rinsed in MilliQ and dried in an oven at 60 °C prior to experiments. In the dead coral controls, one replicate consisted of two colonies of *L. pertusa* and one colony of *M. oculata*, the other of one colony of *L. pertusa* and two colonies of *M. oculata*. Thus, the same controls were used for the two coral species. The restriction to duplicates for the different types of controls and the mixture of dead colonies was done because of space and handling limitations in the temperature-controlled walk-in containers.

Two experiments were performed with non-processed natural seawater (NSW) to assess the potential role of corals for nutrient and organic matter dynamics and

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potential stimulation of the growth of prokaryotes and viruses in incubation water. One experiment was performed with bottom water collected from 590 m at the Clan mounds (NSW1), the other with bottom water from 777 m collected on the Haas mounds (NSW2). Three supplementary experiments were performed with (1) ultrafiltered (i.e. virus- and cell-free-) seawater (UF), (2) a combination of ultrafiltered and virus enriched seawater (VE) and (3) a combination of ultrafiltered and prokaryote enriched seawater (PE). For preparation of these seawater fractions see below (section: Ultrafiltration). These experiments also served to assess the potential role of corals for the dynamics of nutrient and TOC concentrations and potential stimulation of the growth of prokaryotes and viruses in incubation water at varying prokaryotic and viral abundances (long-term, T72h). However, additional questions were addressed using short-term dynamics (T11h) in these prokaryotic and viral manipulation experiments. The UF experiment with its very low initial background abundances of prokaryotes and viruses served to detect potential release of microorganisms from corals. The initial intention to increase the abundances of viruses and (almost) excluding prokaryotes in the VE experiment and increase prokaryotic abundance and reduce viral abundance in the PE experiments was to assess the potential for uptake of viruses (VE) and prokaryotes (PE: see e.g. Houlbrèque et al., 2004); i.e., a reduction of viral abundance in the coral treatments was expected compared to the controls in the VE experiment, and a reduction of prokaryotic abundance in the coral treatments was expected compared to the controls in the PE experiment. However, these expected trends not were observed in the experiments (see below, section: Short-term dynamics in prokaryotic and viral manipulation experiments). Nevertheless, these experimental approaches provided data on the potential release of prokaryotes and viruses (as studied in the UF experiment) and thus, short-term data are presented.

The five experiments were conducted one after the other using new coral microcolonies and new incubation water (for each experiment). Incubation water was ~~subsampled over a duration of~~ 3 days at T0h, T2h, T6h, T11h, T30h, T58h and T72h ~~from all replicates.~~

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2.4 Surface area of corals

The surface area of corals was measured from pictures of micro-colonies used in the experiments and placed on plotting paper with mm^2 grids. The calculation was done using advanced geometry and area equations for geometric shapes and forms (Naumann et al., 2009) considering polyps and coenosteum as cylinders. The total surface area was then divided by the number of polyps, which averaged $373 \pm 48\,mm^2$ for five colonies of *L. pertusa* and $126 \pm 13\,mm^2$ for 5 colonies *M. oculata*. The surface area of the colonies used in the experiments was estimated by using the known amount of polyps.

2.5 Ultrafiltration

Tangential flow filtration was used to obtain different size fractions of seawater (for details see Weinbauer et al., 2009). Water samples (200 l) were filtered through a 20-µm net (Nitex) and 0.8 µm filters (polycarbonate, 143 mm diameter); prokaryotes in the filtrate were concentrated using a Pellicon (Millipore) tangential flow filtration system. This system was equipped with an 0.2 µm filter cartridge (Pellicon, Millipore) that was cleaned before with 0.1 N HCL and flushed with 5 l of MilliQ water and 10 l of sample water before starting concentration. The filtrate containing the majority of the viruses was processed using a 100 kDa polyethersulfone tangential flow cartridge (Prep-Scale™/TFF, $0.23\,m^2$ nominal filter area, Millipore; operated by a peristaltic pump at 1.5 bar) to obtain the viral concentrate and virus-(and cell-)free UF. This cartridge was cleaned with 0.1 N NaOH and rinsed with 5 l of MilliQ water and ca. 5 l of 0.2 µm filtrate prior to the concentration step. The concentration factor for prokaryotic and viral concentrates was ca. 500–1000. Following seawater fractions were made the day a supplementary experiment was set up: In the first experiment (experiment UF), only UF was used, in the second UF was combined with VE (experiment VE) and in the third UF was combined with PE (experiment PE). All filtrations were carried out at in situ temperature (9 °C) in a temperature-controlled ~~walk-in~~ container.

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2.6 Analysis of inorganic nutrients and organic matter

Five ml samples for quantifying nitrogen and phosphorus concentrations were collected in situ and from incubation water, filtered through a 0.2 µm pore-size Acrodisc filter and measured directly onboard. Ammonium, nitrite, nitrate and dissolved inorganic phosphorus (DIP) concentrations were determined using an AxFlow Bran and Luebbe Traacs800 autoanalyzer. The variability is <4% for all nutrients (Herfort et al., 2007). Dissolved inorganic nitrogen (DIN) concentrations were calculated as the sum of all nitrogen species.

For total organic carbon (TOC) analysis, 20 ml samples were filled in precombusted glass ampoules, acidified with 8 drops of concentrated H_2SO_4, sealed and stored at 4 °C until analysis. TOC concentrations were measured by high-temperature combustion on a Shimadzu TOC-5000 as described in Benner and Strom (1993).

2.7 Prokaryotic and viral abundance

Samples for prokaryotic and viral abundance were fixed in glutaraldehyde (0.5% final concentration), kept at 4 °C in the dark for 15 min, flash-frozen in liquid nitrogen and stored at −80 °C until analysis. Bacterial and viral abundance were determined using SYBRGreen II (Invitrogen) and flow cytometry as described elsewhere (Brussaard, 2004; Brussaard et al., 2010; Gasol and del Giorgio, 2000).

2.8 Influence of corals on nutrients dynamics

The nutrient dynamics in the incubations with corals is potentially not only influenced by the release from corals but by the use and transformation of inorganic nutrients by prokaryotes (see below, section: Implications). Thus, release rates *sensu strictu* cannot be calculated. In order to assess the role of corals for the net flux of nutrients in incubation water, the increases in inorganic nutrient concentrations in incubation water with corals were corrected for values in the controls. These increases were typically linear for 6–11 h; linear regression analyses were performed to obtain the net

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flux of nutrients in incubation water with corals. Coral-mediated nutrient flux was then calculated by multiplying these rates by the total volume of the incubation water and then normalizing the values to the surface area of the corals.

2.9 Organic carbon release by corals

In order to estimate organic carbon release from the corals, the increase of TOC measured after 6 h incubation in water with corals were corrected for the corresponding values in the controls. The organic matter release rate by corals was calculated by multiplying the corrected TOC increase with the total volume of the incubation water and then normalizing the values to the coral surface area. This method is similar to the beaker incubation technique, which is used to assess mucus release from corals (Herndl and Velimirov, 1986; Wild et al., 2008). This method is based on the reasonable assumption that prokaryotic activity will not remove considerable amounts of organic matter in short-term incubations.

2.10 Stimulation of prokaryotes and viruses by corals

The net change of prokaryotic and viral abundance in incubation water with corals is due to at least three mechanisms, i.e. direct release from corals, ingestion by corals and growth stimulation of prokaryotes and viruses by nutrient and mucus release. Thus, growth and production rates sensu strictu cannot be calculated for coral treatments. Instead, (net) changes of prokaryotes and viruses were calculated by using linear regressions of log transformed abundance data vs. time. Regressions were calculated from three to seven data points using the highest regression coefficients obtained (p always <0.05). For prokaryotes r^2 values ranged from 0.90–0.98 with the exception of one value of 0.81. For viruses, r^2 values ranged from 0.86 to 0.99. Note that log transformed regression showed slightly higher regressions coefficients than non-transformed data. The slope of the regression was considered as turnover rate. For the controls, this is equivalent to the (net) growth rate. Net change rates (typically a

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stimulation was detected) were calculated by multiplying the turnover rate by the abundances at T0h. In order to estimate the role of corals for the dynamics of prokaryotes and viruses changes, the net change rates were corrected for values in the controls. Stimulation by corals was then calculated by multiplying these rates by the total volume of the incubation water and then normalizing the values to the surface area of the corals.

2.11 Statistical analysis

Kruskal-Wallis tests were use to assess potential differences between types of controls for single experiments. Mann-Whitney U-tests were used to assess for specific time points the difference of parameters between controls and coral treatments (separately for *L. pertusa* and *M. oculata*). Wilcoxon signed rank tests were used to compare parameters across experiments between corals and the controls. A probability (p) of <0.05 was considered significant for all statistical analyses.

3 Results

3.1 In situ data

The water collected at the study sites was similar in nutrient concentrations and prokaryotic and viral abundances (Table 1). The N:P ratio averaged 16.1 ± 0.7, in situ abundance was $4.4 \pm 0.3 \times 10^5$ ml^{-1} for prokaryotes and $7.1 \pm 1.0 \times 10^6$ ml^{-1} for viruses. TOC concentrations are only available for two stations (150 µm at WK13 and 147 µm at WK110).

3.2 General experimental approach

At T0h there was no significant difference of nutrient concentrations and prokaryotic and viral abundances between the three different types of controls (Kruskal-Wallis,

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$p > 0.15$ for all experiments). Thus, a single mean was calculated from the average values of the three types of controls.

At T0h, nutrient concentrations in the control did not differ significantly from values in incubation water with corals (Mann Whitney, $p > 0.05$ or all experiments and both coral species) and were similar compared to in situ conditions, except for nigher ammonium concentrations in experiment NSW2 (Tables 1 and 2). At T0h, prokaryotic and viral abundance did not differ significantly between controls and incubation water with corals (Mann-Whitney, $p > 0.05$ for all experiments and both coral species).

In the UF experiment, prokaryotic and viral abundance was reduced to ca. 10% relative to ambient at the start of the experiment (Table 2). In the VE experiment, viral abundance was 2-fold higher and prokaryotic abundance was reduced to 13%. The presence of prokaryotic abundance in UF and VE is due to regrowth during preparation of seawater fractions. In the PE experiment, prokaryotic abundance was elevated by 2.6 fold and viral abundance reduced to 9%. The viruses in the PE experiment were introduced into the incubations along with the prokaryotic concentrate, since not all viruses pass a 0.2 µm filter. Overall, the experiments resulted in a range of viral and prokaryotic abundances both higher and lower than in situ and thus, experimental intentions were met.

3.3 Dynamics of nutrients and organic carbon in incubations

Data on the dynamics of nutrient concentrations in the experiments are summarized in Table 2 and 3; an example is shown in Fig. 2. Experiments differed in the detailed dynamics and concentrations of nutrients. However, there were also common trends. This is shown in the following mainly for T72h (Table 3), except when short-term incubations showed a different trend. In all experiments, ammonium, nitrate, nitrite and DIN concentrations increased with time (at least during an initial phase). The concentrations of ammonium and DIN were significantly higher in the incubations with corals than in the controls (Mann-Whitney, $p < 0.05$ for all experiments and both coral species; Wilcoxon, $p < 0.05$ across experiments for both species). Nitrate concentrations were

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higher in the coral treatments than in the controls in the UF, VE and PE experiments (Mann-Whitney, $p < 0.05$). In the NSW experiments, nitrate concentrations were only in NSW1 significantly higher in the coral treatment than in the controls for *L. pertusa* (Mann-Whitney, $p < 0.05$). Nitrite concentrations were higher in coral treatments than in controls, however, this trend was only significant in some experiments (Mann-Whitney, $p < 0.05$ for *L. pertusa* in NSW1 and VE and for *M. oculata* in UF and VE). Nevertheless, this trend was significant across experiments for both species (Wilcoxon, $p < 0.05$). The dynamics of DIP differed between experiments. DIP concentrations were in three experiments significantly higher in incubation water with corals than in controls (NSW2, UF, PE) and in one experiment significantly lower (NSW1) (Mann-Whitney, $p < 0.05$). However, when data from short-term incubations (6–11 h) are used, DIP concentrations were significantly higher in the corals treatments than in controls (Wilcoxon, $p < 0.05$ across experiments for both species; data not shown). In all experiments N:P ratios were significantly higher in incubation water with corals than in the controls (Mann-Whitney, $p < 0.05$ for all experiments and both species; Wilcoxon, $p < 0.05$ for both species).

TOC concentrations at T0h were 160 µm in NSW1 and 140 µm in NSW2 and ranged from 71–83 µm in the other experiments (Table 2). In all experiments, TOC concentrations in the controls were similar at T72h compared to T0h (Table 3). However, at T72h, TOC concentrations were significantly higher in incubation water with corals than in the controls (Wilcoxon, $p < 0.05$ for both species).

3.4 Dynamics of prokaryotes and viruses in incubations

Prokaryotic abundance increased with time in all treatments. At the end of the incubations, prokaryotic abundances were significantly higher in incubation water with corals than in controls (Mann-Whitney, $p < 0.05$ for all experiments and both species; Wilcoxon, $p < 0.05$ across experiments for both species; Table 3). Across experiments, prokaryotic turnover rate averaged $0.6 \pm 0.2\,d^{-1}$ in the controls, $1.2 \pm 0.2\,d^{-1}$ for *L. pertusa* and $1.3 \pm 0.3\,d^{-1}$ for *M. oculata* (Fig. 3a); turnover rates were significantly higher

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in incubation water with corals than in the controls (Mann-Whitney, $p < 0.05$ for all experiments and both species; Wilcoxon, $p < 0.05$ across experiments for both species).

With the exception of NSW1, viral abundance was significantly higher in incubation water with corals than in the controls (Mann-Whitney, $p < 0.05$) (Table 3). Across experiments, viral turnover rates averaged $0.25 \pm 0.06\,d^{-1}$ in the controls, $0.43 \pm 0.06\,d^{-1}$ in incubation water with *L. pertusa* and $0.43 \pm 0.12\,d^{-1}$ in incubation water with *M. oculata*. In all experiments, viral turnover rates were higher in incubation water with corals than in incubation water of the controls (Fig. 3b), however, this difference was not significant in the UF experiment (Mann-Whitney, $p > 0.05$). Nevertheless, across experiments, viral turnover rates were significantly higher in coral treatments than in the controls (Wilcoxon, $p < 0.05$ for both species).

3.5 Organic carbon release and stimulation of nutrients, prokaryotes and viruses by corals

The two experiments, where the abundance of prokaryotes and viruses was not manipulated (NSW) were used to assess organic carbon release and the potential for stimulation of the nutrient flux and prokaryotic and viral abundances by corals (Table 4). The estimated organic carbon release rate averaged $23 \pm 6\,mg\,C\,m^{-2}\,h^{-1}$ for *L. pertusa* and $21 \pm 8\,mg\,C\,m^{-2}\,h^{-1}$ for *M. oculata* (Table 4). The DIN flux was on average $0.99 \pm 0.09\,mg\,N\,m^{-2}\,h^{-1}$ for *L. pertusa* and $0.49 \pm 0.15\,mg\,N\,m^{-2}\,h^{-1}$ for *M. oculata.* For DIP, the average flux was $0.037 \pm 0.001\,mg\,P\,m^{-2}\,h^{-1}$ for *L. pertusa* and $0.023 \pm 0.001\,mg\,P\,m^{-2}\,h^{-1}$ for *M. oculata*. The prokaryotic stimulation averaged $6.0 \pm 3.0 \times 10^9$ cells $m^{-2}\,h^{-1}$ for *L. pertusa* and $8.4 \pm 2.9 \times 10^9$ cells $m^{-2}\,h^{-1}$ for *M. oculata*. Viral stimulation averaged $15.6 \pm 12.7 \times 10^9$ particles $m^{-2}\,h^{-1}$ for *L. pertusa* and $4.3 \pm 0.4 \times 10^9$ particles $m^{-2}\,h^{-1}$ for *M. oculata*. Across experiments, coral-mediated ammonium and DIN flux was higher for *L. pertusa* than for *M. oculata* (Wilcoxon, $p < 0.05$).

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Nutrient, prokaryotic and viral stimulation by corals showed no consistent differences between experiments using NSW as incubation water and experiments with incubation water, where the abundances of viruses and prokaryotes was manipulated (Table 4). In contrast organic carbon release was slightly higher in the NSW than in the other experiments.

3.6 Short-term dynamics in prokaryotic and viral manipulation experiments

The short-term dynamics (up to 11 h) of prokaryotic and viral abundance was monitored in the experiments, in which microbial abundances were manipulated. In the UF experiment, prokaryotic abundance remained constant in the controls during the first 2 h, whereas in incubation water with corals, prokaryotic abundance by 50–80% (Fig. 4a). This difference was significant (Mann Whitney, $p < 0.05$ for both species). Viral abundance decreased slightly in the controls and was 3.2-fold higher in incubation water with *L. pertusa* and 2.2-fold higher in *M. oculata* (Fig. 4b) and this difference was significant for both coral species (Mann-Whitney, $p < 0.05$). After 6 h, the differences between controls and incubation water with *L. pertusa* were even more pronounced.

In the VE experiment, prokaryotic abundance was slightly higher at T6h in incubation water with corals than in controls, however, this difference was only significant for incubation water with *M. oculata* (Mann-Whitney, $p < 0.05$; Fig. 4c). Viral abundance decreased during T0–11h in all treatments and the decay was lowest in the coral treatments (Fig. 4d).

In the PE experiment, prokaryotic abundance was for no time-point significantly different between incubation water with corals and the controls (Mann-Whitney, $p > 0.05$; Fig. 4e). From T0h to T6h, viral abundance in incubation water of the controls of experiment PE decreased, whereas viral abundance increased or remained constant in incubation water with corals; between T2h and T11h, values were for all time points significantly higher for *L. pertusa* and *M. oculata* than in the controls (Mann-Whitney, $p < 0.05$; Fig. 4f).

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3.7 Correlation analysis

Correlations were performed from means from all data points per experiments except T0h. In all experiments (except NSW1), viral abundance increased significantly with prokaryotic abundance (r^2 = 0.50–0.78, $p < 0.001$, $n = 18$).

4 Discussion

This study integrates data on ~~(1)~~ the dynamics of nutrients, organic carbon, prokaryotes and viruses in onboard incubations with the cold-water corals *L. pertusa* and *M. oculata*, ~~(2) the estimation of release or stimulation of these parameters by corals and (3) the evaluation of mechanisms responsible for this dynamics~~. To overcome or at least reduce the potential problem of large variation between specimens (van Duyl et al., 2008; Maier et al., 2009), three coral colonies per replicate incubation and several types of controls were used. However, the five experiments could not be performed in parallel thus, variability was introduced in this way. Nevertheless, common trends could be found in incubation with both coral species across 5 experiments. The most consistent trends were a release of DIN, DIP and mucus, an increase of the N:P ratios, potential detachment of prokaryotes and viruses and a stimulation of prokaryotic and viral growth. Also, the finding that the parameters did not differ between the various types of controls (seawater with and without corals skeletons) suggests that the living coral was responsible for the observed trends in the presence of corals (and not e.g. the presence of a skeleton surface).

4.1 Prokaryotic and viral abundance in bottom water

Prokaryotic and viral abundance in situ averaged 4.4×10^5 cells ml^{-1} and 7.1×10^6 viruses ml^{-1}, respectively. Thus, the prokaryotic abundances determined by flow cytometry were similar to the average of 4.9×10^5 ml^{-1} from other stations of the same study site enumerated by epifluorescence microscopy (van Duyl et al., 2008). To the best of our knowledge there are no other data on prokaryotic and viral

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abundances from other CWC reefs. In mesopelagic waters of the North Atlantic, abundances were lower than at the CWC reefs at Rockall Bank (583–777 m) with average values of 1.5×10^5 cells ml^{-1} and 1.8×10^6 viruses ml^{-1} between 250–500 m and 0.5×10^5 cells ml^{-1} and $1.3 - 1.8 \times 10^6$ viruses ml^{-1} between 900–1100 m (Parada et al., 2007). The data suggest that CWC bottom water is enriched with respect to prokaryotic and viral abundance.

4.2 Nutrient dynamics and mucus release

The concentrations of ammonium, nitrite, DIN and DIP were typically higher in incubation water with corals than in controls and the strongest effect was found for ammonium. An enrichment with respect to nitrite, nitrate and DIN has been found in the interstitial water of tropical and temperate corals (Schiller and Herndl, 1989) and in coral reef cavities (Van Duyl et al., 2006; Scheffers et al., 2005). There was no consistent difference in the rates of nutrient flux (ammonium, nitrite, DIN and DIP) between experiments with NSW as incubation water and experiments with incubation water, where viral and prokaryotic abundance was manipulated (Table 4). In two of these experiments (UF and VE) prokaryotic abundances were strongly reduced and thus, have probably not strongly influenced the nutrient dynamics (as estimated in short-term incubations). Thus, the prokaryotic consumption and transformation of nutrients was likely less important than release from corals. As a consequence, the coral-mediated nutrient fluxes given in Table 4 can serve as a first estimation of coral-mediated nutrient release. Overall, the cold-water corals seem to release DIN and DIP into the ambient water. The dominance of ammonium in the experiments with corals compared to the relatively low concentrations in situ suggests rapid dilution of ammonium or rapid nitrification in situ.

Release of mucus has been documented before for temperate (e.g. Herndl and Velimirov, 1986; Schiller and Herndl, 1989), tropical (e.g. Ducklow and Mitchell, 1979b; Wild et al., 2004) and cold-water corals (Wild et al., 2008). Mucus release was 17–29 mg C m^{-2} h^{-1} for *L. pertusa*. This is lower than the mucus release of

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ca. 48 mg C m^{-2} h^{-1} found for the same species collected in a Norwegian Fjord by using a similar approach (Wild et al., 2008). This difference could be due to the different techniques used for assessing coral surface area (geometry vs computer tomography). However, geometry and computer tomography yield similar results including *L. pertusa* (Naumann et al., 2009). Using a correction for relating surface areas of *L. pertusa* as determined by advanced geometry to computer tomography estimates (Naumann et al., 2009) mucus release would be up to 36 mg C m^{-2} h^{-1} in our study and thus, even closer to published values (Wild et al., 2008). The data from our study were from corals collected in 583–777 m depth from the Rockall Bank CWC reefs and incubated onboard, whereas the data from Wild et al. (2008) were from a colony collected in 120 m depth in a fjord off Bergen and kept in an aquarium for six months before the mucus release assay was performed. Considering these differences, the mucus release rates are surprisingly similar in the two studies. Also, first estimates of mucus release are provided for *M. oculata* and show that release rates were similar (12–29 mg C m^{-2} h^{-1}) compared to *L. pertusa*. Overall, the data support the finding that cold-water corals belong to the species with relatively low mucus production rates (Wild et al., 2008).

4.3 Release of prokaryotes and viruses from corals

The UF experiment was designed to reduce background levels of prokaryotes and viruses to be able to detect the potential release of prokaryotes and viruses from corals. Data from the UF (and VE) experiments indicate that prokaryotic abundances were already elevated at T2h in the coral treatments (Fig. 4). The observed increase would correspond to doubling times of as low as 1.1 h in incubation water with corals. However, such doubling times are unrealistic. Prokaryotic turnover times in bottom water from the CWC reef of Rockall Bank as determined by incorporation of radioactively labelled substrates ranged from 2.5–5 days (van Duyl et al., 2008) and in the onboard experiments, they ranged from ca. 0.5–5 days (Fig. 3a). Thus, it is more likely that prokaryotes became detached from corals or were released along with mucus.

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Data from the UF and PE experiments indicate that viral abundances at T2–6h were higher in the coral treatments than in the controls. It is known that stimulated host growth can increase phage production, e.g. by an increased burst size (Parada et al., 2006). Consequently, nutrient and mucus release from the corals (see below, section: Nutrient dynamics and mucus release) could have enhanced the growth of hosts and caused an increase in burst size or phage assembly rate in cells. In situ evidence from diurnal studies suggests that such an increase in burst size is at maximum 2-fold (Parada et al., 2006). Since viral abundance was stimulated at T2h–T6h by up to 3.6-fold in incubation water with *L. pertusa* and by up to 24-fold in incubation water with *M. oculata*, it is unlikely that a stimulated phage production was the only source for the elevated viral abundance in incubation water with corals. It is also conceivable that viruses were released from corals, e.g. by detachment from mucus or along with mucus release. It has been shown that viruses are present in coral mucus (Davy and Patten, 2007) including *L. pertusa* (Weinbauer, unpublished data) and that the VPR was higher over living than over dead corals (Patten et al., 2006). Moreover, electron microscopy pictures indicate release of viruses from heat-stressed corals (Davy et al., 2006). Currently, it is not known whether the released viruses are only from prokaryotes or also from the coral animals.

4.4 Mechanisms stimulating viral and microbial production in incubations with corals

Our data suggest that the holobiont of cold-water corals can be a source of prokaryotes and viruses for the free-living community. However, the stimulation of prokaryotes in incubations with corals was also influenced by other factors such as mucus release. This is supported by the finding that the respiration of microbial plankton was enhanced in incubations with mucus of *L. pertusa* (Wild et al., 2009). Also, stimulation of prokaryotic growth by mucus has been demonstrated before for temperate (Schiller and Herndl, 1989) and tropical corals (Ferrier-Pagès et al., 2000). Inorganic nutrient release as a result of digestion processes by the holobiont could have ensured that the utilization

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of carbon rich mucus was not limited by inorganic nutrients. Indeed, at the end of the experiments nutrient concentrations were >9 µm nitrate and >0.1 µm DIP thus, suggesting no limitation by inorganic N or P. As the concentration of inorganic nutrients is high in situ and thus, potentially not growth limiting in bottom waters of CWC reefs (Table 1), our experimental approach was likely – in this respect – mimicking general CWC bottom water conditions, where mucus release concurs with nutrient replenishment.

Virus were typically also stimulated in incubation water with the corals *L. pertusa* and *M. oculata*. Such experimental data are not available for temperate or tropical corals, although an in situ study suggests that viruses are stimulated close to coral surfaces (Patten et al., 2006), a finding which concurs with our experimental data. The finding that prokaryotic and viral abundance were typically well correlated in the experiments indicates that the stimulation of prokaryotic production also stimulated viral production. As viral infection is a stochastic event (Murray and Jackson, 1992), this is likely a consequence of increased encounter rates of viruses with host cells.

4.5 Implications

The finding of enhanced nutrient turnover, mucus release and growth stimulation of prokaryotes and viruses by corals has several implications. The data suggest that CWC reefs are a source of DIN and increase N:P ratios in surrounding waters. Interestingly, these reefs often occur in depths, where inorganic nitrogen species are converted to molecular nitrogen by denitrification and anammox processes (300–700 m) (Capone and Knapp, 2007; Deutsch et al., 2007) thus, resulting in a N:P ratio lower than the Redfield ratio. Since CWC reefs on continental margins are more frequent than previously thought (Roberts et al., 2006), they might replenish nitrogen species and influence the nitrogen cycle in these areas and counterbalance the low N:P ratios in the sediment.

Work by Wild and co-workers (Wild et al., 2009; Wild et al., 2008) has suggested that CWC reefs could stimulate microbial activity in bottom water by mucus release and potentially exert some control over organic C cycling. Our data confirm mucus

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release and suggest a stimulation for prokaryotic growth and thus, support the idea that CWC reefs sustain microbial activity. This could explain why prokaryotic (and viral) abundances seem to be stimulated in bottom water of CWC reefs (see above, section: Prokaryotic and viral abundance in bottom water). Viruses exert a major influence on the regeneration of organic matter in marine pelagic environments (Middelboe and Lyck, 2002; Motegi et al., 2009; Suttle, 2005). As this influence depends on the rate of viral lysis (Motegi et al., 2009), a stimulation of viral production by corals in bottom water could enhance the remineralization of organic matter and lubricate the microbial food web in bottom water of CWC reefs.

An elevated prokaryotic production could also stimulate the nutrition of corals, since ingestion of prokaryotic cells is a density dependant process (Houlebrèque et al., 2004). Ingestion of viruses has been documented so far for sponges (Hadas et al., 2006) but not for corals. As (most) viruses are per definition part of the DOC pool which is accessible to corals (Sorokin, 1973), one might also expect uptake of viruses. A feed-back loop can be envisioned, where enhanced prokaryotic (and viral) production has a positive upward cascading effect through the trophic levels of the food web and finally fuels zooplankton. This enhanced zooplankton production (and other stimulated trophic levels) could then be cropped by corals thus, closing the trophic loop. This could also explain the apparent contradiction of DIN release (this study) and the finding that CWC reefs preferentially remove nitrogen (Lavaleye et al., 2009). Such a mechanism could result in a reduced loss of elements such as N, P and C from CWC ecosystems. Overall, the study suggests that corals are a strong forcing factor for the microbial food web of the bottom water in CWC reefs via the release of nutrients and mucus.

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Table 1. In situ concentrations of nutrients and abundances of prokaryotes and viruses. Nutrients are given in µM, prokaryotes as 10^5 cells ml^{-1} and viruses as 10^6 viruses ml^{-1}. Exp, experiment; NSW, natural sea water; UF, ultrafiltrate; VE, virus-enriched; PE, prokaryote-enriched; DIN, dissolved inorganic nitrogen; DIP, dissolved inorganic phosphorus.

Sample-ID	Depth (m)	NH_4	NO_3	NO_2	DIN	DIP	N:P	Prokaryotes	Viruses	Exp
WK13	590	0.09	12.8	0.067	12.9	0.76	17.0	4.3	5.7	NSW1
WK42	583	0.20	11.3	0.109	11.6	0.74	15.6	4.3	8.4	UF
WK79	745	0.23	11.6	0.085	11.9	0.75	16.0	4.4	7.5	VE
WK86	770	0.16	11.7	0.093	11.9	0.73	16.3	4.9	6.9	PE
WK110	777	0.18	13.1	0.081	14.1	0.83	16.1	4.0	7.0	NSW2

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Table 2. Nutrient concentrations and prokaryotic and viral abundance in incubation water at the start of the experiments. Nutrient and TOC parameters are given in µM, prokaryotes as 10^5 cells ml^{-1} and viruses as 10^6 viruses ml^{-1}. Data are given as averages ± SE of 3–6 replicates. Exp, experiment; NSW, natural sea water; UF, ultrafiltrate; VE, virus-enriched; PE, prokaryote-enriched; DIN, dissolved inorganic nitrogen; DIP, dissolved inorganic phosphorus.

Exp	NH_4	NO_3	NO_2	DIN	DIP	N:P	TOC	Prokaryotes	Viruses
NSW1	0.30 ± 0.09	13.3 ± 3.3	0.07 ± 0.02	13.7	0.86 ± 0.26	15.9	140 ± 3	4.6 ± 0.7	4.6 ± 0.3
NSW2	1.72 ± 0.41	13.9 ± 4.2	0.10 ± 0.02	15.7	0.99 ± 0.27	15.9	160 ± 24	3.4 ± 0.4	3.6 ± 0.5
UF	0.12 ± 0.03	12.5 ± 2.9	0.09 ± 0.02	12.7	0.75 ± 0.18	16.9	71 ± 2	0.56 ± 0.01	0.07 ± 0.01
VE	0.32 ± 0.03	11.6+0.1	0.11 ± 0.01	12.0	0.74 ± 0.01	16.3	83 ± 3	0.64 ± 0.07	13.9 ± 0.8
PE	0.44 ± 0.10	11.6 ± 2.7	0.10 ± 0.02	12.1	0.72 ± 0.17	16.9	79 ± 1	10.3 ± 0.1	6.4 ± 0.1

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Table 3. Nutrient and total organic carbon (TOC) concentrations and microbial abundances at the end of the experiments. Nutrient and TOC parameters are given in µM, prokaryotes as 10^5 cells ml^{-1} and viruses as 10^6 viruses ml^{-1}. Data are given as averages ± SE of 3 replicates. When SE is not shown, one replicate was not used because the value was ca. twice as high than in the other two replicates. NSW, natural sea water; UF, ultrafiltrate; VE, virus enriched; PE, prokaryote enriched; C, control, Lp; *L. pertusa*; Mo, *M. oculata*; VE, virus enhanced; PE, prokaryote enhanced; DIN, dissolved inorganic nitrogen; DIP, dissolved inorganic phosphorus, NS, not significant (Wilcoxon, $p \geq 0.05$).

Exp.	Treatm.	NH_4	NO_3	NO_2	DIN	DIP	N:P	TOC	Prokaryotes	Viruses
NSW1	C	0.5 ± 0.1	13.4 ± 0.1	0.33 ± 0.04	14.2 ± 0.1	0.67 ± 0.02	21 ± 1	157 ± 25	0.8 ± 0.2	1.3 ± 0.1
	Lp	14.5 ± 3.1*	13.7 ± 0.1*	0.34 ± 0.04	28.5 ± 0.1*	0.24 ± 0.11*	119 ± 13*	220 ± 23	2.9 ± 0.3*	0.9 ± 0.1*
	Mo	3.9 ± 1.8*	13.0 ± 0.2	0.44 ± 0.03*	17.9 ± 0.9*	0.13 ± 0.01*	133 ± 4*	202	4.9 ± 0.9*	0.6 ± 0.1*
NSW2	C	12.7 ± 1.6	14.0 ± 0.1	0.17 ± 0.01	26.9 ± 0.1	1.43 ± 0.07	19 ± 1	154 ± 9	4.3 ± 0.3	1.9 ± 0.8
	Lp	23.0 ± 6.8*	13.9 ± 0.1	0.36 ± 0.06*	37.3 ± 0.1*	1.62 ± 0.06*	23 ± 1*	183	5.1 ± 1.2*	4.1 ± 0.6*
	Mo	25.1 ± 2.0*	14.0 ± 0.1	0.24 ± 0.03*	39.3 ± 0.1*	1.72 ± 0.08*	23 ± 1*	187 ± 12	7.1 ± 0.4*	2.2 ± 0.2*
UF	C	0.2 ± 0.1	10.2 ± 0.3	0.21 ± 0.01	10.7 ± 0.3	0.43 ± 0.05	25 ± 4	103 ± 21	1.0 ± 0.1	0.5 ± 0.2
	Lp	22.6 ± 3.4*	12.7 ± 0.1*	0.33 ± 0.03*	35.6 ± 0.1*	0.71 ± 0.27*	50 ± 14*	178 ± 27	4.2 ± 0.1*	3.5 ± 1.0*
	Mo	6.9 ± 0.2*	12.4 ± 0.1*	0.22 ± 0.01	18.6 ± 0.1*	0.57 ± 0.04*	34 ± 2*	137 ± 29	2.9 ± 0.2*	3.4 ± 0.3*
VE	C	0.2 ± 0.1	9.9 ± 0.3	0.15 ± 0.01	10.2 ± 0.3	0.56 ± 0.05	18 ± 1	79 ± 11	1.1 ± 0.2	1.3 ± 0.1
	Lp	13.6 ± 0.9*	11.7 ± 0.1*	0.17 ± 0.01	25.5 ± 0.1*	0.71 ± 0.08*	36 ± 2*	134	2.2 ± 0.1*	2.0 ± 0.2*
	Mo	2.3 ± 0.5*	11.4 ± 0.1*	0.16 ± 0.01	13.9 ± 0.1*	0.36 ± 0.03	39 ± 2*	125	2.1 ± 0.2*	2.1 ± 0.1*
PE	C	0.2 ± 0.1	9.6 ± 0.4	0.18 ± 0.03	10.0 ± 0.2	0.52 ± 0.02	22 ± 1	82 ± 13.7	2.0 ± 0.1	0.8 ± 0.1
	Lp	36.6 ± 3.4*	11.7 ± 0.1*	0.59 ± 0.07*	48.9 ± 0.1*	1.66 ± 0.08*	30 ± 1*	136	5.2 ± 1.7*	3.4 ± 0.2*
	Mo	14.3 ± 1.1*	11.7 ± 0.1*	0.28 ± 0.09*	26.3 ± 0.1*	1.08 ± 0.23*	24 ± 1*	117 ± 3	3.2 ± 0.4*	1.7 ± 0.3*
Wilcoxon test										
C vs. Lo (p)		< 0.05	NS	< 0.05	< 0.05	NS	< 0.05	< 0.05	< 0.05	NS
C vs. Mo (p)		< 0.05	NS	< 0.05	< 0.05	NS	< 0.05	< 0.05	< 0.05	NS

* significant difference (Mann-Whitney, $p < 0.05$) between coral and control treatment.

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Table 4. Organic carbon release and stimulation of nutrients, prokaryotes and viruses in incubation water with corals (corrected for controls). For calculations see material and methods. Nutrient parameters and TOC are given in mg m^{-2} coral surface h^{-1}, prokaryotes as 10^9 cells and viruses as 10^9 viruses m^{-2} coral surface h^{-1}. NSW, natural seawater; UF, ultrafiltrate; VE, virus-enriched; PE, prokaryote-enriched; DIN, dissolved inorganic nitrogen; DIP, dissolved inorganic phosphorus; ND, not detectable (i.e., statistically not different from or lower than controls).

Exp.	Species	NH_4	NO_3	NO_2	DIN	DIP	TOC	Prokaryotes	Viruses
NSW1	*L. pertusa*	0.97	0.055	ND	1.08	0.040	28.5	3.00	28.2
	M. oculata	0.48	0.129	0.0180	0.63	0.023	29.0	5.50	4.7
NSW2	*L. pertusa*	0.90	ND	0.0105	0.91	0.016	17.2	8.90	2.9
	M. oculata	0.34	ND	0.0032	0.34	0.022	12.3	11.30	3.9
UF	*L. pertusa*	1.53	0.118	0.0019	1.65	0.0234	12.87	10.39	9.42
	M. oculata	0.43	0.014	0.0020	0.45	0.0111	9.73	4.81	7.25
VE	*L. pertusa*	0.50	0.054	0.0116	0.57	0.0116	10.38	2.88	1.71
	M. oculata	0.13	0.047	0.0051	0.18	0.0069	12.44	2.25	1.61
PE	*L. pertusa*	1.54	0.039	0.0120	1.59	0.0300	14.48	8.44	7.05
	M. oculata	0.52	0.014	0.0035	0.54	0.0246	8.40	1.88	1.46
Wilcoxon test *L. pertusa* vs. *M. oculata* (p)		< 0.05	NS	NS	< 0.05	NS	NS	NS	NS

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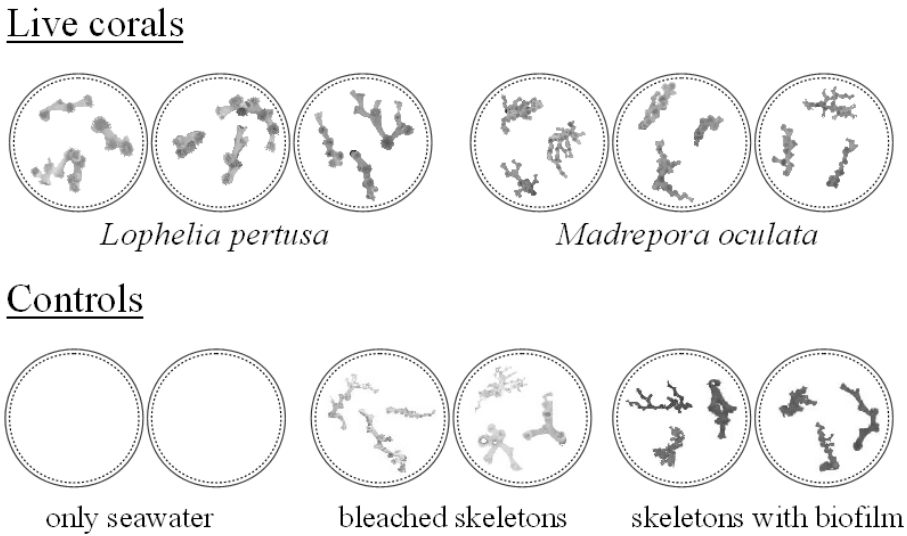

Fig. 1. Experimental set-up.

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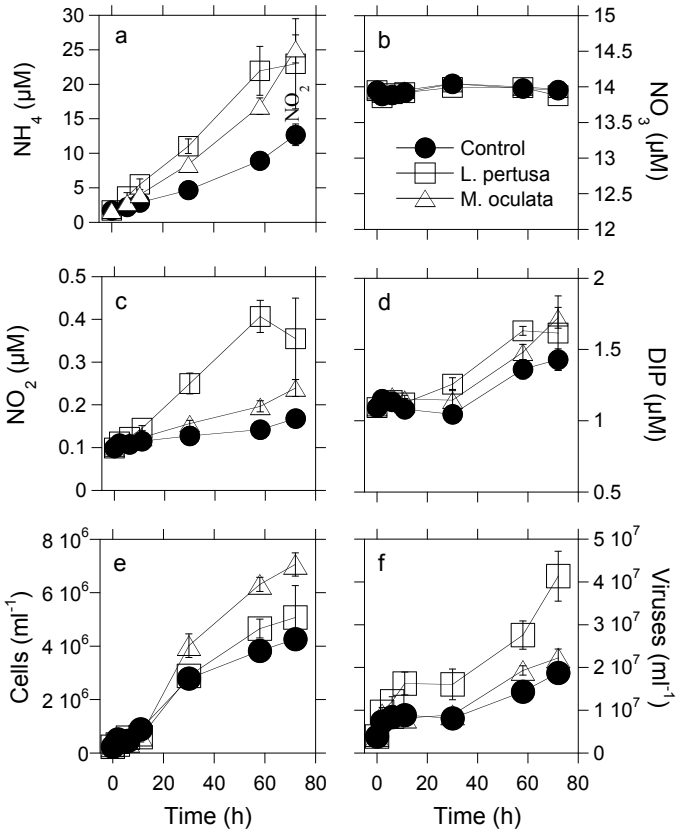

Fig. 2. Dynamics of ammonium, nitrate, nitrite and DIP concentrations, and prokaryotic and viral abundance in experiment NSW2. Note that DIN shows the same trend as ammonium. Data are given as averages ± SE of 3 replicates. When error bars are not visible, they are withing the width of the symbol.

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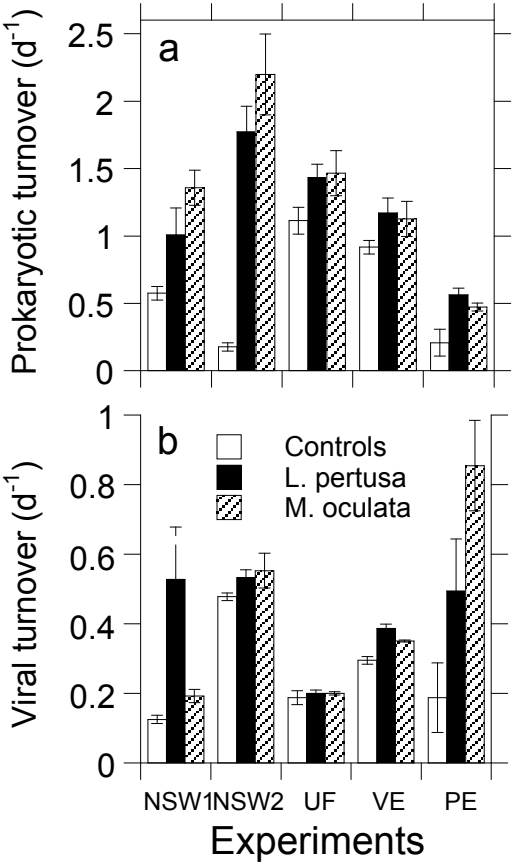

Fig. 3. Prokaryotic and viral turnover rates in the experiments. Data are given as averages ± SE of 3 replicates.

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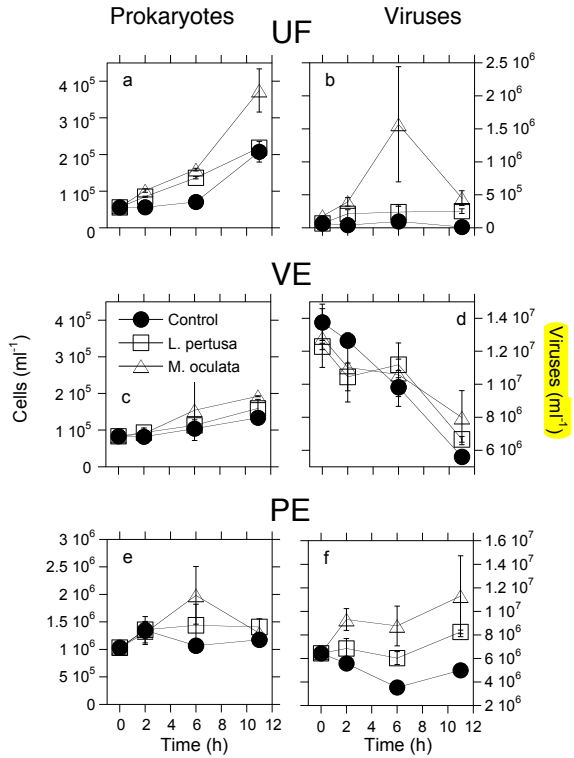

Fig. 4. Short-term dynamics of prokaryotic and viral abundance in the experiments, where prokaryotic and viral abundance was manipulated. Note that axis can be different between experiments. Data are given as averages ± SE of 3 replicates. When error bars are not visible, they are within the width of the symbol. UF, ultrafiltrate; VE, virus-enriched; PE, prokaryote-enriched.

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