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# Microbial abundance in the coelenteron and mucus of the cold-water coral *Lophelia pertusa* and in bottom water of the reef environment

Markus G. Weinbauer<sup>1,2,\*</sup>, Julie Ogier<sup>3</sup>, Cornelia Maier<sup>1,2,3</sup>

<sup>1</sup>INSU-CNRS, Laboratoire d'Océanographie de Villefranche, UMR 7093, BP 28, 06234 Villefranche-sur-Mer Cedex, France

<sup>2</sup>Université Pierre et Marie-Curie-Paris, Laboratoire d'Océanographie de Villefranche, UMR 7093, 06230 Villefranche-sur-mer, France

<sup>3</sup>Department of Biological Oceanography, Royal Netherlands Institute for Sea Research (NIOZ), PO Box 59, 1790 AB Den Burg, Texel, The Netherlands

**ABSTRACT:** Microorganisms play an important role in coral ecology; however, little is known about cold-water coral (CWC) reefs. Microbial abundance in bottom water of *Lophelia pertusa*-dominated CWC reefs in the Skagerrak, North Sea, was elevated by ca. 40% for prokaryotes and 70 to 90% for viruses compared to bottom water where no corals were present. This indicates that CWCs influence the microbial food web in bottom water. Furthermore, results suggest that microorganisms contributed significantly to the stock of organic nitrogen (13%) and organic phosphorus (32%) in bottom water. Prokaryotic abundances in the coelenteron of freshly collected corals were  $(2.0 \pm 0.2) \times 10^7$  cells ml<sup>-1</sup> and viral abundances were  $(4.0 \pm 0.6) \times 10^7$  particles ml<sup>-1</sup>. Prokaryotic abundances in coral mucus were  $(7.5 \pm 0.7) \times 10^7$  cells ml<sup>-1</sup> and viral abundances were  $(1.7 \pm 0.3) \times 10^8$  particles ml<sup>-1</sup>. Onboard incubations with elevated prokaryotic and viral abundances in the incubation water indicated that (1) corals accumulate microorganisms in the coelenteron as a potential food source and (2) control mechanisms exist for the abundance of prokaryotic cells and viral particles in the mucus. Our data suggest that *L. pertusa* controls microbial biomass in the CWC reef environment and coral microhabitats.

**KEY WORDS:** Cold-water coral · Microbial abundance · *Lophelia pertusa* · Viruses · Prokaryotes · Bacteria · Reefs

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## INTRODUCTION

Cold-water corals (CWCs) such as *Lophelia pertusa* and *Madrepora oculata* are more widely distributed than previously assumed and are able to construct reef-like structures (Roberts et al. 2006). However, CWC-microbe interactions are poorly studied. Corals accumulate or grow prokaryotes in the coelenteron (gastral cavity) and digest them when they reach threshold abundances (Sorokin 1973, Herndl et al. 1985, Ferrier-Pagès & Gattuso 1998). In addition, prokaryotic growth is stimulated in ambient water close to corals, e.g. due to the re-

lease of mucus and nutrients (Schiller & Herndl 1989, Maier et al. 2011). Thus, it has been suggested that corals can partly provide for their own nutrition, either directly, by farming prokaryotes as a food source, or indirectly, by attracting microbe-eating zooplankton, which in turn serve as food for corals (Herndl et al. 1985, Schiller & Herndl 1989). An emerging feature of coral-microbe interactions is that a balance or equilibrium exists between corals and their associated prokaryotes (Kline et al. 2006). For example, it has been argued that bacterial diversity in mucus is strongly controlled (e.g. Ritchie 2006) and that mucus-associated bacteria act as a probiotic

\*Email: wein@obs-vlfr.fr

barrier against incoming fowling or pathogenic prokaryotes (Reshef et al. 2006) and as a buffer against environmental change (Rosenberg et al. 2007).

Recent research has shown that viral abundance decreases with distance from the coral (Seymour et al. 2005, Patten et al. 2006) and that the mucus contains an astounding morphological diversity of viruses (Davy & Patten 2007). To our knowledge, no data have been published on the abundance of viruses in the mucus or coelenteron of corals, and the viral ecology of the coral holobiont remains largely unstudied.

This study had 3 objectives: (1) to quantify prokaryotic and viral abundances (and inorganic and organic nutrients) in a CWC reef environment in the Skagerrak, North Sea, (2) to quantify prokaryotic and viral abundance in 2 major microhabitats of *Lophelia pertusa*, viz. the coelenteron and the mucus, and (3) to test whether an increase in the abundance of prokaryotes and viruses in ambient water by ca. 1 order of magnitude can change abundances in the coelenteron and mucus.

## MATERIALS AND METHODS

Samples were obtained during the BIOSYS cruise ([www.nioz.nl/public/dmg/rpt/crs/64pe263.pdf](http://www.nioz.nl/public/dmg/rpt/crs/64pe263.pdf)) on the RV 'Pelagia' in March 2007 to the Skagerrak of Norway at Fjellknausene (59.07° N, 10.74° E) and Soester (59.08° N, 10.76° E), ca. 100 km south of Oslo (Norway). Colonies of *Lophelia pertusa* and water samples were collected at 78 to 128 m depth from a reef site that was dominated by *L. pertusa*. The water column was characterized by a conductivity-temperature-density (CTD) rosette sampler holding Seabird SBE9 sensors for measuring pressure, temperature, salinity and density.

Coral colonies (white variation) were collected with a box corer consisting of a stainless steel cylindrical core (50 cm in diameter and 55 cm in height), which is closed by a lid and a metal blade upon reaching the seafloor. For details of the box core sampling see van Duyl et al. (2008) and Hansson et al. (2009). The advantage of this method of collection is that (1) corals remain in bottom water, (2) corals are protected against exposure to other water layers during the transport through the water column, and (3) bottom water is also collected. Video profiles showed that coral density varied from loose associations to coral thickets. In the box cores, coral density ranged from a few small colonies to a full thicket. A fraction of the colonies was processed

right away, whereas others were used in incubations; only healthy looking coral colonies without epibionts were used. The coelenteron fluid (CF) of *Lophelia pertusa* polyps was sampled by modifying the method of Herndl & Velimirov (1985). Briefly, 5 µl samples (for microorganisms) were taken from the CF using a pipette and 10 µl tips. Rough calculations (C. Maier unpubl.) suggest that ca. 50% of the total CF was sampled. Care was taken to minimize the contact of the pipette tip with the entoderm. These samples were taken immediately after releasing the water from the box cores. For mucus sampling, coral pieces were placed in a hood on a plastic tray covered with aluminium foil. Mucus strings formed within minutes of exposure to air. For the enumeration of microorganisms, samples (5 µl) of mucus strings were immediately collected using a pipette equipped with 10 µl tips. Samples of coelenteron and mucus were mixed with 250 µl TE buffer and vortexed in 1 ml cryovials. Samples were fixed for 30 min in 0.5% glutaraldehyde (pre-filtered through 0.2 µm sterile Acrodisc filters), shock frozen in liquid nitrogen and stored at -80°C until analysis.

Box core (BX) water was collected from all BXs containing living corals (BX with corals) and from BXs that did not contain living corals (BX without corals). For BX without corals, only those samples were used which contained no or only small amounts of sediment. Bottom water was also collected with the CTD rosette sampler holding 12 l no oxygen exchange (NOEX) bottles (CTD water). Water used for keeping corals was sampled from ca. 40 m with an *in situ* pump. For microbial enumeration, 1 ml samples of BX and CTD water were processed as described above.

For onboard incubations, small branches of *Lophelia pertusa* (9 ± 1 polyps) were glued onto Petri dishes using underwater Epoxy®. These microcolonies were kept in 4 acid-cleaned and seawater-rinsed 20 l plastic tanks in a climate chamber (hereafter called 'incubations'), i.e. a temperature-controlled walk-in container laboratory at a density of ca. 4 micro-colonies 100 cm<sup>-2</sup>. The corals were kept for up to 7 d in the dark, and the container was set at ca. *in situ* temperature (7.5°C). One-third of the seawater was replaced every second day with freshly pumped water from ca. 40 m depth (without exposing corals to air), to avoid accumulation of dissolved organic carbon (DOC) and nutrients to levels which can become toxic for corals (Kuntz et al. 2005, Kline et al. 2006). A water flow was generated by small submerged aquarium pumps with a capacity of 250 l h<sup>-1</sup>. Mucus release,

which is a normal process for *L. pertusa* (Wild et al. 2008), can increase prokaryotic and viral production (Maier et al. 2011). This feature was used to expose corals to elevated levels of prokaryotes and viruses and to experimentally assess whether this increased abundance of microorganisms influences their abundances in CF and mucus. It is noteworthy that the colonies we used did not show signs of tissue necrosis and that most of the polyps were extended. The micro-colonies were kept for at least 2 d (and up to 7 d) for acclimation before samples were taken from CF and mucus. Samples from microhabitats in incubations were taken and processed as described above.

Prokaryotic and viral abundances were determined by flow cytometry using SYBRGreen I staining solution (Molecular Probes; 1000-fold dilution of the stock) and a FACSCalibur (Becton Dickinson). Samples were diluted 4- to 100-fold in TE buffer for prokaryotic and 50- to 2000-fold for viral enumeration. For more details, see Gasol & del Giorgio (2000) and Brussaard (2004).

Samples (5 ml) were collected from CTD and BX water for quantifying nitrogen and phosphorus concentrations. Ammonium, nitrite, nitrate, total nitrogen (TN), total inorganic phosphorus (TIP) and total phosphorus (TP) concentrations were determined using an AxFlow Bran & Luebbe Traacs800 auto-analyser (Herfort et al. 2007). Total inorganic nitrogen (TIN) concentrations were calculated as the sum of all inorganic nitrogen species. Total organic nitrogen (TON) and phosphorus (TOP) concentrations were calculated by subtraction of TIN (TIP) from TN (TP). For total organic carbon (TOC) analysis, 20 ml samples were filled in pre-combusted glass ampoules, acidified with 8 drops of concentrated  $\text{H}_2\text{SO}_4$ , sealed and stored at 4°C until analysis. TOC concentrations were measured by high-temperature combustion on a Shimadzu TOC-5000 as described by Benner & Strom (1993).

Carbon, nitrogen and phosphorus in microbial biomass were estimated from prokaryotic and viral abundances and from conversion factors. The conversion factors used were 20 fg C cell<sup>-1</sup> (Lee & Fuhrman 1987), 0.2 fg C virus<sup>-1</sup> (Wilhelm & Suttle 1999), a C:N:P ratio of 45:9:1 for prokaryotes (Goldman et al. 1987) and a C:N:P ratio of 10:4.5:1 for viruses (Hewson et al. 2003). Mann-Whitney *U*-tests were used to assess differences between the microhabitats (i.e. coelenteron and mucus) and ambient water and differences between *in situ* and experimental data. A probability of  $p < 0.05$  was considered significant. All data are given as mean  $\pm$  SE.

## RESULTS AND DISCUSSION

### Water column and bottom water

Average depth of coral sampling was 109 m. Average temperature, salinity and water density values in CTD bottom water (i.e. ca. 5 m above the CWC reef) were 7.5°C, 34.66 and 27.08 kg m<sup>-3</sup>, respectively. Nutrient concentrations and microbial abundances in bottom water (CTD and BX water) are summarized in Table 1. All nitrogen and phosphorus parameters measured and TOC concentrations were higher in BX water with corals than in BX water without corals or CTD water (except for phosphate in BX water without corals). Differences were significant for ammonium in BX water without corals and for ammonium, nitrite, TON, TN and phosphate in CTD water (Mann-Whitney,  $p < 0.05$ ).

TOC concentrations of 160  $\mu\text{M}$ , as observed at the Fjellknausene/Soester reef in the Skagerrak, were similar to TOC or DOC concentrations in CWC reefs at Rockall Bank (van Duyl et al. 2008, Maier et al. 2011) but more than 2 orders of magnitudes lower than DOC concentrations at the Tisler Reef in the Skagerrak (Wild et al. 2009). The reasons for these extremely high DOC concentrations, which surpass DOC or TOC concentrations at all studied tropical reefs (Kline et al. 2006), remain unknown.

In different types of bottom water, prokaryotic abundance averaged  $(3.2 \text{ to } 4.5) \times 10^5$  cells ml<sup>-1</sup> and viral abundance averaged  $(1.0 \text{ to } 1.9) \times 10^7$  particles ml<sup>-1</sup>; the virus to prokaryote ratio (VPR) averaged 32 to 41. The abundance of prokaryotes in bottom water was similar to prokaryotic abundance at the CWC reefs at Rockall Banks, whereas viral abundances were slightly higher in the Skagerrak (Maier et al. 2011). Estimations from abundances indicate that microbial (prokaryotic plus viral) carbon, nitrogen and phosphorus in BX water with corals were 1.6  $\mu\text{M}$ , 1.3  $\mu\text{M}$  and 0.07  $\mu\text{M}$ , respectively, corresponding to a contribution of 1% to TOC, 13% to TON and 32% to TOP. Thus, microorganisms are an important pool of organic nitrogen and phosphorus in bottom water of CWC reefs.

Microbial parameters were significantly higher in BX water with corals than in BX water without corals or CTD water (Mann-Whitney,  $p < 0.05$ ) except for VPR in BX water (Mann-Whitney,  $p > 0.05$ ). It is well documented that corals, including *Lophelia pertusa*, release mucus, which can stimulate the production of microorganisms (Wild et al. 2004a, 2008, 2009, Maier et al. 2011). This could be the reason for the elevated concentrations of prokaryotic abundance in the bot-



Table 2. Literature data of *in situ* prokaryotic abundances in the coelenteron and mucus of corals. Data are given as means  $\pm$  SE (*Lophelia pertusa*) or  $\pm$  SD (all other species). nd: not determined (or *in situ* abundance uncertain); EF: enrichment factor, i.e. ratio of abundance in the coelenteron (or mucus) compared to bottom water

Order/species	Environment	Prokaryotic abundance				Source
		Coelenteron		Mucus		
		Cells ( $10^6$ ml $^{-1}$ )	EF	Cells ( $10^7$ ml $^{-1}$ )	EF	
<b>Scleractinia</b>						
<i>Lophelia pertusa</i>	Skagerrak	19.5 $\pm$ 1.6	55.1	7.5 $\pm$ 0.7	212	This study
<i>Leptopsammia pruvoti</i>	Prvic, Yugoslavia <sup>a</sup>	1.8 $\pm$ 1.1	5.1			Herndl & Velimirov (1985)
<i>Oculina patagonica</i>	Sedot Yam, Mediterranean Sea, Israel, winter			30.0 $\pm$ 20.3	nd	Koren & Rosenberg (2006)
<i>Oculina patagonica</i>	Sedot Yam, Mediterranean Sea, Israel, summer			25.0 $\pm$ 1.07	nd	Koren & Rosenberg (2006)
<i>Acropora</i> sp.	Heron Island, Great Barrier Reef			3.0 $\pm$ 0.2	86	Wild et al. (2004a)
<i>Acropora</i> sp.	Heron Island, Great Barrier Reef			3.5 $\pm$ 0.9	81	Huettel et al. (2006)
<i>Acropora</i> sp.	Heron Island, Great Barrier Reef, end of low tide			1000 $\pm$ 330	23866	Huettel et al. (2006)
<b>Actiniaria</b>						
<i>Stoichactis giganteum</i>	Ras Iwatine, Kenya	2.5 $\pm$ 1.1	2.6			Herndl et al. (1985)
<i>Anemonia sulcata</i>	Ischia, Italy	2.4 $\pm$ 2.3	nd			Herndl & Velimirov (1985)
<b>Zoanthidea</b>						
<i>Parazoanthus axinellae</i>	Prvic, Yugoslavia <sup>a</sup>	1.4 $\pm$ 1.5	3.8			Herndl & Velimirov (1985)

<sup>a</sup>The island Prvic, located off Krk, now belongs to Croatia

phosphorus (nucleic acids)-rich prokaryotes and viruses in the mucus (carbon, 184  $\mu$ M; nitrogen, 45  $\mu$ M; phosphorus, 4.4  $\mu$ M) could, in addition to microorganisms in the water column, represent a nutrient pool potentially usable by corals. Studies on the feeding behavior and general morphology, together with isotope studies of *Lophelia pertusa*, suggest that these corals mainly feed on larger particles such as zooplankton (Kiriakoulakis et al. 2005, Wild et al. 2008). However, microorganisms could also serve as carbon, nitrogen and phosphorus sources to sustain the basic metabolism at times of low zooplankton and detritus availability or provide more spurious substances such as trace elements, vitamins or antibiotics.

In BX coral mucus, prokaryotic abundance was  $(7.5 \pm 0.7) \times 10^7$  cells ml $^{-1}$ . Prokaryotic abundance was significantly higher than in bottom water, and the EF was 212 (Mann-Whitney,  $p < 0.005$ ). Prokaryotic abundance in the mucus of *Lophelia pertusa* was higher than in the tropical coral *Acropora* sp. and lower than in the temperate coral *Oculina patagonica*; however, all abundances were within 1 order of magnitude ( $10^7$  to  $10^8$  cells ml $^{-1}$ ; Table 2). The only exception to this was a special situation in which *Acropora* sp. corals became dry at the end of low tide, resulting in a huge increase in mucus-associated prokaryotes (Huettel et al. 2006).

Viral abundance was  $(1.7 \pm 0.3) \times 10^8$  particles ml $^{-1}$  in the BX mucus and was significantly higher than in BX water with corals (Mann-Whitney,  $p < 0.005$ ); the EF was 13 and thus lower than for prokaryotes in mucus. This resulted in VPRs that were significantly higher in different types of bottom water (32 to 41) than in BX mucus ( $2.7 \pm 0.6$ ; Mann-Whitney,  $p < 0.005$ ). Viral abundance (and VPR) showed lower enrichment factors than prokaryotes. Preliminary data suggest that flow cytometry and epifluorescence counts of viruses (and prokaryotes) in coral mucus do not differ significantly (M. Weinbauer unpubl.). Thus, the low VPR and low EF for viruses could be due to factors such as lower viral production or higher viral decay.

### Onboard incubation

Prokaryotic abundance was ca. 1 order of magnitude higher in incubation water than in BX water (Mann-Whitney,  $p < 0.05$ ). One reason for this was that prokaryotic abundances were higher (prokaryotic abundance,  $[6.1 \pm 0.3] \times 10^6$  ml $^{-1}$ ; viral abundance,  $[1.2 \pm 0.3] \times 10^7$  ml $^{-1}$ ; VPR,  $19 \pm 3$ ) in water that was pumped from 40 m and used for water exchange than in BX water. Also, the release of mucus from

CWCs (Wild et al. 2008) can stimulate prokaryotic biomass accumulation (Maier et al. 2011). An enrichment of prokaryotic abundance to levels of  $10^6$  cells  $\text{ml}^{-1}$  seawater due to release of mucus was previously observed in onboard experiments with *Lophelia pertusa* and did not have detectable effects on coral health (Maier et al. 2011). This feature was used to test whether *L. pertusa* can exert control over the prokaryotic abundance in CF and mucus, when ambient prokaryotic abundance is elevated by an order of magnitude. Viruses were also significantly enriched in incubation water (Mann-Whitney,  $p < 0.05$ ). This is probably linked to higher prokaryotic abundances; a correlation between the increase of prokaryotic and viral abundance in incubations was found previously in onboard incubations with the CWCs *L. pertusa* and *Madrepora oculata* (Maier et al. 2011). Overall, our experimental intentions were met. It is also noteworthy that the salinity of the pumped water (used to maintain corals) was very close to the salinity *in situ* (33.31 versus 34.66). Changing water in the plastic tanks decreased the temperature only by  $<1^\circ\text{C}$ . Thus, the water used for keeping corals probably did not cause stress.

For CF, prokaryotic and viral abundances were significantly higher in incubations than *in situ* (Mann-Whitney,  $p < 0.05$  for both parameters; Fig. 1A,B). These differences were 1.9-fold for prokaryotic abundances and 5.7-fold for viral abundance. VPR in the CF was not significantly different between incubations and *in situ* (Mann-Whitney,  $p > 0.2$ ; Fig. 1C). It has been shown that an increase in prokaryotic abundance in ambient water was followed by an increased prokaryotic abundance in the CF of scleractinian, actinian and zoanthid corals (Herndl et al. 1985, Herndl & Velimirov 1985). Moreover, it has been suggested that corals release bacteriolytic substances at threshold densities of prokaryotes; below this value there is a build-up of prokaryotes (Herndl & Velimirov 1985). In addition, enhanced standing stocks of microorganisms can be cropped by corals (Schiller & Herndl 1989), and uptake of microorganisms by corals is a linear function of the prey abundance in the surrounding water (Houlbrèque et al. 2004). Thus, although the study was not designed as a feeding experiment, the data indicate that *Lophelia pertusa* could use prokaryotes as food. In the CF, viral abundance was elevated in incubations compared to *in situ*, perhaps because prokaryotic and viral abundances in incubation water were stimulated. Corals can take up dissolved organic matter and colloidal material (e.g. Sorokin 1973). Thus, it is possible that corals can also ingest and digest viruses.

Prokaryotic and viral abundance and VPR in coral mucus did not differ significantly between incubations and *in situ* (Mann-Whitney,  $p > 0.05$ ; Fig. 1A–C), although microbial abundances were significantly higher in the incubations than *in situ*. This suggests some coral control of microbial biomass. The range of prokaryotic abundance in the mucus of *Lophelia pertusa in situ* ( $4.8 \times 10^7$  to  $1.2 \times 10^8$   $\text{ml}^{-1}$ ) was similar to the range of  $2.7 \times 10^7$  to  $3.0 \times 10^8$   $\text{ml}^{-1}$  typically found

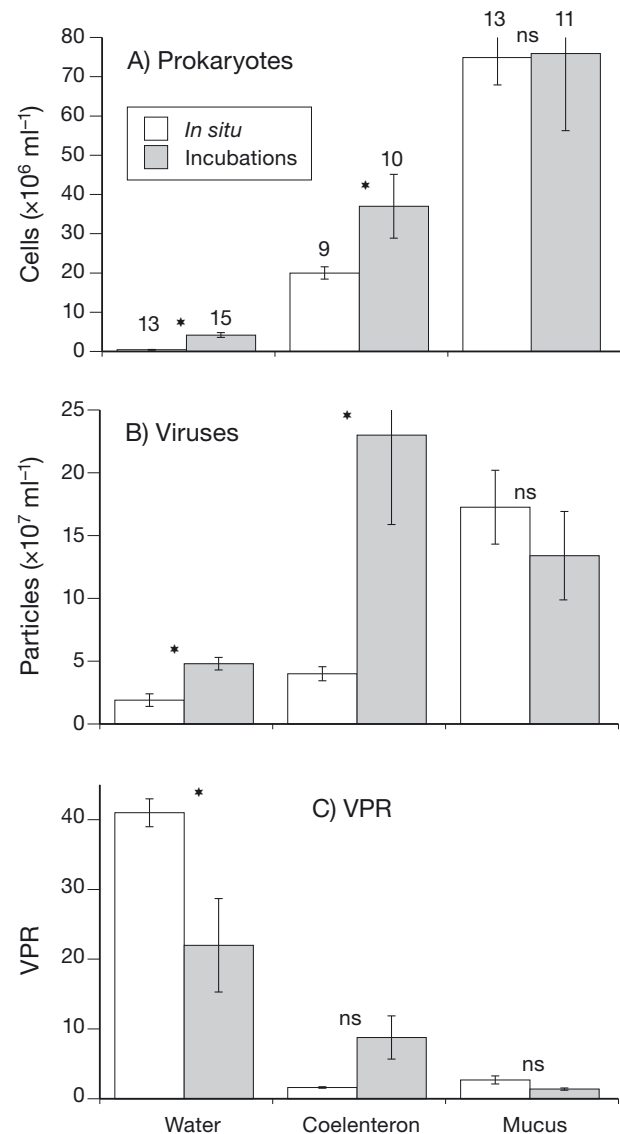


Fig. 1. Prokaryotic and viral abundances in the microhabitats of *Lophelia pertusa in situ* and in the climate container (onboard incubations) in box corer water, the coelenteron and coral mucus. \*Significant difference between *in situ* and onboard incubations (Mann-Whitney,  $p < 0.05$ ); ns: non-significant difference between *in situ* and onboard incubations (Mann-Whitney,  $p > 0.05$ ). VPR: virus to prokaryote ratio. Data are given as means  $\pm$  SE. Numbers above the bars are the number of samples analysed

in other coral species from tropical environments (e.g. Wild et al. 2004b, Huettel et al. 2006, Koren & Rosenberg 2006; see also Table 2). It has also been shown that coral mucus can sustain  $10^8$  cells  $\text{ml}^{-1}$  of isolated bacterial strains (Sharon & Rosenberg 2008). Thus, values of  $10^7$  to  $10^8$  cells  $\text{ml}^{-1}$  are likely close to the carrying capacity of mucus. During extreme conditions such as air exposure of corals, prokaryotic abundance can strongly increase (Huettel et al. 2006). The finding that this effect can rapidly reverse (Huettel et al. 2006) further suggests some coral control of mucus prokaryotes. Our experimental data suggest that corals also exerted control over viral abundance in the mucus. This suggests that corals have mechanisms to control prokaryotic and viral abundances in the mucus of *L. pertusa*, indicating that the microbial landscape of the mucus is not only adaptable in terms of diversity (e.g. Ritchie 2006) but also in a dynamic equilibrium in terms of biomass. Such a control of microbial biomass at the microhabitat and ecosystem level could contribute to the functioning of CWCs and the reef environment.

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