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FEATURE ARTICLE

Bacterial and viral dynamics during a mass coral spawning period on the Great Barrier Reef

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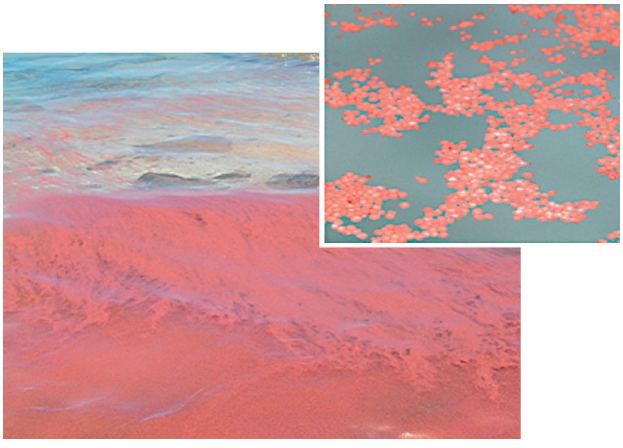
ABSTRACT: Bacterial and virus-like particle (VLP) abundances and physical and chemical parameters were measured in reef water and sediments over a 10 d period, coinciding with mass coral spawning at Heron Island, Great Barrier Reef. Bacterial abundances in reef water increased 2-fold after spawning and remained elevated for 3 d, before declining to below pre-spawning values. Reef water VLP abundances were also elevated 2 d after spawning; however, VLP abundances exhibited a general decline over the study. Dissolved oxygen (DO) and total nitrogen (TN) concentrations appeared to be dominant factors driving reef water bacterial and VLP dynamics. Sediment bacterial and VLP abundances exceeded those in the water column by up to 3 orders of magnitude and exhibited strong positive correlations for all investigated sediment depths. While short-lived peaks in bacterial and VLP abundances within sediments lagged behind water column trends by 2 d, reef water total phosphorus (TP) concentrations were strongly correlated with sediment bacterial and VLP abundances. Shifts in bacterial and VLP abundances in reef water and sediments during the study corresponded with 2 distinct periods: one prior to, and one after the first night of intense spawning. Scavenging by sedimenting coral spawn material is proposed as a direct mechanism contributing to these shifts, by removing bacteria and VLPs from the water column. The input of organic matter and associated nutrients from mass coral spawning, and the immediate and strongly correlated responses of bacteria and VLPs, indicate that viruses are important players in nutrient cycling processes in coral reefs.

KEY WORDS: Virus · Bacteria · Coral reef · Spawning · Sediment



Coral spawn slick washing ashore and (inset) released coral-sperm bundles floating on the seawater surface at Heron Island in 2005. *Photo:* Christiana Damiano

INTRODUCTION

On the Great Barrier Reef (GBR), mass coral spawning is a well-known phenomenon, where over 130 coral species as well as other invertebrates participate in multi-specific synchronous spawning events that occur over a few nights following the full moon, predominantly in November or December (Harrison et al. 1984). Enhanced fertilisation success and predator satiation may result from the mass release of coral gametes (eggs and sperm collectively) (Harrison & Wallace 1990), and degrading gametes represent a significant episodic input of energy rich material to

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the reef system (Wild et al. 2008). A 2.5-fold increase in sedimentary oxygen consumption (Wild et al. 2004a) and rapid oxygen depletion of the water column (Simpson et al. 1993) have been documented in the immediate days following coral spawning. Furthermore, the release of large quantities of particulate organic matter (POM) in the form of gamete material (Wild et al. 2008) can fuel pelagic and benthic autotrophic and heterotrophic activities, resulting in enhanced gross benthic and pelagic primary productivity (Glud et al. 2008), large changes in benthic nitrogen cycling (e.g. enhanced denitrification) and small changes in benthic phosphorus cycling (Eyre et al. 2008). While these studies imply stimulation of microbial processes within reef waters and sediments resulting from the input of a highly labile carbon source (and the associated N and P), there are no studies directly investigating microbial dynamics during a mass coral spawning event.

Bacteria play fundamental roles in the recycling and channelling of dissolved organic matter (DOM) through the microbial loop and to higher trophic levels. In coral reef waters and sediments, bacteria constitute a significant proportion of the microbial biomass and display fluctuations in cell numbers and productivity relative to the availability of organic substrates within the reef system (Moriarty et al. 1985, Hansen et al. 1992, Wild et al. 2004b). Nutrient enrichment studies in coral reefs and other oligotrophic systems have shown significant shifts in bacterial abundance and diversity (Lebaron et al. 1999, Hewson et al. 2007). There is also evidence to suggest that viral production in oligotrophic systems may be nutrient limited (Hewson et al. 2003). If this is also the case in coral reef systems, the input of energy rich coral gamete material may not only influence bacterial dynamics but also the viruses that infect them.

Surprisingly, the roles of viruses in coral reef systems remain relatively unexplored. Virus concentrations in coral reef waters average 10^6 ml^{-1} seawater and exceed bacterial abundances 10-fold (Paul et al. 1993, Seymour et al. 2005, Patten et al. 2006). Important roles for viruses have been proposed in other oligotrophic systems, whereby viral lysis and subsequent uptake of lysis products by non-infected bacteria may limit the flow of nutrients to higher trophic levels (Hewson et al. 2003). Viruses have also been identified as a nutrient-flow pathway in coral reef food webs through sponge predation (Hadas & Marie 2006). In the only known study to date investigating viruses in coral reef carbonate sediments, benthic viral abundances exceeded water column viruses by 2 orders of magnitude (Paul et al. 1993). As benthic bacterial abundances were not determined in that study, relationships between bacteria and viruses are not known. Other sediment types support abundant and dynamic viral populations, which are often positively correlated with bacterial abundance and activity, and benthic viruses are likely to contribute to biogeochemical processes in these sediments (Middelboe et al. 2006). In coral reef microniches, including the sediment–water interface, positive correlations between bacteria and viruses occur, suggesting that bacteria are dominant hosts of viruses in these systems (Seymour et al. 2005). The input of DOM by viral lysis potentially stimulates microbial carbon and nutrient turnover in both pelagic (Riemann & Middelboe 2002) and benthic environments (Siem-Jørgensen et al. in press). Infection and subsequent lysis of prokaryotes and eukaryotes could therefore represent an additional yet overlooked nutrient source contributing to high gross productivity in coral reef systems.

The mass coral spawning phenomenon on the GBR provides a unique opportunity to examine responses of bacteria and viruses to a natural nutrient pulse. The aims of the present study were to document bacterial and virus-like particle (VLP) abundances within reef waters and sediments before, during and after a mass coral spawning event on the GBR, and to determine the dominant physical and chemical parameters influencing bacteria and VLPs during these periods. These results are, to our knowledge, the first quantification of bacterial and VLP abundances simultaneously from reef waters and carbonate coral reef sediments, and provide evidence for dynamic bacterial and viral populations in response to episodic mass coral spawning events.

MATERIALS AND METHODS

Study site. Sampling was conducted at Heron Island reef flat, Southern GBR (23° 27′ S, 151° 55′ E), Australia over 10 d (consecutively; 18 to 27 November) in 2005 to correspond with the predicted timing of mass coral spawning on the GBR (Harrison et al. 1984). Additional complementary experimental work was also completed in 2006 during the mass coral spawning period. Heron Island reef flat covers an area of approximately 26 km^2 and is characterised by coral colonies interspersed with carbonate reef sediments (Fig. 1). The study site was located on the reef flat approximately 150 m from the shore line and 100 m from the reef crest and consisted predominantly of carbonate sediments (Fig. 1). Water depth at the site followed the tidal cycle and ranged between 0.2 and 2.0 m, with many of the corals exposed to air during low tides. Sediment porosity was 0.57 ± 0.01 (v/v, mean ± SE) at the surface and declined gradually to 0.52 ± 0.01 (v/v, mean ± SE) at 10 cm depth (Glud et al. 2008). Sediment permeability

Fig. 1. Heron Island with the surrounding reef (photo by Christian Wild). (*) Location where water and sediment samples were collected

was measured at the beginning of the study, equalling $6.0 \pm 0.25 \times 10^{-11}$ m^{-2} (mean ± SE) and $1.6 \pm 0.19 \times 10^{-11}$ m^{-2} (mean ± SE) for 2 depth intervals, 0 to 0.5 cm and 5 to 10 cm respectively (Glud et al. 2008)

Water column sampling. Physical and chemical parameters (temperature, salinity, pH, water depth, turbidity and dissolved oxygen, DO, concentration) were recorded at the site immediately prior to each sample collection using a calibrated HydroLab q-10™ multiprobe. Seawater was collected in the middle of the water column, using 2 pre-rinsed (3 times) 2 l acid-washed plastic containers in seawater at 05:00 h (dawn), 13:00 h (mid-day) and 17:00 h (dusk) daily. Methods for nutrient analysis follow (Eyre 2000) and are presented in detail in (Eyre et al. 2008). Chlorophyll *a* concentrations were determined fluorometrically (Strickland & Parsons 1972). Duplicate 1 ml subsamples were collected at each sample time for the determination of bacterial and VLP abundances. Subsamples were fixed with glutaraldehyde (0.5% final concentration) for 15 min in the dark at 4°C, frozen in liquid nitrogen and stored at −80°C (Marie et al. 1999).

Benthic sampling. The vertical distribution of bacteria and VLPs within carbonate reef sediments were determined from 3 individual sediment cores (core inner diameter, 5 cm; penetration depth, 10 to 12 cm) spaced 1 m apart and sectioned at 0–0.5, 0.5–1, 1–2, 2–3, 3–4, 4–5, 5–6 and 6–8 cm depths. Sediments from each section were pooled and homogenised, and sterile 0.025 µm filtered seawater (4.5 ml) and glutaraldehyde (2% final concentration) were added to 10 ± 0.05 g sediment and samples stored at 4°C for a maximum of 10 h prior to extraction. To determine any changes in bacterial and VLP abundances in response to the coral spawning event, cores were sampled at 05:00 h daily for the duration of the study. Three sediment cores were sampled as above and each core sectioned for 3 depths: 0–0.5, 3–4 and 6–8 cm. Sediments from each section were pooled, homogenised and fixed as above. Pooling sediments from the 3 cores was done in order to keep the number of samples and analysis at a manageable level. To evaluate the natural spatial variability, on 1 occasion 5 cores spaced 1 m apart were sampled, sectioned and fixed separately (not pooled) as above, and bacteria and VLPs were extracted (see next section). We compared the spatial variance (spatial σ^2) (n = 5) with the total variance (total σ^2), which was calculated from average bacterial and VLP abundances determined from the 3 depths over the study duration (n = 10). Spatial σ^2 represented 22 and 66% of the total σ^2 for bacterial and VLP abundances respectively.

Extraction of bacteria and VLPs from sediment. Bacteria and VLPs were extracted from sediment samples (10 g). Fixed sediment samples were treated with $Na_4P_2O_7$ (sodium pyrophosphate; 10 mM final concentration) for 15 min at 4°C. Samples were sonicated in a sonicator bath (2 cycles for 1 min, samples shaken manually for 10 s between cycles) and centrifuged ($500 \times g$, 3 min, 20°C). The supernatent was transferred to new 50 ml tubes and 4.5 ml seawater (0.025 µm) was added back to the centrifuge tubes containing the original sediment. The sonication–centrifugation step was repeated 3 times. The resulting combined supernatent was vortexed and sub-samples (2×1 ml) were frozen in liquid nitrogen and stored at −80°C until analysis.

Once, we also performed extractions with 1 and 10 g sediment samples because smaller sediment samples have been shown to lead to higher extraction efficiencies (Siem-Jørgensen et al. in press). For 1 g samples, methods for extraction were modified from (Fischer et al. 2005). Samples were treated with sodium pyrophosphate (10 mM final concentration) for 15 min at 4°C. Samples were sonicated using a sonicator probe (50 W, 3 cycles for 20 s on ice, samples shaken manually for 10 s between cycles) and 4.5 ml of 0.025 µm filtered seawater was added. Samples were vortexed and allowed to settle for 30 s, and duplicate 1 ml supernatent sub-samples were frozen in liquid nitrogen and stored at −80°C until analysis. In accordance with Siem-Jørgensen et al. (in press), we observed an increased extraction efficiency using smaller sediment samples. On average, the efficiency for bacteria and VLP increased by a factor of 1.9 ± 0.09 and 3.4 ± 0.12 (mean ± SE), respectively, by reducing the sediment sample from 10 to 1 g. This has been corrected for in the results.

Influence of gamete-enriched sediment on bacteria and VLP abundances. In 2006, surface sediments (0 to 1 cm) were collected the day prior to the first night of coral spawning from the reef flat. Sediments were

transferred into pre-rinsed 500 ml plastic jars and jars were stored at *in situ* seawater temperature in a flow-through outdoor aquarium. Spawned coral gametes were collected from the surface waters of individual aquaria which held individual coral colonies (*Acropora millepora*, *Platygyra daedalea* and *Favia* sp.), within 1 h after these corals had spawned (12 November 2006). Spawned gametes were combined and stored overnight in pre-rinsed plastic jars at *in situ* seawater temperature. Eggs (total volume ~15 ml) were transferred onto Whatman 47 mm GF filters to remove excess seawater, transferred to a 50 ml centrifuge tube and stored at –20°C for 24 h. Sediments (20 g) were added to each of 9 glass screw top vials (30 ml). Thawed egg sub-samples (1 ml) were added to 6 vials, while 3 vials served as controls. Three of the 6 treatment vials and all control vials were filled completely to the top with 0.025 µm filtered seawater and flushed with N_2 gas to ensure anaerobic conditions. The remaining 3 treatment vials were filled with 0.025 µm filtered seawater to 1 cm below the vial rim to allow oxygen exchange between water and air in the vials (aerobic conditions). Vials containing sediments and seawater were continuously mixed on a temperature controlled mixing table (24°C). Sediment slurry sub-samples (1 g) were removed from vials (ensuring predominantly sediment, and not seawater, was collected) at 0, 3, 6, 12, 24 and 36 h and fixed in glutaraldehyde (2% final concentration) with the addition of 4.5 ml 0.025 µm filtered seawater. Following removal of sediment slurry sub-samples, vials were filled with 0.025 µm filtered seawater as above, and anaerobically treated vials flushed with N_2 gas to maintain anaerobic conditions. Bacteria and VLPs were extracted according to the 1 g extraction method described above.

Flow cytometric analysis of bacteria and VLPs. Flow cytometry was used to enumerate bacteria and VLPs (Marie et al. 1999). All water and sediment samples were diluted in TE buffer (10 mM Tris, 1 mM EDTA, pH 7.5) (1:5 for seawater and 1:50 to 1:100 for sediment) and stained with SYBR Green I (5:100 000 dilution) (Molecular Probes). Stained samples were incubated in a water bath at 80°C in the dark for 10 min (Marie et al. 1999). Flow cytometric analysis was performed using a Becton-Dickinson FACSCanto flow cytometer, with phosphate buffered saline solution used as the sheath fluid. Fluorescent 1 µm diameter beads (Molecular Probes) were added to all samples as an internal reference (~10^5 beads ml^{-1}) and flow cytometric parameters were normalised to bead fluorescence and concentration. Acquisition was run for 2 min (400 to 800 events s^{-1}, ~40 μl^{-1} min^{-1}) and data were collected as list-mode files. Bacteria and VLPs were discriminated according to variations in green (SYBR I) fluorescence (indicative of nucleic acid content) and side scatter (indicative of cell size) using Win Midi 2.8© (Joseph Trotter) flow cytometry analysis software (Marie et al. 1999).

Statistical analyses. As the temporal distributions for bacteria and VLPs were not normal, and due the low number of measurements, non-parametric tests were employed to investigate trends and relationships between parameters. Kruskal-Wallis tests and post-hoc multiple comparisons were used to determine whether bacteria and VLP abundances in the water column and sediments differed significantly between days. Differences were considered significant when $p < 0.05$. To detect any local changes in bacterial and VLP abundances, the cumulative sums method was employed (Ibañez et al. 1993). For this calculation, the mean value taken over the series was subtracted from the mean value for each day, and the residuals were successively added to form a cumulative function. Successive positive residuals produce an increasing slope; successive negative residuals produce a decreasing slope, and values not very different from the mean show a slope close to zero. Correlations between bacteria and VLPs in water and sediments were determined using Kendall's coefficient of rank correlation, τ (Kendall & Stuart 1966). To assess which physical–chemical parameters could best explain variations in bacterial and VLP abundances within the water column and sediments, the BIOENV procedure was applied (Clarke & Ainsworth 1993). Bray-Curtis similarity matrices were constructed for the biological and physical–chemical data and compared by calculating Kendall's τ. Kendall's τ was used in preference to Spearman's coefficient of correlation, ρ, because disagreements in ranks are weighted equally, while Spearman's ρ gives greater weight to pairs of ranks that are further apart. All statistical analyses were performed using SPSS (version 14) apart from the BIOENV procedure, which was performed using Primer 5 (version 5.2.9).

RESULTS

Mass coral spawning observations

In November 2005, coral spawning occurred over 4 consecutive nights (20 to 23). The major nights of coral spawning occurred on 21 and 22 November as determined from observations of spawning corals on the reef flat and in aquaria. After the first night of major coral spawning, eggs were observed in surface waters. Following the second night of major spawning, spawn slicks accumulated on the water surface. In the 2 d following these mass coral spawning events, sinking of degrading gamete material through the water column

Table 1. Minimum, maximum and mean values for physical–chemical parameters in reef water at Heron Island, Great Barrier Reef, over the period 18 to 27 November 2005 (summarised from Eyre et al. 2008, Glud et al. 2008)

Parameter	Min.–Max.	Mean
Depth (m)	0.2–2.1	1.0
Temperature (°C)	22.4–31.1	26.4
Dissolved O_2 (µmol l^{-1})	2.1 –13.10	7.42
Salinity (ppt)	37.3–37.8	37.4
pH	7.9–8.3	8.1
Chl *a* (µg l^{-1})	0.1–1.4	0.5
Total N (µmol l^{-1})	6.0–18.5	12.1
Total P (µmol l^{-1})	1.2–1.8	1.34

turned reef water turbid, concomitant with the formation of a massive benthic bloom dominated by dinoflagellates *Prorocentrum* sp. (Glud et al. 2008). While not determined during this coral spawning period, it has been estimated that 310 t C and 18 t N are released as eggs during coral spawning at Heron Island reef (Wild et al. 2004a).

Physical–chemical parameters

A summary of physical-chemical parameters is provided in Table 1 (summarised from Eyre et al. 2008, Glud et al. 2008). Water temperature and chlorophyll *a* concentrations increased over the duration of the study. DO exhibited the largest variations on 23 November following the 2 nights of major coral spawning (Glud et al. 2008).

Flow cytometric discrimination of bacteria and VLPs within reef water and sediment

Based on characteristics of side scatter, SYBR green fluorescence, red and orange fluorescence and comparisons with other studies in oligotrophic environments (Marie et al. 1999, Seymour et al. 2005) the flow-cytometrically defined bacterial community (Fig. 2) was composed predominantly of heterotrophic bacteria. VLPs were distinguished from bacteria by lower side scatter and SYBR green signals (Fig. 2). As discrete sub-populations could only be discerned in a few instances, only total abundances for bacteria and VLPs are reported.

Bacteria and VLPs in reef water

Bacterial abundance in reef water exhibited a gradual decline from 0.86×10^6 ml^{-1} at the start of the study to 0.45×10^6 ml^{-1} on 22 November (Fig. 3A). A 2.0-fold significant increase ($p < 0.05$) and a subsequent plateau in bacterial abundance occurred from 22 to 25 November. A significant 2.4-fold decline in bacterial abundance followed from 24 November to the end of the study ($p < 0.05$). Cumulative sums plots for bacterial abundance confirmed this trend, whereby successive values exhibited a positive increase from 22 to 25 November, indicating that these values were higher than the series mean (Fig. 4A). Following nights of major spawning, VLP abundances were significantly lower than pre-spawning values (all $p < 0.05$). VLP abundance in reef water expressed a significant decline over the course of the study from 3.27×10^6 ml^{-1} to 1.55×10^6 ml^{-1} ($\tau = -0.69$, $p < 0.01$, $n = 10$) (Fig. 3A). However, a slight but non-significant increase in VLP abundance occurred on 24 November ($p < 0.05$) and lagged (by 1 d) behind increased bacterial abundances. Cumulative sum plots for VLP abundance showed 2 distinct periods over the course of the study

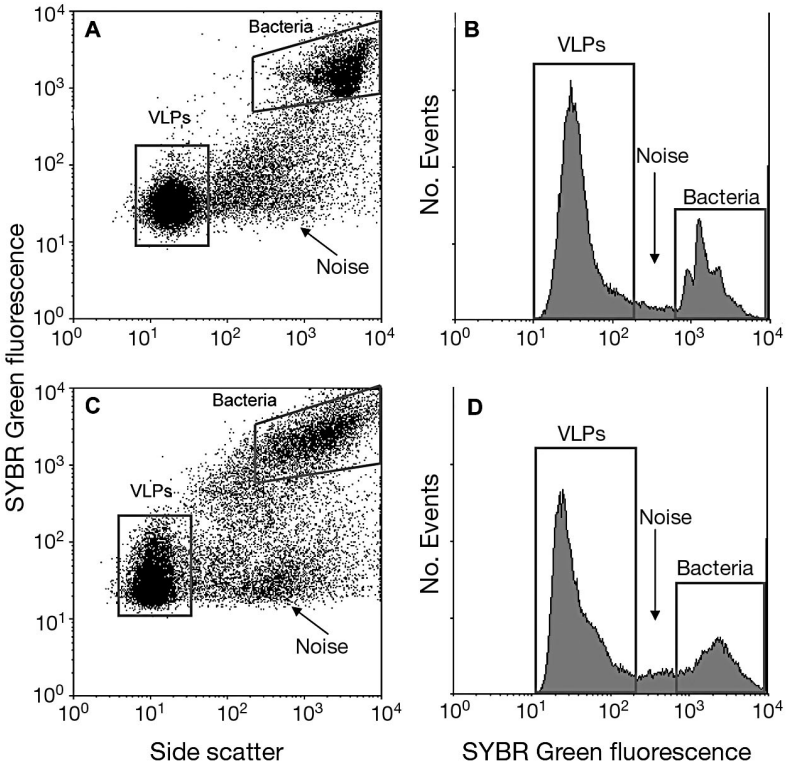

Fig. 2. Characteristic cytograms from flow cytometric analysis showing bacteria and virus-like particles (VLPs). (A,C) Dot plots of side scatter vs. SYBR Green I for (A) reef water sampled on 23 November 2005 and (C) sediment sampled on 27 November 2005. (B,D) Frequency distributions of SYBR Green I fluorescence for (B) the reef water sample and (D) the sediment sample

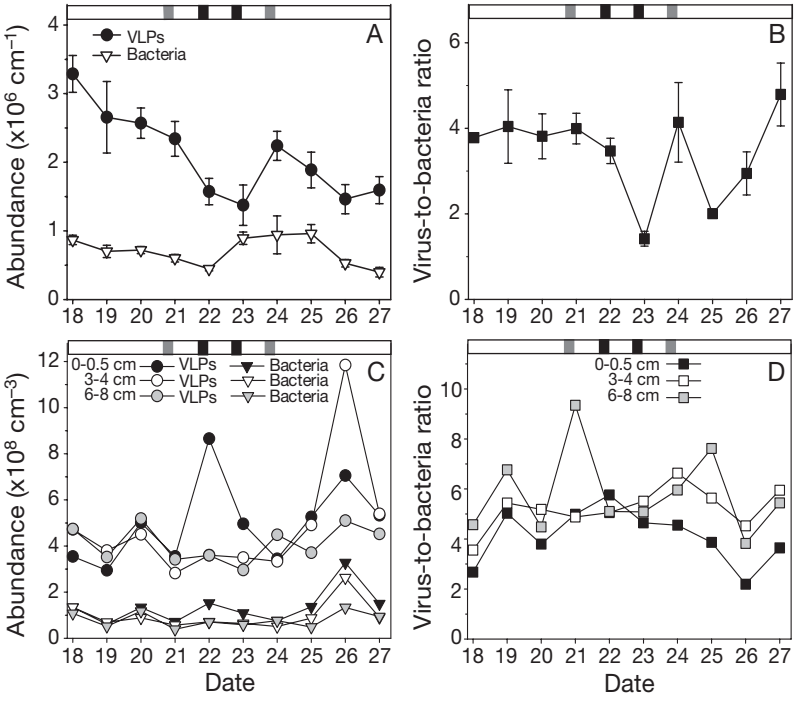

Fig. 3. Temporal abundances of bacteria and virus-like particles (VLPs) and virus-to-bacteria ratios (VBR) in reef water and sediments in November 2005. (A) Abundances of bacteria and VLPs in reef water. (B) VBR in reef water. Error bars are ±SE (n = 6). (C) Abundances of bacteria and VLPs in 0–0.5, 3–4 and 6–8 cm sediment depth horizons. (D) VBR in 0–0.5 cm, 3–4 cm and 6–8 cm sediment depth horizons. Grey/black bars above the graphs represent nights where minor/major spawning occurred

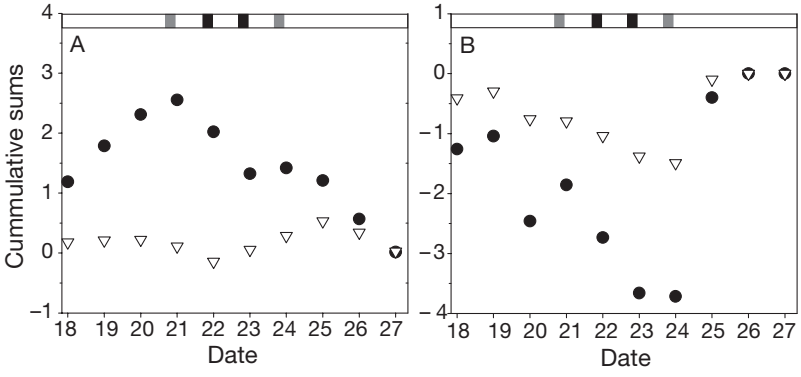

Fig. 4. Cumulative sums plots for abundances of bacteria (▽) and virus-like particles (●) in (A) reef water and (B) sediments in November 2005. Grey/black bars above the graphs represent nights where minor/major spawning occurred

(Fig. 4A). The first period (18 to 21 November) was characterised by values above the mean and the second period (22 to 27 November) showed values close to or below the series mean. Values for virus-to-bacteria ratios (VBR), employed here to document relative shifts occurring between the viral and bacterial communities (e.g. Wommack & Colwell 2000), ranged from 1.4 to 4.8 in reef water, with lowest VBR occurring in the days following the nights of intense coral spawning (Fig. 3B).

Reef water bacteria and VLP abundances were not correlated over the duration of the study, even when shifting the respective temporal dynamic forward or backward (all $p > 0.05$). However, from 18 to 22 November a significant positive correlation between bacteria and VLPs occurred ($\tau = 0.80$, $p < 0.05$, $n = 5$), further indicating shifts in bacterial and VLP dynamics following the spawning period. The BIOENV analysis showed that the highest correlation between bacteria and VLPs in reef water occurred for a combination of 2 or 3 environmental parameters (Table 2). Bacterial abundances were linked to changes in chlorophyll *a* and total phosphorus (TP) concentrations, while VLP abundances were best explained by the depth of the water column and total nitrogen (TN) concentrations. However, complex interactions were further revealed when reef water bacteria and VLPs were combined, with DO and TN appearing to be dominant factors driving reef water bacterial and VLP dynamics.

Bacteria and VLPs in reef sediment

The vertical microscale distribution of bacteria and VLPs within sediment varied by 1.5- and 2.1-fold, respectively; however, there were no clear trends in bacterial or VLP abundances with depth (data not shown). In general, all 3 sediment horizons remained at the same level during the entire study period (Fig. 3C). However, on 2 occasions, short-lived peaks in bacterial and VLP abundances followed soon after nights of most intense coral spawning (Fig. 3C). These peaks exceeded reef water bacterial and VLP abundances by more than 3 orders of magnitude. For example, on 22 November, VLP abundance in surface sediments exceeded pre-spawning values by up to 2.4-fold (Fig. 3C). Similarly, bacterial and VLP abundances exceeded pre-spawning background levels by up to 4.6- and 4.2-fold, respectively, in the 3 to 4 cm depth horizon on 26 November (Fig. 3C). These elevated bacterial and VLP abundances lagged 2 to 3 d behind maximum deposition rates of matter (Wild et al. 2008).

Table 2. Results from BIOENV analysis of bacterial and virus-like particle (VLP) abundances and physical–chemical parameters at different grouping levels. Kendall's coefficient (τ) is given for the best single or combination of physical–chemical parameter(s), marked by ×. DO = dissolved oxygen, Chl *a* = chlorophyll *a*, TN = total nitrogen, TP = total phosphorous, *T* = temperature (°C), Depth = water column depth (m)

Grouping	τ	DO	Chl *a*	TN	TP	*T*	Depth
Bacteria & VLPs in water & sediments	0.52	×		×	×		
Bacteria & VLPs in seawater	0.47	×		×			
Bacteria & VLPs in sediments	0.39				×		
VLPs in water	0.47			×			×
VLPs in sediments	0.42				×		
Bacteria in water	0.32		×		×		
Bacteria in sediments	0.31				×		

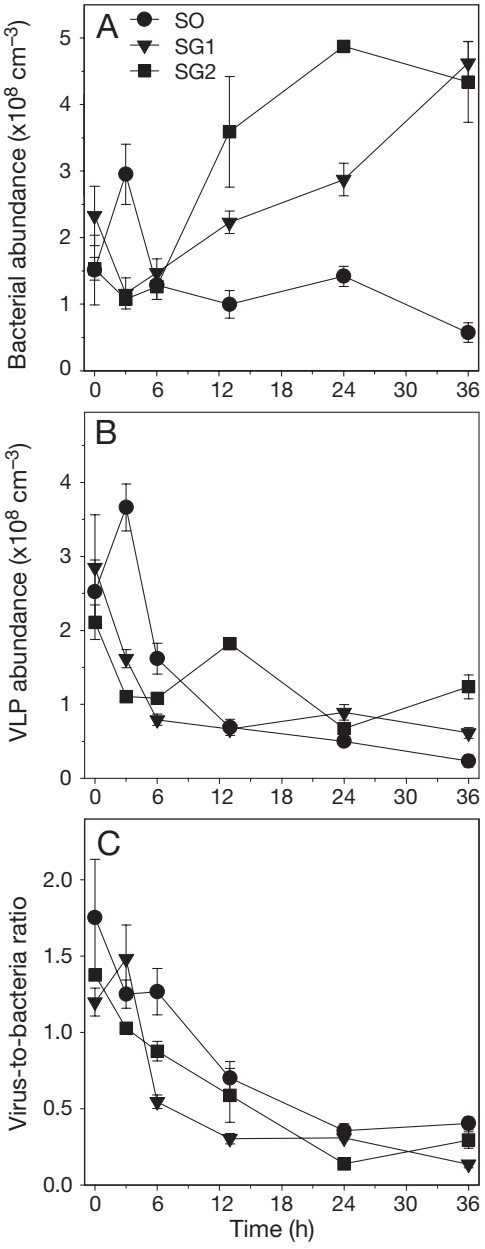

Fig. 5. Changes in abundances of (A) bacteria and (B) virus-like particles (VLPs), and (C) virus-to-bacteria ratios in sediments during the gamete addition experiments. SO = sediment only (controls), SG 1 = gamete addition to sediments in anaerobic conditions, SG 2 = gamete addition to sediments in aerobic conditions. Error bars are ±SE (n = 3)

Cumulative sums plots of sediment bacteria and VLP abundances further revealed distinct inflection points on 24 November, and cumulative values following 24 November were significantly higher than the overall series means (Fig. 4B). VBR in sediments ranged between 2.2 and 9.4 (Fig. 3D). No clear trends could be distinguished even when VBR were averaged over the 3 sediment depths.

Overall and for each investigated sediment depth, bacteria and VLP abundances exhibited highly significant positive correlations (all $\tau > 0.69$, all $p < 0.01$). However, it appears that surface sediments exhibited stronger bacterial and VLP dynamics than deeper layers (Fig. 3C). The BIOENV analysis showed that the highest correlation occurred when a single environmental factor, TP, was correlated with sediment bacteria and VLP abundance data (Table 2).

Combined reef water and benthic trends

Neither bacteria nor VLPs within sediments were significantly correlated with bacteria or VLPs in the water column (all $p > 0.05$). The BIOENV analysis revealed that for all water and sediment parameters measured, the highest correlation occurred with a combination of DO, TN and TP ($\tau = 0.52$, $n = 10$) (Table 2). The second best correlation was similar ($\tau = 0.50$, $n = 10$) and was achieved when depth, DO and TP were combined.

Response of bacteria and VLPs to gamete enrichment

Bacterial abundances increased approximately 4-fold with the addition of gamete material, while the bacterial abundance decreased 2-fold in the controls (Fig. 5A). In contrast, VLP abundances in sediment slurries decreased 3- to 4-fold over 36 h with the addition of gamete material and up to 10-fold in controls (Fig. 5B). These responses were reflected in VBR changes, with approximately 11-fold declines in VBR in gamete-enriched sediments and a 4-fold decline in VBR in control sediments over 24 to 36 h (Fig. 5C).

DISCUSSION

Coral spawning-induced shifts in bacteria and VLP populations

Nutrient availability and viral infection have been hypothesised as 2 major factors shaping pelagic and benthic bacterial populations (Hewson et al. 2001a, Hewson et al. 2003, Schwalbach et al. 2004). Previous studies addressing these hypotheses have involved nutrient enrichment experiments and manipulation of VLP concentrations in confined settings (Hewson et al. 2001b, 2003, Schwalbach et al. 2004). As such, it has been difficult to extrapolate these results to natural environments. The mass coral spawning event on the GBR provides for a natural nutrient enrichment experiment in an otherwise oligotrophic system (Eyre et al. 2008). The input of highly labile organic matter induced significant shifts in bacterial and VLP abundances within reef water and sediments. In the following discussion, we describe the sequence of events and potential processes that directly and indirectly affected bacterial and VLP abundances and dynamics at Heron Island.

Flow cytometric enumeration of bacteria and VLPs

To our knowledge, the present study is the first to use flow cytometry as a tool to enumerate bacteria and VLPs within carbonate reef sediments, and only the second to use the technique for sediments in general (see Duhamel & Jacquet 2006). Here, flow-cytometrically enumerated water column and benthic bacteria and VLPs fell within reported ranges for other studies in oligotrophic environments (Table 3). Low VBR occurred within carbonate sediments, but values were similar to VBR from both shallow and deep sea sediments (Hewson et al. 2001a, 2003, Mei & Danovaro 2004).

Water column

Highest VLP abundances occurred within the first few days of the study and coincided with shallowest water depths at the site (<0.5 m). This may be explained by accumulation of VLPs directly through *in situ* infection of planktonic microorganisms and/or resuspension of benthic VLPs with changing tidal regimes. Alternatively,

Table 3. Comparison of virus-like particle (VLP) abundances and virus-to-bacteria ratios (VBR) within seawater ($\times 10^6$ ml^{-1}) and sediments ($\times 10^8$ cm^{-3}) from different oligotrophic and eutrophic marine environments. FCM = flow cytometry; TEM = transmission electron microscopy; ND = not determined

Environment and location	VLP abundance	VBR	Method	Source
Seawater				
Oligotrophic				
Heron Island Reef (Great Barrier Reef)	1–5	1–7	FCM[a]	Present study
Myrmidon Reef (Great Barrier Reef)	14	3	FCM[a]	Patten et al. (2006)
Magnetic Island (Great Barrier Reef)	6	5	FCM[a]	Seymour et al. (2005)
Florida Bay (USA)	1–5	ND	TEM	Paul et al. (1993)
Coral Sea (Australia)	1–5	12	EFM[a]	Hewson et al. (2001a)
Eutrophic				
Brisbane River (river mouth) (Australia)	<1	10	EFM[a]	Hewson et al. (2001a)
Los Angeles Harbour (USA)	80	39	EFM[a]	Hewson & Fuhrman (2003)
Adriatic Sea (coastal) (Mediterranean)	<1	3	EFM[a]	Corinaldesi et al. (2003)
Marine sediments				
Oligotrophic				
Heron Reef (<2 m) (Great Barrier Reef)	3–12	2–9	FCM[a]	Present study
Noosa River (river mouth) (<2 m) (Australia)	2–5	3–6	EFM[a]	Hewson et al. (2001a)
Adriatic Sea (Palombina) (<50 m)	2	<1	EFM[a]	Mei & Danovaro (2004)
Florida Keys (<10) (USA)	5	ND	TEM	Paul et al. (1993)
Big Fisherman's Cove (<1 m) (USA)	2	98	EFM[a]	Hewson & Fuhrman (2003)
San Pedro Channel (~900 m)(USA)	2	11	EFM[a]	Hewson & Fuhrman (2003)
Eutrophic				
Brisbane River (river mouth) (Australia) (<2 m)	20–50	35–65	EFM[a]	Hewson et al. (2001a)
Port of Ancona (~8 m) (Italy)	25	<1	EFM[a]	Mei & Danovaro (2004)
Gulf of Thermaikos (~50 m) (Italy)	6	<1	EFM[a]	Mei & Danavoro (2004)
Los Angeles Harbour (>20 m) (USA)	2	10	EFM[a]	Hewson & Fuhrman (2003)
Sagami Bay (~1400 m) ((Japan)	1–23	5–35	EFM[a,b]	Middelboe et al. (2006)
Sagami Bay (cold seep station) (1200 m) (Japan)	≤1–9	<1–8	EFM[a,b]	Middelboe et al. (2006)

[a]SYBR Green I
[b]SYBR Green II

detachment of reef and coral surface-associated VLPs (Davy & Patten 2007) when water is advected through the reef framework may have indirectly contributed to increased water column viral loads.

In contrast, bacterial abundances appeared to be less affected by tidal flushing and more responsive to the input of labile organic matter from the spawning event. This was evidenced by bacterial abundances increasing 2.1-fold 3 d following the first night of major spawning and that they remained elevated for 3 d. These elevated bacterial abundances coincided with a planktonic algal bloom and with elevated water column chlorophyll *a* concentration. As such, the link between enhanced bacterial abundances and enhanced phototrophic activity can be best explained by microbial exploitation of phytoplankton extracellular release rather that direct prokaryotic mineralization of the spawning material (Glud et al. 2008).

The rapid decrease in both bacterial and VLP abundances from 24 to 27 November coincided with calmest weather conditions, low tidal exchanges and maximum deposition rates of particulate matter (Wild et al. 2008). We hypothesise that scavenging by sinking sedimenting spawning material is a dominant mechanism removing free bacteria and viral particles from the water column following spawning. In aquatic environments, there is limited information about the extent to which VLPs associate with organic aggregates. However, a significant fraction of water column VLPs may attach to organic aggregates over a continuum of size scales from transparent exopolymeric particles (~1 to 100 µm) to marine snow (~300 to >1000 mm), and these aggregates may represent hotspots of viral activity (Proctor & Fuhrman 1991). Within coral reef waters, up to 50% of bacteria have been observed attached to organic matter >3 µm (Moriarty 1979), and 30% of total bacterial productivity may occur on these particles (Moriarty et al. 1985). The 3- to 11-fold increase in POM in the shallow water column (Wild et al. 2008), and the highly charged colloidal properties of VLPs, and to a lesser extent bacteria, which readily adsorb to particulate matter (Bitton & Mitchell 1974), are consistent with a considerable loss of free-living bacteria and VLPs to the post-spawning POM pool. Direct feeding on, or filtering of, bacteria and VLPs by coral reef organisms (Pile et al. 2003, Hadas & Marie 2006) and grazing by flagellates (Gonzalez & Suttle 1993, Caron 2000) may also have led to the loss of microbes from the water column. Indirect removal of gamete-attached bacteria, and VLPs via intense fish grazing (Pratchett et al. 2001) may have further contributed to the observed loss.

Whether degrading coral spawn material would provide a favourable micro-niche for viral infection remains to be determined. The enzymatic activity within degrading material may facilitate the breakdown of viral capsids or provide a micro-niche for enhanced host-virus contact and/or enhanced viral survivorship (Proctor & Fuhrman 1991). In coral reef systems, viral activity on aggregates may link the free-living and particle-associated bacteria because cell debris from viral lysis acts as a binding agent further enhancing aggregate formation (Proctor & Fuhrman 1991). Alternatively, it may lead to the dissolution of aggregates, essentially resulting in the conversion of POM into DOM within the water column (Mari et al. 2007). The time taken for spawning material to reach carbonate reef sediments is short when compared to particle flux in oceanic environments (i.e. days compared to months). While this would require further investigations, both viral lysis and protozoan grazing might occur on degrading coral spawn material and contribute to microbial loop processes within coral reef ecosystems.

Carbonate reef sediments

High sediment permeability permits rapid incorporation of organic material into deeper sediments through current and wave driven advection (Wild et al. 2004b). Compared to the water column, where any dramatic shifts in bacterial and VLP dynamics were presumably masked by tidal and pelagic–benthic exchange, 2 distinct spawning-associated bacterial and VLP peaks occurred in sediments, which exceeded those in the water column by 3 orders of magnitude. Two processes are hypothesised to account for these increased sediment bacterial and VLP loads. The first one involves the direct importation of coral spawn material and associated particle-attached bacteria and VLPs, to the sediment floor. Spawned material reaches the sediment floor via direct sedimentation, indirectly through fish and zooplankton faecal deposition, and through the release of phyto-detritus following nutrient stimulated phototrophic activity (Eyre et al. 2008, Glud et al. 2008, Wild et al. 2008). The 2.4- and 1.5-fold decline in water column bacteria and VLPs, respectively, between 24 and 27 November, co-occurred with a 4-fold increase in POM in sediments traps (Wild et al. 2008). This sedimentation process may then dominate over the tidal flushing mechanism proposed to influence water column VLPs pre-spawning, because degrading spawned material sinks rapidly through the shallow water column (Wild et al. 2004a). Indeed, sediments close to shore were covered with coral spawn material following nights of intense spawning. The second process is likely to involve percolation of coral spawn through permeable carbonate sediments (Wild et al. 2004a) and subsequent decomposition by benthic microbial mineralisation. This is consistent with the observed enhanced bacterial and

VLP abundances within the upper 4 cm in the days post major coral spawning.

While the gamete addition experiment supported observed *in situ* stimulation of bacteria by highly labile coral spawn material, VLPs did not show a similar response (Fig. 5). Different responses of VLPs between the *in situ* and gamete addition experiment may be due to enhanced degradation of VLPs by enzymatic activity (Proctor & Fuhrman 1991), or proliferation of phage-resistant bacteria following enrichment (Middelboe 2000). It is also possible that bottle experiments do not reflect *in situ* conditions (Ferguson et al. 1984). Slurry incubations are known to stimulate bacterial activity above that of non-manipulated sediments, and the effect of slurry conditions on viral production is not clear (Middelboe & Glud 2006). Such stimulated conditions may shift the balance of the system by favouring the growth of bacterial subpopulations, which are not exposed to a severe viral infection pressure initially, causing a decrease in the original viral assemblage due to a reduction in susceptible host cells. Over a slightly longer time scale, new viral populations would be expected to propagate and infect the developing bacterial assemblage, leading to increased viral abundances in the incubations. Further experiments involving whole core incubations are however needed to estimate bacterial and viral production in carbonate sediments.

Bacteria and VLPs in aquatic sediments are shown to be either strongly correlated (Middelboe et al. 2006) or show no relationship (Hewson et al. 2001a). In this study, strong positive correlations between sediment-associated bacteria and VLPs occurred over dynamic temporal shifts in bacterial and VLP abundances, suggesting that benthic bacteria are dominant hosts for viruses. Elevated bacterial and VLP abundances within carbonate sediments should promote high host–phage contact rates. In this way, viral-mediated conversion of POM (bacteria) to DOM through cell lysis, and subsequent liberation and utilisation of lysis products by non-infected prokaryotes and eukaryotes, could further contribute to the heightened phototrophic and heterotrophic activities within carbonate reef sediments post-spawning, and may have subsequently prolonged the water column response (Eyre et al. 2008, Glud et al. 2008, Wild et al. 2008).

Pelagic–benthic coupling and ecological implications of the coral spawning event on microbial loop processes

Shallow coral reef environments exhibit intense pelagic–benthic coupling, due to tidal movement and wave actions (Wild et al. 2004b), and processes occurring within the sediments and overlying water were not mutually exclusive during this study. On average, 150 and 250% of bacteria and VLPs, respectively, were lost from the water column following mass coral spawning. Assuming that the loss was due solely to adsorption onto coral spawn material, and that the carbon content of 1 bacteria and virus particle is 20 and 0.2 fg C, respectively (Wilhelm & Suttle 1999), then for a reef rim area of 26.4 km^2 with an average water depth of 1.5 m, reef water bacteria and VLPs would contribute to 1.4% of the estimated 11.7 g C m^{-2} released as coral eggs (Glud et al. 2008) during the spawning period at Heron Island. While it is likely that some proportion of this total carbon is advected offshore into inter-reef areas, there are still important implications for the removal of bacteria and VLPs from the water column, and the potential deposition of water column bacteria and VLPs into sediments. Firstly, the loss of bacteria and VLPs from the water column, in essence, leads to a reduction in the efficiency of pelagic microbial loop nutrient cycling processes. This in turn could then lead to increased transfer of carbon to higher pelagic trophic levels. The occurrence of a planktonic phytoplankton bloom 1 d prior to the dramatic decrease in bacteria and VLP abundances provides some evidence for this. Secondly, in addition to carbon, bacterial cells contain nitrogen, phosphorous and iron (Vrede et al. 2002), while VLPs can be rich in phosphorous (Maruyama et al. 1993). Deposition and subsequent degradation of bacteria and VLPs within sediments may then further contribute to the nutrient pool within sediments. If virus survivorship is enhanced within sinking coral spawn material and there is overlap in viral hosts between the water column and sediments, then viral mediated mortality of bacteria on particles and within sediments may further contribute to nutrient release, permitting enhanced water–benthic coupling. Large changes in dissolved organic nitrogen fluxes and small changes in other nitrogen and phosphorus fluxes occurred following coral spawning (Eyre et al. 2008); however, it is unknown how much of this change can be attributed to viral-mediated mortality of bacteria.

The complex biological and physical–chemical interactions observed in this study have hindered attempts to unravel the direct roles of viruses in this coral reef system. In future, a combination of bulk *in situ* observations with mesocosm experiments could better permit the elucidation for the roles of VLPs in coral reef systems and during mass coral spawning events. Nonetheless, the input of a large fraction of organic matter over a period of a few days (Wild et al. 2008), and the immediate and strongly correlated responses of bacteria and VLPs, indicate that viruses are potentially important agents contributing to nutri-

ent cycling in coral reefs. Similar yet unresolved roles for viruses are also envisaged when more variable sources of organic matter such as coral mucus (Wild et al. 2004b) and detritus (Hansen et al. 1992) are imported into the reef system.

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