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 ten day period, coinciding with mass coral spawning at Heron Island, Great Barrier Reef (GBR). Bacterial abundances in reef water increased 2-fold after spawning and remained elevated for 3 days, before declining to below pre-spawning values. Reef water VLP abundances were also elevated 2 days after spawning, however, VLP abundances exhibited a general declined 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 three orders of magnitude. In contrast to no relationship occurring between reef water bacteria and VLPs, bacteria and VLPs in sediments exhibited strong positive correlations for all investigated depths. While short-lived peaks in bacterial and VLP abundances within sediments lagged behind water column trends by 2 days, reef water total phosphorous (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 two 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 viruses are important players in nutrient cycling processes in coral reefs.

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 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. in press), large changes in benthic nitrogen cycling (e.g. enhanced denitrification) and small changes in benthic phosphorus cycling (Eyre et al. in press). 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 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 ten-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 (Middelboe et al. 2006). Subsequently, benthic viruses are likely to contribute to biogeochemical processes in these sediments. In coral reef micro-niches, including the sediment-water interface, positive correlations between bacteria and viruses occur, suggesting bacteria are dominant hosts of viruses in these systems (Seymour et al. 2005). The input of dissolved organic matter (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 (23º27'S, 151º55'E), Australia over 10 consecutive days (18^{th} – 27^{th} 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 was comprised predominantly of carbonate sediments (Fig. 1). Water depth at the site followed the tidal cycle and ranged between 0.2 m and 2.0 m, with many of the corals exposed to air during low tides. Sediment porosity was 0.57 ± 0.02 (v/v) at the surface and declined gradually to 0.52 ± 0.02 (v/v) at 10 cm depth (Glud et al. in press). Sediment permeability was measured at the beginning of the study, equalling $6.0 \pm 0.7 \times 10^{-11}$ m^{-2} and $1.6 \pm 0.6 \times 10^{-11}$ m^{-2} for two depth intervals, 0 – 0.5 cm and 5 – 10 cm respectively (Glud et al. in press)

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 two 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. in press). Chlorophyll *a* concentrations were determined fluorometrically (Strickland & Parsons 1972). Duplicate 1 ml sub-samples were collected at each sample time for the determination of bacterial and VLP

abundances. Sub-samples were fixed with glutaraldehyde (0.5% final concentration) for 15 minutes in the dark at 4ºC, frozen in liquid nitrogen and stored at 80ºC (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 – 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) added to 10 ± 0.05 g sediment and samples stored at 4ºC for a maximum of 10 hours 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 three 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 three cores was done in order to keep number of samples and analysis at a manageable level. To evaluate the natural spatial variability, on one occasion 5 cores spaced 1 m apart were sampled, sectioned and fixed separately (not pooled) as above and bacteria and VLPs were extracted (see below). 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 $Na_4P_20_7$ (sodium pyrophosphate; 10 mM final concentration) for 15 minutes at 4ºC. Samples were sonicated in a sonicator bath (2 cycles for 1 min, samples shaken

169 manually for 10 s between cycles), centrifuged (500 × *g*, 3 min, 20ºC), the supernatent
170 transferred to new 50 ml tubes and 4.5 ml seawater (0.025 µm) was added back to the centrifuge
171 tubes containing the original sediment. The sonication-centrifugation step was repeated three
172 times in total. The resulting combined supernatent was vortexed and sub-samples (2 × 1 ml)
173 were frozen in liquid nitrogen and stored at -80ºC until analysis.

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

186 **Influence of gamete enriched sediment on bacteria and VLP abundances**

187 In 2006, surface sediments (0 – 1 cm) were collected the day prior to the first night of
188 coral spawning from the reef flat. Sediments were transferred into pre-rinsed 500 ml plastic jars
189 and jars were stored at in situ seawater temperature in a flow-through outdoor aquarium.
190 Spawned coral gametes were collected from the surface waters of individual aquaria which held
191 individual coral colonies (*Acropora millepora*, *Platygyra daedalea* and *Favia* sp.), within 1 h of
192 these corals spawning (12th 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 -20ºC for 24 h. Sediments (20 g) were added to each of nine 30 ml glass screw top vials. Thawed egg sub-samples (1 ml) were added to 6 vials, while 3 vials served as controls. Three of the six 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 vials (aerobic conditions). Vials containing sediments and seawater were continuously mixed on a temperature controlled mixing table (24ºC). 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 [10mM Tris, 1mM EDTA, pH 7.5] (1:5 for seawater and 1:50 - 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 80ºC 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 – 800 events per second, ~ 40 ul^{-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 (after Nemenyi) 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

241 weighted equally while Spearman's ρ gives greater weight to pairs of ranks that are further apart.
242 All statistical analyses were performed using SPSS (version 14) apart from the BIOENV
243 procedure, which was performed using Primer 5 (version 5.2.9).
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RESULTS

Mass coral spawning observations

In November 2005, coral spawning occurred over 4 consecutive nights (20^{th} – 23^{rd}). The major nights of coral spawning occurred on the 21^{st} and 22^{nd} 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 days following these mass coral spawning events, sinking of degrading gamete material through the water column turned reef water turbid, concomitant with the formation of a massive benthic bloom dominated by dinoflagellates (*Prorocentrum* sp.) (Glud et al. in press). While not determined during this coral spawning period, it has previously 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 Glud et al. in press and Eyre et al. in press). Water temperature and chlorophyll *a* concentrations increased over the duration of the study. DO exhibited the largest variations on 23^{rd} November following the two nights of major coral spawning (Glud et al. in press).

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 was composed predominantly of heterotrophic bacteria (Fig. 2). 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.

289 **Bacteria and VLPs in reef water**

290 Bacterial abundance in reef water exhibited a gradual decline from 0.86×10^6 ml^{-1} at the
291 start of the study to 0.45×10^6 ml^{-1} on November 22nd (Fig. 3A). A 2.0-fold significant increase
292 and the subsequent plateau in bacterial abundance occurred from the 22nd to 25th November. A
293 significant 2.4-fold decline in bacterial abundance followed from the 25th November to the end
294 of the study. Cumulative sums plots for bacterial abundance confirmed this trend whereby
295 successive values exhibited a positive increase from the 22nd to 25th November, indicating these
296 values were higher than the series mean (Fig. 4A). Following nights of major spawning, VLP
297 abundances were significantly lower than pre-spawning values. VLP abundance in reef water
298 expressed a significant decline over the course of the study from 3.27×10^6 ml^{-1} to 1.55×10^6
299 ml^{-1} ($\tau = -0.69$, $p < 0.01$, $n = 10$; Fig. 3A). However, a slight but non-significant increase in VLP
300 abundance occurred on the 24th November and lagged one day behind increased bacterial
301 abundances. Cumulative sum plots for VLP abundance showed two distinct periods over the
302 course of the study (Fig. 4A). The first period (18th to 21st November) was characterised by
303 values above the mean and the second period (22nd and 27th November) showed values close to
304 or below the series mean. Values for virus-to-bacteria ratios (VBR), employed here to document
305 relative shifts occurring between the viral and bacterial communities (e.g. Wommack & Colwell
306 2000), ranged from 1.4 to 4.8 in reef water, with lowest VBR occurring in the days following the
307 nights of intense coral spawning (Fig. 3B).

308 Reef water bacteria and VLP abundances were not correlated over the duration of the
309 study, even when shifting the respective temporal dynamic forward or backward (all $p > 0.05$).
310 However, from the 18th to the 22nd a significant positive correlation between bacteria and VLPs
311 occurred ($\tau = 0.80$, $p < 0.05$, $n = 5$), further indicating shifts in bacterial and VLP dynamics
312 following the spawning period. The BIOENV analysis showed that the highest correlation

between bacteria and VLPs in reef water occurred for a combination of two or three environmental parameters (Table 2). Bacterial abundances were linked to changes in chlorophyll *a* and total phosphorous (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-fold and 2.1-fold respectively, however there were no clear trends in bacterial or VLP abundances with depth (data not shown). In general all three sediment horizons remained at the same level during the entire study period (Fig. 3C). However on two 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 the 22nd 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-fold and 4.2-fold respectively in the 3 – 4 cm depth horizon on the 26th November (Fig. 3C). These elevated bacterial and VLP abundances lagged 2 to 3 days behind maximum deposition rates of matter (Wild et al. 2008). Cumulative sums plots of sediment bacteria and VLP abundances further revealed distinct inflection points on the 24th November and cumulative values following 24th 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 three 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 appeared that surface sediments exhibited stronger bacterial and VLP dynamics than deeper layers (Fig. 3C). The BIOENV analysis showed the highest correlation occurred when one 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

Viral abundances in sediment-slurries decreased by 3 to 4-fold over 36 hours with the addition of gamete material and up to 10-fold in controls (Fig. 5A). In contrast, bacterial abundances increased approximately 4-fold with the addition of gamete material, while the bacterial abundance decreased 2-fold in the 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 – 36 hours (Fig. 5C).

DISCUSSION

Coral spawning-induced shifts in bacteria and VLP populations

Nutrient availability and viral infection have been hypothesised as two major factors shaping pelagic and benthic bacterial populations (Hewson et al. 2001a, Hewson et al. 2003, Schwalbach et al. 2004). To date, previous studies addressing these hypotheses have involved nutrient enrichment experiments and manipulation of VLP concentrations in confined settings (Hewson et al. 2001b, Hewson et al. 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 provided for a natural nutrient enrichment experiment in an otherwise oligotrophic system (Eyre et al. in press). The input of highly labile organic matter induced significant shifts in bacterial and VLP abundances within reef water and sediments. Below we describe the sequence of events and potential processes that directly and indirectly affected bacterial and VLP abundances and dynamics.

Flow cytometric enumeration of bacteria and VLPs

To our knowledge, this was the first study to use flow cytometry as a tool to enumerate bacteria and VLPs within carbonate reef sediments, and only the second time for sediments in general (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, Hewson et al. 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

384 VLPs directly through *in situ* infection of planktonic microorganisms and/or resuspension of
385 benthic VLPs with changing tidal regimes. Alternatively, detachment of reef and coral surface
386 associated VLPs (Davy & Patten 2007) when water is advected through the reef framework may
387 have indirectly contributed to increased water column viral loads it the water column.

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

396 The rapid decrease in both bacterial and VLP abundances from the 25th to the 27th
397 November coincided with calmest weather conditions, low tidal exchanges and maximum
398 deposition rates of particulate matter (Wild et al. 2008). We hypothesise that scavenging by
399 sinking sedimenting spawning material may have been a dominant mechanism removing free
400 bacteria and viral particles from the water column following spawning. In aquatic environments,
401 there is limited information for the extent by which VLPs associate with organic aggregates.
402 However, a significant fraction of water column VLPs may attach to organic aggregates over a
403 continuum of size scales from transparent exopolymeric particles (~ 1 to 100 µm) to marine
404 snow (~ 300 – >1000 mm), and these aggregates may represent hotspots of viral activity (Proctor
405 & Fuhrman 1991). Within coral reef waters up to 50 % of bacteria have been observed attached
406 to organic matter >3 µm (Moriarty 1979), and 30 % of total bacterial productivity may occur on
407 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. 1997, Hadas & Marie 2006) and grazing by flagellates (Caron 2000, Gonzalez & Suttle 1993), 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 alternatively, 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 nonetheless 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, two 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 (Wild et al. 2008, Eyre et al. in press, Glud et al. in press). The 2.4-fold and 1.5-fold decline in water column bacteria and VLPs respectively, between the 24th and 27th 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

456 experiments do not reflect *in situ* conditions (Ferguson et al. 1984). Slurry incubations are
457 known to stimulate bacterial activity above that of non-manipulated sediments, and the effect of
458 slurry conditions on viral production is not clear (Middelboe & Glud 2006). Such stimulated
459 conditions may shift the balance of the system by favouring the growth of bacterial
460 subpopulations, which are not exposed to a severe viral infection pressure initially, causing a
461 decrease in the original viral assemblage due to a reduction in susceptible host cells. Over a
462 slightly longer time scale, new viral populations would be expected to propagate and infect the
463 developing bacterial assemblage, leading to increased viral abundances in the incubations.
464 Further experiments involving whole core incubations are however needed to estimate bacterial
465 and viral production in carbonate sediments.

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

477 **Pelagic-benthic coupling and ecological implications of the coral spawning event on**
478 **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 were not mutually exclusive from the overlying water during this study. On average 150 % and 250 % of bacteria and VLPs respectively were lost from the water column following mass coral spawning. Assuming the loss was due solely to adsorption onto coral spawn material, that the carbon content of one 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. in press) during the spawning period at Heron Island. While it is likely that some proportion of this total carbon is advected offshore into inter-reefal 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 one day 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. in press), but 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 nutrient 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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TABLES

Table 1. Minimum, maximum and mean (in brackets) values for physical-chemical parameters in reef water at Heron Island, Great Barrier Reef (GBR), over the duration of study period, November 18th – November 27th, 2005 (summarised from Glud et al. in press and Eyre et al. in press).

Parameter	Min – Max	(Mean)
Depth (m)	0.2 – 2.1	(1.0)
Temperature (ºC)	22.4 – 31.1	(26.4)
Dissolved 0_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)
Chlorophyll *a* (μg l^{-1})	0.1 – 1.4	(0.5)
Total Nitrogen (μmol l^{-1})	6.0 – 18.5	(12.1)
Total Phosphorus (μmol l^{-1})	1.2 – 1.8	(1.34)

Table 2. Results from BIOENV analysis of bacterial and virus-like particle (VLP) abundances and physical-chemical parameters at different grouping levels. Kendall's coefficients (τ) are given for the best single or combination of physical-chemical parameter(s). The best correlation for single or a combination of physical-chemical parameters is marked by 'x'. DO = dissolved oxygen, Chl *a* = chlorophyll *a*, TN = total nitrogen, TP = total phosphorous, Temp = temperature, Depth = water column depth.

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

749
750 Table 3. Comparison of virus-like particle (VLP) abundances and virus-to-bacteria ratios (VBR) within
751 seawater ($\times 10^6$ ml^{-1}) and sediments ($\times 10^8$ cm^{-3}) from different oligotrophic and eutrophic marine
752 environments. FCM = flow cytometry; TEM = Transmission Electron Microscopy; [a] = SYBR Green I, [b] =
753 SYBR Green II.

Environment and location	VLP abundance	VBR	Method	Reference
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. 2005a
Florida Bay (United States)	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 (United States)	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) (United States)	5	ND	TEM	Paul et al. 1993
Big Fisherman's Cove (< 1m) (United States)	2	98	EFM[a]	Hewson & Fuhrman 2003
San Pedro Channel (~900m)(United States)	2	11	EFM[a]	Hewson & Fuhrman 2003
Eutrophic				
Brisbane River (river mouth) (Australia) (< 2m)	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) (United States)	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

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FIGURES LEGENDS

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

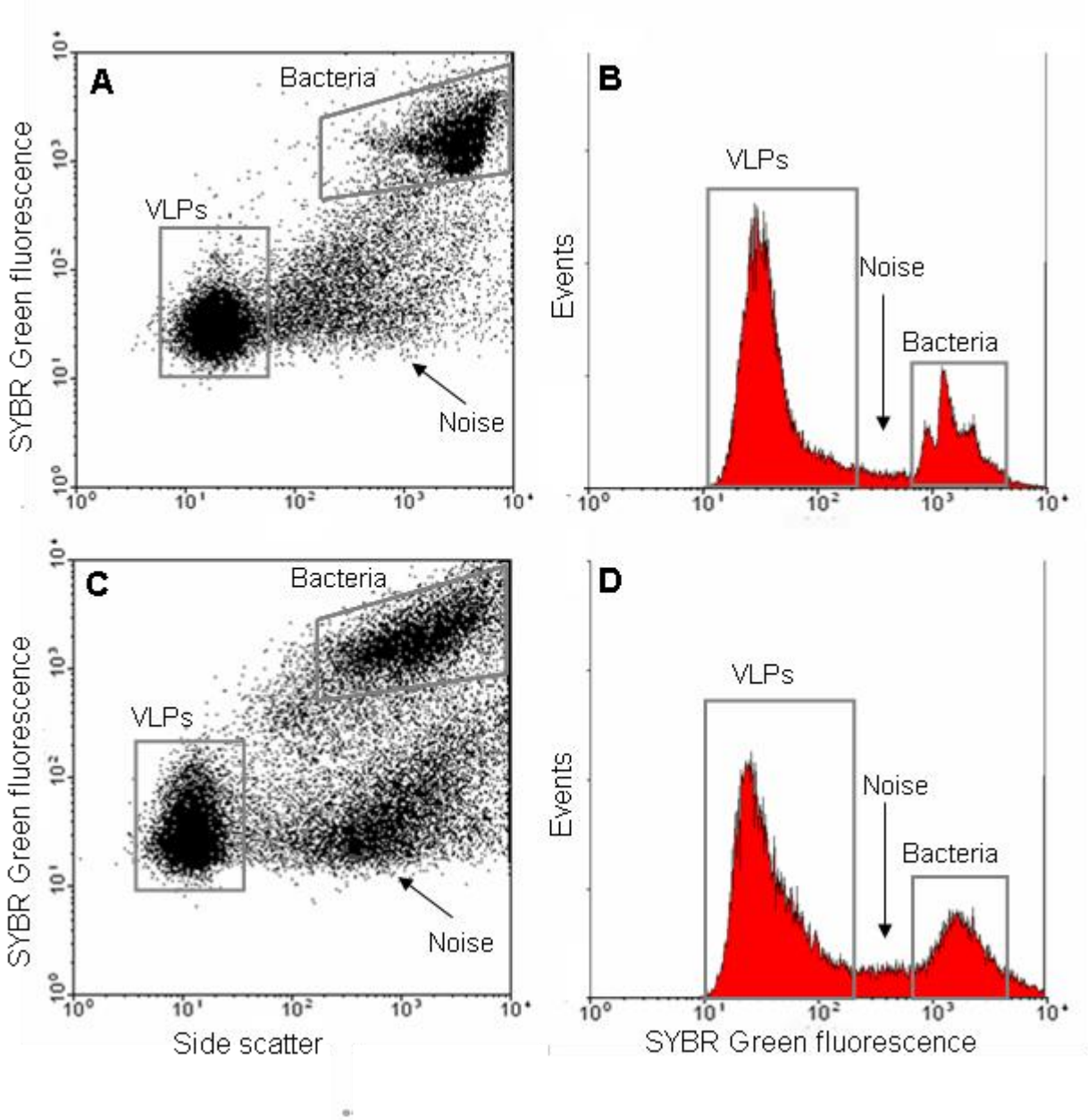
Fig. 2. Characteristic cytograms from flow cytometric analysis showing bacteria and virus-like particles (VLPs). (A) Dot plot of side scatter vs SYBR Green I and (B) Frequency distribution of SYBR Green I fluorescence for a reef water sampled on 23rd November; 2005 (C) Dot plot of side scatter vs SYBR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

Fig. 3. Temporal abundances of bacteria and virus-like particles (VLPs) and virus-to-bacteria ratios (VBR) in reef water and sediments. (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 cm, 3 – 4 cm and 6 – 8 cm sediment depth horizons. (D) VBR in 0 – 0.5 cm, 3 – 4 cm and 6 – 8 cm sediment depth horizons. Vertical grey bars above the graphs represent nights where minor spawning occurred and black bars represent nights where major spawning occurred.

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

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

Fig. 1.



821

822 Fig. 2.

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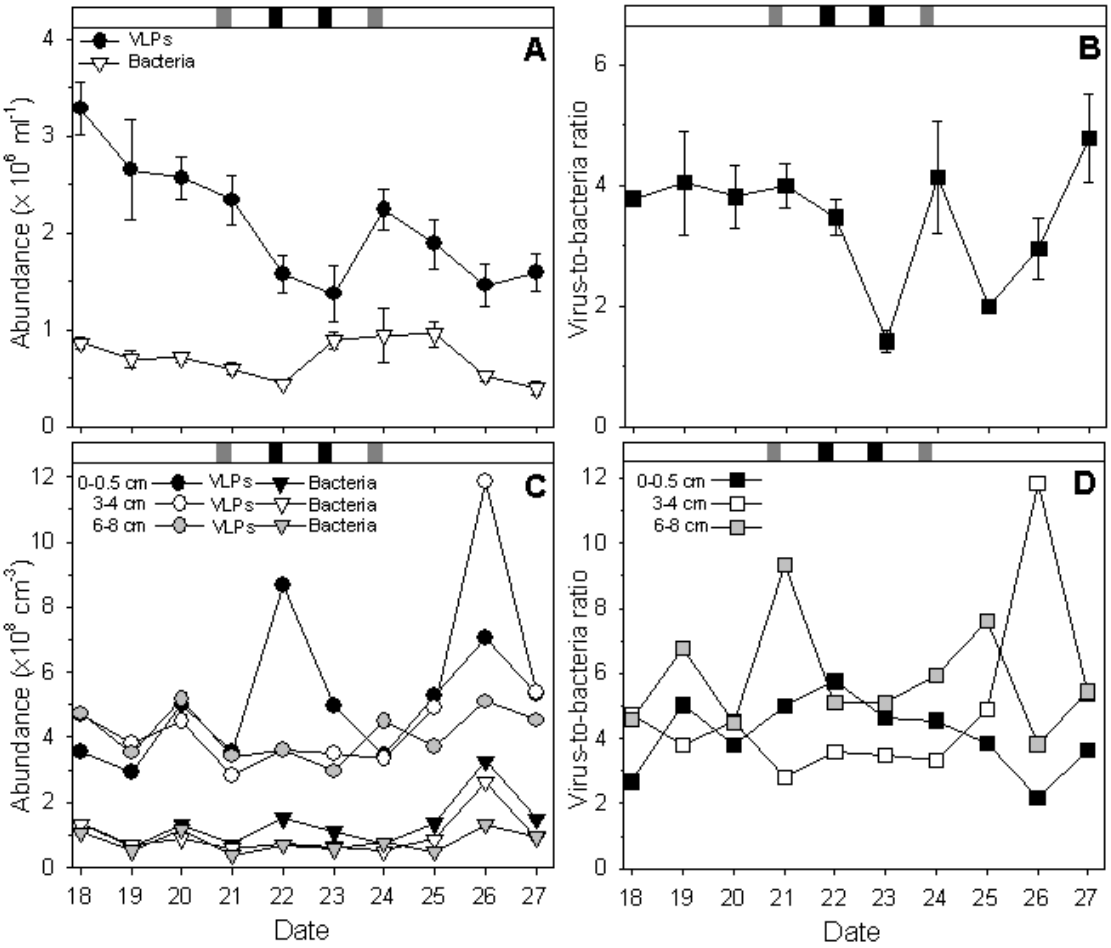

833
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835 Fig. 3.

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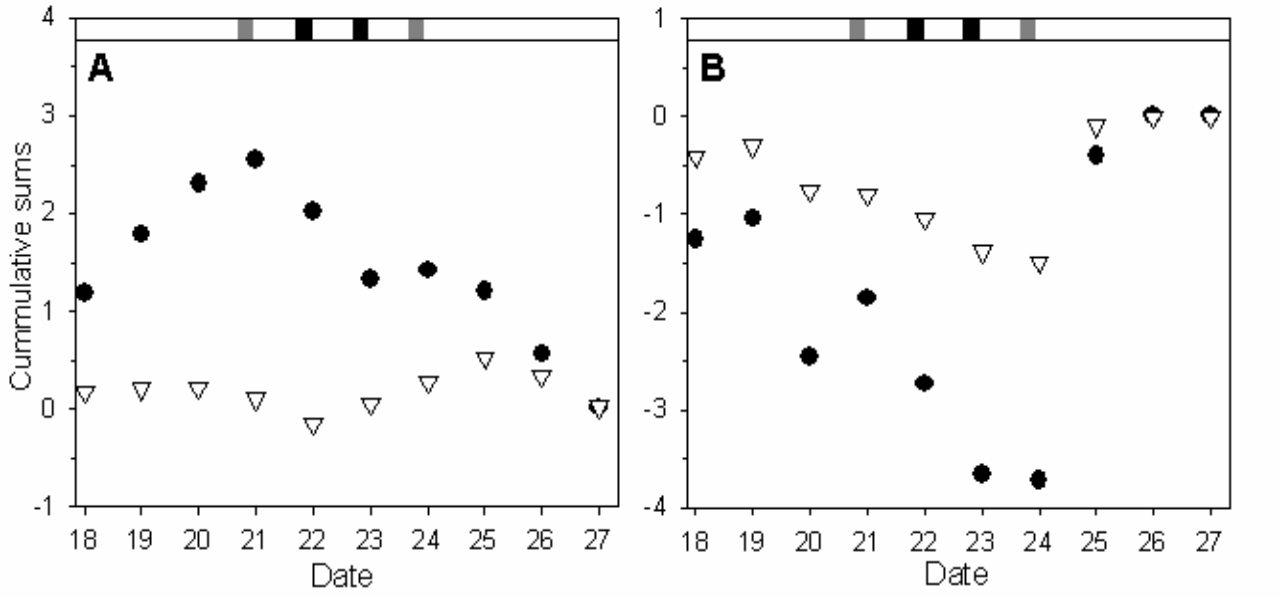
A
B
Cummulative sums
Date
Date
4
3
2
1
0
-1
1
0
-1
-2
-3
-4
18
19
20
21
22
23
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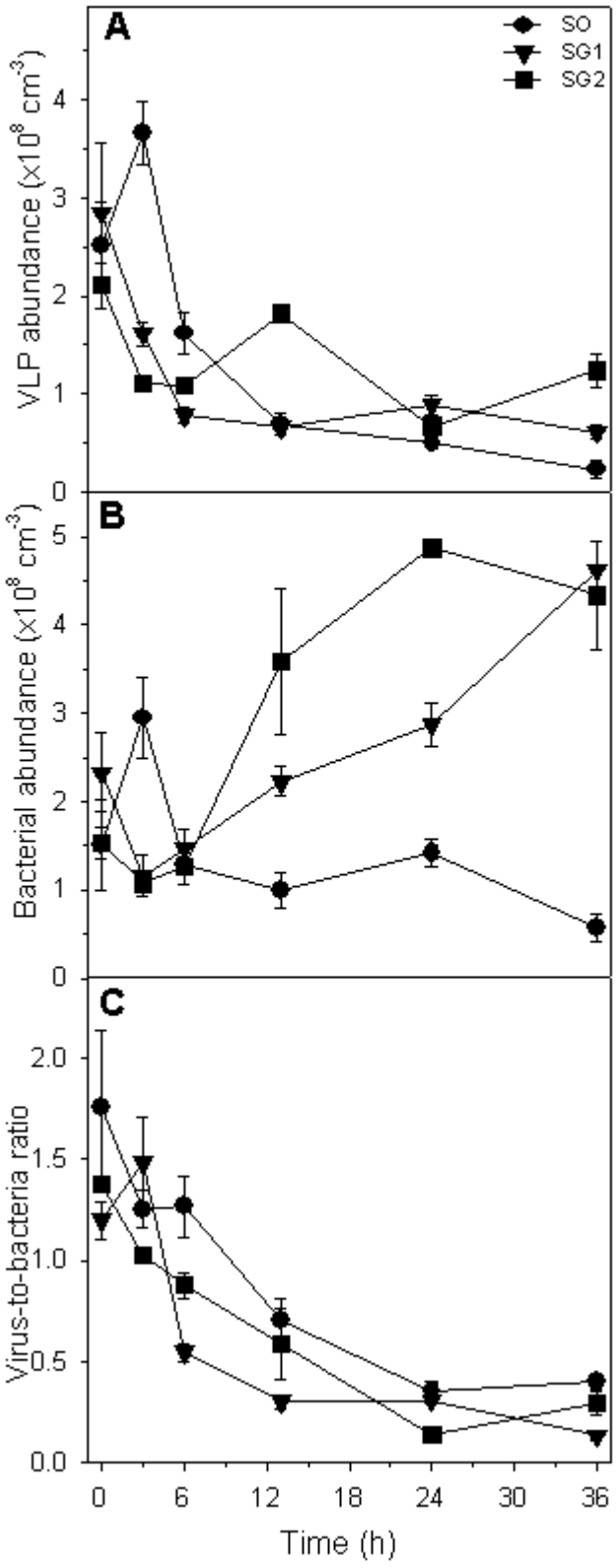
Fig. 4.


A
SO
SG1
SG2
VLP abundance (×10^8 cm^-3)
0
1
2
3
4
B
Bacterial abundance (×10^8 cm^-3)
0
1
2
3
4
5
C
Virus-to-bacteria ratio
0.0
0.5
1.0
1.5
2.0
0
6
12
18
24
30
36
Time (h)

853

854 Fig. 5