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Influence of local environmental variables on the viral consortia associated with the coral *Montipora capitata* from Kaneohe Bay, Hawaii, USA

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ABSTRACT: Coral-associated viruses are a component of the coral holobiont that have received attention only relatively recently. Given the global increase in the prevalence of coral disease, and the lack of positively identified etiological agents for many diseases, these virus consortia require increased investigation. Little is known about the viruses that are naturally associated with coral reefs and how they are affected by the local environment. In the present study, a short-term analysis of viral consortia associated with the coral *Montipora capitata* in Kaneohe Bay, Hawaii, USA, was carried out to determine the environmental factors influencing their composition. Coral surface microlayer (CSM) and seawater samples collected at 4 sites with a range of environmental characteristics were analyzed using transmission electron microscopy (TEM), and relative abundances of virus-like particle (VLP) morphotypes were correlated with environmental measurements. Relative proportions of several CSM-associated VLP types, including phages and filamentous VLPs, were correlated with water temperature, turbidity and chlorophyll *a* levels. In seawater samples, turbidity and temperature showed the strongest correlation, altering the proportion of *Podoviridae*-like, *Geminiviridae*-like and putative Archaeal viruses, among others. Overall VLP consortium composition differed significantly between the CSM and seawater only at the more degraded sites, suggesting that human activity may be affecting coral reef-associated virus consortia.

KEY WORDS: Corals · Virus · Environmental drivers · Turbidity · Chlorophyll

INTRODUCTION

Coral reefs are the most diverse ecosystems in the ocean, with estimates of diversity ranging from 1 to 9 million species worldwide (Pelley 2004). A major contributor to this diversity is the vast array of coral-associated microbes, including dinoflagellates of the genus *Symbiodinium* (Douglas & Smith 1989), endolithic algae (Wanders 1977, Shashar 1992), fungi (Bentis et al. 2000), archaea (Kellogg 2004, Wegley et al. 2004) and both heterotrophic and autotrophic bacteria (Ducklow & Mitchell 1979, Rohwer et al. 2002, Lesser et al. 2004). One component of this coral holobiont (Rohwer et al. 2002) which has received limited examination until recently is the coral-associated virus consortium. Viruses are increasingly seen as important members of marine communities, often having significant effects at the level of the individual host and on the ecosystem and wider environment (Munn 2006, Suttle 2007, Sandaa 2008, Rohwer

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& Vega Thurber 2009). Viruses are known to infect numerous taxa within the coral reef ecosystem, including bacteria, archaea, fungi, algae and fish (Vega Thurber & Correa 2011). Viruses were first reported from within the tissues of a scleractinian coral in 2005 (Wilson et al. 2005), but there have only been ~20 subsequent studies of viruses associated with reef-building corals. Viruses have been found in the gastrodermis, epidermis and coral surface microlayer (CSM, or mucus layer) of scleractinian corals, and in their intracellular dinoflagellate symbionts (*Symbiodinium* spp.) (reviewed by Vega Thurber & Correa 2011).

It is uncertain whether viruses are responsible for any of the ~30 currently recognised coral diseases (Willis et al. 2004); however, there is some evidence that viruses may play a role in several of them. Patten et al. (2006) found that virus abundance and the virus:bacteria ratio (VBR) increased with proximity to acroporid corals suffering from white syndrome, suggesting an increase in non-phage viruses associated with the diseased corals. Further evidence for viral diseases of cnidarians and their dinoflagellate symbionts comes from *in vitro* experiments, such as those of Wilson et al. (2001) and Davy et al. (2006), which showed that temperature stress of otherwise healthy *Symbiodinium* cells and cnidarians resulted in an increase in viral abundance within the dinoflagellates and possibly the cnidarian host. In both studies, virus-like particles (VLPs) induced by heat stress were capable of lysing healthy *Symbiodinium* cells in the absence of stress, providing strong evidence that viruses can indeed cause disease in these dinoflagellates. Microscopic examination of coral-associated VLPs has also provided evidence that viruses may play a role in 2 diseases of poritid corals: *Porites* tissue loss (Lawrence et al. 2014) and *Porites* white patch syndrome (Lawrence et al. 2015). Recently, several studies have taken a molecular approach to assessing viral associations with coral diseases. For example, Vega Thurber et al. (2008) found, using metagenomics, that several environmental stressors (reduced pH, elevated nutrient levels and thermal stress) caused increases in herpes-like virus abundance in *Porites compressa*. Further, Littman et al. (2011) found viral sequences associated with bleaching corals on the Great Barrier Reef, and Soffer et al. (2014) recently reported on single-stranded DNA virus sequences associated with white plague disease of the coral *Montastraea annularis*. Soffer et al. (2014) also found that sequences belonging to nucleocytoplasmic large DNA viruses (NCLDVs) were more common in bleached *M. annularis* than in healthy samples. It seems likely that, with further research, viruses will be identified as the etiological agents of some coral diseases.

Despite some viruses potentially causing disease and disruption of the coral holobiont, it is probable that the majority of coral-associated viruses are in fact a relatively stable and important component of the holobiont. VLP abundance and VBR in seawater have been shown to increase with proximity to healthy corals (Seymour et al. 2005), albeit not to the same extent seen with diseased corals by Patten et al. (2006). Marhaver et al. (2008) carried out metagenomic analyses of viral consortia associated with the coral *Diploria strigosa* and they were found to be highly diverse (healthy colonies were predicted to contain over 28 000 viral types). A diverse viral consortium is to be expected, given the range of potential hosts within the coral holobiont. The huge network of interactions arising from this vast assemblage of eukaryotes, prokaryotes and viruses makes determining the roles of individual viruses, and their collective effect on the coral holobiont, all the more difficult. However, in spite of the difficulties involved, the identity and roles of coral-associated viruses deserve investigation, as they represent the least well-known component of a holobiont and an ecosystem that are increasingly at risk of massive alteration via disease (Harvell et al. 2002, Bourne et al. 2009), temperature-induced bleaching (Hoegh-Guldberg 1999, Baker et al. 2008), ocean acidification (Hughes et al. 2003, Hoegh-Guldberg et al. 2007) and other anthropogenic impacts (Knowlton 2001, Pandolfi et al. 2003).

In the present study, we examined the VLP consortia associated with the CSM of the scleractinian coral *Montipora capitata*. The CSM acts as a selective barrier, keeping the coral free of sediment (Hubbard & Pocock 1972) while trapping food particles (Lewis & Price 1976). Additionally, the CSM harbours a diverse microbial community, with bacterial abundance of up to 100 times that of surrounding seawater (Ritchie & Smith 2004). This bacterial community is thought to be coral species-specific (Ritchie & Smith 1997, Kvennefors et al. 2010) and there is evidence that these endemic bacteria prevent invasion of the coral by potentially harmful microbes (Reshef et al. 2006, Ritchie 2006, Nissimov et al. 2009). This community can, however, shift to a predominantly pathogenic state under stress conditions (Vega Thurber et al. 2009, Mao-Jones et al. 2010, Rypien et al. 2010). Though not as well studied, the viral consortium present in the CSM also appears to differ from that in the surrounding seawater (Davy & Patten

2007). With this in mind, the present study used transmission electron microscopy (TEM) to assess the morphological diversity of VLP consortia in the CSM of *M. capitata* and in overlying seawater at several locations in Kaneohe Bay, Hawaii, USA, to determine how these viral consortia differ between the CSM and surrounding seawater, and in response to differing environmental parameters.

MATERIALS AND METHODS

Sampling

Sampling was conducted between 26 May and 14 June 2009 at 4 sites on the reef crest of the northern and eastern flanks of Coconut Island, Kaneohe Bay, Oahu, Hawaii, USA (21° 26′ N, 157° 47′ W; see Fig. 1 for sampling locations). The distance from shore was between 50 and 200 m, and the maximum depth at low tide was approximately 4.5 m. Samples were collected at low tide from 2 viral 'habitats': the coral surface microlayer (CSM) and the surrounding seawater. Sample collection was performed by divers using snorkels, and consisted of 5 mucus samples from the CSM of separate, healthy *Montipora capitata* colonies, and 5 seawater samples, from each of the 4 sites. The 4 sampling sites were selected on the basis of environmental data collected previously (Williams et al. [2010] and unpublished data from a survey carried out in October and November 2007), which provided a range of environmental variables (turbidity, chlorophyll *a* [chl *a*], salinity, temperature and depth) within the sampling area. Furthermore, at the time of sample collection, there appeared to be a difference in reef quality (i.e. proportional coral cover and species diversity) among the sites, with Sites A and B featuring a more 'healthy' reef structure, and Sites C and D showing signs of degradation. Although not quantified during the present sampling, a survey carried out in June 2008 found that Sites A and B had 72 and 87% live coral cover, respectively, while Sites C and D had 63 and 66%, respectively (G. J. Williams unpubl. data). CSM samples (25 ml) were harvested using sterile syringes, with care being taken to ensure that the syringe tip remained in contact with the coral surface at all times. Seawater samples (50 ml) were collected in centrifuge tubes 10 cm above the surface of each sampled coral colony. All samples were fixed in 2% glutaraldehyde within 2 h of collec-

Fig. 1. Locations of the 4 sampling sites around Coconut Island, Kaneohe Bay, Hawaii, USA. Map adapted from Jokiel et al. (1993); aerial image of Coconut Island sourced from Google Earth

tion and stored in the dark at 4°C until processing for electron microscopy.

Five environmental variables (temperature, chl *a* fluorescence, turbidity, depth and salinity) were measured at each of the 4 sampling sites over a 48 h period using RBR XR-420 submersible CTD thermistors. The CTDs were set at 1 min sampling intervals and their placement was randomised among the sampling sites throughout the sampling period. Mean values of each parameter measured over this period were used in environmental data analysis.

Virus isolation and electron microscopy

Samples of seawater (50 ml) and coral mucus (25 ml) were centrifuged at 1500 × *g* for 10 min at 4°C to remove coral tissue, bacteria and other non-viral material. Viruses were pelleted from the supernatant by centrifugation at 146 000 × *g* for 2 h at 4°C and resuspended in 100 µl of supernatant. Pioloform-coated 200 µm mesh copper grids were carbon coated and rendered hydrophilic by high voltage glow discharge, before being floated on 20 µl aliquots of the viral suspensions for 1 h. Grids were negative-stained with 3% aqueous uranyl acetate for 30 s and viewed on a Philips CM100 transmission electron microscope (TEM; 80 kV) at 33 000 to 66 000 × magnification. The size (length of filamentous, rod-shaped and beaded VLPs; maximum capsid width of all others) and morphology of the first 100 VLPs observed on each grid were determined and assigned to 1 of 5 major morphological groups (tailed phages, icosahedral/spherical tail-less VLPs, filamentous VLPs [FVLPs], lemon-shaped VLPs, and other miscellaneous VLPs), and then assigned to 26 further subgroups within these major morphological groups.

Data analysis

To test for differences in virus consortium structure between the CSM and surrounding seawater within and between sites, a 2-factor multivariate regression model was applied under permutation of dissimilarities, using the program DISTLM (Anderson 2001, McArdle & Anderson 2001). In cases of significant interaction between factors, simple effects tests were carried out using the denominator mean square from the interaction model.

A dissimilarity matrix of the virus morphotype frequencies was constructed using the semi-metric Bray-Curtis measure. This was done using the vegdist function of the vegan package in R (R Development Core Team 2006, Oksanen et al. 2010). The appropriately coded predictor (X_{SITE}, $X_{HABITAT}$ and X_{SxH}) and full (X_{FULL}) matrices were constructed using the program XMATRIX (Anderson 2003). Orthogonal contrasts were used due to unbalanced replication, as 2 of the electron microscope grids had degraded and were unusable.

In order to illustrate relative site differences within each habitat type (i.e. CSM and seawater), a non-metric multidimensional scaling (NMDS) ordination of site and species scores (i.e. the 26 VLP subgroups) was carried out using the metaMDS function of the MASS package in R (R Development Core Team 2006, Oksanen et al. 2010), with points representing viral morphotypes and field sites.

To determine how well patterns of virus consortium composition correlated with measured environmental parameters across the 4 sites, and in which direction they were correlated, a subset of environmental variables was chosen based on maximum correlation with consortium dissimilarities. For each habitat type, environmental variables were fitted and projected as vectors onto the ordination diagrams using the envfit procedure in the vegan package in R (R Development Core Team 2006, Oksanen et al. 2010). The goodness of fit for each vector was assessed using the squared correlation coefficient (r^2) test statistic based on 4999 permutations of the environmental variables and a 5% significance level.

RESULTS

Environmental variables

The 4 reef sites sampled were chosen to include a range of environmental attributes. While salinity and chl *a* values remained relatively constant across sampling locations, depth, temperature and turbidity showed notable differences (Table 1). In particular, Sites C and D were much more turbid than Sites A and B, consistent with these former sites appearing more degraded.

Viral morphology

In this study, 3700 VLPs were counted, belonging to 5 major groups and further categorised into 26 subgroups (Table 2). Examples of VLP morphologies found in the CSM and seawater are shown in Fig. 2.

Table 1. Mean values (±SE) of environmental variables recorded over sampling period. STU: standard turbidity units

Site	Depth (m)	Chl *a* (µg l^{-1})	Salinity (ppt)	Temperature (°C)	Turbidity (STU)
A	4.1 ± 0.1	16 ± 0.2	35.01 ± 0.01	26.83 ± 0.07	1.9 ± 0.2
B	2.45 ± 0.05	1 ± 0.5	35.09 ± 0.01	27.23 ± 0.08	1.5 ± 0.3
C	1.3 ± 0.1	2.3 ± 0.9	34 ± 1.3	26.57 ± 0.12	4.5 ± 1
D	1.62 ± 0.07	1.7 ± 0.2	35.16 ± 0.04	26.02 ± 0.06	8 ± 2

The numerically dominant VLP group in all samples was the icosahedral/spherical VLPs (constituting 56.4% of the total VLP count), of which 72.2% were smaller than 50 nm. Large icosahedral/spherical VLPs (>100 nm), which may have included members of the *Herpesviridae* and Nucleocytoplasmic Large DNA Viruses (NCLDVs) such as the *Phycodnaviridae*, constituted 2.9% of the total VLP count. The various tailed phages were present in high numbers in all samples (making up 30.1% of the total VLP count), with *Podoviridae*-like VLPs making up 11.1%, *Myoviridae*-like VLPs 12.6%, and *Siphoviridae*-like VLPs 6.4% of the total. Filamentous VLPs, ranging in size from <50 nm to >2 µm in length, comprised 7.2% of the total VLP count, while lemon-shaped VLPs made up 2.3% of the count. In 2 of the CSM samples, from Sites A and D, VLPs classed as 'Other' (including mushroom-shaped and *Polydnaviridae*-like VLPs) were seen in low numbers (<0.5% of the total count). Proportions

Table 2. Morphological diversity and proportions of virus-like particles (mean % VLPs ± SE) from the coral surface microlayer (CSM) of *Montipora capitata* and in surrounding seawater (SW), from 4 sites in Kaneohe Bay, USA (n = 5 except for Site B CSM, Site C SW and Site D SW, where n = 4). Dashes indicate that a VLP size/morphological group was not present in samples from a habitat/site

Viral morphptype	Site A		Site B		Site C		Site D	
	CSM	SW	CSM	SW	CSM	SW	CSM	SW
Podoviridae-like								
<100 nm	14 ± 1	13 ± 2	11 ± 1	13 ± 3	6 ± 2	7.5 ± 0.9	6 ± 1	9 ± 2
>100 nm	2 ± 1	0.4 ± 0.2	0.2 ± 0.2	1.1 ± 0.6	1.4 ± 0.6	0.3 ± 0.3	0.4 ± 0.2	4 ± 1
Elongate *Myoviridae*-like								
<100 nm	0.4 ± 0.4	–	0.7 ± 0.5	0.8 ± 0.6	–	–	–	–
100–200 nm	0.8 ± 0.4	0.2 ± 0.2	1.2 ± 0.7	1 ± 0.3	0.4 ± 0.2	0.3 ± 0.3	1.1 ± 0.5	1.7 ± 1.7
>200 nm	0.8 ± 0.5	0.4 ± 0.5	1.3 ± 0.5	–	–	0.8 ± 0.5	0.8 ± 0.8	–
Isometric *Myoviridae*-like								
<100 nm	6 ± 11	7 ± 3	10 ± 4	4 ± 1	3 ± 1	2 ± 0.4	1.9 ± 0.5	3 ± 1
100–200 nm	8 ± 3	5 ± 0.8	7 ± 1	5 ± 1	4 ± 1	8 ± 2	5 ± 2	7 ± 2
>200 nm	1 ± 0.5	0.6 ± 0.4	–	–	0.2 ± 0.2	2 ± 0.7	0.2 ± 0.2	0.7 ± 0.5
Siphoviridae-like								
<100 nm	3 ± 1	4 ± 1	3 ± 2	8 ± 6	0.6 ± 0.2	0.5 ± 0.3	0.8 ± 0.4	4.4 ± 0.5
100–200 nm	2 ± 1	3 ± 1	2.2 ± 0.5	4 ± 1	1.8 ± 0.7	2.3 ± 0.6	2.6 ± 0.6	3 ± 2
>200 nm	0.2 ± 0.2	0.6 ± 0.2	0.5 ± 0.3	0.4 ± 0.2	0.6 ± 0.2	2.5 ± 0.6	0.4 ± 0.2	0.7 ± 0.2
Icosahedral/spherical								
<50 nm	32 ± 3	42 ± 2	32 ± 6	41 ± 6	56 ± 6	52 ± 3	39 ± 10	32 ± 4
50–100 nm	12 ± 3	7 ± 2	14 ± 3	6 ± 2	12 ± 1	8 ± 1	15 ± 4	28 ± 8
>100 nm	1 ± 0.3	0.4 ± 0.2	2 ± 1	6 ± 5	2 ± 0.3	0.8 ± 0.5	8 ± 4	2 ± 1
Lemon-shaped								
<100 nm	6 ± 4	0.2 ± 0.2	2 ± 0.4	0.2 ± 0.2	3 ± 1	0.5 ± 0.5	1.1 ± 0.6	0.7 ± 0.5
>100 nm	1.2 ± 0.8	–	2 ± 1	–	1 ± 0.3	–	0.5 ± 0.4	0.8 ± 0.8
Filamentous								
<100 nm	1 ± 1	1.2 ± 0.4	3.2 ± 0.5	2 ± 1	0.2 ± 0.2	–	–	1.4 ± 0.5
100–500 nm	3 ± 2	2.6 ± 0.6	3 ± 0.7	3 ± 1	1.6 ± 0.6	1.8 ± 0.5	3.2 ± 0.7	1.2 ± 0.7
500 nm–1 µm	0.6 ± 0.2	3 ± 1	1.7 ± 0.6	1.5 ± 0.6	1.6 ± 0.7	4 ± 1	4.0 ± 0.8	0.3 ± 0.3
>1 µm	1.8 ± 0.5	2 ± 2	0.7 ± 0.5	1.6 ± 0.8	0.4 ± 0.2	2.3 ± 0.8	3.2 ± 0.9	–
Geminiviridae-like	0.8 ± 0.2	0.6 ± 0.4	1.0 ± 0	0.9 ± 0.4	2 ± 0.5	1 ± 0.4	3 ± 1	0.2 ± 0.2
beaded	1 ± 0.6	2.4 ± 0.7	1.5 ± 0.6	0.2 ± 0.2	1.6 ± 0.7	–	0.2 ± 0.2	0.2 ± 0.2
Rod-shaped	0.8 ± 0.2	2.2 ± 0.5	0.5 ± 0.3	0.2 ± 0.2	0.8 ± 0.4	2.3 ± 0.5	3 ± 1	–
Cube-shaped	0.4 ± 0.2	2 ± 0.3	1 ± 0.4	0.6 ± 0.4	–	–	0.5 ± 0.4	1 ± 0.4
Hook-shaped	–	–	–	–	–	–	0.2 ± 0.2	–
Other	0.4 ± 0.4	–	–	–	–	–	0.5 ± 0.5	–

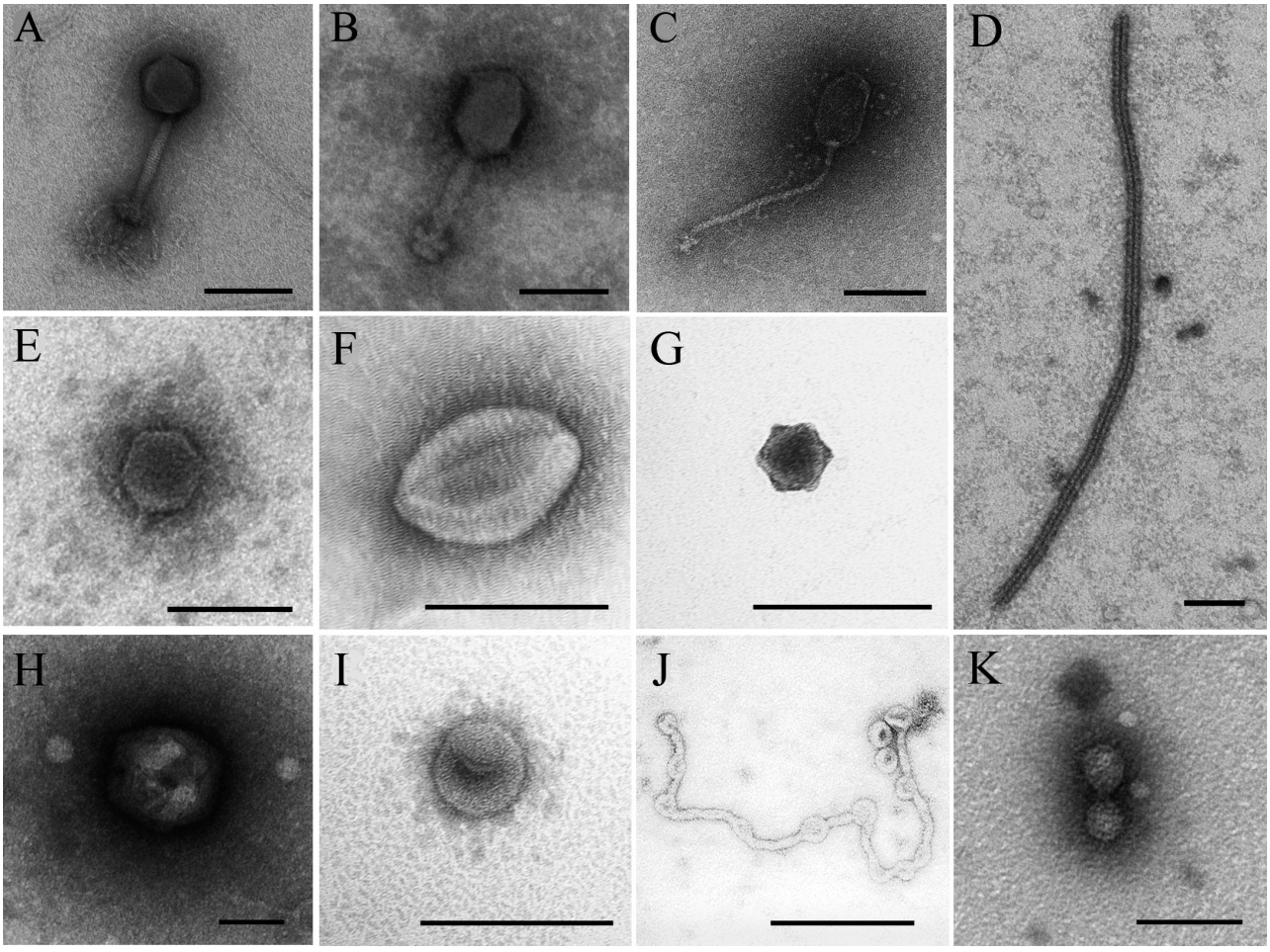

Fig. 2. Transmission electron micrographs of virus-like particles (VLPs) isolated from the coral surface microlayer (CSM) of *Montipora capitata* and surrounding seawater. (A) Isometric *Myoviridae*-like VLP; (B) elongate *Myoviridae*-like VLP; (C) *Siphoviridae*-like VLP; (D) filamentous VLP; (E) *Podoviridae*-like VLP; (F) lemon-shaped VLP; (G) small polyhedral VLP; (H) large, phycodnavirus-like polyhedral VLP; (I) spherical VLP; (J) beaded VLP; (K) geminivirus-like VLP. Scale bars are 100 nm

of each VLP group in both CSM and seawater from the 4 sites are documented in Table 2.

Effect of habitat type on VLP consortia

Combining data from all 4 sites, similar levels of VLP diversity were seen in the CSM and surrounding seawater (permutation test, $p > 0.05$; Shannon-Wiener index ± SE = 2.00 ± 0.09 and 1.92 ± 0.05 for CSM and seawater samples, respectively), though hook-shaped VLPs and those classed as 'Other' were found exclusively in the CSM (Table 2).

Non-parametric analysis of variance revealed a significant multivariate interaction between habitat type (CSM and seawater) and sampling site in determining the composition of the VLP assemblage associated with *Montipora capitata* (permutation $p = 0.007$). Simple effects tests showed a significant difference in VLP consortium composition T between sites in both habitat types (permutation $p = 0.004$ and 0.001 for CSM and seawater samples, respectively). At the 2 sites with relatively good water quality (Sites A and B), there was no significant difference between the CSM and seawater (permutation $p > 0.05$ for both sites). Consortium composition did, however, differ significantly between the 2 habitat types at the sites with poorer water quality (permutation $p = 0.021$ and 0.036 for Sites C and D, respectively).

Effect of the environment on VLP consortia

The envfit variable selection analysis indicated that the environmental variables that had the most influence on VLP consortium composition in the CSM of

M. capitata were temperature, turbidity and chl *a* concentration (permutation p = 0.004, 0.001 and 0.012, respectively). VLP consortia in the surrounding seawater were affected by temperature and turbidity (permutation p = 0.013 and 0.007, respectively).

Projection of environmental variables onto NMDS ordinations indicated a negative correlation between turbidity and temperature, as shown by their near-antiparallel vectors (Fig. 3). Subsets of the CSM and seawater VLP consortia were distributed along this gradient, with several individual morphotypes having their distribution mode centered in either a positive or negative direction along the axis. In the seawater ordination (Fig. 3B), the near-orthogonal chl *a* vector indicated that the concentration of chl *a* was not correlated with either temperature or turbidity, i.e. increased turbidity was associated with lower temperature, but chl *a* concentration was unrelated to either turbidity or temperature.

Effect of habitat type and environment on individual VLP morphotypes

The ordination diagrams (Fig. 3) revealed several patterns in the association between VLP consortia, habitat type and environmental variables at the level of individual virus morphology. In the seawater samples (Fig. 3A), large (>100 nm) *Podoviridae*-like VLPs and elongate *Myoviridae*-like VLPs, as well as lemon-shaped VLPs of both size groups, showed a positive correlation with turbidity. In contrast, the relative abundance of *Geminiviridae*-like, beaded and rod-shaped VLPs, along with the 3 largest size groups of FVLPs, showed a positive association with temperature. A different pattern was apparent in the CSM samples (Fig. 3B), where the relative abundances of all 4 groups of small (<100 nm) tailed phages, small lemon-shaped VLPs, FVLPs and beaded VLPs were positively correlated with increased temperature. The abundance of the numerically dominant, small (<50 nm) polyhedral/spherical VLPs in the CSM samples appeared to be positively correlated with chl *a* concentration.

DISCUSSION

In this study, we examined the virus consortia associated with the CSM of *Montipora capitata* and with the surrounding seawater at locations experiencing different environmental conditions. VLP diversity based on TEM examination was high across all sites and habitats (CSM and seawater), in agreement with previous reports from coral reefs (Davy & Patten 2007, Dinsdale et al. 2008) and other oligotrophic waters (Hewson et al. 2006). While the results of the present study were largely in agreement with those of Davy & Patten (2007), who examined the VLP consortia associated with the corals *Acropora muricata* and *Porites* spp. on the Great Barrier Reef (GBR), there were several notable differences in the consortia seen here. All samples examined here were dominated by tailed phages and small (<100 nm) icosahedral VLPs (many of which were likely also phages). Conversely, Davy & Patten (2007) observed much higher abundances of filamentous VLPs and large (>100 nm diameter) polyhedral/spherical VLPs in all samples. Without knowing the identities of the filamentous and polyhedral/spherical VLPs observed in each study, it is difficult to comment on what might be driving these differences, but the greater proportion of phage-like VLPs seen here suggests higher bacterial abundances in the Kaneohe Bay samples than in the samples from Heron Reef on the GBR used by Davy & Patten (2007). Increased bacterial abundance may reflect the greater water residence time in southern Kaneohe Bay (13 to >30 d; Smith et al. 1981, Lowe et al. 2009) than at Heron Reef (15 to 60 h; Mongin & Baird 2014), or a greater level of human impact in Kaneohe Bay (Hunter & Evans 1995, Stimson et al. 2001). Alternatively, it may be a result of the different sampling times; samples for the current study were collected in summer, whereas those used by Davy & Patten (2007) were collected in autumn. The CSM is known to harbour a wide array of bacteria (Ritchie & Smith 2004), and the increased seawater temperature in summer may lead to episodes of thermal stress, resulting in increased mucus production (Jokiel & Coles 1990) and, therefore, higher bacterial abundance (Segel & Ducklow 1982). This hypothesis requires further study however.

In addition to these dominant groups, numerous other viral morphotypes were present in relatively low proportions. While the specific hosts of these VLP groups cannot be positively identified without further research, several resembled viruses known to infect Archaea, plants, fungi and animals. Viral abundance was not measured in the present study, but recent data from the same sites and the CSM of the same host species indicate that virus numbers are approximately twice as high in the surrounding seawater as they are in the CSM (Arlidge 2012)

Similar levels of viral diversity were seen among the field sites sampled. This may, in part, be due to the limited resolution of electron microscopy in terms

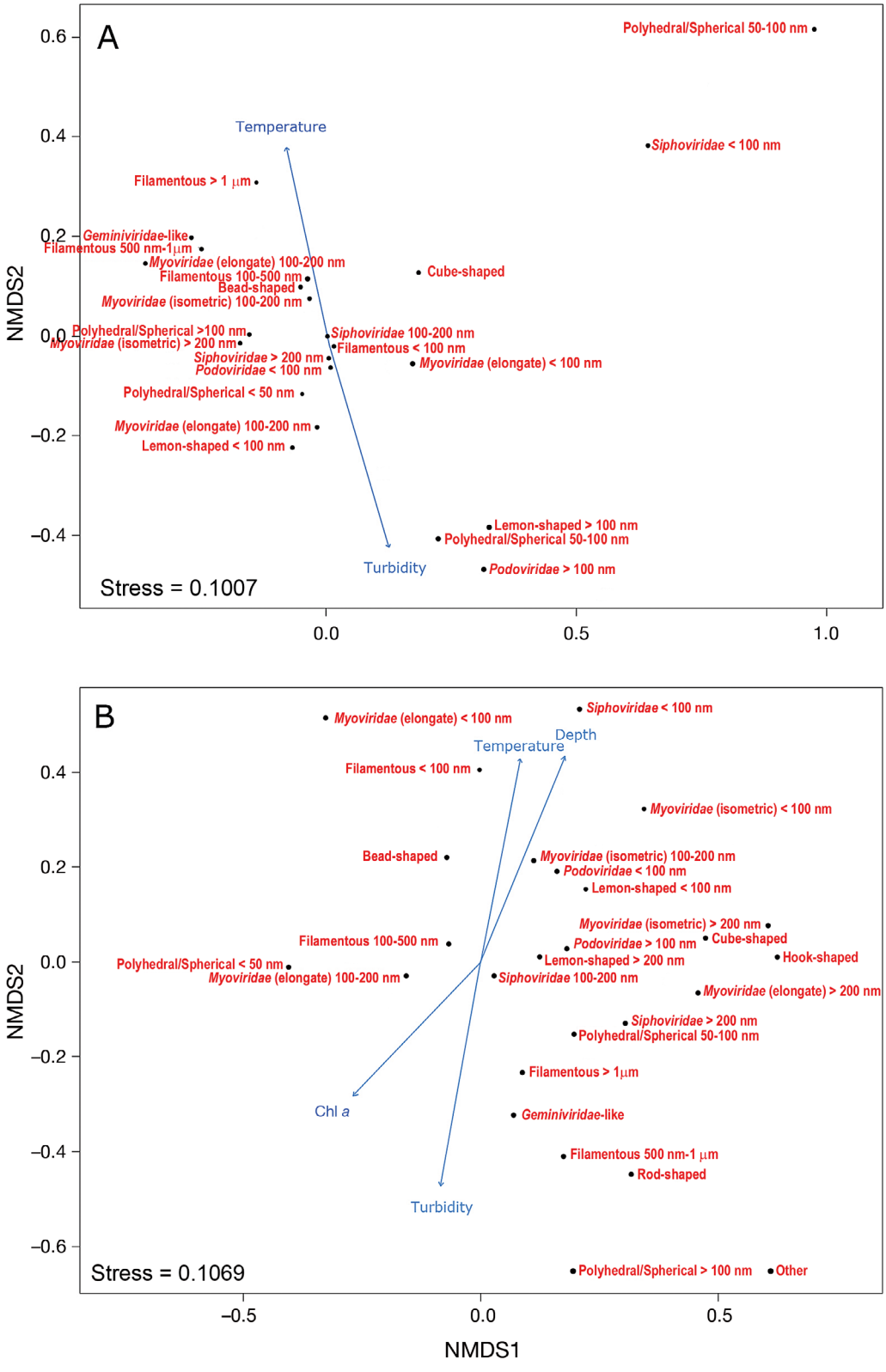

Fig. 3. (A) VLP consortia of seawater and (B) CSM of *Montipora capitata*, showing the patterns of variation in consortium composition explained by environmental variables. Red labels are approximate relative centers of VLP morphotype distributions and blue arrows represent the relative size and direction of fitted environmental gradients

of viral identification (highlighted by the fact that in the present study, VLPs were sorted into just 26 morphological/size groups); molecular analysis would be required to determine the true diversity of these samples. Despite this limitation, several patterns at the level of individual viral morphotypes were observed in relation to environmental variables.

Within the CSM, temperature, turbidity and chl *a* concentration correlated with viral consortium composition. Small phages and filamentous VLPs (<100 nm) were positively correlated with temperature; small polyhedral/spherical VLPs were positively correlated with chl *a* concentration; and geminivirus-like, large filamentous and rod-shaped VLPs were positively correlated with turbidity. As noted earlier, stressors such as elevated temperature or sedimentation can lead to increased mucus production and increased bacterial abundance, which may explain the correlation between phage abundance and temperature. Additionally, temperature stress can cause induction of lysogenic prophages (Jiang & Paul 1996, Cochran & Paul 1998), which could lead to an increase in phage particle abundance in the CSM. The CSM has been shown to contain a bacterial community which is distinct from that of the surrounding seawater (Cooney et al. 2002), which further explains the differing response of the CSM and seawater phage consortia to increased temperature. It is more difficult to explain the positive correlation between small filamentous VLPs and temperature, but slightly larger (~200 nm length) filamentous VLPs have previously been found in stressed *Symbiodinium* (Lohr et al. 2007), hinting that these smaller filamentous VLPs may have algal hosts. Certainly, there is no shortage of potential algal hosts within the CSM and the outer layers of the coral, including the symbiotic *Symbiodinium*, endolithic algae and phytoplankton that have been trapped by the mucus layer. Lohr et al. (2007) provided evidence that these larger filamentous VLPs were in fact latent viruses, induced to enter the lytic cycle via stress to the host. Whether a similar phenomenon is occurring here is unclear, however, as the mean temperatures differed among sites in the present study by only 1.2°C (26.02 to 27.23°C), a much smaller temperature increase than those used previously to induce such viruses. While no long-term data exist for the sites sampled here, these temperatures are within the range reported previously for Kaneohe Bay (e.g. Williams et al. 2010, Apprill & Rappé 2011). Temperature data collected in June 2008 (i.e. 1 yr prior to the present study) at the 4 sites sampled here showed that temperatures at all sites were similar to each other (mean temperature ± SE: 26.58 ± 0.005°C [Site A], 26.53 ± 0.002°C [Site B], 26.72 ± 0.003°C [Site C], 26.21 ± 0.003°C [Site D]) and similar to the temperatures recorded here, with the exception of Site B, which was approximately 0.8°C warmer during the present study (G. J. Williams unpubl. data). Whether the association between viral consortium composition and temperature is due to thermal stress-induced viral induction, therefore, remains unknown, but if the increased temperature at Site B in 2009 was an anomalous occurrence, this association may represent a short-term change in the viral consortia, rather than a more permanent difference between sites. Longer-term sampling would be required to determine if this is the case.

Without more precise identification, it is difficult to determine what the small polyhedral/spherical VLPs in the CSM are infecting; this morphological/size group includes viruses of bacteria, plants, invertebrates and vertebrates (Fauquet et al. 2005). The positive correlation with chl *a* concentration suggests that they may be infecting algae or cyanobacteria within the mucus, but further investigation would be needed to confirm this. Moreover, it is important to note that several studies have documented the effects of increased nutrient levels (for which chl *a* is often used as a proxy) on the general health of the coral holobiont (Bruno et al. 2003, Voss & Richardson 2006, Vega Thurber et al. 2014), and on the dynamics of coral-associated viral consortia (Vega Thurber et al. 2008).

The increased prevalence of large filamentous and rod-shaped VLPs at the more turbid sites is an interesting finding, as such viruses normally infect plants (Fauquet et al. 2005), and resemble VLPs found in experimentally stressed *Symbiodinium* cells, which were hypothesized to belong to the *Closteroviridae* (Lohr et al. 2007). Similar levels of turbidity to those seen here have been shown to increase mucus production and reduce net photosynthesis in other coral species at other locations (Telesnicki & Goldberg 1995), while sedimentation (linked to turbidity) has resulted in bleaching (Acevedo & Morelock 1988) and loss of *Symbiodinium* cells from corals (Philipp & Fabricius 2003). It is therefore possible that turbidity-related stress is causing induction of latent viruses in *Symbiodinium* or other algae within the CSM. This is further supported by the fact that seawater chl *a* concentrations were not closely correlated with turbidity, i.e. the increase in putative algal viruses was not simply due to an increase in planktonic algae. The increased proportion of *Geminiviridae*-like VLPs at the more turbid sites is also of interest. These viruses normally infect plants, but geminivirus-like particles

(Davy & Patten 2007) and DNA sequences (Vega Thurber et al. 2008) have been found associated with corals. While this may also be a case of stress-related viral induction, further investigation is again required for confirmation.

At the time of this study, temperature and turbidity appeared to be the predominant environmental factors affecting seawater virus consortia on Kaneohe Bay reefs. This was a similar response to that seen in the CSM samples, where temperature, turbidity and chl *a* concentration were associated with consortium composition. Large (>100 nm) *Podoviridae*-like, 50 to 100 nm sized polyhedral/spherical and large (>100 nm) lemon-shaped VLPs were positively correlated with turbidity, and geminivirus-like and intermediate and large-sized filamentous VLPs were positively associated with temperature. The increase in *Podoviridae*-like and polyhedral VLPs (many of which may have been phages) may simply be due to an increase in bacterial abundance at the more turbid sites. Likewise, the increased proportion of lemon-shaped VLPs (possibly belonging to the Archaea-infecting *Fuselloviridae* or *Salterproviridae*) at the turbid sites may be due to an increase in Archaea abundance at these sites. Although Archaea are typically thought of as extremophiles, they are known to be relatively abundant in the plankton (DeLong 1992) and the CSM (Kellogg 2004, Wegley et al. 2004), and putative archaeal viruses have previously been observed in the CSM (Davy & Patten 2007). The increase in geminivirus-like and large filamentous VLPs at sites with higher temperatures may be due to induction of latent algal viruses, as was suggested for the CSM virus consortia. Although it is questionable whether an increase of ~1°C would cause sufficient stress for viral induction in either the seawater or CSM samples, this would explain the response of these virus groups to temperature and not to chl *a* concentration (i.e. the viruses are being produced from within the existing algal population, rather than increasing in prevalence due to increased host abundance).

CONCLUSIONS

We have shown in this study that environmental variables, including those that may be associated with anthropogenic impact, such as increased turbidity and chl *a*, play a role in structuring the highly dynamic virus consortia associated with the coral *Montipora capitata*. Furthermore, the observed relationships between viral consortium composition and environmental variables were different in CSM and seawater samples, indicating that there is an interaction between habitat type and environment in structuring viral consortia. Whether these changes represent a negative feedback loop, where an increase in coral pathogens is met by an increase in those viruses which infect them, or a direct increase in pathogenic groups of viruses, remains unknown, and is an interesting avenue of further research. Given the apparent prevalence of coral-associated viruses, future research should also try to determine whether the patterns observed here are replicated in other coral species at other locations, particularly over longer time periods. If the observed relationships between coral-associated viral consortia and environmental parameters potentially associated with anthropogenic impacts (including increased temperature, turbidity and chl *a*) are a common occurrence, this has implications for the management of coral reefs into the future.

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