Changes of viral and prokaryote abundances in a high rate algal pond using flow cytometry detection

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ABSTRACT

High rate algal ponds (HRAPs) are shallow, mixed systems for wastewater treatment, which use sunlight exposure for disinfection. Little is known regarding the relationships between the bacteria and viruses within HRAP systems. Uniquely, flow cytometry permits the rapid identification of bacterial and viral populations in wastewater samples, separating populations based on genome and particle size. Treated wastewater samples were collected from an HRAP at Kingston on Murray, South Australia. Flow cytometry analysis detected bacterial populations and discriminated virus-like particles (VLP) and large VLP (LVLP). Rapid, short term, fluctuations in the abundance of all three populations were observed. Changes in the abundance of these populations was compared; wastewater composition was used as metadata for the comparisons. Linear regression determined relationships in abundances between bacteria and LVLP (R^2 0.2985); LVLP and VLP (R^2 0.5829) and bacteria and VLP (R^2 0.5778) all with p-values of <0.001. Bacterial, LVLP and VLP abundance positively correlated with each other, indicating potential microbial interactions. Overall, the results suggest a parasitic relationship was occurring and driving the abundances of bacteria and viruses within the system.

Key words | bacteria, flow cytometry, high rate algal ponds, virus-like particles, wastewater treatment

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HIGHLIGHTS

- Uses flow cytomerty to characterise viruses and bacteria in high rate algae ponds.
- Discovered high fluctuations in viral and bacterial populations that can occur over hours.
- Suggests that this was the result of parasitic interactions between the two.

INTRODUCTION

Disposal of waste in rural communities is challenging, most resort to using on-site septic tanks for primary wastewater treatment (Buchanan *et al.* 2018). This can be problematic as poorly treated septic water can pool at the surface potentially increasing the risk of human exposure to pathogens by contaminating surface water and food crops or moving through porous soils, contaminating groundwater used for drinking (Lusk *et al.* 2014; Ternes *et al.* 2015).

High rate algae ponds (HRAPs) are cost effective wastewater treatment systems with low installation costs and maintenance requirements, short retention time and minimal environmental impacts (Buchanan *et al.* 2018). HRAPs are shallow (0.3–0.5 m) raceway systems that use a paddlewheel to mix the wastewater creating homogenous conditions of temperature, pH and dissolved oxygen. Mixing also enhances the exposure of the wastewater to sunlight for disinfection (Buchanan *et al.* 2018). They are well suited for use as wastewater treatment plants in rural communities. HRAPs depend upon a combination of algae and bacteria to remediate excess nutrients, including nitrogen,

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phosphorus and carbon from the wastewater (Buhr & Miller 1983). HRAPs are exposed, outdoor systems permitting introduction of environmental microbes in addition to those that enter through the system inlet (Borowitzka 1999; Young *et al.* 2016).

Microbes, including bacteria and viruses, rely on interacting with each other to increase their chances of survival (Ghoul & Mitri 2016). These interactions can come at a benefit and/or cost to others in the system by producing energy resources or matter which can be used by other microbes in the system (Faust & Raes 2012). Interactions can be positive, neutral, or negative depending on the environmental conditions and spatial separation (Ramanan *et al.* 2016). Types of interactions include parasitism, predation, mutualism, commensalism, amensalism and competition (Faust & Raes 2012). Parasitism and predation in systems can have a potentially negative relationship on functional organisms including the algae and bacteria used to treat the wastewater, with a contrasting positive effect on parasites or predators (Faust & Raes 2012). Competition can negatively affect the functional organisms and the competitor by contending for the same resources and tends to dominate in nutrient-rich environments (Ramanan *et al.* 2016). Mutualism and commensalism could be beneficial to the functional microbes as they could offer nutrients or resources; however, mutualistic relationships are rare in high nutrient environments (Ramanan *et al.* 2016). As algae and bacteria are required to treat the wastewater, understanding how these 'functional' microbes interact with other non-functional microbes in the system could help optimise these systems and develop target strategies for managing functional microbial abundances.

A known parasitic relationship between bacteria is with viruses (Faust & Raes 2012). Viruses have the ability to influence the growth of microorganisms adversely by lysis and positively by cycling the nutrients available, changing microbial dynamics (Paterson *et al.* 2012). Viruses of prokaryotes (bacteriophages) have been proposed as the main driving force of bacterial mortality in aquatic environments (Fuhrman 1999). Bacteriophages infect and lyse prokaryotes by coming into contact through passive diffusion with their host organisms and have the ability to control prokaryotic populations in the systems (Fuhrman 1999). The wastewater within HRAPs is mixed by a paddlewheel increasing turbulence, which together with the presence of lytic bacteriophages has been shown to affect the aggregation and abundance of prokaryotes in other aquatic systems (Maltis & Weinbauer 2009).

The bacterial abundance in HRAPs has been investigated using agar plating and plaque assays. However, these techniques fail to determine the diversity and abundance of bacteria and viruses, as less than 1% of bacterial species can be cultured and plaque assays require the isolation and culture of the specific host. Pande & Kost (2017) indicated that over 99% of all bacteria are unculturable as they depend on metabolites provided by symbiotic partners, so the remaining 1% are over-represented in cultured samples as only they can survive independently. Flow cytometry is a novel approach for determining microbial abundance in HRAPs, which can detect culturable and non-culturable microorganisms (van der Merwe *et al.* 2014). Flow cytometers use lasers to detect fluorescence of DNA, a proxy for DNA content, and scattered light from the particle as a proxy for particle size (Paterson *et al.* 2012). Flow cytometry has been shown to have a standard error of 5% for population abundances, compared to 2–58% using culturing and epifluorescence sampling in bacterial samples (Joachimsthal *et al.* 2003).

The results are reported here of a study using flow cytometry to observe and quantify changes in bacterial and viral abundance in an HRAP treating wastewater from the rural community of Kingston on Murray, South Australia. This project used flow cytometry and time series analysis to identify bacterial and viral population dynamics in the HRAP.

METHODS

Study site

Samples were collected from the Kingston on Murray HRAP in South Australia (34°14′33.19″S, 140°19′45.06″E). The 200 m^2 HRAP consisted of a single loop raceway (length 30 m, channel width 2.5 m), which was mixed (surface velocity 0.2 $m\,s^{-1}$) using an eight-bladed paddlewheel (Buchanan *et al.* 2018). The HRAP received wastewater from the community of 300 persons, pre-treated in on-site septic tanks. The total influent flow rate was 12 $m^3 day^{-1}$, delivered by six controlled pumping events each of approximately 2 m^3. The HRAP was operated at 0.3 m depth at a hydraulic retention time of 4.5 days.

Collection of wastewater samples

HRAP wastewater samples were collected twice daily at 03:00 and 15:00, each consisting of 150 mL, between the dates of 01/09/2017 (early spring) and 01/11/2017 (late

spring) close to the paddlewheel. These were refrigerated (4 °C) using an auto sampler (ISCO 5800 Teledyne, ISCO, Lincoln, NE, USA) and stored for up to 12 days before retrieval and transportation on ice for subsequent analysis.

Inlet wastewater samples were collected to determine the abundance of bacteria and viruses entering the HRAP system. Inlet samples were collected using grab samples at each collection trip. Inlet sample collection occurred at 09:00, 11:00, and 13:00 and was dependent on when wastewater was pumped to the treatment plant.

HRAP wastewater *in situ* monitoring of temperature, dissolved oxygen (DO), and pH

Temperature, DO and pH (Hach sc 100^{tm}) were measured continuously adjacent to the paddlewheel and the auto sampler suction tube. Data was collected every 15 minutes from 01/09/2017 to 01/11/2017 and logged using HOBOware software (HOBOware®).

Data for air temperature, light exposure and daily rainfall was obtained from the Bureau of Meteorology. Air temperature was obtained from the Renmark station (30.0 km distant). Light exposure and daily rainfall were obtained from the Kingston on Murray station (0.8 km; Australian Bureau of Meteorology 2017).

Analysis of wastewater

Ammonia (NH_4-N), and nitrite/nitrate (NO_x-N) were analysed, following 1:10 dilution of filtered wastewater samples with reverse osmosis water (Millipore Q), using a Foss Fiastar 5000 nutrient analyser (Foss Pacific Pty Ltd, North Ryde, NSW, Australia) and methods described in *Standard Methods for the Examination of Water and Wastewater* (Greenberg *et al.* 1992).

Flow cytometry

The abundance and changes in microbial population in the inlet and HRAP wastewaters were analysed using flow cytometry. Samples were mixed, and three 1 mL aliquots were taken and added to cryovials; 20 μL of glutaraldehyde (final concentration 0.5%) was added to fix samples. Cryovials were stored on ice for 15 minutes and snap frozen in liquid nitrogen. Samples were stored at −80 °C until required (Dann *et al.* 2016). Prior to analysis samples were thawed in warm water, diluted 1:10 for inlet wastewater samples and 1:100 for HRAP wastewater samples using 0.02 μm filtered TE buffer (10 mM Tris, 1 mM EDTA buffer, pH 7.4) and stained with SYBR-1 Green (Molecular Probes) 1:20,000 dilution using concentrated SYBR-1 Green stock and 0.02 μm filtered TE buffer (Dann *et al.* 2016). Three flow cytometry control tubes consisting of 0.5 mL of TE buffer and SYBR-1 Green were prepared to determine the background contamination of the reagents. SYBR-1 Green treated samples and the flow cytometry controls were incubated for 10 minutes at 80 °C in the dark. Two hundred microlitres of samples and flow cytometry controls were placed inside a microtiter plate. Fluorescent latex beads (1 μm diameter; Molecular Probes) were prepared using concentrated yellow-green bead stock and 0.02 μm filtered Millipore Q water at a final concentration of 10^5 beads mL^{-1}. Beads were used for calibrating the size of viruses and bacteria, and normalising bead fluorescence and determination of particle concentration (Paterson *et al.* 2012). All samples were analysed using a Cytoflex S flow cytometer (Beckman Coulter) for 2 minutes each on a low flow setting, approximately 10 μL min^{-1}. Results were recorded in CytExpert software (Beckman Coulter).

Data management and statistical analysis

Flow cytometry data was entered into CytExpert for approximate identification of bacterial and viral populations and exported into FlowJo software (© Tree Star) for further analysis. Viral and bacterial populations were determined via their position on each cytogram based on their intensity of violet side-angle scatter height, which is a proxy for particle size, and SYBR-1 Green fluorescence, which is a proxy for the amount of nucleic acid present in each particle (Paterson *et al.* 2012).

Three populations were identified in FlowJo and characterised as bacteria, virus-like particles (VLP) and large VLP (LVLP) based on SYBR-1 Green fluorescence and violet side-angle scatter height ratio. Raw data was entered as a single time series into Microsoft Excel (2016). Data for bacterial, LVLP and VLP abundance was $\log_{10}$ transformed to facilitate visualisation and comparison. Particle numbers ($\log_{10}$) were plotted in Microsoft Excel against time (h) to visualise the change in microbial abundance over the study period.

Pearson correlations and linear regression plots were conducted using the IBM Statistical Package for Social Sciences SPSS (SPSS Statistics for Windows, Version 25.0, released 2017. IBM Corp., Armonk, NY, USA)

RESULTS AND DISCUSSION

The objective of this project was to quantify bacterial and viral populations in an HRAP treating wastewater using flow cytometry and to determine wastewater quality and environmental factors influencing their change in abundance. Currently, there are few studies on microbial abundance in wastewater HRAPs. This study uniquely applied flow cytometry to determine changes in microbial abundance in an HRAP. The paucity of comparative data required comparisons to be made with other systems, including marine and fresh waters, to confirm the identity of the populations observed by the flow cytometry.

In Figure 1(a), two populations, bacteria and VLP, were identified in the inlet; significantly, the LVLP population was not detected. In Figure 1(b), three distinct regions were defined in the HRAP cytograms with VLP occurring in the bottom left corner, LVLP bottom right, and bacteria top right. A similar LVLP population (Figure 1(b)), has not been observed in marine or fresh water in previous studies (Paterson *et al.* 2012; Dann *et al.* 2016). Since an LVLP population was not observed in the inlet wastewater (Figure 1(a)) it has subsequently developed in the outdoor HRAP. The appearance of populations, which have grown in the HRAP and were not introduced in the inlet, was expected as it is inevitable that in an outdoor system microbial populations would be introduced from the external environment (Ibekwe *et al.* 2017).

Figure 2 shows that the bacterial, LVLP, and VLP population abundances fluctuated at the same rate over the time series suggesting these populations may have been interacting. This was expected as viruses are known to drive mortality of bacteria (Fuhrman 1999). At the sampling rate chosen for this experiment it was not possible to determine which population was 'leading' or 'lagging' in the relationship as these changes were occurring in under an hour (Seymour *et al.* 2005). Previous studies have shown both are possible as a high abundance of bacteria can allow greater infection by VLP thereby increasing VLP abundance (Jasna *et al.* 2019), and an increase in VLP can increase mortality in bacteria, reducing bacterial abundance (Brown *et al.* 2019).

There was a high rate of fluctuation in abundance in the bacterial, LVLP, and VLP samples, especially in the 0–600 h range, as the abundance counts between adjacent samples increased or decreased by 1.5 $\log_{10}$. Modelling reported by Thingstad (2000), suggested that this change in abundance could be driven by the viral host ratio reducing bacterial abundance in the system. As samples were taken twice daily these changes are occurring within a 24-hour period suggesting these abundances are dynamically changing. Sampling less than twice daily could miss important viral and bacterial interactions. Future investigations are required to determine the frequency of changes in relative microbial abundances.

During the majority of the time series the LVLP population had the lowest abundance except in the 900–1,400 h region when the LVLP particle abundance surpassed the bacterial abundance. However, from the data reported here the reason for this high LVLP abundance is unclear.

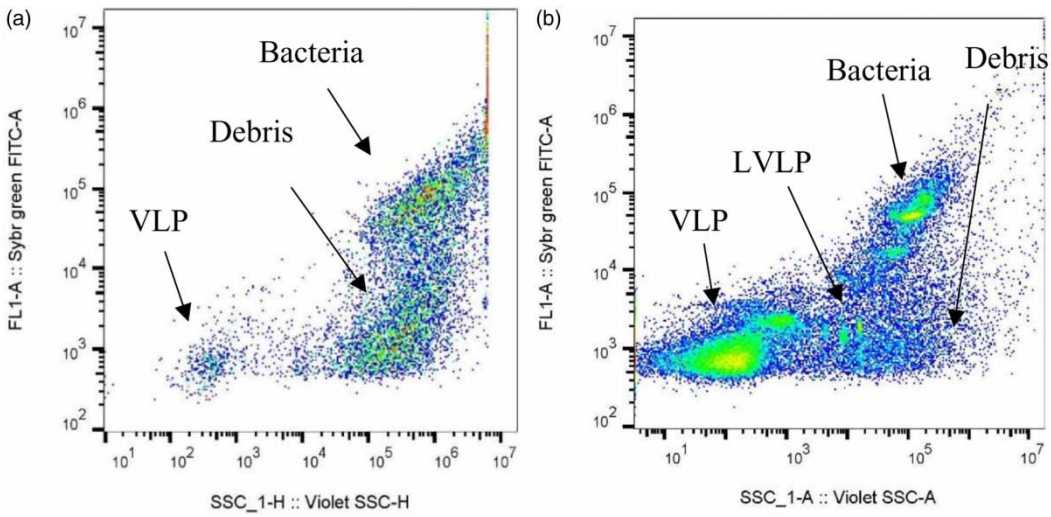

Figure 1 | (a) Example of a cytogram from a 1:10 dilution of inlet wastewater (12/09/2017) and (b) 1:100 dilution of HRAP wastewater (03:00, 23/09/2017) at Kingston on Murray. SYBR-1 Green is a proxy for quantifying DNA and violet side-angle scatter height (SSC-H) is a proxy for the particle size. Colour determines the abundance of particles in an area. Dark grey (blue in online version), low number of particles, to light grey (red in online version), high number of particles. Please refer to the online version of this paper to see this figure in colour: http://dx.doi.org/10.2166/wst.2020.379.

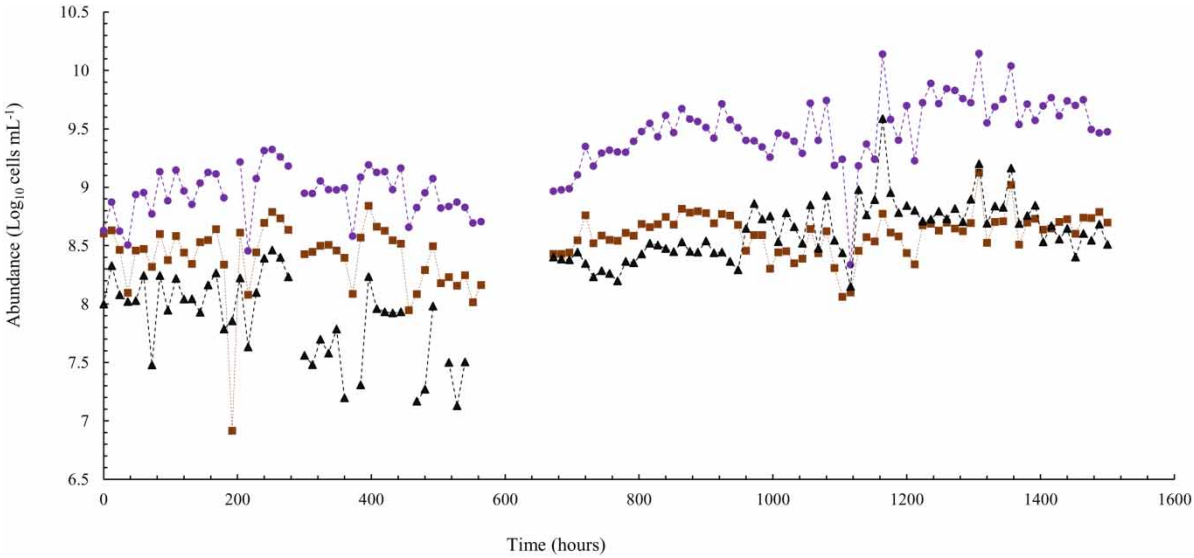

Figure 2 | Time series data for HRAP wastewater (01/09/2017–01/11/2017). Log_{10} transformed data cell counts. Bacteria (■), LVLP (▲), and VLP (●). Samples were taken twice daily at 03:00 and 15:00. The gap in data (564–672 h) was due to malfunction of autosampler.

At the same time (900–1,400 h) there were three events when the VLP abundance increased to 10^{10} particles mL^{-1}. This increase in VLP and LVLP abundance and decrease in bacterial abundance might be due to a shift in the other microbial populations within the system. Changes in microbial composition favouring viruses can result in the interference of microbial interactions and a reduction in metabolite production through the infection and/or eradication of bacterial and algal taxa (Murray & Jackson 1992). Due to the absence of any assessment of algal abundance it was not possible to determine if an algal interaction may have influenced this relationship. More research is required to understand the nature of the VLP increase and shift in bacteria and LVLP particle abundance.

The VLP had the highest abundance compared to bacteria and LVLP, ranging from 10^8 to 10^{10} in the HRAP system over the time series, which was expected as viruses usually are the most dominant microbe in environmental systems (Wommack & Colwell 2000). The high VLP abundance might be a result of either the prevalence of hosts including algae and bacteria (Coutinho *et al.* 2017), or an influx of host bacteria from the inlet (Ibekwe *et al.* 2017). However, in this HRAP system, although a significant seed population, there does not appear to be an influx of host bacteria coming in from the inlet as the bacterial abundance was significantly lower than the range in fluctuation. The average influx of bacteria was 6.96 log_{10} mL^{-1} and 6.31 VLP log_{10} mL^{-1}, suggesting this increase of VLP abundance was the result of growth of a host within the system.

In Figure 3 significant correlations were observed between bacterial, LVLP, and VLP abundance. The highest correlation (R^2 0.583; $p = <0.001$) identified was between the VLP and the abundance of the LVLP particles suggesting these populations may have similar microbial interactions within the system. The LVLP particle population, which appears to respond to a host within the system, was defined on genome and particle size and their positioning in the cytograms which was closer to previously observed VLP regions (Paterson *et al.* 2012; Dann *et al.* 2016). The LVLP population was not likely to have originated from the inlet wastewater, where it was not detected. The lowest correlation out of the three populations (R^2 0.299; $p = <0.001$) was between the LVLP and bacteria, from which it may be implied that this LVLP population was not a bacteriophage since bacteriophages have a close correlation with their host (Coutinho *et al.* 2017) and can account for up to 67% of the variance of bacterial abundance (Fuhrman 1999). However, the LVLP population still appears to have a microbial interaction with the bacteria, but this relationship may not be a direct relationship. Previous studies have shown that algae and bacteria have close interactions ranging from mutualistic and sharing resources (Šimek *et al.* 2011) to parasitic with the bacterium consuming resources the algae need (Ramanan *et al.* 2016). If the LVLP were an algal virus this could explain this correlation with the bacteria. Future investigation into this LVLP population is required.

The VLP and bacteria abundance significantly correlated (R^2 0.578; $p = <0.001$) suggesting some of the VLP

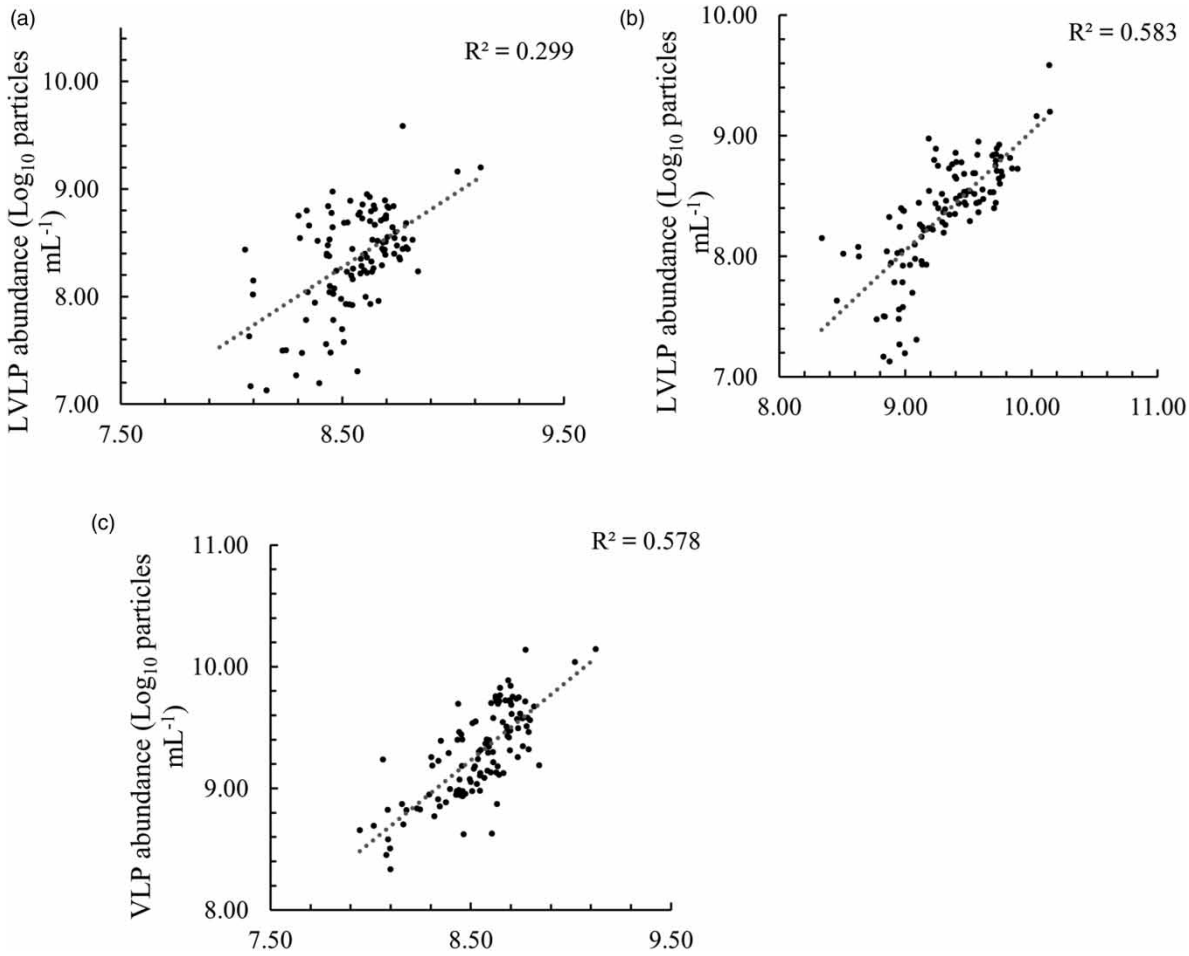

Figure 3 | Linear regression plot of population abundances in HRAP wastewater determined by flow cytometry (01/09/2017–01/11/2017). (a) Bacteria abundance (Log_{10} cells mL^{-1}) compared to LVLP particle abundance (Log_{10} particles mL^{-1}). (b) VLP abundance (Log_{10} particles mL^{-1}) compared to LVLP particle abundance (Log_{10} particles mL^{-1}). (c) Bacterial abundance (Log_{10} cells mL^{-1}) compared to VLP abundance (Log_{10} particles mL^{-1}).

present in the HRAP were bacteriophages expressing a potentially a parasitic relationship. Bacteriophages have a close relationship with their hosts and are known to drive host abundance (Coutinho *et al.* 2017). They could directly infect function microbes within the system, or they can form an indirect relationship by infecting the mutualistic or commensal bacteria partners and reducing their abundance. Future investigation should determine if this is a direct or indirect interaction.

Significant Pearson correlations ($p = <0.0009$) were observed between pH, DO and temperature, and bacterial, LVLP and VLP abundance (Supplementary data 1 and 2 in the Supplementary Information). Interestingly, only the viral particles showed a significant positive correlation with solar irradiance, which may imply mediation by photosynthetic microalgae. An increase of bacteria, LVLP and VLP abundance was observed during periods of high pH. Elevated pH and DO within HRAPs is associated with high rates of algal photosynthesis, suggesting this increase in abundance may be associated with an increase in other microbial groups' abundance including algae. Furthermore, high pH and DO values are associated with inactivation of faecal indicator organisms used as surrogates for pathogenic bacteria and viruses (Fallowfield *et al.* 1996). An increase in mutualistic or commensal partners could have facilitated an increase in the bacterial abundance (Ramanan *et al.* 2016), a consequent increase in host abundance resulting in an increase in LVLP and VLP population abundance (Fuhrman 1999). The highest abundances of bacteria, LVLP and VLP were observed during periods of low DO suggesting the increased abundance of bacteria and other microbes within the system was associated with lower DO availability.

Microbial assemblages have previously been shown to have shifts in community composition associated with changes in the concentration of nutrients and water chemistry of the system including NO_2-N, NO_3-N, NH_4-N, pH, temperature and DO (Newton *et al.* 2011). However, the Pearson correlation between bacterial and LVLP, VLP and LVLP, and bacterial and VLP abundance suggests there was a stronger influence between microbial populations than any of the environmental parameters measured within the system. In the literature changes in microbial abundance appear to explain more of the variation between population abundance than do environmental parameters (Fuhrman 1999).

The LVLP and VLP populations had more similar correlations with environmental parameters than the bacterial population, supporting analogous parasitic relationships. A significant negative Pearson correlation was observed between the combined concentration of nitrite and nitrate and the abundance of both the LVLP and VLP. This may be due to these nutrients mediating changes in potential hosts, including algae, within the wastewater (Buchanan *et al.* 2018). Temperature and light exposure had a significant positive Pearson correlation with the LVLP and VLP, from which it may similarly be inferred that light was mediating the abundance of a host, e.g. microalgae. No relationship was identified between the abundance of bacteria and combined nitrite and nitrate concentration, HRAP temperature or light exposure. Future investigation into the relationship algae have with these bacterial, LVLP, and VLP abundances could help identify the LVLP population and identify algae as another potential host in the HRAP system.

CONCLUSION

Flow cytometry successfully identified bacterial and VLP populations in the inlet system, and bacterial and viral populations present in the HRAP. In the HRAP an LVLP population of particles was also observed and considered to be a distinct VLP population. Bacterial, LVLP and VLP populations significantly correlated with each other and the relationships were probably driving their abundances; however, it was unable to be confirmed nor was it possible to identify which population was driving abundance in the system. It was implied from the results of the study that members of the VLP were bacteriophage which had formed a parasitic relationship with the bacteria in the HRAP. Bacteria, LVLP, and VLP correlated with certain environmental parameters suggesting microbial interactions and environmental parameters are jointly controlling the abundances of microbes. However, the highest correlations were observed with abundance suggesting these populations had a greater influence on each other's total abundance than did other factors within the system.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

REFERENCES

Australian Bureau of Meteorology 2017 *Weather Station Directory*. Available from: http://www.bom.gov.au/climate/data/stations/ (accessed 24 January 2018).

Borowitzka, M. A. 1999 Commercial production of microalgae: ponds, tanks, and fermenters. *Progress in Industrial Microbiology* **35**, 313–321.

Brown, M. R., Baptista, J. C., Lunn, M., Swan, D. L., Smith, S. J., Davenport, R. J., Allen, B. D., Sloan, W. T. & Curtis, T. P. 2019 Coupled virus–bacteria interactions and ecosystem function in an engineered microbial system. *Water Research* **152**, 264–273.

Buchanan, N. A., Young, P., Cromar, N. J. & Fallowfield, H. J. 2018 Performance of a high rate algal pond treating septic tank effluent from a community wastewater management scheme in rural South Australia. *Algal Research* **35**, 325–332.

Buhr, H. O. & Miller, S. B. 1983 A dynamic model of the high-rate algal-bacterial wastewater treatment pond. *Water Research* **17** (1), 29–37.

Coutinho, F. H., Silveira, C. B., Gregoracci, G. B., Thompson, C. C., Edwards, R. A., Brussaard, C. P., Dutilh, B. E. & Thompson, F. L. 2017 Marine viruses discovered via metagenomics shed light on viral strategies throughout the oceans. *Nature Communications* **8**, 15955.

Dann, L. M., Paterson, J. S., Newton, K., Oliver, R. & Mitchell, J. G. 2016 Distributions of virus-like particles and prokaryotes within microenvironments. *PloS ONE* **11** (1), e0146984.

Fallowfield, H. J., Cromar, N. J. & Evison, L. M. 1996 Coliform die-off rate constants in a high rate algal pond and the effect of operational and environmental variables. *Water Science & Technology* **34**, 141–147.

Faust, K. & Raes, J. 2012 Microbial interactions: from networks to models. *Nature Reviews Microbiology* **10** (8), 538.

Fuhrman, J. A. 1999 Marine viruses and their biogeochemical and ecological effects. *Nature* **399** (6736), 541–548.

Ghoul, M. & Mitri, S. 2016 The ecology and evolution of microbial competition. *Trends in Microbiology* **24** (10), 833–845.

Greenberg, A., Clesceri, L. & Eaton, A. 1992 *Standard Methods for the Examination of Water and Wastewater*. American Public Health Association/American Water Works Association/ Water Environment Federation, Washington, DC, USA.

Ibekwe, A. M., Murinda, S. E., Murry, M. A., Schwartz, G. & Lundquist, T. 2017 Microbial community structures in high rate algae ponds for bioconversion of agricultural wastes from livestock industry for feed production. *Science of the Total Environment* **580**, 1185–1196.

Jasna, V., Parvathi, A., Aswathy, V., Aparna, S., Dayana, M., Aswathy, A. & Madhu, N. 2019 Factors determining variations in viral abundance and viral production in a tropical estuary influenced by monsoonal cycles. *Regional Studies in Marine Science* **28**, 100589.

Joachimsthal, E. L., Ivanov, V., Tay, J. H. & Tay, S. T. L. 2003 Flow cytometry and conventional enumeration of microorganisms in ships' ballast water and marine samples. *Marine Pollution Bulletin* **46** (3), 308–313.

Lusk, M., Toor, G. S. & Obreza, T. 2014 *Onsite Sewage Treatment and Disposal Systems: Viruses1. Series of the Soil and Water Science Department, UF/IFAS Extension*.

Maltis, A. & Weinbauer, A. G. 2009 Effect of turbulence and viruses on prokaryotic cell size, production and diversity. *Aquatic Microbial Ecology* **54**, 243–254.

Murray, A. G. & Jackson, G. A. 1992 Viral dynamics: a model of the effects of size, shape, motion and abundance of single-celled planktonic organisms and other particles. *Marine Ecology Progress Series* **89** (2/3), 103–116.

Newton, R. J., Jones, S. E., Eiler, A., McMahon, K. D. & Bertilsson, S. 2011 A guide to the natural history of freshwater lake bacteria. *Microbiology and Molecular Biology Reviews* **75** (1), 14–49.

Pande, S. & Kost, C. 2017 Bacterial unculturability and the formation of intercellular metabolic networks. *Trends in Microbiology* **25** (5), 349–361.

Paterson, J. S., Nayar, S., Mitchell, J. G. & Seuront, L. 2012 A local upwelling controls viral and microbial community structure in South Australian continental shelf waters. *Estuarine, Coastal and Shelf Science* **96**, 197–208.

Ramanan, R., Kim, B. H., Cho, D. H., Oh, H. M. & Kim, H. S. 2016 Algae–bacteria interactions: evolution, ecology and emerging applications. *Biotechnology Advances* **34** (1), 14–29.

Seymour, J. R., Seuront, L. & Mitchell, J. G. 2005 Microscale and small-scale temporal dynamics of a coastal planktonic microbial community. *Marine Ecology Progress Series* **300**, 21–37.

Šimek, K., Kasalický, V., Zapomělová, E. & Horňák, K. 2011 Algal-derived substrates select for distinct betaproteobacterial lineages and contribute to niche separation in *Limnohabitans* strains. *Applied and Environmental Microbiology* **77** (20), 7307–7315.

Ternes, T., Joss, A. & Oehlmann, J. 2015 Occurrence, fate, removal and assessment of emerging contaminants in water in the water cycle (from wastewater to drinking water). *Water Research* **72**, 1–2.

Thingstad, T. F. 2000 Elements of a theory for the mechanisms controlling abundance, diversity, and biogeochemical role of lytic bacterial viruses in aquatic systems. *Limnology and Oceanography* **45** (6), 1320–1328.

van der Merwe, R., Hammes, F., Lattemann, S. & Amy, G. 2014 Flow cytometric assessment of microbial abundance in the near-field area of seawater reverse osmosis concentrate discharge. *Desalination* **343**, 208–216.

Wommack, K. E. & Colwell, R. R. 2000 Virioplankton: viruses in aquatic ecosystems. *Microbiology and Molecular Biology Reviews* **64** (1), 69–114.

Young, P., Buchanan, N. & Fallowfield, H. 2016 Inactivation of indicator organisms in wastewater treated by a high rate algal pond system. *Journal of Applied Microbiology* **121** (2), 577–586.

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