Small- and large-scale distribution of microbes and biogeochemistry in the Great Barrier Reef

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Microbial communities distribute heterogeneously at small-scales (mm-cm) due to physical, chemical and biological processes. To understand microbial processes and functions it is necessary to appreciate microbes and matter at small scales, however, few studies have determined microbial, viral, and biogeochemical distribution over space and time at these scales. In this study, the small and large-scale spatial and temporal distribution of microbes (bacteria and chlorophyll *a*), viruses, dissolved inorganic nutrients and dissolved organic carbon were determined at five locations (spatial) along the Great Barrier Reef (Australia), and over 4 consecutive days (temporal) at a coastal location. Our results show that: 1) the parameters show high small-scale heterogeneity; 2) over the large scale the coastal location showed the highest average concentrations/abundances; 3) none of the parameters measured explained the bacterial distributions at these scales spatially or temporally; 4) chemical (ammonium, nitrate/nitrite, phosphate, dissolved organic carbon, and total dissolved nitrogen) and biological (chl *a*, bacteria, and viruses) measurements did not reveal significant relationships at the cm scale; and 5) differences were found between sites/days but without a clear pattern.

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Abstract

Microbial communities distribute heterogeneously at small-scales (mm-cm) due to physical, chemical and biological processes. To understand microbial processes and functions it is necessary to appreciate microbes and matter at small scales, however, few studies have determined microbial, viral, and biogeochemical distribution over space and time at these scales. In this study, the small and large-scale spatial and temporal distribution of microbes

(bacteria and chlorophyll *a*), viruses, dissolved inorganic nutrients and dissolved organic carbon were determined at five locations (spatial) along the Great Barrier Reef (Australia), and over 4 consecutive days (temporal) at a coastal location. Our results show that: 1) the parameters show high small-scale heterogeneity; 2) over the large scale the coastal location showed the highest average concentrations/abundances; 3) none of the parameters measured explained the bacterial distributions at these scales spatially or temporally; 4) chemical (ammonium, nitrate/nitrite, phosphate, dissolved organic carbon, and total dissolved nitrogen) and biological (chl *a*, bacteria, and viruses) measurements did not reveal significant relationships at the cm scale; and 5) differences were found between sites/days but without a clear pattern.

Introduction

Marine bacterioplankton and phytoplankton and their associated functions are the primary controls of energy and material cycling in the global ocean. How they interact with the environment is therefore of pivotal importance for understanding ocean food web structure and biogeochemical processes (Wiens, 1989). Depending on the process to be studied, the scale of spatial resolution has to be adjusted accordingly. As microbes interact at the cellular level, it is essential to describe microbial community ecology at small scales (mm to cm) to capture the microbial functions and productivity in marine environments (Azam and Malfatti, 2007). There is evidence that microbes distribute heterogeneously at small scales in marine environments (Azam and Long, 2001), which has been linked to biological factors (e.g., grazing, lysis), and interactions between microbes and the environment (e.g., organic matter, aggregates) (e.g., Seymour et al., 2006;Stocker et al., 2008). Viruses are estimated to kill between 20-40% of the prokaryotic community every day, with major consequences for the microbial diversity and carbon cycling (Suttle, 2005). However virus-microbes's relationship

is not always straightforward. Viruses are typically tightly coupled with bacterial communities when large datasets are used (Wigington et al., 2016), however when small datasets or small volumes are used, bacteria and viruses are not coupled (Bouvy et al., 2012;Carreira et al., 2013). This difference is probably a result of the time lag between infection and replication which is easier to observe at smaller scales and volumes (Carreira et al., 2013). It has also been demonstrated that prokaryotes can move towards a chemical cue (chemotactic behaviour), as a response to point sources of organic and inorganic matter (Malmcrona-Friberg et al., 1990;Hütz A, 2011). This chemotactic behaviour has been suggested to increase the microbial degradation of dissolved organic matter (DOM) (Fenchel, 2002), and heterogeneous environments are suggested to have higher phytoplankton production than found under homogeneous condition (Brentnall et al., 2003). Such findings have implications for the way we frame marine biogeochemical cycling by microbes. Models have been used previously to understand the interaction between microbes and organic matter at small scales (e.g., Datta et al., 2015), while other studies have used controlled experiments (e.g., Brumley et al., 2019), and measured microbial distribution at small scales in a natural ecosystem (e.g., Seymour et al. 2005). But none of these have measured the chemical (organic and inorganic) components interacting with the microbes at small scales in a natural ecosystem.

The Great Barrier Reef (GBR) is situated on the continental shelf and slope of Australia's north-eastern coast and is the largest contiguous coral reef system in the world. The GBR has a total of ~ 3700 reefs which are mainly located away from shore; with the open water body separating the reef matrix from the mainland known as the GBR lagoon. The system is characterized by stable high temperatures, oligotrophic, sunlit, and alkaline waters (Furnas et al., 2011;Uthicke et al., 2014). The microbial patchiness at the cm scale has been studied by Seymour et al. (2005) on one reef, demonstrating a 2- to 3.5-fold changes in the

viral and bacterial concentrations over a distance of 12 cm above coral colonies. This microbial heterogeneity suggests that small-scale interactions could be important in understanding the microbial ecology and biogeochemistry of this system. But it remains to be understood how representative these single measurements are for other locations in the GBR and how this might vary over temporal scales.

In this study we determined the spatial and temporal variability in the small-scale distribution of microbes (bacteria, and chlorophyll *a* – a proxy for phytoplankton biomass), and viruses, as well as biogeochemical variables (dissolved inorganic nutrients and dissolved organic carbon) at five locations along the GBR, and over 4 consecutive days at a coastal location.

Material and methods

Study sites and sampling

Samples were collected at six sites spanning from coast to the outer reef in the Great Barrier Reef (GBR; Fig 1; Table 1). Sites 1, 2, 3, and 5 were at coral reefs, site 4 was in the Coral Sea, and site 6 was at the Australian Institute of Marine Science (AIMS) harbour (Bowling Green Bay; Fig 1). Site 4 (Coral Sea) was used as a reference non-coral site. Site 6 (Bowling Green Bay) is located in the inner zone of the GBR, has no coral coverage and is dominated by a nearby saltmarsh and small river. All coral reef sites showed clumps of floating *Trichodesmium* spp. at the surface (Carreira pers. observ.) at the time of sampling. To determine the spatial variability in the small-scale distribution of microbes, viruses and biogeochemical measurements, surface water samples were collected once at sites 1 to 5 (Fig 1, Table 1; 17 to 22 December 2014). The temporal variability in the small-scale distribution was determined at site 6 with samples collected during high tide every 24h over four

consecutive days (Fig 1, Table 1; 12 to 15 January 2015). Although 24h is not a temporal small scale, the objective was only to understand the changes in spatial small scale over time. Niskin bottle samples collected at site 6, at the same time and days as the temporal study (days 1 to 4), were used as controls for the standard sampling method.

Samples were collected with a purpose built device consisting of 25 inlets (5 x 5) (Fig 2). With the help of a lever, all samples were collected manually, at the same time from 0.5 m below the sea surface with the sampling taking about 5 seconds. As our objective was to understand small-scale heterogeneity in the coral reef system, samples from sites 1, 2, 3, and 5 were taken above the coral reefs, but not in the proximity of a coral as done by Seymour et al. (2005). Each inlet was connected to a 25 mL syringe each separated by 7 cm, representing a total sampling area of 784 cm^2. This distance between the syringes was calculated to account for the volume necessary for all measurements (25 mL) without interfering with neighbouring sampling volumes. In this calculation we assume that the rapid intake of water by the syringes is similar in shape to a turbulent jet (Pope, 2000). The following equations were used for the calculation:

$$V = \pi \times r^2 \times \frac{h}{3} \quad (1)$$

$$Tan\ \theta = \frac{r}{h} \quad (2)$$

$$r = \left(\frac{3 \times V \times tan\ \theta}{\pi}\right)^{\frac{1}{3}} \quad (3)$$

we combined equations 1 and 2 to obtain the minimum distance (r) between syringes (equation 3, Fig S1). Equation (1) calculates the volume of a cone (V), which is the water rapidly sucked up (‘turbulent jet’) by the syringe and equation (2) takes into account its shape. This allowed to calculate the distance (r) using a known angle of 11.8° (equation (3); Fig S1)

125 (Pope, 2000;Cushman-Roisin, 2019). This angle is always the same independent of the fluid
126 used (Cushman-Roisin, 2019).
127 At sites 1 to 5 temperature and salinity were recorded using a conductivity-
128 temperature-depth (CTD) profiles (Seabird SBE19Plus). At site 6 salinity samples were
129 collected and analysed in the laboratory with a Portasal Model 8410A, while temperature was
130 measured manually. Salinity and temperature varied between 32.0 and 35.6, and 28.7 and 32.0
131 ºC, respectively (Table 1). From each syringe samples were collected for dissolved inorganic
132 nutrients (ammonium - NH_4^+, nitrate/nitrite - NO_3^-/NO_2^-, and phosphate - HPO_4^{2-}), dissolved
133 organic carbon (DOC), total dissolved nitrogen (TDN), chlorophyll *a* (chl *a*), and bacterial
134 and viral counts. A precombusted (450°C, 4 h) GF/F filter (13 mm diameter) was used to
135 filter 5 mL for inorganic nutrients analysis, and 10 mL of seawater for DOC and TDN
136 analysis. DOC and TDN samples were fixed with 50 µL of 25 % H_2PO_4 and kept at 4 ºC,
137 whereas inorganic nutrients were filtered and kept at -20 ºC until analysed. The GF/F filters
138 used for collecting inorganic nutrients, DOC, and TDN samples were snap-frozen in liquid
139 nitrogen and kept at -20 ºC for chl *a* extraction. All syringes, filter-holders and inorganic
140 nutrient sample tubes were acid-washed in 10 % HCl for 24 h, and then washed three times
141 with Milli-Q water before use.
142 For bacterial and viral counts, unfiltered subsamples of 1 mL, were collected in sterile
143 2 mL Eppendorf tubes and fixed with 0.5 % glutaraldehyde final concentration (25 % EM-
144 grade, Merck) for 15 min at 4 °C, after which samples were snap-frozen in liquid nitrogen and
145 stored at -80 ºC until analysis by flow cytometry (FCM).
146
147 **Samples analysis**
148 Inorganic nutrients (NH_4^+, NO_3^-/NO_2^- and HPO_4^{2-}) were determined by standard
149 segmented flow analysis (SFA) as described in Hansen & Koroleff (1999). As all the

150 determined NH_4^+ concentrations were below the detection limit of the method (<0.02 µmol L^{-1}) the data is not shown. The detection limit and precisions for the other parameters were: 0.02 µmol L^{-1} for NO_3^-/NO_2^- and 0.001 µmol L^{-1} for HPO_4^{2-}. Please note that the HPO_4^{2-} concentrations at site 2 were also below the detection limit and therefore the data is not shown. DOC and TDN concentrations were measured using a Shimadzu TOC-L carbon analyser coupled in series with a nitric oxide chemiluminescence detector according to Lønborg et al. (2018). Three to five replicate injections of 150 µL were performed per sample. Concentrations were determined by subtracting a Milli-Q blank and dividing by the slope of a daily standard curve of potassium hydrogen phthalate and glycine. Using the deep ocean reference (Sargasso Sea deep water, 2600 m) we obtained a concentration of 45.6 ± 1.8 µmol L^{-1} (average ± SD) for DOC and 22.0 ± 1.5 µmol L^{-1} for TDN. Please note that the TDN measurements for day 2 and 3 of the temporal study are not reported due to problems with the gas supply for the nitric oxide chemiluminescence detector during these specific sample runs. The detection limit for DOC and TDN were 8 µmol L^{-1} and 0.02 µmol L^{-1}, and the precisions were ± 1 µmol L^{-1} and ± 0.3 µmol L^{-1}, respectively.

Chl *a* determinations were made by extracting the GF/F filters in ethanol (96 %) for 8 h. Samples were analysed spectrophotometrically according to Strickland & Parsone (1972). The dectection limit and precision for the chl *a* method were 0.005 µg L^{-1} and ± 0.05 µg L^{-1}, respectively. Flow cytometric (FCM) enumeration of bacteria and viruses was carried out using a standard bench top Becton-Dickinson FACSVerse FCM, equipped with an air-cooled argon laser (excitation 488 nm, 15 mW power) according to Gasol et al. (1999) and Brussaard (2004) for bacteria and viruses, respectively. Samples were diluted (5-60 times) in TE buffer (Tris 10 mM, EDTA 1 mM, pH 8.0), stained with SYBR Green I (Molecular Probes®, Invitrogen Inc., Life Technologies™, NY, USA) to a final concentration of 10^{-4} of the commercial stock solution. Bacterial samples were incubated at ambient temperature, whereas

175 viral samples were incubated at 80 ºC (Brussaard, 2004), both in the dark for 10 min. The
176 trigger was set for green fluorescence and the data was analysed using Flowing Software
177 2.5.1. (freeware; http://flowingsoftware.btk.fi). The event rate was 300 bacteria s^{-1} and
178 between 300-800 viruses s^{-1} to avoid coincidence (Gasol et al., 1999;Brussaard, 2004). We
179 would like to note that recent research (e.g., (Forterre et al., 2013) has suggested that viral
180 counts might also include gene transfer agents (GTAs), membrane -derived vesicles (MVs),
181 or even cell debris that might be confused with viruses. However currently there is no method
182 to distinguish between all these particles, therefore, we assumed that the viral counts made by
183 FCM are viruses.
184 Inorganic nutrients, DOC, TDN, and chl *a* concentration, and bacterial and viral
185 abundances data were plotted using Surfer 9.0.
186
187 **Statistical analyses**
188 To measure small-scale heterogeneity within each site/day it was used the coefficient
189 of variation (CV) calculated as the (Standard deviation/Mean) × 100.
190 To understand differences in concentrations/abundance between sites/days, boxplots
191 were made using the average values for each site/day. To compare the distributions of the
192 independent samples, the non-parametric Kruskall-Wallis tests were performed for the spatial
193 and temporal data sets because for each variable at least one subgroup (for site or day) failed
194 the normality condition for parametric tests (Agresti, 2007), i.e., the values of each variable
195 did significantly change over the locations. Multiple comparison tests were also performed to
196 understand which pairs of sites/days had the biggest differences. For these pairwise
197 comparisons two tests were performed: Nemenyi tests with Chi-squared approximation and
198 the Dunn´s tests for multiple comparisons with the Bonferroni adjustment method (Dun,
199 1964).

To identify which variables were more linearly correlated, and determine the correspondent magnitude, Pearson correlation coefficient were calculated, considering each site and all sites combined as well as each day and all days combined.

To understand the relation between the parameters, independent of the site and day, factor analysis was applied to the data. A factor analysis is used to describe an eventual correlation between several observed variables in regard to another group of non-observed variables, of smaller dimension, named factors (Johnson and Wichern, 2007). To perform the factor analysis all variables were considered, regardless of site, as there were no significant correlations between the variables, according to Bartlett's test of sphericity (p value<0.001). In order to classify the variables cluster analyses were performed in the spatial and temporal data. For the temporal data cluster analysis was tested, but without meaningful results. For the statistical analyses R (1.1.442) and SPSS (v25) software were used.

Results

Small scale variability

Using the coefficient of variability (CV) as a measure of heterogeneity, generally, in the spatial and temporal studies, there was a high small-scale heterogeneity (up to 76 % for chl *a*) for chl *a*, NO_3^-/NO_2^- and HPO_4^{2-} and lower heterogeneity for DOC, bacteria and viruses. With the exception of chl *a*, the chemical variables were more variable, than the biological within each site and day (Table 2 and 3). Next is given a description of the small-scale variability for each parameters for the sites and days measured.

In the spatial study of NO_3^-/NO_2^-, site 5 showed the lowest heterogeneity (10 %), while site 1 had the highest (37 %; Table 2, Fig. 3). In the temporal study, the highest variability was observed at day 1 (45 %) and lowest at day 3 (10 %) (Table 3, Fig 5).

225 Maximum differences observed between two nearby points in the spatial and temporal studies
226 were of 2.6 x and 3.6 x, respectively (Fig 3 and 5). Also site 1 showed the highest
227 heterogeneity in HPO_4^{2-} concentrations (27 %), and sites 4 and 5 the lowest (20%; Table 2,
228 Fig 3). In the temporal study the heterogeneity was highest at day 4 (26 %), and lowest at day
229 3 (10 %; Table 3, Fig 5). The maximum variability between nearby points was of 2.4 x, both
230 spatially and temporally. DOC concentrations showed the highest heterogeneity at sites 2 and
231 3 (13 %; Table 2, Fig 3), while the lowest was found at site 5 (6 %). DOC showed the lowest
232 heterogeneity of all measured parameters at all sites. The DOC concentrations in the temporal
233 study were higher than in the spatial study, but the heterogeneity was lower. The highest
234 heterogeneity was of 5 % at day 3, and the lowest just 4 % all other days (Table 3, Fig 5). A
235 maximum variability of 1.5 x and 1.2 x between two nearby point was found spatially and
236 temporally. Finally, TDN varied most at site 3 (24 %) and least at site 4 (9 %; Table 2, Fig 3).
237 A maximum variability of 3 x was found between points. In the temporal study, TDN was
238 only measured on days 1 and 4, and the heterogeneity was low in those two days measured (9
239 and 6 %).

240 Chl *a* showed the highest variability of all parameters, with the highest heterogeneity
241 at site 5 (68 %) and the lowest at site 2 (43 %; Table 3, Fig 6). In the temporal study the
242 heterogeneity was higher than found in the spatial study, with a maximum at day 3 (76 %).
243 Differences between nearby points were 7.9 x and 25.5 x, spatially and temporally.

244 Bacterial and viral abundances showed generally similar and low heterogeneity both
245 spatially and temporally, with viral abundances being nearly 1 order of magnitude higher than
246 bacteria. Bacterial abundances showed similarly low heterogeneity at site 2 and day 1 (4 %
247 and 5 %), and high at site 3 and day 3 (15 % and 19 %; Table 2 and 3, Fig 4 and 6). Viral
248 abundances showed lowest heterogeneity at sites 1 and day 1 (6 % and 9 %), while highest
249 heterogeneity was found at sites 2 and 3, and day 2 (15 % and 13 %; Table 2 and 3, Fig 4 and

250 6). Finally, the VBR showed low heterogeneity at sites 4 and 5 (9 %) and day 1 (10 %), and
251 highest heterogeneity was observed at site 3 (15 %) and day 3 (20 %; Table 2 and 3, Fig 4 and
252 6).

253 Overall, although no clear pattern emerged, site 3 (furthest north) and day 3 (high
254 nutrient concentrations) had most parameters with highest heterogeneity, while site 5 (furthest
255 south) and day 1 (low nutrient concentrations) had most parameters with the lowest
256 heterogeneity.

257

258 **Large scale variability**

259 All sites showed comparable concentrations/abundances overall, with the exception of
260 bacterial and viral abundances at the non-coral site 4 (Coral Sea) that were on average 1.8 x
261 and 2.6 x lower compared to the other sites (Table 2, Fig 4). Over the four days, nutrient
262 concentrations increased while bacterial and viral abundances decreased, and chl *a* and VBR
263 showed no differences (Table 3, Fig 6, Fig S2). The inorganic nutrients (NO_3^-/NO_2^- and
264 HPO_4^{2-}) showed comparable average concentrations between the outer reef and Coral Sea
265 sites (Table 2, Fig 3), and to site 6 (temporal study; Table 3; Fig 5), except for day 4 when
266 concentrations increased by 11.0 x and 1.4 x compared to day 1 (lowest concentrations, but
267 still comparable to the sites). DOC was slightly higher at site 6 (average range over the 4
268 days: 100 ± 4 µmol L^{-1} to 118 ± 6 µmol L^{-1}; Table 3, Fig 5), compared to the coral sites
269 (average range over sites 1, 2, 3, and 5: 83 ± 11 µmol L^{-1} to 90 ± 9 µmol L^{-1}) and Coral Sea
270 site (89 ± 8 µmol L^{-1}; Table 2, Fig 3). TDN showed slightly higher concentrations at site 6
271 (particularly day 4) compared to the other sites (Table 2 and 3, Fig 3 and 5). Chl *a* was on
272 average lower at site 6 compared to all other sites (Table 2 and 3, Fig 4 and 6). Bacterial and
273 viral abundances were on average higher at site 6 (total average of the 4 days: 18.1 ± 3.1 x 10^5
274 mL^{-1} and 104.8 ± 17.3 x 10^5 mL^{-1}, respectively; Table 3, Fig 6), compared to the coral sites

(average range: 7.8 ± 0.5 x 10^5 mL^{-1} to 12.5 ± 1.9 x 10^5 mL^{-1}; and 41.3 ± 2.7 x 10^5 mL^{-1} to 58.2 ± 8.8 x 10^5 mL^{-1} respectively; Table 2, Fig 4). Overall site 6 (temporal study) showed either similar or slighly higher concentrations/abundances when compared to the other sites (coral sites and non-coral - Coral Sea), but these results should be taken with cautions as there was no temporal follow-up at sites 1 to 5.

Comparing the concentrations and abundances obtained with a Niskin bottles during the temporal study (Table S5) with the range of values for each parameter over the 4 days (Table 3), the values are generally within these ranges, hence also showing the increase in nutrients and decrease in bacterial and viral abundances over the 4 days.

For each variable (NO_3^-/NO_2^- , HPO_4^{2-} DOC, Chl *a*, TDN, bacteria, viruses) pairwaise comparisons between sites and days were performed. The Kolmogorov-Smirnov tests showed that the spatial and temporal data are not normally distributed for each variable. Concerrning each variable in the study, the Kruskall-Wallis test revealed that at least one of the samples for each site/day is significantly different from the others. However, the pairwise comparisons using suitable non-parametric tests for multiple comparisons (Nemenyi-tests with Chi-squared approximation and Dunn´s tests) did not point to a common pattern, nonetheless the p values are shown in the supplement material (Table S3 and S4). Overall, statistically significant differences were found, but there were no clear patterns spatially or temporally as determined by non-parametric tests, i.e., no site and day or combinations of sites and days showed a trend or similar behaviour for all the parameters or a subsection of these (Fig 7 and 8). DOC showed the least statistical differences between sites (Fig 7, Table S3), while chl *a* showed the least differences between days (Fig 8, Table S4).

Correlations were determined between all parameters within a site/day and between sites/days without any clear results (Table S1 and S2). Most correlations did not exhibit a strong linear relationship between the variables in study (Table S1 and S2). However it can be

highlighted that spatially, bacteria correlated negatively with HPO_4^{2-} and viruses (n = 25, R^2 = -0.54 and 0.55, respectively) and temporally bacteria correlated negatively with NO_3^-/NO_2^- and HPO_4^{2-} (n = 25, R^2 = -0.75 and -0.50). The correlation between all bacterial and all viral abundances (spatial and temporal) showed a positive correlation (n = 325, R^2 = 0.75; Fig 9). This correlation showed an intercept not significantly different from zero, indicating a tight link between viruses and bacteria.

Although no relations were found between the parameters at the different sites and days, factor analysis was applied to understand the relation between the parameters. The factor analysis showed that the variables can be decomposed into two factors, the chemical (NO_3^-/NO_2^- and DOC) and biological (bacteria, chl *a* and viruses) groups (Fig. S3). It should be noted that HPO_4^{2-} and TDN were excluded from this analysis because the correspondent anti-image matrices values were smaller than 0.5 (0.340 and 0.372, respectively) meaning that we are discarding these variables since its partial correlation values are considered too small to apply factorial analysis. Overall these results show: 1) that the chemical variables (NO_3^-/NO_2^- and DOC) are more related to each others than to the biological variables (bacteria, chl *a* and viruses), and likewise for the biological variables, 2) the chemical variables do not explain the bacterial distribution, and 3) given the biological variables are more related to each others, there is a higher likelihood they could explain the bacterial distribution, but the results are insufficient to make a firm conclusion. Cluster analysis showed (Fig. S4A) a clear grouping between the biological (bacteria, viruses and chl *a*) and the chemical (NO_3^-/NO_2^-, HPO_4^{2-}, DOC and TDN) between all sites, whereas the classification was less clear between the days (Fig. S4B). Here bacteria and viruses grouped together, while DOC, chl *a*, NO_3^-/NO_2^- and HPO_4^{2-}) clustered. Please note that the TDN data was not included in this analysis as there was no data for days 2 and 3. These results suggest that the measured scales (cm) are

most probably not appropriate to understand the relation between nutrients (organic and inorganic), and the microbial and viral communities.

Discussion

A major challenge in microbial ecology is to understand how microbial communities are influenced by changing environmental conditions. To date, however, most studies have explored these links using both larger volumes (litres) and spatial scales (km), ignoring that the water column is in fact heterogeneous at smaller scales (Azam and Malfatti, 2007). Previous theoretical and laboratory based studies have suggested that both microbes and their growth substrates (DOC, inorganic nutrients) have a variable distribution at small scale (Blackburn et al., 1998;Blackburn and Fenchel, 1999), but few studies have actually determined this combined heterogeneity in field studies (Seymour et al., 2006). Our study shows the first *in-situ* heterogeneous 2-dimensional distribution of chemical (ammonium, nitrate/nitrite, phosphate, dissolved organic carbon, and total dissolved nitrogen) and biological (chl *a*, bacteria, and viruses) variables at the cm scale over spatial and temporal scales. Nonetheless the authors are aware that the small scale of observation used in this study (cm) does not fully replicate the differences in scale of interactions between the biogeochemistry and microbes. However this study is intended as a first approach to understand these interactions and more detailed studies at smaller scales are therefore needed. Furthermore, we have used this large dataset with 25 replicates per site/day to compare the data between sites and days.

At the resolution of our measurements none of the variables (NO_3^-/NO_2^-, HPO_4^{2-}, DOC, TDN, chl *a*, or viruses) explained the small-scale distribution of bacteria at the studied sites and days (Table S1 and S2). Nonetheless site 3 (furthest north) showed more parameters with higher heterogeneity, compared to site 5 (furthest south). At site 6 (Bowling Green Bay),

day 3 showed more parameters with higher heterogeneity compared to day 1. The increase of nutrients in Bowling Green Bay, observed at day 3, could explain the higher heterogeneity observed at this day, perhaps as a results of chemotactic behaviour by the microbes in search of food (Malmcrona-Friberg et al., 1990;Hütz A, 2011).

The high variability of chl *a* (indicative of phytoplankton biomass) and nutrients found in both the spatial and temporal studies could be caused by distinct heterogeneous microenvironments created by 'Phycosphere' patches (nutrient rich areas surrounding phytoplankton cells resultant from their excretion), suggested to be hotspots for bacterial growth (Stocker and Seymour, 2012). However, bacterial growth was not measured in our work, and no clear link between chl *a* or nutrients and bacterial abundances was observed at the scale sampled. Additionally, the low variability observed for bacteria, viruses and DOC might suggest that the sample sizes collected for analysis (1 and 10 mL) is too large to determine heterogeneity, but it could also be due to that most DOC is refractory and large proportions of cells may be dormant (Giorgio and Scarborough, 1995;Lønborg et al., 2018). Furthermore, the distribution of biological and chemical variables in the ocean are known to be impacted by processes occurring at a range of scales; for example, at the centimetre scale marine snow formation is important, while at kilometre scales fronts and eddies can shape the distribution of both chemical and biological variables (Kiørboe, 2001;Jickells et al., 2008;Baltar and Aristegui, 2017).

The concentrations of the chemical parameters and chl *a* were comparable to previous studies in the GBR (Furnas et al., 2005;Lønborg et al., 2018). Viral and bacterial abundances found in our study were within the estimates found for middle shelf reef surface waters in the GBR (Alongi et al., 2015), but about 4 - 6 and 5 - 7 x higher than those determined from a coastal coral reef in the GBR (Seymour et al., 2005), respectively. The Niskin bottle samples taken at site 6 (temporal study) also showed concentrations and abundances within the ranges

of the small-scale sampling. However, sampling using a Niskin bottle clearly misses the high small-scale heterogeneity determined using the cm scale device from the present study. Overall, the average concentrations from the temporal study were higher than in the spatial study, which was expected as the temporal station was closer to shore.

On the whole, no pattern could be statistically detected, suggesting that the controlling factors and dynamics were different between sites and days. Some variability in the spatial study could be attributed to the differences in sampling time as our temporal study also showed differences. However the differences observed between days (Table 3; Fig S2) are comparable to the differences observed between sites (Table 2), suggesting that differences between sites cannot solely be due to different sampling times. In the temporal study differences in the variability became more obvious over time, with nutrient concentrations increasing, while bacteria and viruses showed an overall decrease in abundances. Previous studies have also suggested that the heterogeneous distribution of microbes could be linked with chemical (e.g., substrate), physical (e.g., turbulence) and/or biological (e.g., viral lysis) processes or a combination of these (Stocker et al., 2008;Durham et al., 2013;Carreira et al., 2015). A likely explanation for the spatial differences could be the variability in the quality and type of substrate, with one previous study showing spatial differences (km scale) in the concentrations of potential microbial substrate (i.e., carbohydrates and proteins) in the GBR (Lønborg et al., 2017). Another important factor to consider for both the spatial and temporal variability is turbulence, which increases the heterogeneity of swimming phytoplankton by 10-fold (Durham et al., 2013). Other studies have found spatial differences in the composition of the microbial communities (both phytoplankton and bacteria) in the GBR, which could have impacted the results in our spatial component (Revelante et al., 1982;Angly et al., 2016). Grazing by microzooplankton could also have influenced the spatial and temporal variability, particularly of phytoplankton, as shown by the high mortality rates (75%) by

399 microzooplankton of phytoplankton in tropical/subtropical regions (Calbet and Landry 2004).
400 Cell lysis might also have impacted the distributions of phytoplankton and bacteria, but we
401 currently lack sufficient data to conclude if this is a cause for the variability found in this
402 study. Specifically, for the temporal study, which took place at an inshore station, the daily
403 differences in nutrient level could also have been caused by variable inputs from the nearby
404 river and/or sediment resuspension, which both have been shown to increase nutrients
405 concentrations in inshore parts of the GBR (Lambrechts et al., 2010).
406 The negative correlations found between nutrients (NO_3^-/NO_2^- and HPO_4^{2-}) and
407 bacteria could be explained by a discrepancy between the timescales of nutrient uptake and
408 bacterial growth, or bacterial growth could be limited by other factors than N and P (e.g.,
409 carbon, iron) and they therefore did not take up these nutrients (Pinhassi et al., 2006). No
410 correlations were found between bacterial and viral abundances for individual sites or days (n
411 = 25), but when combining all data, a relationship was observed (n = 325, R^2 = 0.75; Fig. 9).
412 A lack of relationship between viruses and bacteria has previously been found in another
413 study in a reef system with a small sample size (n = 36) (Bouvy et al., 2012), suggesting that
414 small datasets might capture mismatched communities. This effect is then averaged out when
415 larger datasets are pulled together (Wigington et al., 2016). Likewise the lack of relations
416 between bacteria and organic and inorganic nutrients could result from a discrepany between
417 assimilation and observable changes. Thus, as most oceanographic studies collect larger
418 samples (e.g., litres) or areas (e.g., kilometres) the interactions between microbes and viruses
419 at small scales will not be included. Likewise, we also show that “similar” sites (reef sites)
420 show a high degree of heterogeneity between them. Our results therefore indicate that caution
421 is necessary when using one site or time point as a reference and it is important to consider
422 the scale of observation to obtain an accurate understanding of microbial interactions.

In conclusion the spatial study showed: 1) high small-scale variability across coral and non-coral sites; 2) lower bacterial and viral abundances in the Coral Sea compared to coral sites, and 3) the Northern most site had more heterogeneous parameters than the southernmost site. The temporal study showed: 1) persistent high small-scale heterogeneity over time, 2) 24h is not an appropriate measure of temporal change, instead, shorter time periods should be used, 3) day 3, with higher nutrient concentration, also showed more heterogeneous parameters, compared to day 1 with lower nutrients concentrations, 4) Niskin bottle samples showed a similar variability, but missed the heterogeneity observed using the device presented, and 5) variability observed across days is comparable to that across sites, hence differences between sites cannot only be attributed to different sampling times.

Overall, this study shows that at the cm scale measured in the GBR: 1) parameters show high small-scale heterogeneity, 2) over the large scale the coastal location showed the highest average concentrations/abundances; 3) none of the parameters could explain the small- or large- scale distribution of bacteria spatially or temporally; 4) at the scales measured no significant relation were found, and 5) statistical differences were found for the measured parameters between sites and days.

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568

Table 1(on next page)

Sampling sites information

Great Barrier Reef (Australia) location names, sites identification number, latitude, longitude, sampling date, time, and the in-situ salinity and temperature. Sites 1 to 5 were each sampled once for the spatial study, while site 6 was sampled over 4 days for the temporal study.

Location	Site	Latitude	Longitude	Date	Time	Salinity	Temperature (°C)
Rudder Reef	1	-16.20139	145.76722	17/12/2014	8:00 AM	35.5	30.1
Irene Reef	2	-15.64772	145.68234	17/12/2014	2:50 PM	n.d.	n.d.
Osterlund Reef	3	-15.55405	145.45964	18/12/2014	11:00 AM	35.6	29.4
Coral Sea	4	-15.55972	145.97222	19/12/2014	11:20 AM	35.3	28.8
Flora Reef	5	-17.22020	146.25450	22/12/2014	11:00 AM	35.5	28.7
Bowling Green Bay	6	-19.27602	147.05744	12-15/01/2015	High Tide	32 - 33	31 - 32

1

Table 2(on next page)

Spatial study data

Total and per site average (± standard deviation, SD), minimum (Min) and maximum (Max) values for nitrate/nitrite (NO_3^-/NO_2^-), phosphate (HPO_4^{2-}), dissolved organic carbon (DOC), total dissolved nitrogen (TDN), chlorophyll *a* (chl *a*), bacterial and viral abundances, and virus to bacteria ratio (VBR) measured at the sites 1 to 5 included in the spatial study in the Great Barrier Reef (Australia); n.d. - not determined.

Site	Calculation	NO_3^-/NO_2^- (µmol l^{-1})	HPO_4^{2-} (µmol l^{-1})	DOC (µmol l^{-1})	TDN (µmol l^{-1})	Chl a (µg l^{-1})	Bacteria (x10^5 ml^{-1})	Viruses (x10^5 ml^{-1})	VBR
1	**Average ± SD**	0.07 ± 0.02	0.06 ± 0.02	90 ± 9	8.4 ± 0.9	0.44 ± 0.21	7.8 ± 0.5	51.9 ± 3.4	6.7 ± 0.7
	Min - Max	0.06- 0.05	0.04 - 0.09	75 - 106	6.9 - 10.2	0.11 - 0.88	7.3 - 9.6	45.6 - 60.6	3.5 - 7.4
	CV (%)	37	27	10	11	47	7	6	10
2	**Average ± SD**	0.06 ± 0.01		83 ± 11	6.5 ± 0.8	0.50 ± 0.21	9.6 ± 0.3	58.2 ± 8.8	6.1 ± 1.0
	Min - Max	0.05 - 0.08	n.d.	72 - 112	5.3 - 8.6	0.14 - 0.79	8.8 - 10.2	35.6 - 72.8	3.5 - 7.4
	CV (%)	20		13	12	43	4	15	16
3	**Average ± SD**	0.08 ± 0.01	0.05 ± 0.01	85 ± 11	6.8 ± 1.6	0.51 ± 0.23	12.5 ±1.9	47.0 ± 6.9	3.8 ± 0.6
	Min - Max	0.05 - 0.10	0.04 - 0.07	72 - 112	2.1 - 10.1	0.06 - 1.12	11.2 - 20.5	37.7 - 67.2	2.8 - 4.7
	CV (%)	15	21	13	24	46	15	15	15
4	**Average ± SD**	0.06 ± 0.01	0.07 ± 0.01	89 ± 8	6.5 ± 0.6	0.34 ± 0.18	5.5 ± 0.3	18.9 ± 1.1	3.4 ± 0.3
	Min - Max	0.05 - 0.11	0.04 - 0.11	77 - 104	5.6 - 7.7	0.04 - 0.69	5.2 - 6.5	17.4 -21.4	2.9 - 4.1
	CV (%)	21	20	9	9	54	5	6	9
5	**Average ± SD**	0.05 ± 0.01	0.04 ± 0.01	86 ± 5	8.1 ± 1.1	0.25 ± 0.17	10.1 ± 0.6	41.3 ± 2.7	4.1 ± 0.3
	Min - Max	0.05 - 0.07	0.04 - 0.08	78 - 102	6.1 - 10.4	0.05 - 0.62	9.4 - 12.5	36.2 - 45.3	3.2 - 4.7
	CV (%)	10	20	6	13	68	6	7	9
Total	**Average ± SD**	0.06 ± 0.02	0.06 ± 0.02	86 ± 9	7.3 ± 1.3	0.41 ± 0.22	9.1 ± 2.5	43.4 ± 14.5	4.8 ± 1.5
	Min - Max	0.05 - 0.16	0.04 - 0.11	72 - 112	2.1 - 10.4	0.04 - 1.12	5.2 - 20.5	17.4 - 72.8	2.8 - 8.3

Table 3(on next page)

Temporal study data

Total and per day average (± standard deviation, SD), and minimum (Min) and maximum (Max) values for nitrate/nitrite (NO_3^-/NO_2^-), phosphate (HPO_4^{2-}), dissolved organic carbon (DOC), total dissolved nitrogen (TDN), chlorophyll *a* (chl *a*), bacterial and viral abundances, and virus to bacteria ratio (VBR) measured during the 4 days of the temporal study at Bowling Green Bay (site 6) in the Great Barrier Reef (Australia); n.d. - not determined.

Day	Calculation	NO_3^-/NO_2^- (µmol l^{-1})	HPO_4^{2-} (µmol l^{-1})	DOC (µmol l^{-1})	TDN (µmol l^{-1})	Chl a (µg l^{-1})	Bacteria (x10^5 ml^{-1})	Viruses (x10^5 ml^{-1})	VBR
1	**Average ± SD**	0.06 ± 0.03	0.08 ± 0.01	107 ± 4	8.9 ± 0.8	0.27 ± 0.18	20.3 ± 0.9	98.9 ± 9.2	4.9 ± 0.5
	Min - Max	0.05 - 0.18	0.06 - 0.09	99 - 114	7.2 - 10.5	0.02 - 0.67	18.8 - 23.0	81.6 - 126.0	4.0 - 6.4
	CV (%)	45	12	4	9	65	5	9	10
2	**Average ± SD**	0.08 ± 0.0	0.07 ± 0.01	101 ± 4		0.24 ± 0.15	19.8 ± 2.0	121 .1 ± 15.7	6.2 ± 1.0
	Min - Max	0.05 - 0.11	0.05 - 0.09	95 - 110	n.d.	0.01 - 0.63	17.8 - 25.3	90.2 - 155.0	4.4 - 8.4
	CV (%)	19	13	4		63	10	13	16
3	**Average ± SD**	0.18 ± 0.02	0.09 ± 0.01	118 ± 6		0.16 ± 0.12	17.9 ± 3.3	111.0 ±12.4	6.4 ± 1.3
	Min - Max	0.15 - 0.21	0.07 - 0.11	109 - 135	n.d.	0.05 - 0.67	13.0 - 24.1	94.8 - 160.0	4.0 - 8.2
	CV (%)	10	10	5		76	19	11	20
4	**Average ± SD**	0.63 ± 0.09	0.11 ± 0.03	100 ± 4	11.3 ± 0.7	0.20 ± 0.10	14.5 ± 0.9	88.1 ± 10.6	6.1 ± 0.7
	Min - Max	0.47 - 0.82	0.07 - 0.16	94 - 113	10.2 - 12.7	0.07 - 0.48	12.1 - 16.4	68.8 - 108.0	5.0 - 7.3
	CV (%)	15	26	4	6	52	6	12	12
Total	**Average ± SD**	0.24 ± 0.24	0.09 ± 0.02	106 ± 8	10.1 ± 1.4	0.22 ± 0.14	18.1 ± 3.1	104.8 ± 17.3	5.9 ± 1.1
	Min - Max	0.05 - 0.82	0.05 - 0.16	94 - 135	7.2 - 12.7	0.01 - 0.67	12.1 - 25.3	68.8 - 160.0	4.0 - 8.4

Figure 1

Sampling locations

Map showing the sampled location for study sites along the Great Barrier Reef (Australia). Larger map is a representation of the square indicated in the smaller map of Australia.

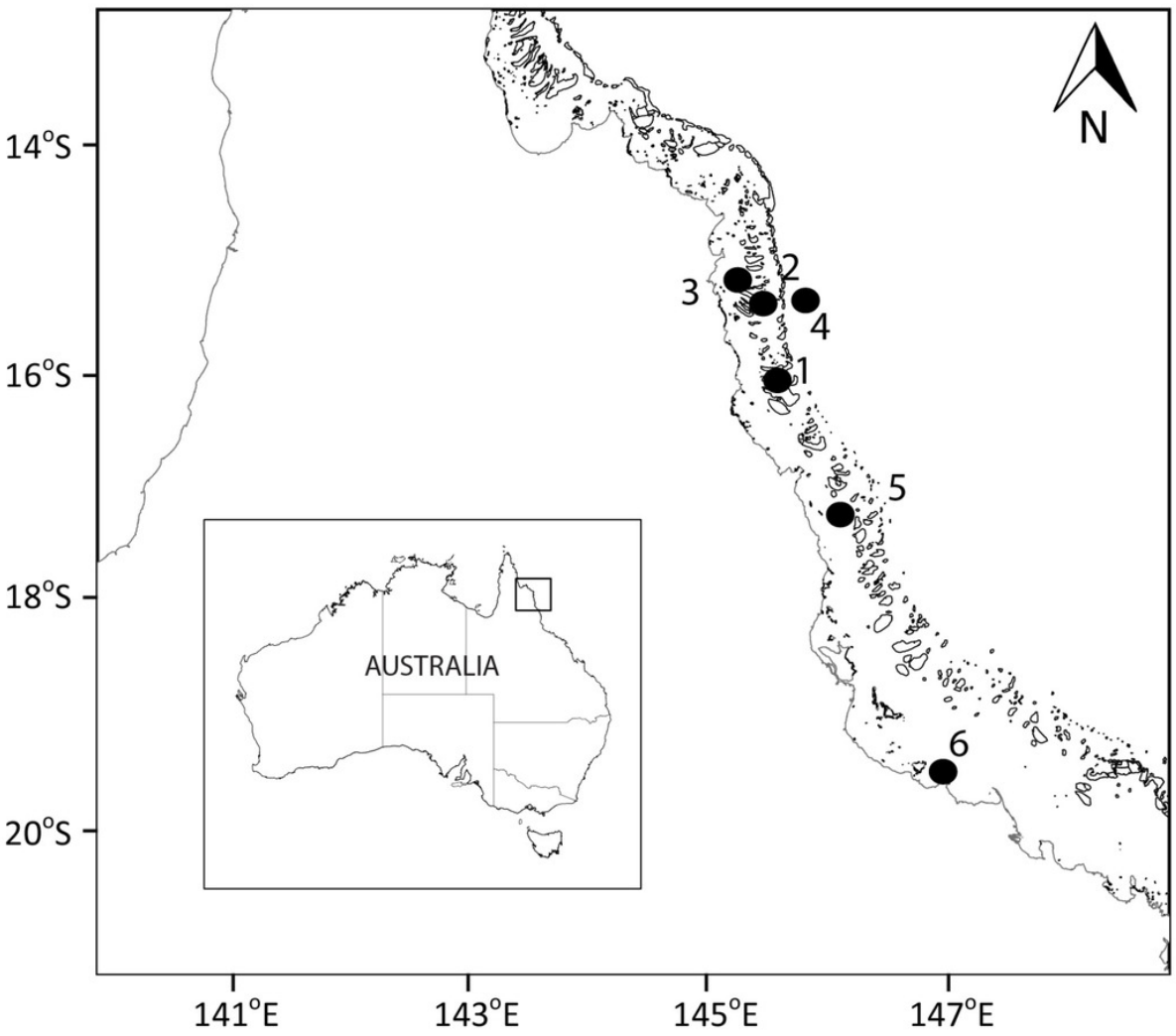

N
14°S
16°S
18°S
20°S
141°E
143°E
145°E
147°E
1
2
3
4
5
6
AUSTRALIA

Figure 2

Sampling device

Two-dimensional device used for sampling consisting of 25 inlets (5 x 5) connected to a 25 mL syringe, each separated by 7 cm with a total sampling area of 784 cm^2.

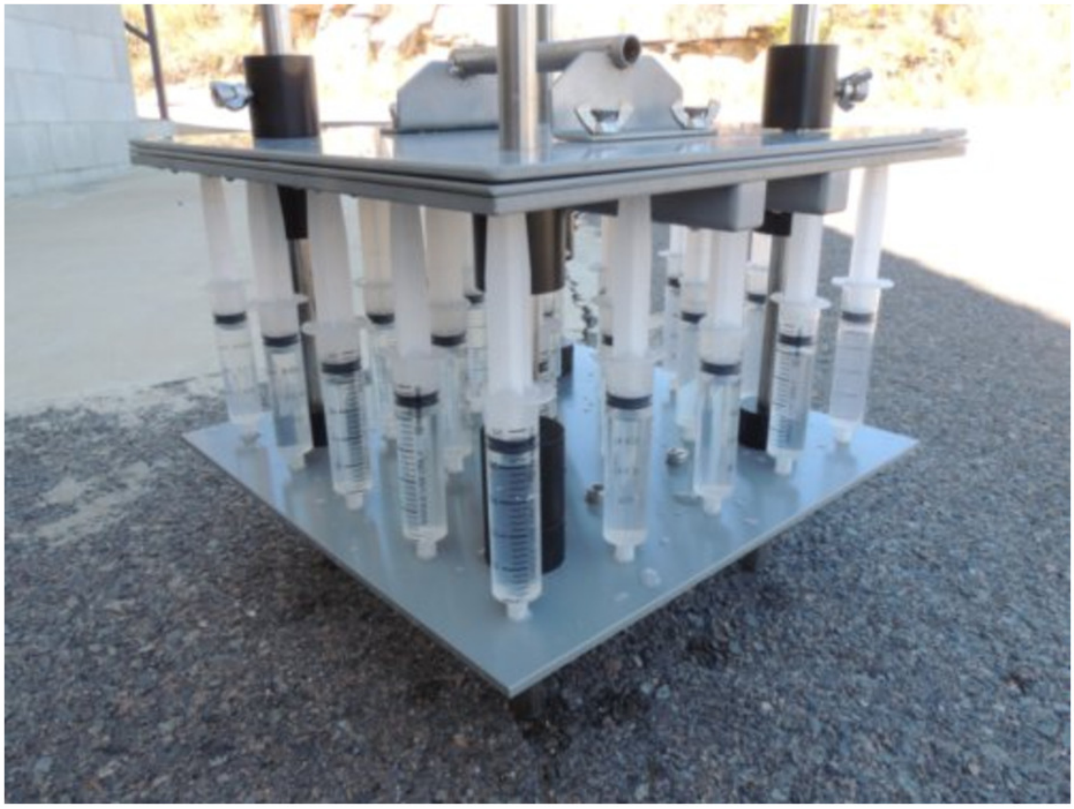


Figure 3

Spatial distribution of chemical parameters

Small-scale spatial distribution of nitrate/nitrite (NO_3^-/NO_2^-), phosphate (HPO_4^{2-}), dissolved organic carbon (DOC), total dissolved nitrogen (TDN; top to bottom) measured at the 5 sites (left to right) of the spatial study in the Great Barrier Reef (Australia). The grey scale represents the range of concentrations for each parameter, with white being the lowest concentration and black the highest. The axes represent the 28 cm spatial array used for sampling.

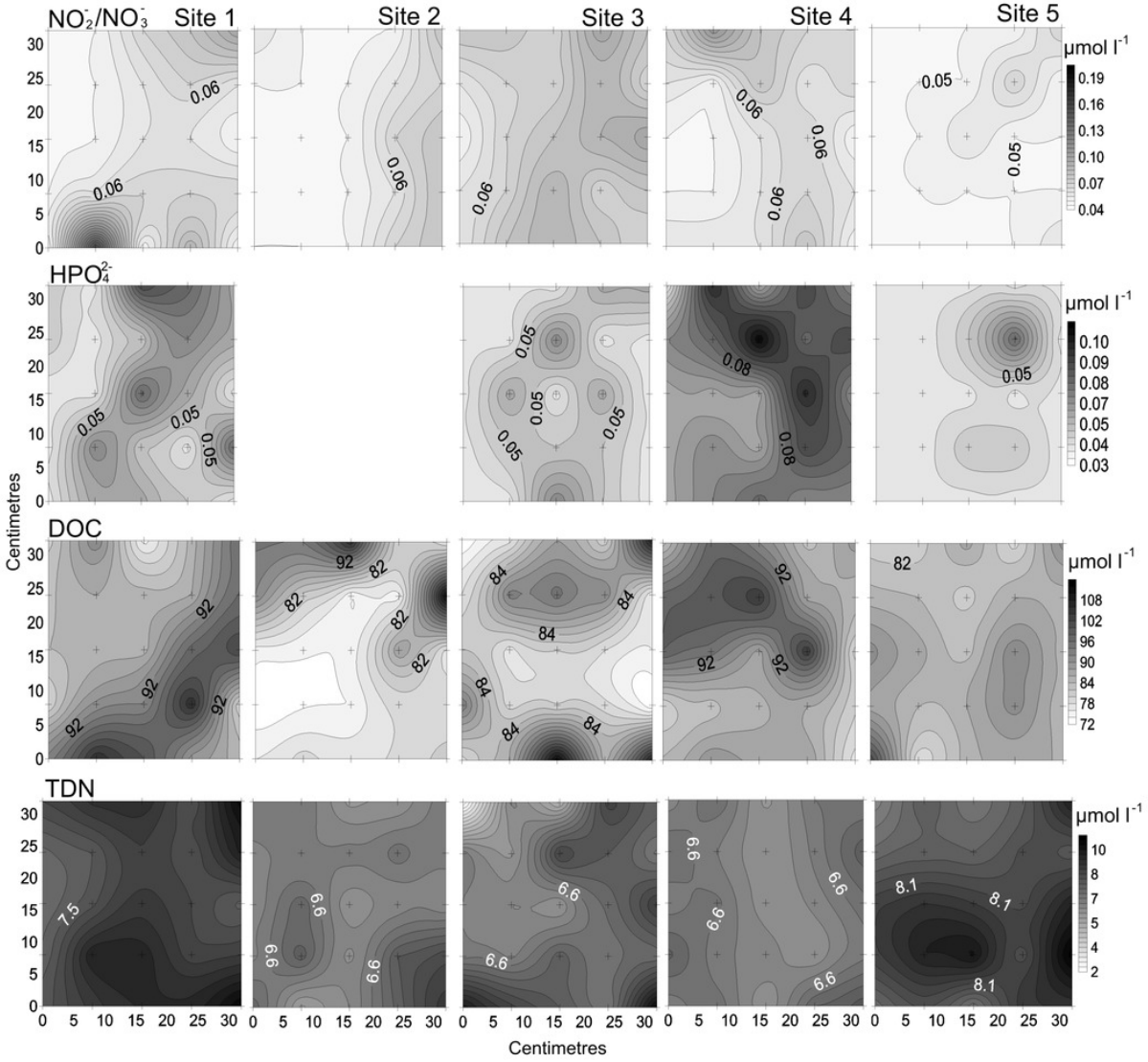

NO2-/NO3- Site 1
Site 2
Site 3
Site 4
Site 5
µmol l-1
HPO42-
DOC
TDN
Centimetres
Centimetres

Figure 4

Spatial distribution of biological parameters

Small-scale spatial distribution of chlorophyll *a* (chl *a*), bacteria, viruses, and virus-to bacteria ratio (VBR; top to bottom) measured at the 5 sites (left to right) of the spatial study in the Great Barrier Reef (Australia). The grey scale represents the range of concentrations for each parameter, with white being the lowest concentration and black the highest. The axes represent the 28 cm spatial array used for sampling.

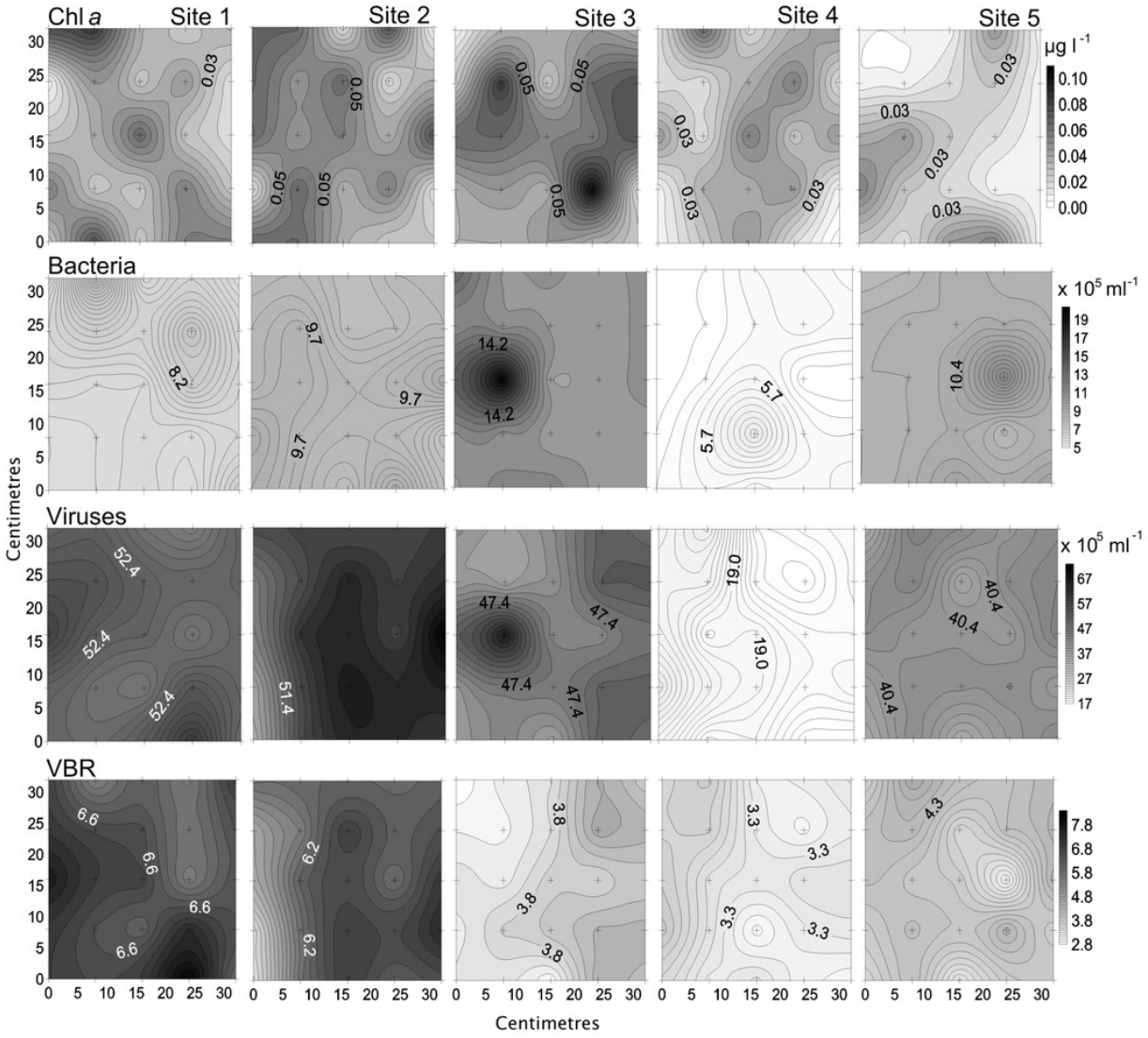

Chl a
Site 1
Site 2
Site 3
Site 4
Site 5
µg l-1
0.10
0.08
0.06
0.04
0.02
0.00
Bacteria
x 10^5 ml^-1
19
17
15
13
11
9
7
5
Viruses
x 10^5 ml^-1
67
57
47
37
27
17
VBR
7.8
6.8
5.8
4.8
3.8
2.8
Centimetres
Centimetres

Figure 5

Temporal distribution of chemical parameters

Small-scale spatial distribution of nitrate/nitrite (NO_3^-/NO_2^-), phosphate (HPO_4^{2-}), dissolved organic carbon (DOC), total dissolved nitrogen (TDN; top to bottom) measured during the 4 days (left to right) of the temporal study in the Great Barrier Reef (Australia). The grey scale represents the range of concentrations for each parameter, with white being the lowest concentration and black the highest. The axes represent the 28 cm spatial array used for sampling.

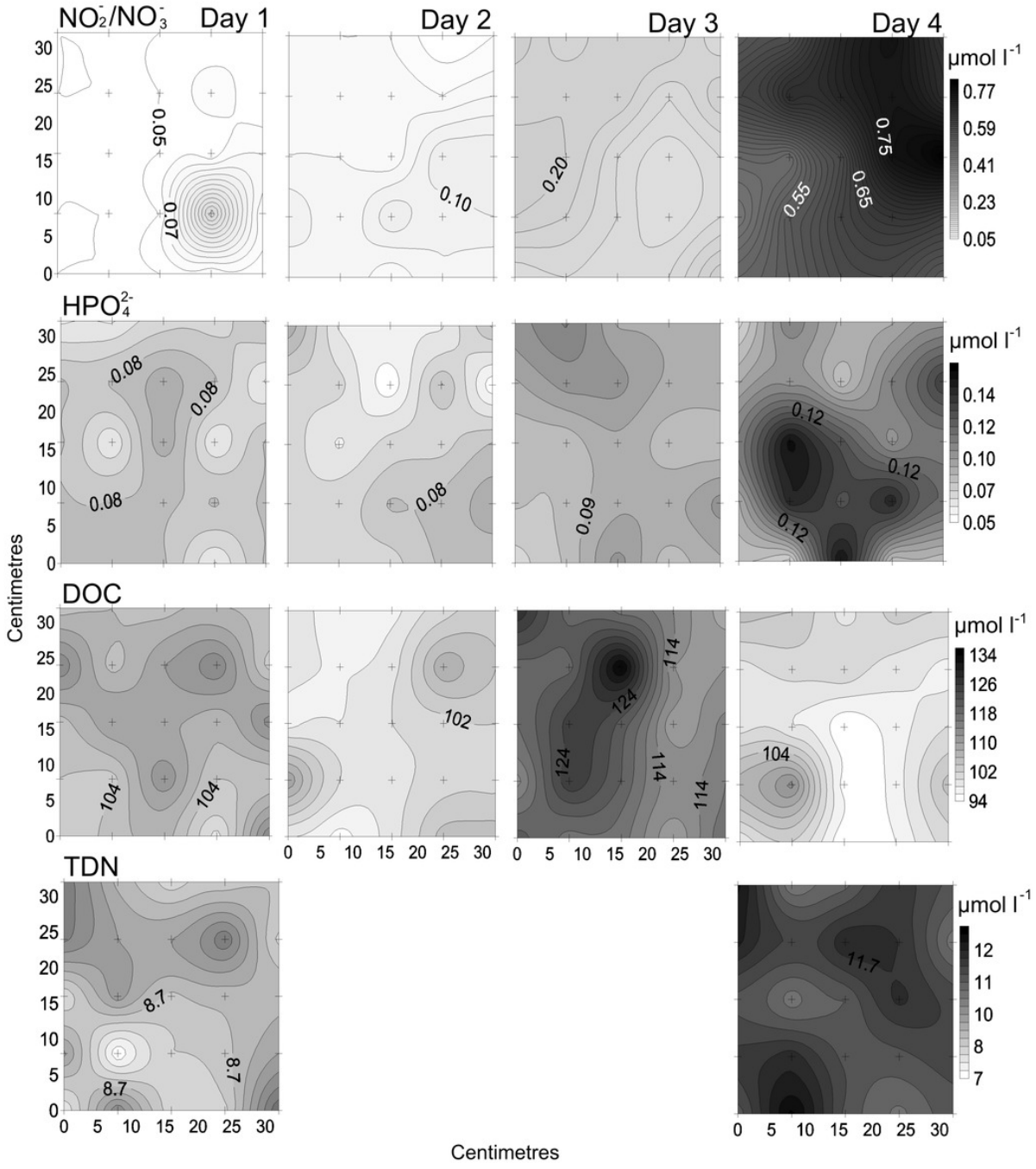

NO2-/NO3- Day 1
Day 2
Day 3
Day 4
µmol l-1
0.77
0.59
0.41
0.23
0.05
0.05
0.07
0.10
0.20
0.75
0.65
0.55
HPO4 2-
µmol l-1
0.14
0.12
0.10
0.07
0.05
0.08
0.08
0.08
0.08
0.09
0.12
0.12
0.12
DOC
µmol l-1
134
126
118
110
102
94
104
104
102
124
114
124
114
114
104
TDN
µmol l-1
12
11
10
8
7
8.7
8.7
8.7
11.7
Centimetres
Centimetres
0 5 10 15 20 25 30

Figure 6

Temporal distribution of biological parameters

Small-scale spatial distribution of chlorophyll *a* (chl *a*), bacteria, viruses, and the virus to bacteria ratio (VBR; top to bottom) measured during the 4 days (left to right) of the temporal study in the Great Barrier Reef (Australia). The grey scale represents the range of concentrations for each parameter, with white being the lowest concentration and black the highest. The axes represent the 28 cm spatial array used for sampling.

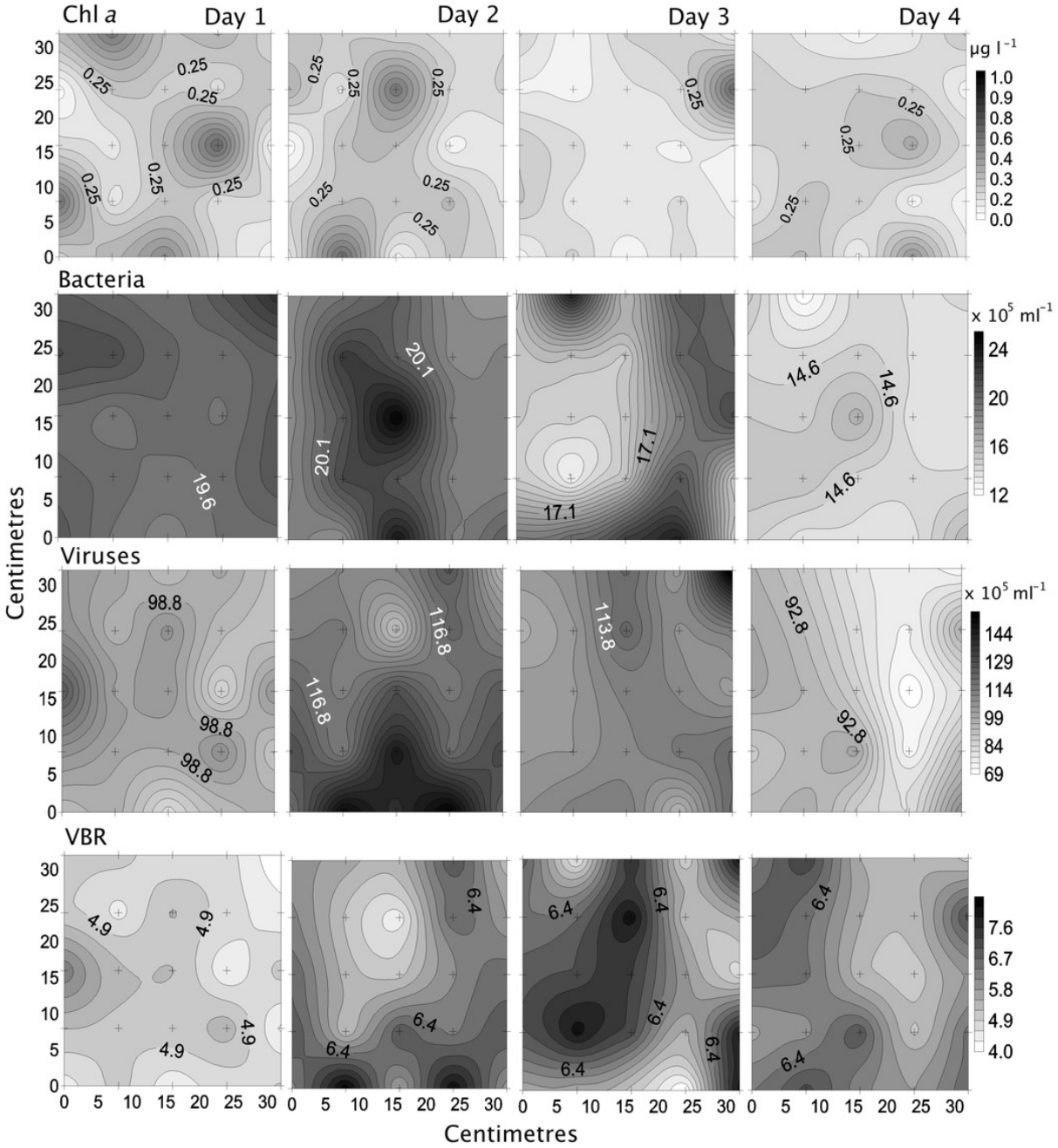

Chl a
Day 1
Day 2
Day 3
Day 4
µg l⁻¹
1.0
0.9
0.8
0.6
0.4
0.3
0.2
0.0
Bacteria
x 10⁵ ml⁻¹
24
20
16
12
Viruses
x 10⁵ ml⁻¹
144
129
114
99
84
69
VBR
7.6
6.7
5.8
4.9
4.0
Centimetres
Centimetres

Figure 7

Spatial distribution of biological parameters at large-scale

Boxplots of each chemical (A-nitrate/nitrite - NO_3^-/NO_2^-, B-phosphate - HPO_4^{2-}, C-dissolved organic carbon - DOC, and D-total dissolved nitrogen - TDN), and biological parameter (E-chlorophyll *a* - chl *a*, F-bacteria, and G-viruses) for the sampled sites (1, 2, 3, 4, and 5) in the Great Barrier Reef (Australia). Error bars represent the 10^{th} and 90^{th}percentiles, with 50 % of the data inside the box. The solid line inside the box represents the median. Each site had a sample size of n = 25. Boxplots showing the same letter are not significantly different ($P < 0.05$).

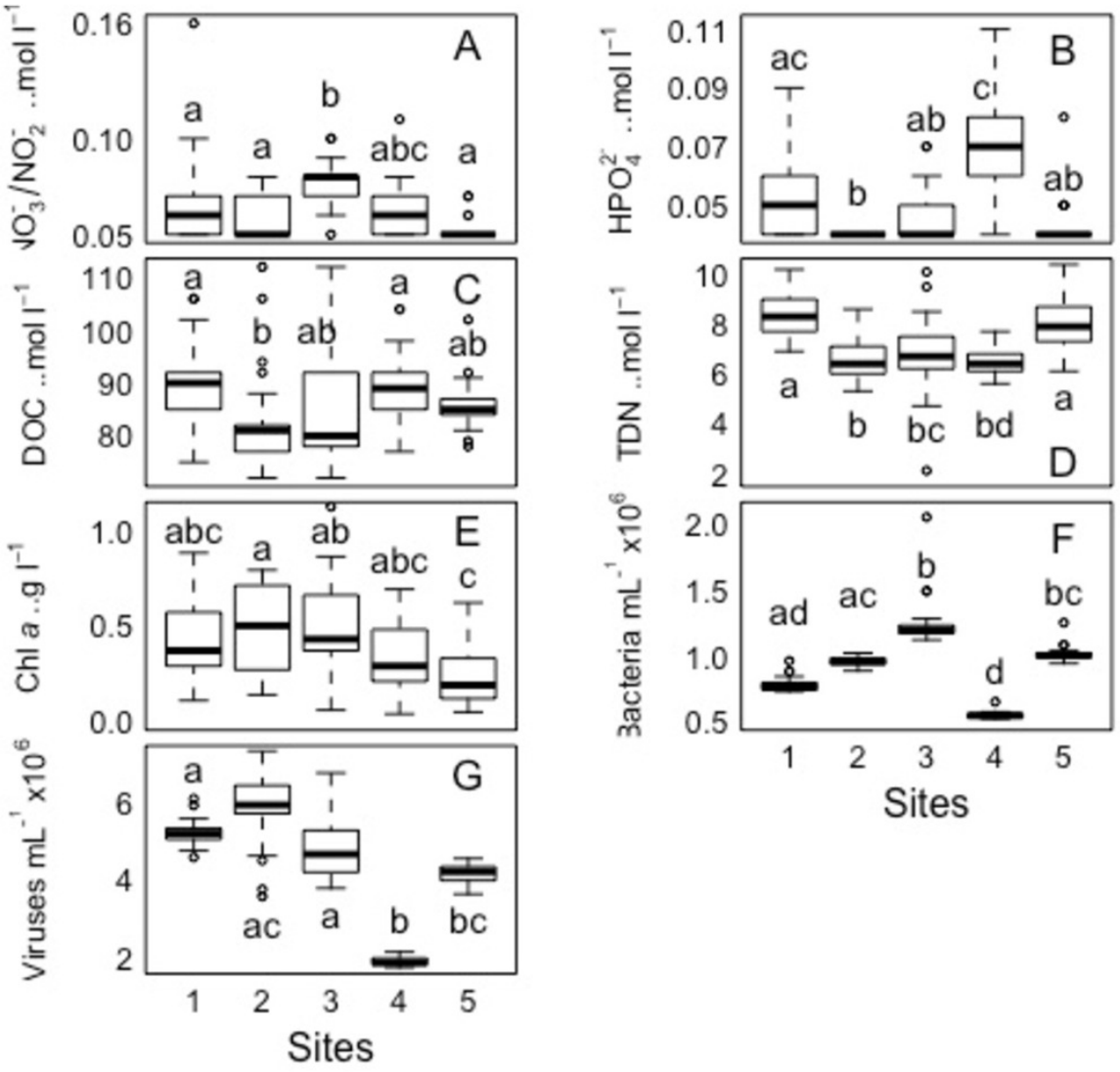

A
B
C
D
E
F
G
Sites
1
2
3
4
5

Figure 8

Temporal distribution of biological parameters at large-scale

Boxplots of each chemical (A-nitrate/nitrite - NO_3^-/NO_2^-, B-phosphate - HPO_4^{2-}, and C-dissolved organic carbon - DOC), and biological parameter (D-chlorophyll *a* - chl *a*, E-bacteria, and F-viruses) for the sampled days (1, 2, 3, and 4) in the Great Barrier Reef (Australia) at Bowling Green Bay (site 6). Error bars represent the 10^{th} and 90^{th}percentiles, with 50 % of the data inside the box. The solid line inside the box represents the median. Each day had a sample size of n = 25. Boxplots showing the same letter are not significantly different ($P < 0.05$).

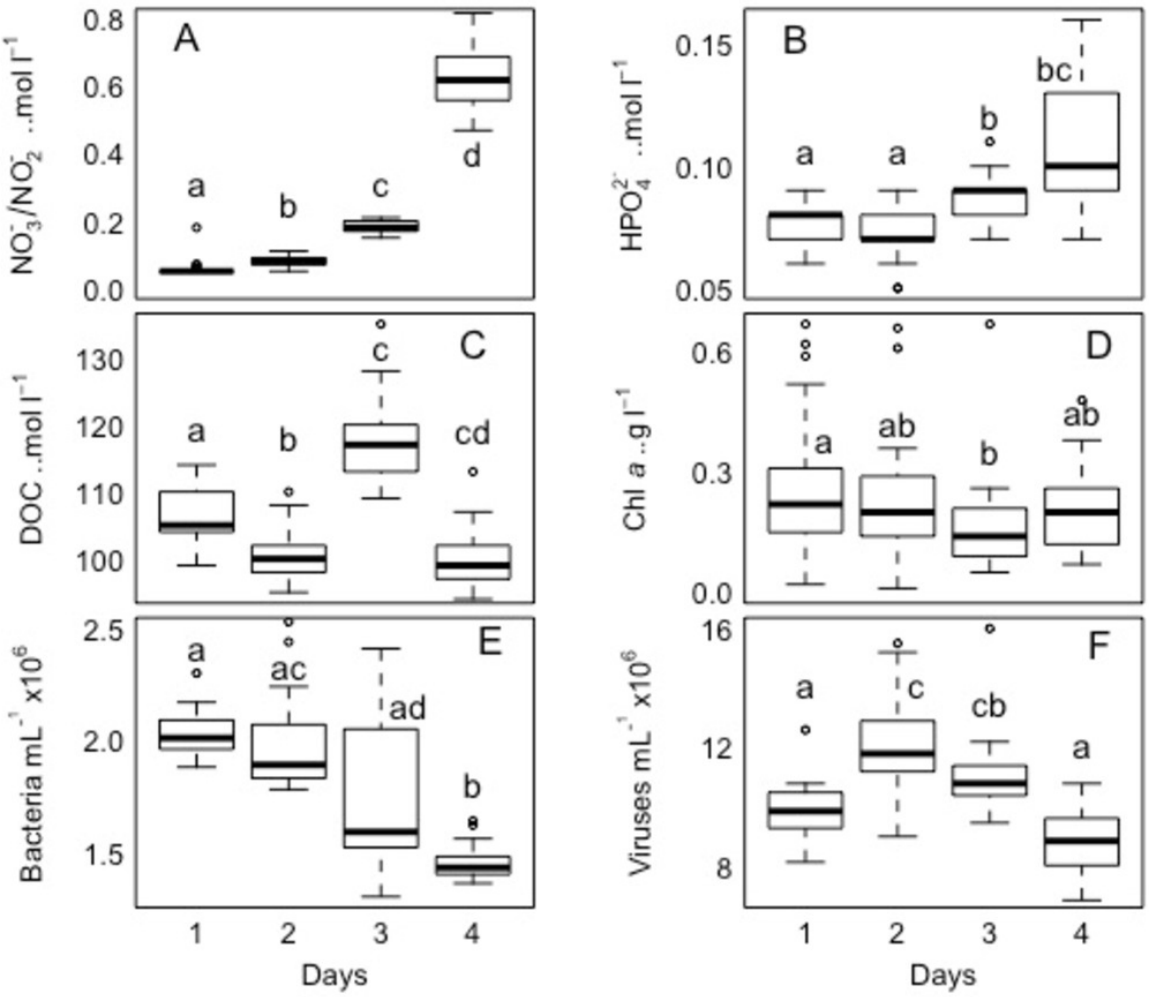
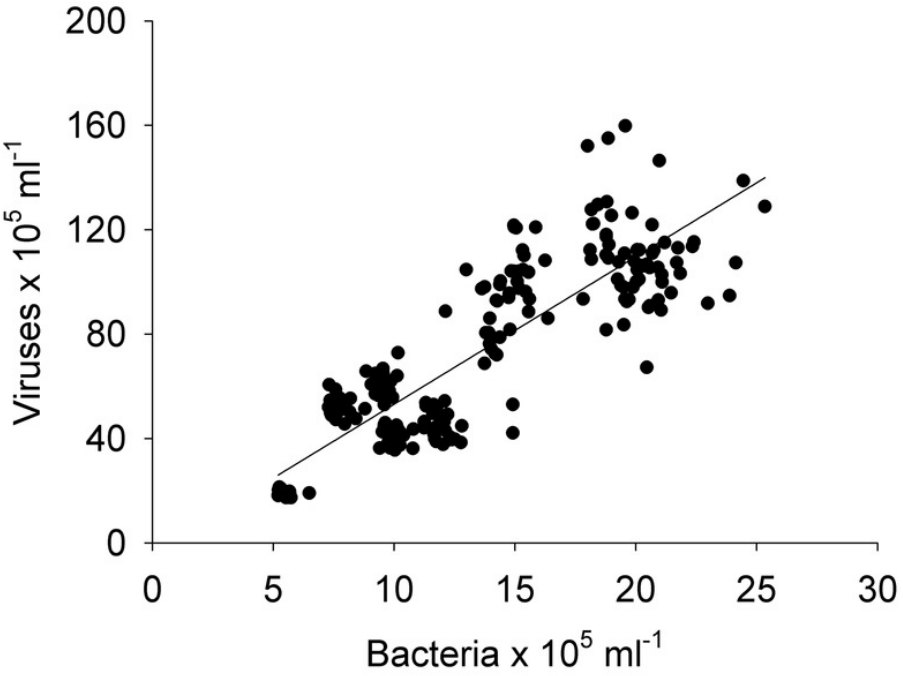

A
B
C
D
E
F
NO_3^-/NO_2^- ..mol l^{-1}
HPO_4^{2-} ..mol l^{-1}
DOC ..mol l^{-1}
Chl *a* ..g l^{-1}
Bacteria mL^{-1} x10^6
Viruses mL^{-1} x10^6
Days
Days

Figure 9

Linear regression

Between the abundances of bacteria and viruses from all the sites (1, 2, 3, 4, and 5) and days (1,2, 3, and 4) measured in the Great Barrier Reef (Australia) ($n = 325$; $R^2 = 0.75$; $p < 0.0001$; Vir = 5.4 ± 0.3 Bact).


200
160
120
80
40
0
Viruses x 10^5 ml^-1
0
5
10
15
20
25
30
Bacteria x 10^5 ml^-1