Do human activities affect the picoplankton structure of the Ahe atoll lagoon (Tuamotu Archipelago, French Polynesia)?

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Marc Bouvy [1*], Christine Dupuy [2], Marc Pagano[3], Aude Barani[4], Loic Charpy[5]

[1] UMR 5119, ECOSYM, Ecologie des systèmes marins côtiers, (UM2, CNRS, IRD, Ifremer, UM1). Université Montpellier 2, Place Eugène Bataillon, Case 093, 34095 Montpellier cedex 5, France

[2] Littoral, Environnement et SociétéS (LIENSs), Université de La Rochelle, UMR 6250 CNRS-ULR, 2 rue Olympe de Gouges, 17000 La Rochelle cedex, France

[3] IRD - UMR MIO (Mediterannean Institut of Oceanography), Campus de Luminy Case 901 13288 Marseille Cedex 09, France.

[4] PRECYM platform, UMS 2196, Campus de Luminy, Case 901, 13288 Marseille Cedex 09, France.

[5] UMR MIO, Center of Tahiti, BP 529, 98713, Papeete, French Polynesia

*To whom correspondence should be addressed. E-mail: marc.bouvy@ird.fr

Abstract

The spatial variations of the picoplankton (photoautotrophic and heterotrophic microorganisms) in the Ahe atoll lagoon were studied in May and October 2008 to assess whether they were affected by human activities along the atoll. Spatial patterns were studied using 10 sampling stations chosen according to the location of the anthropogenic activities (pearl farming, harbor). Experiments were also carried out to determine whether bacterial growth, with or without predators, was limited by inorganic (N and P) substrates. The results showed that heterotrophic bacterioplankton abundance was superior to the photoautotrophic organisms, especially in May. Significant increases in bacterial abundance were observed in May after 24 hours incubation with +P and +N (but not in October). All samples complied with the quality levels for fecal indicator bacteria (FIB) defined by the European Union and there was no evidence that human sewage had any impact on picoplankton over the whole atoll.

1. Introduction

Human activities have a major impact on marine ecosystems. The pressures exerted are diverse and result from a wide range of activities such as coastal engineering, sediment dredging, pollution, fishing, aquaculture, urban development, maritime transport, tourism, mining, oil extraction, transport and refining of oil, agricultural and industrial activities (Islam and Tanaka, 2004; Halpern et al., 2008). All these activities have an effect on the components of the marine food web, from microorganisms to top animal predators. A recent report analyzed the ecological impact of anthropogenic activities in the oceans worldwide, focusing on stressors that can be evaluated at global scale (Halpern et al., 2008). All the analyzed ocean ecosystems (coral reefs, mangroves, seagrass meadows, seamounts, rocky reefs, soft shallow areas, continental shelf areas, slope areas, pelagic waters and the deep sea) can be considered to be affected by anthropogenic activities, although to different degrees (Nogales et al., 2011).

In a context of eutrophication, the composition and structure of microbial communities are also basic indicators of ecosystem status, including phytoplankton bloom and the heterotrophic activity of aerobic and anaerobic bacteria (Paerl et al., 2002; Bouvy et al., 2010). Nutrient availability (bottom-up control), predation by protozoa (top-down control) and viral lysis are the most important factors regulating bacterial communities. Organic carbon has usually been considered to be the main factor limiting the growth of pelagic heterotrophic bacteria. However, studies on nutrient limitation of such communities have shown that mineral limitation of growth rate is widespread in various marine ecosystems (Torreton et al., 2000; Carlsson and Caron, 2001). Nutrient enrichment is a direct consequence of eutrophication, modifying the biological abundance and activity at each trophic level and, for example, increasing bacterial standing stocks and production (Ducklow and Shiah, 1993). Nutrient enrichment bioassays are the most direct method for assessing the nutrient status of phytoplankton and bacterioplankton communities and this method has been widely used (Torreton et al., 2000; Bouvy et al., 2004).

Although viral infection is now considered to be one of the major structuring processes in the dynamics of marine microbial communities (Furhman, 1999), grazing by heterotrophic nanoflagellates (HNF) has been identified as the main limiting factor affecting bacteria and has been shown to hinder bacterial production, thus regulating bacterial biomass in a large number of pelagic ecosystems (Solic and Krstulovic, 1994; Christaki et al., 1999; Ferrier-Pagès and Furla, 2001). Size-

selective grazing coupled with resource availability has been shown to be a shaping force in both the taxonomic and phenotypic structures of bacterial communities (Jürgens and Matz, 2002). However, although factors controlling bacterial communities in temperate and tropical areas have been studied, little research has been undertaken to study these microorganisms in atolls (Gonzalez et al., 1998; Ferrier Pages and Furla, 2001).

In addition to their role in the organic matter mineralization and in the diet for heterotrophic nanoflagellates, micro-organisms are effective descriptors for evaluating and predicting the environmental impact of human activities. Continental waters are often polluted by pathogenic microorganisms from recreational marinas, sewage disposal sites, septic tanks, rainfall runoff from urban areas and many other sources (Lipp et al., 2001; Aslan-Yilmaz et al., 2004). Fecal indicator bacteria (FIB) including thermo tolerant coliforms (TTC) and fecal streptococci (FS) are used as surrogates for human and animal pathogens for assessing water quality. In epidemiological studies, FIB are documented as being associated with an increased risk of contracting gastrointestinal and respiratory illnesses after contact with waters with high concentrations (Haile et al., 1999). Their origin has always been presumed to be anthropogenic (e.g. sewage, agricultural and urban runoff). An important criterion for assessing the potential health risk for recreational waters is the FIB density. Although FIB do not necessarily induce illness, they are often associated with pathogenic bacteria, viruses and parasites in domestic sewage. Microbial water quality varies according to the magnitude of inputs and the flow and dispersion of organisms as a result of near-shore hydrodynamics such as tides and currents (Davies-Colley et al., 1994).

French Polynesia made up of several lagoons are of great importance to the economy of the region, where farming of pearl oyster, *Pinctada margaritifera,* is the major source of export earnings, especially from Ahe atoll (Thomas et al., 2010 ; Andréfouët et al., this issue). The main objective of this study was to estimate the impacts of human activities along the Ahe atoll by determining fecal indicator bacteria (FIB) and by describing the abundance of heterotrophic and autotrophic picoplankton in the lagoon. For this, spatial patterns (10 sampling stations) and temporal patterns (before and after the rainy season) were studied using 10 biological indicators. Bacterial responses to nutrient enrichment based on bioassay experiments at one station were also studied for the two periods.

2. Material and Methods.

2.1. Study site and sample collection

This study was conducted in the Ahe atoll, 500 km northeast of Tahiti, in the north of the Tuamotu Archipelago (Fig. 1; see details in Thomas et al., 2010). Ahe lagoon is 142 km^2 in area, with a maximum depth close to 70 m, and can be defined as a semi-enclosed atoll. There is one passage to the west of the lagoon and there are several reef-flats (less than 50 cm depth) along the reef rim.

This study was conducted at the end of dry season (May 2008, temperature: 28.61°C ±0.04°C) and at the end of the rainy season (October 2008, temperature: 27.12°C ±0.06°C). Ten stations (S1 to S10) were sampled, near pearl farms mostly found along the shoreline of the atoll. Samples were taken at a depth of 0.5 m, using 2 liter sterile acid-cleaned bottles.

2.2. Biological analyses

Heterotrophic bacteria and picoautotrophic cells were fixed with 0.2 µm filtered formaldehyde (final concentration 2%) and frozen in liquid nitrogen. All samples were analyzed using a MoFlo™ flow cytometer (Beckman-Coulter) equipped with a dual line, water-cooled, Coherent™ argon laser (351 and 488 nm). All data were collected in log scale, stored in list mode and analyzed with the Summit™ software package (Beckman-Coulter, Miami, FL). Standard protocols were used to enumerate phytoplankton and heterotrophic prokaryotes (Marie et al., 1999). A 1 ml formaldehyde-fixed subsample was incubated with DAPI at a final concentration of 1/10,000 for 15 min at room temperature in the dark. Fluorescent beads (2 µm Fluoresbrite™ Polysciences Inc., Warrington, PA, USA) were added to each sample to normalize and control the flow cytometer settings. For heterotrophic bacteria analysis, the side (SSC) light scatter of the photons from the 488 nm laser beam was used as trigger signal and DAPI fluorescence, excited by the UV laser, was collected in the range 405/30nm (FL4). Combining DAPI fluorescence and light scattering unambiguously distinguishes cells from inorganic particles, detritus and free DNA (Marie et al., 1999). Picophytoplankton (*Prochlorococcus sp.* and *Synechococcus sp.* cells) and autotrophic picoeukaryotes counts were performed using the red fluorescence emission (from the 488 nm laser beam) as trigger signal. Cells were detected and enumerated according to their SSC and FSC properties, and their orange fluorescence (580/30 nm) and red fluorescence (>640 nm) from phycoerythrin and chlorophyll pigments, respectively.

The net bacterial production was estimated from the DNA synthesis rates measured by (^{3}H-methyl) thymidine (^{3}H-TdR) incorporation by microcentrifuge (Smith and Azam, 1992). A subsample was added to a sterile polystyrene snap cap tube containing a final saturating concentration of 20 nM of ^{3}H-TdR (specific activity 53 Ci $mmol^{-1}$, Amersham). Triplicate live samples and a control were run

for each assay. The bacterial growth was measured in the dark at *in situ* temperature for a short incubation time (no longer than 1 h). Incorporation was terminated by adding TCA (5% final concentration) and samples were then stored for at least 2 h at 4°C. After centrifugation, the precipitates were rinsed three times with 5% TCA and then resuspended in a liquid scintillation cocktail (Ultima Gold LLT, Perkin Elmer) before the radioactivity was determined using a liquid scintillation counter (Beckman LS 6500). The results were expressed as µgC l^{-1} h^{-1} using the conversion factors determined by Guntersen et al (2002) of 14fC per bacteria and our unpublished data of 8.6 x 10^{17} cells per mole of thymidine incorporated.

The chlorophyll concentrations were determined by fluorometry after filtration of samples onto Whatman GF/F fiberglass filters, directly extracted using methanol (Welschmeyer, 1994).

2.3. Fecal indicator bacteria

For FIB, a membrane filtration method was used to count thermo tolerant coliform (TTC) and fecal streptococci (FS) as colony forming units per 100 ml of the sample. TTC were counted as the number of blue colonies of 1-2 mm developed on mFC medium (Sartorius) after 24 hours incubation at 44.5°C. Small red to reddish-brown colonies of FS (~1 mm) were counted on Slanetz and Bartley medium (Sartorius) after 48 hours incubation at 37°C.

2.4. Enrichment bioassay

Samples for the bioassays were taken from station S1 in front of the laboratory in the northeast of the atoll. Fresh water samples were taken at 0.5 m depth. Two bioassay series were conducted to compare the responses of the bacterial community to inorganic enrichment with 100% of predators (using <50 µm-filtered water) and with 1% (using 99% of 0.22 µm-filtered water and 1% of <50 µm-filtered water) bacterial predators. Each of these was homogenized and distributed equally into 2 series of 4 x 100 ml Whirl-Pak® polyethylene sterile bags which allow the transmission of 70% UV radiation. At time zero (t0), inorganic nitrogen (mixture of 20 µM as NH_4Cl and 20 µM as NO_3) and inorganic phosphorus (2 µM as NaH_2PO_4) were added alone or in combination: +N, +P, +NP; nothing was added to the controls called C. All assays were performed in triplicate with a total of 24 Whirlpaks per experiment. All Whirlpaks were incubated for 24 hours in a floating enclosure held at 2m below the surface of the water. The water temperature stayed fairly constant during the experiments (28.6±0.55°C in May and 27.03±0.37°C in October). Subsamples were removed at time zero (t0) and after 24 h (t24) incubation to measure the bacterial abundance. The bacterioplankton

growth rates in each triplicate were calculated as $\mu = (\ln N24 - \ln N0)/t$, where t was the incubation time and N24 and N0 were the bacterial concentration at the end and at the beginning of the incubation time. The grazing rates were calculated as the difference between apparent growth rate ($\mu+g$) and net growth rate (μ). The ingestion rate of HNF (I, bact HNF^{-1} h^{-1}) was calculated according to Davies and Sieburth (1984):

$I = g \times N_{bact} / N_{HNF}$ where g was the grazing rate (h^{-1}) and N_{bact} and N_{HNF} were the average concentrations of bacteria and HNF. To enumerate the nanoplanktonic cells, water samples (25 ml) were fixed and preserved with paraformaldehyde (1% final concentration). Samples were concentrated to 10 ml using a filtration tower with 0.8 µm pore size black polycarbonate filters (Nuclepore) and stained with DAPI (2.5×10^{-4} $g.l^{-1}$ final concentration). The stained nanoplanktonic cells were enumerated under UV light excitation on at least 15 randomly selected fields, at a magnification of x1000. The method used in this study made it possible to distinguish pigmented nanoflagellates (PNF) from heterotrophic nanoflagellates (HNF) by repeatedly interchanging the filter sets (Caron 1983): phototrophic cells (crimson under UV 365 nm excitation and red colored under green 450 to 490 nm excitation) and heterotrophic cells (blue under UV excitation and invisible under green excitation) were enumerated separately. Ciliates were enumerated using the Utermöhl method.

2.5. Data processing

The relationships between chlorophyll *a*, bacteria and picoautotroph abundances, fecal coliforms and streptococci were studied using multivariate analysis. All data was $x \rightarrow \log(x+1)$ transformed and a centered Principal Component Analysis (PCA) was performed to identify the major sources of temporal and spatial variability in the microbial communities. The data was processed using ADE-4 (Thioulouse et al., 1997). The differences between dates and stations for all parameters studied were tested using the non-parametric Mann-Whitney U-test. Differences were considered as significant when $p < 0.05$ (Sigma Stat version 3.5).

3. Results

The highest values for chlorophyll-a were recorded in May 2008 (0.55 µg l^{-1} at station S7), with a mean value of 0.34 µg l^{-1}, significantly different from the mean recorded in October (0.21 µgl^{-1}, p=0.013, Table 2). The total abundance of bacteria (BACT-A) was higher in May than in October

($p<0.001$) with the highest values generally observed in the north of the atoll (Stations S9 and S10). However, the mean values for bacterial production (BACT-P) were similar in both surveys, with the highest values recorded in May at station S9 (15.22 µgC l^{-1} d^{-1}). Of the FIB, thermo-tolerant coliforms (TTC) were abundant in some stations with maximum values recorded at stations S9 and S10 in May (94 and 200 CFU 100 ml^{-1}, respectively). No significant differences were noted between the two surveys. A different pattern was observed for fecal streptococci (FS), with values significantly higher in May ($p=0.003$), which were present in 80% of samples. The FS concentrations measured in October did not exceed 2 CFU 100 ml^{-1} in two stations. No significant correlation was observed between TTC and SF concentrations for any of the data ($r=0.045$; $n=20$). The average values were all below the EU guide level for marine water quality of 500 CFU 100ml^{-1} for TTC and 100 CFU 100ml^{-1} for FS.

Among the phytoplankton, there were two groups of cyanobacteria, *Synechococcus* (SYNE) and *Prochlorococcus* (PROC). *Synechococcus* abundances were similar in both surveys (9.6 x 10^4 cells ml^{-1} and 8.4 x 10^4 cells ml^{-1}), whereas *Prochlorococcus* concentrations were significantly higher in May (mean of 0.5 x 10^4 cells ml^{-1}) than in October (mean of 3.1 x 10^4 cells ml^{-1}; $p<0.001$), with the highest values recorded at stations S8-S10 (Table 2). Picoeukaryotes (PICO) populations were similar for the two surveys, with no distinct distribution pattern irrespective of the season. Ratio between heterotrophic bacteria and picoautotroph abundances was significantly higher in May (mean ratio of 7.72) than in October (ratio of 1.64) ($p<0.001$). In May, maximum values were recorded at stations S3, S9 and S10 (15.5, 11.5 and 11.2, respectively) revealing a dominance of heterotrophic bacteria, whereas values decreased to 0.6 at stations S5 and S8 in October with a dominance of picoautotrophs.

During the 24 hours of bioassay experiments, lower values of ciliate abundances were counted at the beginning of the experiments (236 and 130 ciliates l^{-1} in May and October, respectively). As no ciliates were found after 24 h of incubation, these potential bacterial grazers were not taken into account in these bioassays. The density of heterotrophic and pigmented nanoflagellates ranged from 1.9 x 10^4 cells ml^{-1} in May to 4.4 x 10^3 cells ml^{-1} in October. The nanoflagellate abundance did not increase significantly in the control or in the different assays ($p > 0.05$, ANOVA). The number of bacteria increased significantly in May in the samples with only 1% of the predators and the increase was more marked in presence of nutrients, whatever the nutrient added (Figure 2). In October, the bioassay experiment did not reveal any significant increase in the number of bacteria in the samples with 1% of the predators, except when NP was added. Thus, removing 99% of bacterivorous

predators caused a higher bacterial growth rate compared to the growth rate in the presence of 100% of the predators (Table 3). Higher net growth rates (μ) were observed with nutrients in May compared to the results obtained in October with similar growth rates, for all the treatments except the NP combination (Table 3). Clearly, there was a close correspondence between growth- and grazing rate values. In the presence of nutrients, grazing rates were significantly higher in May than in October (for example, 0.059 *versus* 0.010 h^{-1} with +N; 0.061 *versus* 0.052 h^{-1} with +NP). Typically, nanoflagellates (PNF and HNF) ingested between 7.23 (with +N) and 9.43 bacteria h^{-1} (with + P) in May whereas the values observed in October were lower (0.84 and 2.54 h^{-1}, respectively) (Table 3).

The two PCAs were performed on the independent datasets (10 descriptors and 10 stations). The first two eigenvalues of the PCA analysis accounted for 72.0% of the total variability in May and 69.1% in October 2008. The analysis, therefore, only considered these two first axes to highlight the relationship between descriptors along a spatial distribution of samples. In May, the variable PROC was opposed to all other variables linked to the phytoplankton (AUTO, PICO, SYNE and Chlor-A) on the first axis, as also demonstrated by the significant correlations between them (Table 4). Bacterial variables (BACT-A, BACT-P, SF and TTC) were linked to the variable H/A (heterotrophic bacteria *versus* picoautotrophs) and opposed to the variable PROC. In October, the same correlations were observed among the phytoplankton descriptors, with a clear opposition of the descriptor PROC with all the other variables. However, unlike the situation described in May, the TTC concentrations did not seem to be linked to the bacterial abundances in October. The ratio H/A was always linked to the bacterial variables and not to the autotrophic variables, as also demonstrated by the significant correlations between them (Table 4).

For both sets of measurements, the location of each station in the atoll was clearly differentiated on the first axis. In May, there were high concentrations of autotrophic cells except *Prochlorococcus* cells (PROC) at stations S6 and S9. However, the highest abundances of *Prochlorococcus* were found at stations S2 to S5 (Fig. 3A). Stations S7 and S10 had high values of all the bacterial variables studied, especially the FIB concentrations. Station S7 was close to a large pearl farm, whereas station S10 was near Bird Island. In November, the distribution pattern at the stations was quite different from that observed in May. However, station S6 was always associated with high concentrations of AUTO, SYNE, PICO and Chlor-A and very low concentrations of PICO. Station S2 had the highest values of BACT-A and BACT-P but did not have high concentrations of TTC. Stations S9 and S10 near the passage had the highest values of PROC (Fig 3B).

4. Discussion

The production of black Tahitian pearls is of key importance for the economy of the Tuamotu Archipelago (French Polynesia) but since the 2000s intensified farming has caused a reduction in quality and a collapse in prices. A multidisciplinary research program was funded by the EDF (European Development Fund) to analyze the causes of the crisis. One of the major objectives of this program was to analyze the ecological environment of the pearl-oyster, *Pinctada margaritifera*, in Ahe atoll and its relationship with the pelagic trophic network. As explained by Thomas et al. (2010), the Ahe atoll seems to be comparable to closed atolls, mainly because of its high abundance of picophytoplankton. In fact, despite the existence of a channel and several open reef-flat spillways allowing exchanges with the surrounding ocean, the large depth of the lagoon could contribute to the reduction in water exchange. These conditions explained the increase in phytoplankton concentrations, especially of lagoonal communities like picoeukaryotes in the 11 atoll lagoons studied by Charpy and Blanchot (1998). The highest chlorophyll values were observed at Station 1, in the more confined, shallow area of the lagoon where pearl farming is more intensive. These high chlorophyll levels were related to high phytoplankton production linked to the recycling of nutrients by pearl oysters (Loret et al., 2000). The bacterial densities recorded during the two surveys were between 5.7 x 10^4 ml^{-1} (S5 in October) and 1.1 x 10^6 ml^{-1} (S10 in May), slightly lower than in previous studies conducted in the Ahe atoll (from April 2007 to March 2008; Thomas et al., 2010). All values were almost in the same range as values previously recorded in Takapoto (Sakka et al., 2000) and the nearby Tikehau atolls (Torréton and Dufour, 1996).

Bacterial production was close to that determined in the Tikehau and Takapoto atolls (4 to 5 µgC l^{-1} d^{-1}; Torréton and Dufour, 1996) and within the range of values reported in 11 other Tuamotu atolls (1.00 to 7.74 µgC l^{-1} d^{-1}; Torréton et al., 2002). Bacterial production (BACT-P) and abundance (BACT A) were correlated in October but not in May ($r=0.601$; $p<0.05$; Table 4), suggesting that bacterial communities were more controlled by resources in October (Billen et al., 1990; Bouvy et al., 1998). However, when compared to the control, no increase of bacterial numbers following the addition of nutrients was observed in the enrichment experiment performed on one single station,. Others studies should be pay special attention in the future about the availability of inorganic nutrients for bacterial consumption. Top-down factors (grazing by nanoflagellates) seemed to be more responsible for the regulation of bacterial dynamics in May (see below). Picophytoplankton abundances were in the same range as values recorded by Thomas et al. (2010), except for

Prochlorococcus concentrations. They reported mean values from 6.3 x 10^4 ± 4.0 x 10^4 cells ml^{-1} (April – May) to 13.6 x 10^4 ± 4.4 x 10^4 cells ml^{-1} (February – March) at 5m depth, in 12 stations in the lagoon, whereas the values measured in this study were all below 46 x 10^3 cells ml^{-1}, possibly as the stations were very shallow, located near the coast line and *Prochlorococcus* seems more abundant in deep lagoons (Charpy and Blanchot, 1998). However, as also observed by Thomas et al (2010), the highest values were found in October suggesting that oligotrophic situation occurs during this period in the Ahe atoll, and can be explained by a marine influence. Indeed, *Prochlorococcus* is considered to be an oceanic marker whereas picoeukaryotes are considered to be lagoon markers (Charpy, 1996). Thomas et al. (2010) reported *Synechococcus* abundances ranging from 7.7 x 10^4 ± 3.9 x 10^4 (November) to 12.2 x 10^4 ± 4.8 x 10^4 (April – May) cells ml^{-1}. These values are within the range of *Synechococcus* abundances observed during this study (Table 2). Picoeukaryote abundances found during this study were close to those measured by Thomas et al. (2010). Their abundances varied with respect to *Synechococcus* and *Prochlorococcus* concentrations in October (r=0.981 and 0.609, respectively; Table 4) but not in May. These potential differences point out the variation of composition of type of resources encountered in the system, as reported in many studies (e.g. Binder et al., 1996).

In terms of water quality, one of the aims of this study was to assess the impact of human sewage by monitoring the input and dispersion of fecal indicator bacteria (FIB). Fecal indicator bacteria (FIB) such as TTC and FS are widely accepted as being useful indicators of fecal contamination in the aquatic environment, generally associated with an increased risk of contracting gastrointestinal and respiratory illnesses (Haile et al., 1999). The analytical and statistical approaches adopted provided the first data on the trophic, health and sanitary status of the Ahe atoll. The major sewage source in the Ahe atoll was found at S9 and S10 in May, with the highest TTC and FS concentrations observed in the near shore area. In S9, the construction of a small harbor near a farm (close to twenty inhabitants) is certainly a potential source of contamination. It is also important to mention that a bird island is located near S10 (see Figure 1); bird guano has a potential effect that was not considered in this study but that may explain some of the high FIB concentrations, as also described in some atolls located in the central Pacific by Dinsdale et al. (2008). Local currents may also explain the presence of FIB at these stations. In October, FIB concentrations were very low, especially for FS. Despite unfavorable environmental conditions encountered in the Ahe atoll (high salinity, high direct illumination), FIB concentrations remained relatively high at S9 and S10 (94 and 200 CFU $100ml^{-1}$ of TTC in May). This suggests a large potential source of FIB contamination with

an input from sewage discharges high enough to overcome the effects of dilution. Values of bacterial production confirm the presence of dissolved organic matter from effluent, with the highest value at S9 in May, and also at S2 in October, and suggest a temporal/spatial variability in dissolved organic matter coming from episodic human activities (pearl farming, harbor). At atoll scale, bacterioplankton abundances and productions were significantly correlated with TTC concentrations in May, and with SF in October, respectively (Table 4; Figure 3). This may result from the direct discharge of heterotrophic bacteria and the stimulation of the autochthonous marine community by the release of sewage-derived organic substrates, as demonstrated by Cunha and Almeida (2006) in the coastal region off Aveiro (NW Portugal). The data presented here showed that the dilution and the mortality processes were not sufficient to avoid the presence of FIB in these stations. These observations corroborate previous studies in tropical zones (Senegal, West Africa) demonstrating the presence of FIB far from the sewage source (Troussellier et al., 2004; Bouvy et al., 2008). Standards and recommended guidelines based on indicators such as bacteria concentrations have been drawn up to prevent the public being exposed to pathogenic enteric microorganisms. There are no guidelines or water directives in Tuamotu Archipelago for bathing waters and so the values indicated in the EU directive were applied. The results obtained in this study show that all samples complied with the guideline value of 500 CFU $100ml^{-1}$ for TTC (maximum limit of 2000 CFU $100ml^{-1}$) and 100 CFU $100ml^{-1}$ for FS defined in the European Union bathing water quality directive 76/160/EEC. This study can, therefore, conclude that no evidence of human sewage was apparent at atoll scale, except for the station S9. The contamination observed at the station S10 seems to be linked to the concentration of bird guano, as reported by Choi et al. (2003). Indeed, sources of fecal bacteria other than human (i.e. birds, pets, wildlife, etc..) should not be disregarded and can introduce microbial pathogens into the marine environment (Nogales et al., 2011).

The enrichment experiments were performed in nutrient conditions comparable to those observed in 12 Tuamotu atolls (Dufour et al., 2001). In October 2010, Charpy et al (this volume) reported NH_4-N concentrations below 0.05 µmol l^{-1}, NO_3-N ranging from 0.05 to 0.10 µmole l^{-1} and concentrations of reactive orthophosphate averaging 0.26 ± 0.01µmol l^{-1}. Thus, in this oligotrophic environment, the growth of the heterotrophic bacterial populations in the Ahe atoll can be expected to be limited by phosphorus and/or nitrogen availability, given that mineral limitation of growth rates is widespread in various marine ecosystems, including atoll systems (e.g. Torréton et al., 2000). A nutrient is considered to limit bacterial growth when the availability of this nutrient is low relative to the

demand of the bacterial cells (Cotner et al., 1997). This was expressed as an increase in bacterial growth after the addition of the nutrient alone or in combination with other nutrients. In May, bacterial community dynamics were affected by a single addition of N and P or in combination N+P, especially without predators, whereas in October the responses were not significant, except with addition of NP. This contrast revealed a clear change in environmental conditions in the Ahe lagoon, explained in May by the high ratio of heterotrophic bacteria to picoautotrophs within the picoplankton compartment (mean of 7.7 *versus* 1.64 in October). In this context, carbon limitation can be also advanced in May knowing that the values of the ratio between heterotrophs and autotrophs are high compared to values in October. Another hypothesis can be advanced on the major role played by the virioplanktonic community in the aquatic food web (Fuhrman, 1999; Bouvy et al., 2011). In October, viral lysis can explain the absence of increase of bacterial numbers with addition of N and P, but the absence of data did not permit to conclude on this hypothesis. However, virus and bacteria dynamics has been studied in August 2009 in Ahe atoll, and patterns observed confirm that virus communities are abundant within coral reefs but viral infection of bacterial cells seems rare (Bouvy et al, this volume).

Previous studies in atolls have always shown that bacterial communities (from 10 lagoons) responded in various ways to nutrient additions (C, N and P), mainly linked to the *in situ* concentration, the atoll morphology and benthic nitrogen fixation in the deepest open lagoons (Torreton et al., 2000). As already discussed by Thingstad et al. (1998), the hypothesis of an inorganic nutrient limitation on heterotrophic bacteria has important conceptual and functional consequences. Heterotrophic bacteria have been shown to compete successfully with phytoplankton for phosphate owing to their higher affinity for this nutrient (Kirchman, 1994). This competition suggests that nutrient limitation of primary production may have occurred in May and may have been reinforced by the bacterioplankton demand. Torréton et al. (2000) concluded that bacteria are net consumers of inorganic nitrogen and that this nitrogen is recycled by bacterioplankton grazers.

With the limited data set obtained during this study on bacterioplankton, we can only speculate that the *in situ* concentrations reflect the nutrient status of bacterioplankton in October (except with +NP), whereas phosphorus and nitrogen stimulate bacterial growth rates in May. It is not possible to state that there is an overall limitation in the Ahe atoll since the concentrations probably vary over short time-scales, responding to variations in nutrient concentrations driven by small-scale physical phenomenon such as wind (Thomas et al., 2010).

This study also investigated the growth rates of bacterial communities with and without bacterivorous predators. It is now commonly accepted that nanoflagellates are the most important grazers of bacteria in most environments (e.g. Sanders et al., 2000; Tsai et al., 2011). The ingestion rates of nanoflagellates (HNF and PNF) on natural populations of bacteria in the Ahe atoll (1.36 and 3.61 cells nanoflagellate^{-1} h^{-1} in May and October, respectively) are comparable to data in the literature (e. g. Seong et al, 2006; Tsai et al., 2011). However, our ingestion rate values are lower than those reported by Sakka et al. (2000) for the Takapoto atoll (107.6 ±44.5 cells HNF^{-1} h^{-1}). For the treatment with NP, the mean values for grazing on bacteria were high, compared to the control, with 1.46 d^{-1} in May and 1.25 d^{-1} in October, suggesting that nitrogen and phosphorus deficiency can affect in different ways the growth and metabolism of bacteria, as suggested by Torreton et al. (2000). Sakka et al. (2000) reported an average protozoan grazing of 28% of bacterial growth. In this study, a maximum of 15.3% (May) and 63.4% (October) of the bacterial stock were ingested per day for the total nanoflagellate community (PNF and HNF) in the controls. However, in May with phosphorus addition, 66.3 % of the bacterial stock was ingested per day, suggesting that, simultaneously to the increase of the bacterial growth rate due to nutrient enrichment, grazing rates also increased. In our experiment, it is obvious that grazing rates of bacteria by PNF and HNF were higher in May, concomitant with the bacterial growth rates, which provided the evidence for the close coupling of bacterial and nanoflagellate dynamics at this station. Water temperature and prey abundance are usually considered among the most important factors regulating the grazing activity of HNF (Choi, 1994). In the tropical conditions encountered in the Ahe atoll, water temperature is certainly not limiting, but the oligotrophic conditions (Charpy et al., this volume) may stimulate the ingestion of particles by PNF (pigmented nanoflagellates) (Unrein et al., 2007). Flagellates, as expected, seem to be the major protistan bacterivores in Ahe. Moreover, the alternative source of bacterial mortality, such as lysis by viruses, does not seem very important in this environment (Bouvy et al., this volume). Very few studies have been carried out on viruses in atoll lagoons (Seymour et al., 2005; Dinsdale et al., 2008; Patten et al., 2011) and nothing is known about the response of atoll lagoon bacterioplankton to the presence of viruses.

Based on sub-surface sampling, this study revealed distinctive spatial and temporal patterns in the Ahe atoll, which were characterized by microbial variables (chlorophyll-a, picoplankton structure, etc). In marine environments, substrate availability and water temperature are considered to be important factors that regulate plankton dynamics, especially bacterioplankton (Pomeroy and Wiebe, 2001). Similar temperature data recorded in May and October cannot explain the difference between

the patterns observed during the two periods. It may, therefore, be argued that the major regulator of picoplankton distribution is the substrate supply rather than the temperature. The direct influence of the ocean on the lagoon picoplankton structure was insignificant as also reported by Thomas et al. (2010). These authors explained that wind can be the main factor driving spatial and daily variability of phytoplankton and bacterioplankton. However, station S6 (harbor village) was always associated with high concentrations of *Synechococcus* and picoeukayotes and low concentrations of *Prochlorococcus*, reflecting the possible impact of human activities with inputs from the land. The positive ratio between heterotrophic bacteria and autotrophic cells (H/A) observed during both periods reflects the high heterotrophic content in the microbial network, especially in May. According to Duarte and Agusti (1998), our result show that Ahe atoll belongs to the unproductive aquatic system, without high external inputs of inorganic nutrients issuing from human activities. Higher heterotrophic influence could be related to changes (i) in the relative contribution of the various picophytoplankton components (such as higher *Prochlorococcus* abundance) or (ii) in the nutrient concentrations (no data available to assess this hypothesis). This study, therefore, supports the idea that the planktonic communities in the Ahe atoll can act as CO_2 sources, according to Duarte and Agusti (1998). Unlike static analyses producing indices similar to those of the OECD (Organization for Economic Cooperation and Development), enrichment bioassays appear to provide a good understanding of bacterial dynamics, especially given potential nutrient input, as is the case of the Ahe atoll. Hotspots of FIB presence were found in the vicinity of some farms but the concentrations were too low to draw any conclusions on any possible impact on the microbial food web or, more generally, on the health of the oysters farmed (*Pinctada margaritifera*).. However, it will be useful if the authorities take measures to limit human sewage runoff into atoll waters as these waters are used for the pearl industry which is essential for the economy of French Polynesia.

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Legends of the figures

Figure 1: Location of the Tuamotu Archipelago and of the ten stations studied in Ahe atoll. Location of the pass (between S8 and S9) is noted.

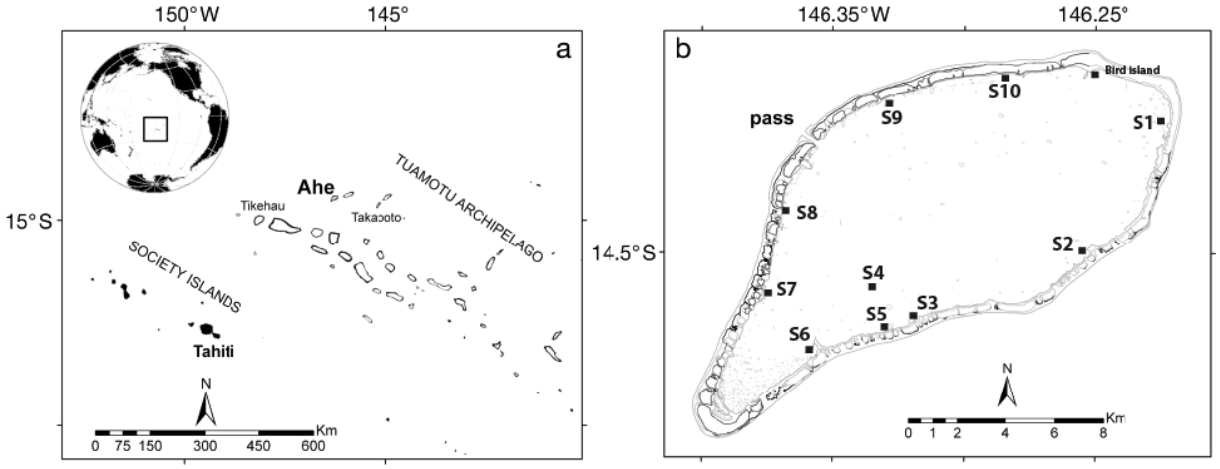
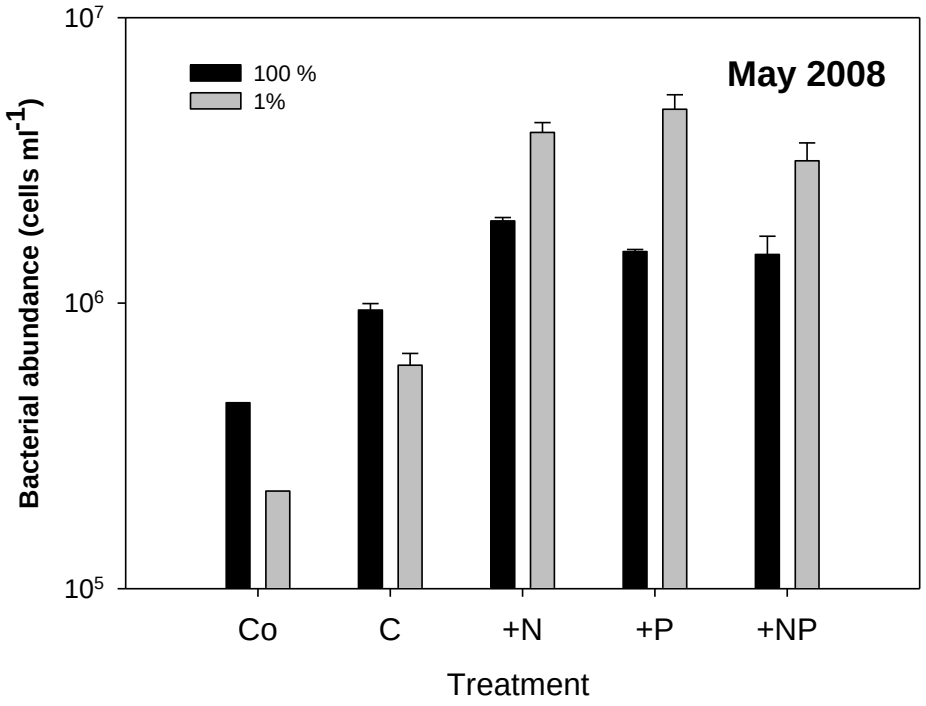


Figure 2: Changes in bacterial abundance following different nutrient treatments (N, P and NP) between beginning of experiment (control at t = 0; C0) and end of incubation (24h) conducted with (Serie 100%) and without (Serie 1%) bacterial predators. C = control at t = 24 hours.



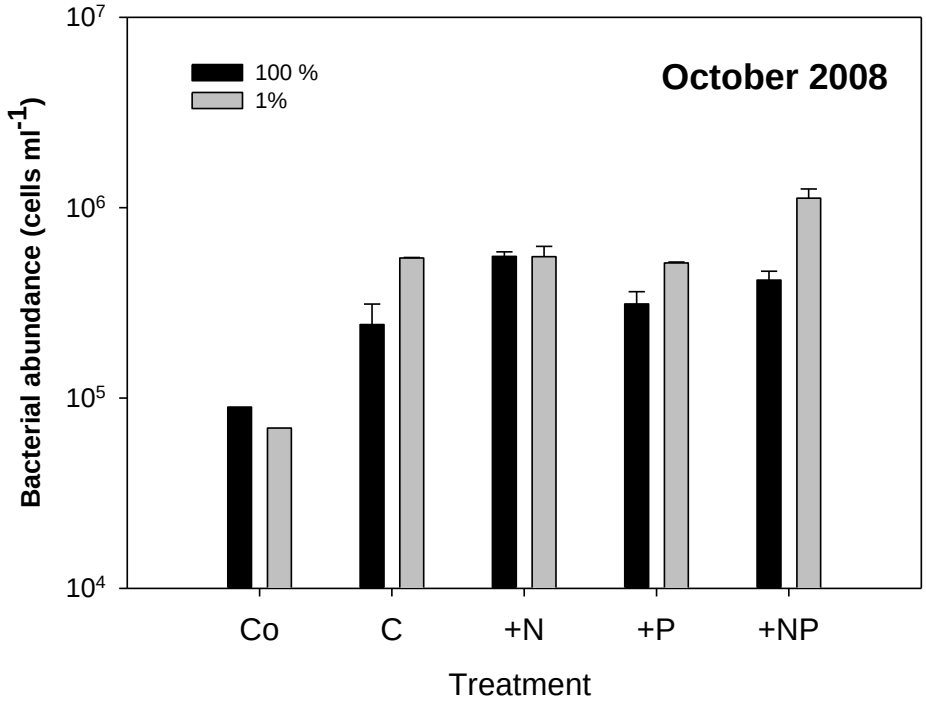
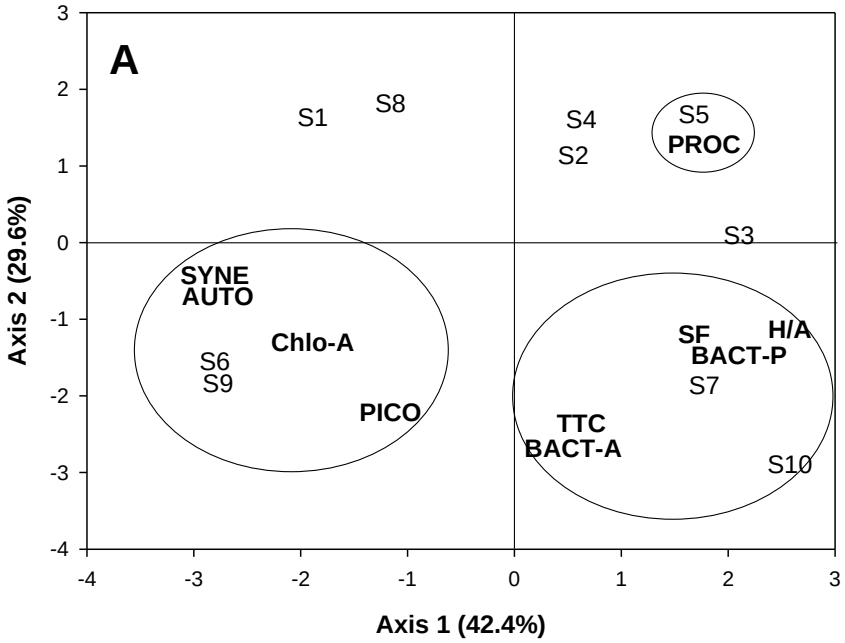


Figure 3: Principal component analysis (PCA) on the two first axes in May (A) and October (B) 2008. Eigenvalues for each axis of the PCA are reported. Stations are identified as in Table 1. Abbreviations: Chlo-a: chlorophyll-a; Bact-A: bacterial abundance; Bact-P: bacterial production; TTC: concentrations of thermotolerant coliforms; FS: concentration of faecal streptococci; SYNE: Synechococcus abundance; PROC: Prochlorococcus abundance; PICO: Pigmented picoeukaryote abundance; AUTO: Picoautotroph abundance; H/A: ratio between heterotroph (bacteria) and picoautotroph abundance.



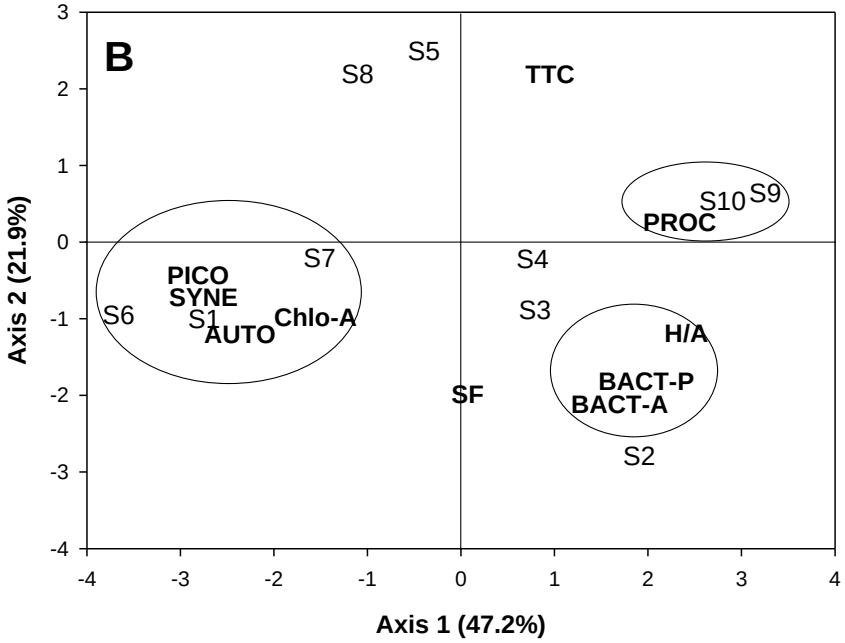


Table 1 : List of stations studied in Ahe atoll with their geographical coordinates. Maximum depth and average temperature of the water column are reported for the two surveys (May and October 2008).

station code	station name	latitude South	longitude West	depth m
1	Raita pension	14°27' 10"	146°13'18"	2.0
2	Motu Tahiri farm	14°30'39"	146°15'53"	3.7
3	Maruata farm	14°31'18"	146°18'32"	2.5
4	Aito pearl farm	14°31'28"	146°19'03"	1.8
5	Apeang farm	14°31'14"	146°19'44"	3.4
6	Harbour village	14°32'17"	146°21'24"	2.5
7	Manuia farm	14°31'17"	146°22'51"	1.7
8	Kamoka farm	14°28'53"	146°22'67"	10.0
9	Mamaha farm	14°26'29"	146°19'38"	1.8
10	Pang Fat Kyn farm	14°25'57"	146°16'23"	3.8

Table 2 : Mean and standard deviation (STD) for the parameters studied at each station during the two surveys (May and October 2008). Differences between surveys were tested using the non-parametric Mann-Whitney U-test. (ns. non significant = p > 0.05). Abbreviations: Chlo-a: chlorophyll-a; Bact-A: bacterial abundance; Bact-P: bacterial production; TTC: concentrations of thermotolerant coliforms; FS: concentration of faecal streptococci; SYNE: Synechococcus abundance; PROC: Prochlorococcus abundance; PICO: Pigmented picoeukaryote abundance; AUTO: Picoautotroph abundance; H/A: ratio between heterotroph (bacteria) and picoautotroph abundance. nd: non determined

station	CHLOR	BACT-A	BACT-P	TTC	SF	SYNE	PROC	PICOEUK	AUTO	Hete/Auto
				CFU	CFU					
code	µg l^{-1}	cells ml^{-1}	µgC l^{-1} d^{-1}	100 ml^{-1}	100 ml^{-1}	cells ml^{-1}	cells ml^{-1}	cells ml^{-1}	cells ml^{-1}	
May 2008										
S1	0.39	$4.9x10^5$	1.04	0	0	$9.9x10^4$	$0.3x10^4$	$4.1x10^3$	$1.1x10^5$	4.58
S2	0.23	$6.3x10^5$	0.58	1	5	$12.0x10^4$	$0.6x10^4$	$5.0x10^3$	$1.3x\ 10^5$	4.67
S3	0.35	$9.2x10^5$	7.38	0	23	$5.2\ x10^4$	$0.5x10^4$	$2.3x10^3$	$5.9x10^4$	15.50
S4	0.33	$6.3x10^5$	1.04	0	2	$10.0x10^4$	$0.5x10^4$	$3.0x10^3$	$1.1x10^5$	5.54
S5	0.11	$5.5x10^5$	4.56	2	43	$6.1x10^4$	$0.8x10^4$	$3.5x10^3$	$7.3x10^4$	7.46
S6	0.39	$9.0x10^5$	4.42	13	9	$13.0x10^4$	$0.3x10^4$	$5.5x10^3$	$1.4x10^5$	6.46
S7	0.55	$8.1x10^5$	2.80	6	9	$18.0x10^4$	$0.3x10^4$	$7.3x10^3$	$1.9x10^5$	4.29
S8	0.37	$5.2x10^5$	0.42	7	0	$7.2x10^4$	$0.9x10^4$	$4.0x10^3$	$8.5x10^4$	6.11
S9	0.26	$7.9x10^5$	15.22	94	12	$5.8x10^4$	$0.6x10^4$	$5.4x10^3$	$6.9x10^4$	11.49
S10	0.37	$1.1x10^6$	3.5	200	8	$8.6x10^4$	$0.5x10^4$	$9.8x10^3$	$1.0x10^5$	11.13
mean	0.34	$7.4x10^5$	4.10	32	11	$9.6x10^4$	$0.5x10^4$	$5.0x10^3$	$1.1x10^5$	7.72
std	0.12	$2.1x10^5$	4.49	66	13	$3.9x10^4$	$0.2x10^4$	$2.2x10^3$	$3.9x10^4$	3.75
October 2008										
S1	nd	$1.1x10^5$	0.48	4	1	$11.0x10^4$	$2.4x10^4$	$4.6x10^3$	$1.4x10^5$	0.80
S2	0.26	$2.3x10^5$	12.13	0	2	$6.5x10^4$	$4.3\ x10^4$	$2.5x10^3$	$1.1x10^5$	2.09
S3	0.31	$2.9x10^5$	2.22	0	0	$6.8x10^4$	$2.3\ x10^4$	$2.6x10^3$	$9.4x10^4$	3.13
S4	0.24	$1.8x10^5$	3.46	0	0	$6.2x10^4$	$2.5\ x10^4$	$2.4x10^3$	$8.9x10^4$	2.03
S5	0.17	$0.6x10^5$	0.03	11	0	$6.3x10^4$	$3.0\ x10^4$	$2.3x10^3$	$9.6x10^4$	0.60
S6	0.32	$1.5x10^5$	2.16	0	0	$18.0x10^4$	$2.2\ x10^4$	$8.9x10^3$	$2.1x10^5$	0.72
S7	0.21	$1.7x10^5$	0.58	0	0	$10.0x10^4$	$2.3\ x10^4$	$4.4x10^3$	$1.3x10^5$	1.28
S8	0.15	$0.8x10^5$	0.05	40	0	$8.3x10^4$	$3.1\ x10^4$	$4.3x10^3$	$1.2x10^5$	0.66
S9	0.10	$2.5x10^5$	6.71	55	0	$5.4x10^4$	$4.6\ x10^4$	$1.1x10^3$	$1.0x10^5$	2.49
S10	0.11	$2.5x10^5$	4.09	15	0	$5.5x10^4$	$3.9\ x10^4$	$9.8x10^2$	$9.6x10^4$	2.63
mean	0.21	$1.8x10^5$	3.19	13	0	$8.4x10^4$	$3.1x10^4$	$3.4x10^3$	$1.2x10^5$	1.64
std	0.08	$0.8x10^5$	3.79	20	1	$3.8x10^4$	$0.9x10^4$	$2.3x10^3$	$0.4x10^5$	0.94
test	***0.013***	***<0.001***	***ns***	***ns***	***0.003***	***ns***	***<0.001***	***ns***	***ns***	***<0.001***

Table 3 : Net bacterial growth rates (µ) and grazing rates of bacteria by predators (g) in May and October 2008 from dilution experiments (1 and 100%) without (control, C) and with nutrient (N, P and NP). Experiments were performed in triplicates. Ingestion rates (I) were calculated from the formula described in M&M.

MAY				
dilution	100%	1%		
variable	µ +g	µ	g	I
unit	(h^{-1})	(h^{-1})	(h^{-1})	Bact $HNF^{-1}h^{-1}$
C	0.031	0.042	0.011	1.36
N	0.061	0.121	0.059	7.23
P	0.051	0.128	0.078	9.43
NP	0.050	0.111	0.061	7.44
OCTOBER				
dilution	100%	1%		
variable	µ +g	µ	g	I
unit	(h^{-1})	(h^{-1})	(h^{-1})	Bact $HNF^{-1}h^{-1}$
C	0.042	0.086	0.044	3.61
N	0.076	0.086	0.010	0.84
P	0.052	0.083	0.031	2.54
NP	0.064	0.116	0.052	4.24

Table 4: Mann-correlation coefficients between the 10 biological variables studied during the two surveys. May and October 2008. Significant values are given in bold (* p< 0.05; ** p<0.01; *** p<0.001). Chlo-a: chlorophyll-a; Bact-A: bacterial abundance; Bact-P: bacterial production; TTC: concentrations of thermotolerant coliforms; FS: concentration of faecal streptococci; SYNE: Synechococcus abundance; PROC: Prochlorococcus abundance; PICO: Pigmented picoeukaryote abundance; AUTO: Picoautotroph abundance; H/A: ratio between heterotroph (bacteria) and picoautotroph abundance.

May 2008										
	Chlo-a	*Bact-A*	*Bact-P*	*TTC*	*SF*	*SYNE*	*PROC*	*PICO*	*AUTO*	*H/A*
Chlo-a	1.000	0.328	-0.214	0.024	-0.572	**0.615***	**-0.708***	0.381	**0.602 ***	-0.162
Bact-A		1.000	0.379	**0.688 ***	0.050	0.062	-0.380	0.025	0.078	**0.606 ***
Bact-P			1.000	0.339	0.362	-0.448	-0.027	0.013	-0.452	**0.676 ***
TTC				1.000	-0.077	-0.216	0.009	0.772	-0.173	0.445
SF					1.000	-0.406	0.313	-0.246	-0.406	0.403
SYNE						1.000	**0.595 ***	0.410	**0.998*****	**-0.683****
PROC							1.000	-0.280	-0.561	0.076
PICO								1.000	0.454	-0.042
AUTO									1.000	**-0.686***
H/A										1.000

October 2008										
	Chlo-a	*Bact-A*	*Bact-P*	*TTC*	*SF*	*SYNE*	*PROC*	*PICO*	*AUTO*	*H/A*
Chlo-a	1.000	0.144	0.048	**-0.766***	0.250	0.555	**-0.633***	0.569	0.453	-0.001
Bact-A		1.000	**0.601***	-0.027	0.088	0.335	0.356	-0.407	-0.291	**0.950*****
Bact-P			1.000	0.054	**0.659***	-0.337	**0.727****	-0.356	-0.196	0.532
TTC				1.000	-0.283	-0.340	**0.610***	-0.328	-0.228	0.042
SF					1.000	-0.051	0.311	-0.044	0.022	0.010
SYNE						1.000	-0.573	**0.981*****	**0.979*****	-0.578
PROC							1.000	**0.609***	-0.394	0.384
PICO								1.000	**0.951*****	**-0.629***
AUTO									1.000	-0.556
H/A										1.000