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Bacterial community composition and potential controlling mechanisms along a trophic gradient in a barrier reef system

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ABSTRACT: Bacterial abundance and community composition were investigated along trophic gradients in the barrier reef lagoon of Noumea, New Caledonia. Bacterial abundance and the percentage of high nucleic acid (%HNA) bacteria (a potential indicator for bacterial production) increased from offshore waters towards the head of the bays. 16S rRNA gene PCR and denaturing gradient gel electrophoresis (DGGE) were used as genetic fingerprints for assessing differences in bacterial community composition. Sequences of DGGE bands were assigned to (1) the genera *Rugeria* and *Roseobacter* (*Rhodobacteriaceae*), (2) the SAR11 cluster, (3) other *Alphaproteobacteria*, and (4) the genus *Alteromonas*. Removal of the operationally defined attached bacteria by prefiltration did not affect community profiles in offshore waters but had a strong influence in the bays, probably due to the much higher particle load and thus, attached bacteria in the bays. For the free-living community, the number of bands decreased linearly with increasing water residence time, chlorophyll *a* concentration, and viral abundance. Specific bands were found for offshore waters and the 2 investigated semi-enclosed bays, whereas the lagoon showed no specific bands. A similarity analysis showed specific clusters for offshore water, the lagoon, and the bays. A principle component analysis together with cluster and correlation analysis indicated that water residence time, viruses, and a complex top-down cascading effect of ciliate grazers on flagellates influenced community composition. Also, data from fingerprints of the total and free-living communities suggest that the free-living and the attached community are controlled by different mechanisms.

KEY WORDS: Diversity · Virus · Protist · Flagellate · Ciliate · *Roseobacter* · SAR11

INTRODUCTION

Genetic fingerprinting (sometimes including sequencing) and cloning have been used to assess changes in bacterial community composition and potential controlling mechanisms. Genetic fingerprints have been used to characterize gradients such as depth (Lee & Fuhrman 1991, Giovannoni et al. 1996, Acinas et al. 1999), salinity (Bouvier & del Giorgio 2002, Crump et al. 2004), or productivity (Reinthaler et al. 2005, Winter et al. 2005). Also, the dynamics of bacterioplankton diversity during phytoplankton blooms have been studied (Arrieta & Herndl 2002, Arrieta et al. 2004) and the results used to argue that there is seasonality in oceanic bacterio-

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plankton composition (Pinhassi & Hagström 2000, Fuhrman et al. 2006). Although the number of studies on bacterial diversity has increased in recent years, many environments remain poorly investigated, including the water column systems of tropical coral reefs (Dinsdale et al. 2008).

A variety of mechanisms influence bacterial community composition, including UV radiation (Winter et al. 2001), turbulence (Malits & Weinbauer 2009), nutrient and dissolved organic matter composition (Arrieta & Herndl 2002), grazing (Pernthaler 2005, Pernthaler & Amann 2005), and viral infection (Hewson et al. 2003, Winter et al. 2004). Also, general ecological patterns such as the species-area relationship (Horner-Devine et al. 2004b), the increase of diversity with system ('island') size (Bell et al. 2005, Reche et al. 2005), or the existence of latitudinal gradients (Fuhrman et al. 2008) appear to apply to bacteria. While one can expect that such influences and patterns are also operational in coral reef systems, no studies are available. In coral reef systems, the release of nutrients and mucus by corals stimulates the production of bacteria in nearby waters (Herndl & Velimirov 1986, Schiller & Herndl 1989) and fuels the food web of lagoon systems (Wild et al. 2004). This likely explains observations that the microbial loop is predominant and the community is often heterotrophic in the water column part of reef systems (Ferrier-Pagès & Gattuso 1998). Typically, lagoon bacteria are more active than bacteria in the open ocean, and usually only a moderate fraction of bacteria (ca. 10%) is attached to particles in the open ocean and reef lagoons (Rath et al. 1993, Torréton & Dufour 1996, Torréton 1999, Torréton et al. 2002). However, attached bacteria are often more active than their free-living counterparts (Simon et al. 2002, Grossart et al. 2007). It is also well documented that the diversity of prokaryotes attached to organic aggregates can be very different from free-living prokaryotes (DeLong et al. 1993, Acinas et al. 1999), although this has not been investigated yet in coral reef systems.

Water residence time is an important concept to understand the hydrodynamically restricted aquatic systems and its control of ecological processes in the plankton. Water residence time is thought to be the most important physical control factor of ecological processes in estuaries (Jouon et al. 2006). In atoll and lagoon systems, it has been shown that chlorophyll *a* (chl *a*) concentrations, primary production, and bacterial production increase with water residence time (Delesalle & Sournia 1992, Charpy et al. 1997, Torréton et al. 2007). Water residence time also influences the dynamics of dissolved organic carbon and transparent exopolymeric particles (TEP; Mari et al. 2007b). Water residence time also determines whether a specific bacterial community can develop; thus, for the development of a specific bacterial community, the water residence time must exceed that of bacterial growth. Indeed, a study in an estuary showed that specific communities developed with high bacterial production and long water residence time (Crump et al. 2004).

A recent study of organic matter reactivity along eutrophic gradients in the lagoon of New Caledonia revealed that the transfer efficiency of organic matter from the dissolved to the particulate phase via aggregation processes was reduced when the residence time of the water increased (Mari et al. 2007b). Such a reduction in organic matter reactivity may, in addition to water residence time itself, influence the distribution and fate of attached and free-living prokaryotes. The lagoon of Noumea represents a steep, 20 km long gradient from the oligotrophic open ocean to mesotrophic semi-enclosed bays with a negligible freshwater input from terrestrial runoff. Here we present data on the community composition of bacterioplankton in the water column of various lagoon habitats by using genetic fingerprints and relate these diversity patterns to potential controlling factors such as water residence time and mortality factors.

MATERIALS AND METHODS

Study site and sampling. The SW lagoon of New Caledonia is an enclosed, relatively shallow site (average depth: 20 m), surrounded by oligotrophic oceanic water. In contrast to the oligotrophy observed near the coral barrier, the near-shore environment is subject to terrestrial and, especially in the bays around the city of Noumea, to both industrial and urban inputs that increase the general productivity in these areas. Eutrophication in Grande Rade Bay is mainly of industrial origin, due to the close proximity of a large nickel smelter, while in Sainte Marie Bay, eutrophication is mostly of urban origin, i.e. due to wastewater outfalls from the Sainte Marie area. For more details see Mari et al. (2007b).

Seawater samples were collected during November and December 2004 at 10 stations distributed along 2 transects in the SW lagoon of New Caledonia, going from 2 semi-enclosed bays in the city of Noumea (~130 000 inhabitants) to a station outside the reef barrier (Fig. 1). Both transects were sampled twice, on 22 and 24 November 2004 (from Grande Rade Bay to the outer edge of the coral reef) and 29 November and 1 December 2004 (from Sainte-Marie Bay to the outer edge of the coral reef). Samples were obtained from 5 m depth using a Teflon pump. CTD casts were used on each sampling occasion to determine the vertical distribution of physical parameters. All stations were sampled within 1 h; water samples were stored in acid-

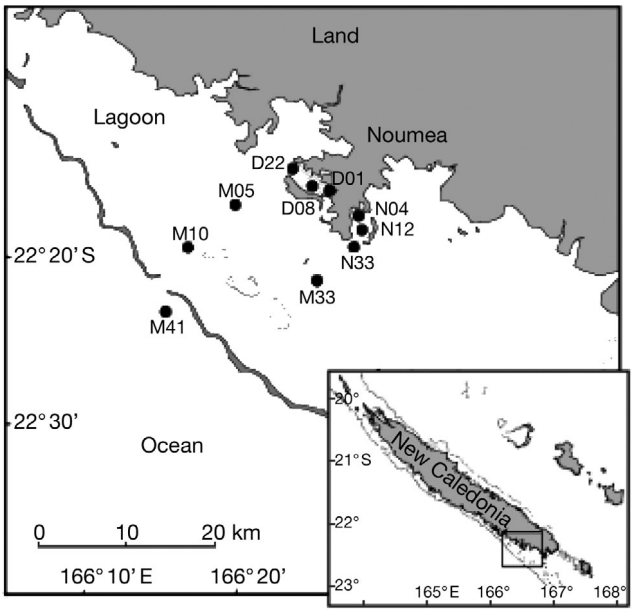

Fig. 1. Study area showing sampling stations. Stns D and N are in Grande Rade Bay and Sainte Marie Bay, respectively, whereas Stns M are in the lagoon proper and offshore

cleaned polycarbonate bottles and brought back to the laboratory within 1 to 2 h after sampling.

Residence time of the water masses. The parameter used to depict the 'residence time' of the water mass is the Local e-Flushing Time (LeFT; in days). The LeFT is defined as the time required for a tracer mass contained within the control volume (station) to be reduced by a factor 1/e by waters coming from outside the lagoon and, thus, it describes the replacement efficiency of water masses in the study area (Jouon et al. 2006): the shorter the LeFT, the faster the water masses at the location will definitely be replaced and, thus, renewed. The annual average LeFT at the different stations was calculated from a hydrodynamic model taking into account topographic constraints, average wind condition, and tidal cycle (Jouon et al. 2006). During the sampling period, wind conditions were similar to those used as input parameters in the hydrodynamic model (i.e. well established trade winds of about 8 to 10 m s^{-1}). The LeFT was 0 at the offshore station (input parameter) and it was calculated for each station to range from 0.4 to 5.6 d in the lagoon stations, 12 to 17 d in Sainte Marie Bay, and 31 to 47 d in Grande Rade Bay (Mari et al. 2007b; Table 1).

Enumeration of microbes. Water samples were preserved with glutaraldehyde (0.5% final concentration) for 30 min at 4°C, then flash frozen in liquid nitrogen and stored at −80°C until analysis. Viruses and bacteria were stained with SYBRGreen I and counted by flow cytometry using the protocol of Gasol & del Giorgio (2000) for bacteria and Brussaard (2004) for viruses. *Synechococcus*, *Prochlorococcus*, and autotrophic picoeukaryotes were counted as described by Jacquet et al. (2006). Samples for viruses were diluted 100-fold in TE (10 mM Tris, 1 mM EDTA, pH 8.0) buffer. Based on fluorescence intensity (and side scatter) 2 bacterial populations were distinguished: low (LNA) and high nucleic acid (HNA) bacteria.

Flagellate and ciliates were stained with DAPI, collected onto 0.8 µm pore-size Nuclepore filters, and counted using an epifluorescence microscope. It should be noted that ciliate counts obtained by this method can be underestimates. Heterotrophic (HNF) and autotrophic nanoflagellates (ANF) were distinguished based on autofluorescence. Total flagellate (TNF) abundance is the sum of HNF and ANF. Chl *a* was determined fluorometrically from duplicate 200 ml subsamples filtered onto 25 mm Whatman GF/F filters, and data were obtained from Mari et al. (2007b).

Table 1. Bacterial and environmental characteristics of the sampling stations. Local e-flushing time (LeFT) and chlorophyll *a* (chl *a*) data are from Mari et al. (2007b) and are presented to characterize the trophic gradient. Each value corresponds to the average (range in parentheses) of 2 or 4 measurements (except free-living communities, FLC). The numbers of denaturing gradient gel electrophoresis (DGGE) bands are for total bacterial communities (TC) and FLC. HNA: high nucleic acid bacteria; ND: not determined

Trophic group	Stn	LeFT (d)	Chl *a* (µmol l^{-1})	No. of bands TC	No. of bands FLC	Bacteria (10^6 ml^{-1})	% HNA bacteria
Head of bays	D01	46.9	1.64 (1.50–1.82)	14.5 (14–15)	12	1.27 (1.20–1.34)	80 (79–82)
	N04	17.1	1.72 (0.97–2.46)	16.0 (16)	ND	1.70 (1.44–1.97)	69 (62–75)
Middle of bays	D08	40.8	0.88 (0.85–0.92)	13.5 (13–14)	13	1.08 (1.03–1.14)	77 (73–81)
	N12	12.9	1.12 (0.89–1.36)	16.0 (16)	ND	1.42 (1.32–1.52)	67 (66–69)
Mouth of bays	D22	31.0	0.47 (0.43–0.52)	12.5 (12–13)	13	1.13 (1.11–1.16)	62 (62)
	N33	12.4	0.38 (0.35–0.41)	11.5 (11–12)	ND	1.10 (1.07–1.14)	60 (59–62)
Middle of lagoon	M05	5.6	0.31 (0.28–0.34)	12.0 (12)	13	1.04 (1.03–1.06)	51 (51)
	M33	0.5	0.30 (0.31–0.31)	11.5 (11–12)	ND	1.26 (1.10–1.34)	57 (57–58)
Near barrier	M10	0.4	0.31 (0.25–0.36)	12.8 (12–13)	14	0.99 (0.82–1.22)	60 (52–64)
Open ocean	M41	0	0.19 (0.10–0.31)	15.3 (14–16)	15	0.67 (0.61–0.73)	54 (51–60)

DNA extraction. Cells from 0.5 to 1 l samples (total community) were recovered on a 0.2 µm pore-size polycarbonate filter (diameter 47 mm; Whatman). A replicate sample (except for the Sainte-Marie Bay transects) was prefiltered through 1 µm polycarbonate filter (diameter 47 mm; Whatman) to remove mainly attached bacteria, and the filtrate containing mainly the free-living community was collected. Nucleic acids were extracted from the filters and purified as described elsewhere (Winter et al. 2004). Briefly, after 4 freeze-thaw cycles (–196°C to +37°C), an enzyme treatment was performed with lysozyme (1.25 mg ml^{-1} final concentration; Fluka BioChemika) for 30 min at 37°C followed by a digestion with Proteinase K (100 µg ml^{-1} final concentration; Fluka BioChemika) and 1% sodium dodecyl sulfate for 2 h at 55°C. In contrast to the phenol-chloroform extraction step from the original protocol, nucleic acids were extracted with 4.5 M $NaCl_2$, followed by 100% isopropanol precipitation. This modified procedure avoids the use of a toxic chemical and yields genetic fingerprints identical to those obtained by the original method (Malits & Weinbauer 2009). The pellets were re-suspended in 60 µl of 0.5× TE buffer (10 mM Tris, 1 mM EDTA [HCl, pH 8.0]).

PCR. Conditions of the touchdown PCR and chemicals were as described by Schäfer & Muyzer (2001). One to 4 µl of cleaned nucleic acid extract were used as template in a 50 µl PCR reaction (1.5 mM $MgCl_2$, 0.25 µM of each primer, and 2.5U *Taq* polymerase; Sigma) together with a positive and a negative control. A fragment of the 16S rRNA gene was amplified using the primer pairs 341F-GC/ 907R and 344F-GC/ 915R for *Bacteria* and *Archaea,* respectively (Schäfer & Muyzer 2001). The bacterial primers had the modifications suggested to also detect *Gammaproteobacteria* (Sanchez et al. 2007). Archaeal PCR products could either not be obtained or were often too weak to perform denaturing gradient gel electrophoresis (DGGE). Therefore, we conclude that *Archaea* were not abundant at the study site and we use the term 'bacteria' in the following.

DGGE. DGGE procedures followed those described by Schäfer & Muyzer (2001). PCR products (500 ng) were separated into bands by electrophoresis for 18 h at 100 V on acrylamide/bis-acrylamide (6%) gels prepared using a gradient of 30 to 70% (urea and formamide) using an INGENYphorU DNA Mutation Detection System (Ingeny International). A standard made from a 200 l bacterioplankton extraction was loaded to allow for comparison of bands within and between gels. DGGE gels were photographed with a gel documentation system (GelDoc EQ; Bio-Rad) after 15 min of staining with a 10× SYBR Gold solution (Molecular Probes). Analysis of band patterns between lanes was performed with the Quantity One Software (Bio-Rad) using a variety of exposure times.

Selected samples covering all detected bands were rerun on a DGGE gel, and bands were excised. The DNA was eluted overnight in autoclaved Milli Q water at 4°C, checked for purity by re-running it on a gel, and sequenced (MWG-Biotech). Sequences were edited using the freeware program 4Peaks 1.6 (www.mekentosj.com). Up to approximately 500 bp of the 16S rRNA gene were used in a BLAST search.

Statistical analyses. Correlation and regression analyses were performed using a probability (p) < 0.05 as significant. Data were log or arcsine transformed (for percentages) to meet statistical requirements. Bacterial fingerprints were analyzed using a Simple Match similarity index. The difference in the number of bands was compared between offshore, lagoon, and bay waters using an analysis of variance (ANOVA). Data comparison between the 2 bays was done by Student's *t*-test.

Potential relationships among variables were tested by linear pairwise correlations (Spearman correlation analysis). Key parameters were used to perform principal component analysis (PCA). Data were log(+1) transformed to satisfy the requirements of normality and homogeneity of variance necessary for parametric statistics. Only factors with an eigenvalue >1 were retained. All statistical analyses were performed with the JMP program.

RESULTS

Characterization of sampling sites

A physical and chemical characterization of the sampling stations at the sampling dates can be found in Mari et al. (2007b). Briefly, salinity and temperature varied only slightly between sampling sites and dates, and no stratification of the water column was observed within the barrier reef. Nutrient concentrations were highest at the head of the bays and gradually decreased towards the mouth of the bays, the distant parts of the lagoon, and the open ocean. All sampling stations during the sampling period were characterized by inorganic nitrogen limitation as indicated by N:P ratios <5 (Mari et al. 2007b).

Microbial abundance

Bacterial abundance ranged from 0.6×10^6 to 2.0×10^6 ml^{-1}, and in all 4 transects, bacterial abundance was lowest at the open ocean station and highest at the head of the bays (Table 1). Bacterial abundance was generally higher in Sainte Marie Bay than in Grande Rade Bay. HNA ranged from 51 to 82% and increased from 54% in the open ocean to 80% at the head of Grande Rade Bay.

The abundance of *Synechococcus* ranged from 0.8 to 19.8×10^4 ml^{-1}. The abundance was highest in Grande Rade Bay, similar in the lagoon and Sainte Marie Bay, and much lower offshore (Table 2). *Prochlorococcus* was not detectable in most bay stations and showed low abundances in the lagoon and very high abundances offshore (on average 1.5×10^5 ml^{-1}). Thus, *Prochlorococcus* dominated the cyanobacterial community offshore, whereas *Synechococcus* dominated in the lagoon and bays. Abundances of *Cyanobacteria* (i.e. *Synechococcus* plus *Prochlorococcus*) did not change much between study sites. Picoeukaryotes ranged from 1.6 to 12.4 $\times$ 10^3 ml^{-1} and generally showed higher abundances in the bays than at other stations.

HNF abundance ranged from 0.6 to 13.4×10^3 ml^{-1}. HNF abundance was higher at the head of the bays than in the open ocean, although the lowest values were found at a station in the middle of the lagoon and in the middle and mouth of Sainte Marie Bay (Table 3). With the exception of a single sampling, ANF abundance was lower than HNF abundance. ANF abundance and its relative contribution to total abundance were lowest in the open ocean and highest in the bays. Ciliate abundance ranged from 0.1 to 19 cells ml^{-1}, and abundance was on average lowest in the open ocean. The distribution of viral abundance in the lagoon of Noumea has been described elsewhere (Mari et al. 2007a). Briefly, viral abundance ranged from 0.8 to 3.2×10^7 ml^{-1} and was highest at the head of the bays and lowest in the open ocean.

Bacterial community composition

Fourteen different bands were successfully sequenced (Table 4). The identity of bands could be confirmed by identical sequences. Five major groups of phylotypes were detected: (1) sequences related to *Rhodobacteri-*

Table 2. Characteristics of *Cyanobacteria* and autotrophic picoeukaryotes at the sampling stations. Each value corresponds to the average (range in parentheses) of 2 or 4 measurements taken for each station. ND: not detectable. *Cyanobacteria* is the sum of *Synechococcus* and *Prochlorococcus*

Trophic group	Stn	*Synechococcus* (10^4 ml^{-1})	*Prochlorococcus* (10^3 ml^{-1})	*Cyanobacteria* (10^4 ml^{-1})	Picoeukaryotes (10^3 ml^{-1})
Head of bays	D01	16.8 (16.0–17.7)	3.7 (ND–7.3)	17.1 (16.7–17.8)	6.3 (5.6–7.1)
	N04	12.4 (11.2–12.8)	ND	12.0 (11.2–12.8)	10.7 (10.1–11.4)
Middle of bays	D08	19.1 (18.5–19.8)	ND	19.1 (18.5–19.8)	5.3 (5.1_5.5)
	N12	12.9 (12.2–13.6)	ND	12.9 (12.2–13.6)	11.6 (10.7–12.4)
Mouth of bays	D22	18.3 (17.4–19.5)	0.7 (ND–1.3)	18.6 (17.5–19.5)	3.9 (3.7–4.0)
	N33	10.5 (10.2–10.8)	ND	10.5 (10.2–10.8)	5.4 (5.0–5.9)
Middle of lagoon	M05	12.5 (11.1–13.9)	10.1 (5.5–14.6)	13.5 (11.2–15.4)	3.2 (2.3–4.4)
	M33	13.2 (12.5–14.0)	6.1 (5.0–7.2)	13.9 (13.3–14.5)	3.3 (2.4–4.2)
Near barrier	M10	12.2 (8.2–15.2)	20.7 (9.2–41.4)	13.6 (9.5–17.1)	4.8 (2.0–8.1)
Open ocean	M41	2.2 (0.8–2.8)	145.7 (130.7–167.7)	16.8 (15.0–19.4)	3.4 (1.6–4.3)

Table 3. Protistan and viral characteristics at the sampling stations. Virus data are from Mari et al. (2007a). Each value corresponds to the average (range in parentheses) of 2 or 4 measurements taken for each station. HNF: heterotrophic nanoflagellates; ANF: autotrophic nanoflagellates; TNF: total nanoflagellates

Trophic group	Stn	HNF (10^3 ml^{-1})	ANF (10^2 ml^{-1})	TNF (10^3 ml^{-1})	Ciliates (ml^{-1})	Viruses (10^7 ml^{-1})
Head of bays	D01	3.0 (2.7–3.2)	7.8 (2.4–13.2)	3.8 (3.4–4.1)	2.1 (2.0–2.2)	2.8 (2.8–2.9)
	N04	5.5 (4.7–6.3)	6.8 (2.5–11.0)	6.2 (5.8–6.6)	3.1 (1.0–5.1)	2.7 (2.2–3.2)
Middle of bays	D08	1.5 (0.6–2.3)	3.5 (0.5–6.5)	1.8 (1.3–2.4)	0.9 (0.9–1.0)	2.9 (2.8–3.0)
	N12	8.5 (3.7–13.4)	4.0 (1.8–6.3)	8.9 (4.3–13.5)	9.6 (0.5–18.6)	2.6 (2.3–2.9)
Mouth of bays	D22	1.6 (1.5–1.6)	3.5 (2.2–4.8)	1.9 (1.8–2.0)	1.2 (0.4–1.9)	2.5 (2.4–2.7)
	N33	2.7 (1.5–4.0)	16.3 (2.2–30.5)	4.4 (4.2–4.6)	0.9 (0.6–1.1)	2.4 (2.3–2.6)
Middle of lagoon	M05	1.4 (0.8–1.9)	1.6 (1.1–2.1)	1.5 (0.9–2.1)	2.3 (0.1–4.5)	2.0 (1.7–2.2)
	M33	2.8 (2.0–3.6)	1.7 (1.2–2.3)	3.0 (2.1–3.9)	1.7 (1.1–2.3)	2.6 (2.4–2.8)
Near barrier	M10	2.1 (1.4–4.0)	1.4 (0.7–3.1)	2.3 (1.6–4.1)	1.7 (1.2–2.4)	2.0 (1.4–2.6)
Open ocean	M41	2.5 (1.4–3.5)	1.4 (0.4–2.5)	2.6 (1.4–4.1)	0.6 (0.3–0.8)	1.2 (0.8–1.7)

Table 4. Phylogenetic affiliation of sequences from denaturing gradient gel electrophoresis (DGGE) bands with closest uncultured and cultured matches. The number of bases used to calculate sequence similarity is given in parentheses in the third column. Note that the last 2 sequences were from bands not included in the analysis of the community composition because they were below the threshold set for the detection of a band

Band	Closest match (environmental or culture)	% Sequence similarity (no. bases)	Taxonomic group
Noumea04-3	*Roseobacter* sp. MED001	98 (413)	*Alphaproteobacteria/Rhodobacteriaceae*
Noumea04-4	*Roseobacter* sp. MED001	96 (369)	*Alphaproteobacteria/Rhodobacteriaceae*
Noumea04-7	Clone VH-PA7-59	97 (457)	*Gammaproteobacteria/Alteromonadaceae*
Noumea04-9	Clone 2_B6 or 2_C6	99 (433)	*Alphaproteobacteria/Rhodobacteriaceae*
Noumea04-10	Clone SIMO-846	99 (420)	*Alphaproteobacteria/Rhodobacteriaceae*
Noumea04-11	Clone 2_C6	98 (434)	*Alphaproteobacteria/Rhodobacteriaceae*
Noumea04-12	Clone SIMO-846	98 (470)	*Alphaproteobacteria/Rhodobacteriaceae*
Noumea04-13	*Alphaproteobacterium* IMCC10404	99 (279)	*Alphaproteobacteria*
Noumea04-14	*Alphaproteobacterium* IMCC10417	97 (281)	*Alphaproteobacteria*
Noumea04-16	*Proteobacterium* MS-B-38 or MS-F-42	96 (250)	*Proteobacteria*
Noumea04-18	Clone VH-PA7-59	98 (445)	*Gammaproteobacteria/Alteromonadaceae*
Noumea04-21	Marine eubacterial sp. (FL1)	93 (242)	*Alphaproteobacteria*/SAR11
Noumea04-22	SAR11 cluster *Alphaproteobacterium*	86 (268)	*Alphaproteobacteria*/SAR11
Noumea04-27	*Prochlorococcus marinus* Str. MIT 9215	98 (364)	*Cyanobacteria/Prochlorococcus*

aceae (e.g. genera *Rugeria* and *Roseobacter*), (2) sequences related to the SAR11 cluster, (3) sequences related to other *Alphaproteobacteria*, (4) sequences related to *Alteromonadaceae* (genus *Alteromonas*), and (5) a sequence related to cyanobacterial sequences. The band corresponding to cyanobacterial sequences was not included in the analysis of the community composition. It is noteworthy that no plastid sequences were detected.

Duplicate filters yielded identical community profiles (data not shown). Bacterial community composition changed strongly along trophic gradients. Twenty different bands were detected, and 13 bands were present at all stations. The number of detected bands ranged from 14 to 16 in offshore waters, from 11 to 13 in the lagoon and the mouth of the bays, and from 13 to 15 in Grande Rade Bay, and it was 16 in Sainte Marie Bay (middle and head of bays). Thus, the highest number of bands of total bacterial communities was found in the upper part of the bays and at the open ocean station (Fig. 2). An ANOVA and post hoc tests showed that the number of bands of the total community in the lagoon was significantly different from bay and offshore stations. The number of bands of the free-living community showed a different trend and decreased from the open ocean towards the head of the bays. An ANOVA showed that offshore, lagoon, and bay waters were significantly different with respect to the number of bands of the free-living community.

Using band identity to describe environments, 3 specific bands were found for offshore waters, 1 band was specific for the middle and head station of Sainte Marie Bay, and 1 band was specific for Grande Rade Bay. An additional band occurred only in the 2 bays, whereas no specific band was detected for the lagoon stations. A similarity tree of the DGGE profiles (presence versus absence of bands) showed that the free-living community in Grande Rade was different from all others (Fig. 3). The offshore community was different from all other remaining lagoon and bay communities. Within the lagoon and bay communities, 3 clusters were found: 1 consisting of the head and center of Sainte Marie Bay, 1 of the mouth of Sainte Marie Bay and some lagoon stations (total community), and 1 of the

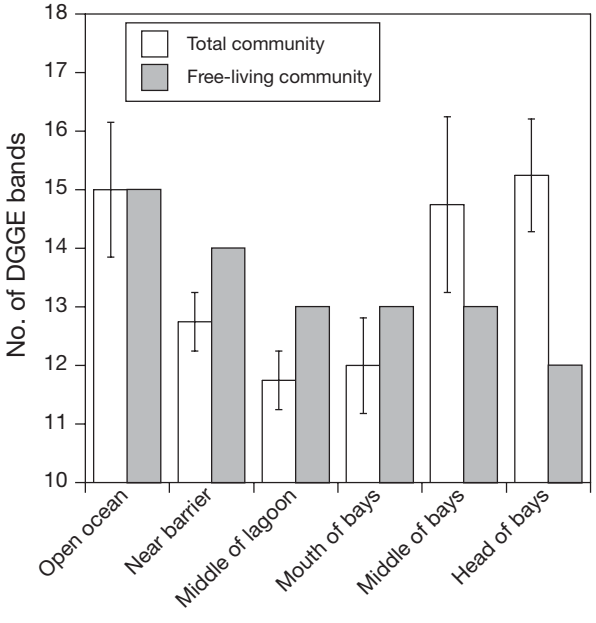

Fig. 2. Number of detected bands denaturing gradient gel electrophoresis (DGGE) of total and free-living communities in the southwest lagoon of New Caledonia as a function of environment. For the free-living community, only data from Grande Rade Bay transects are available

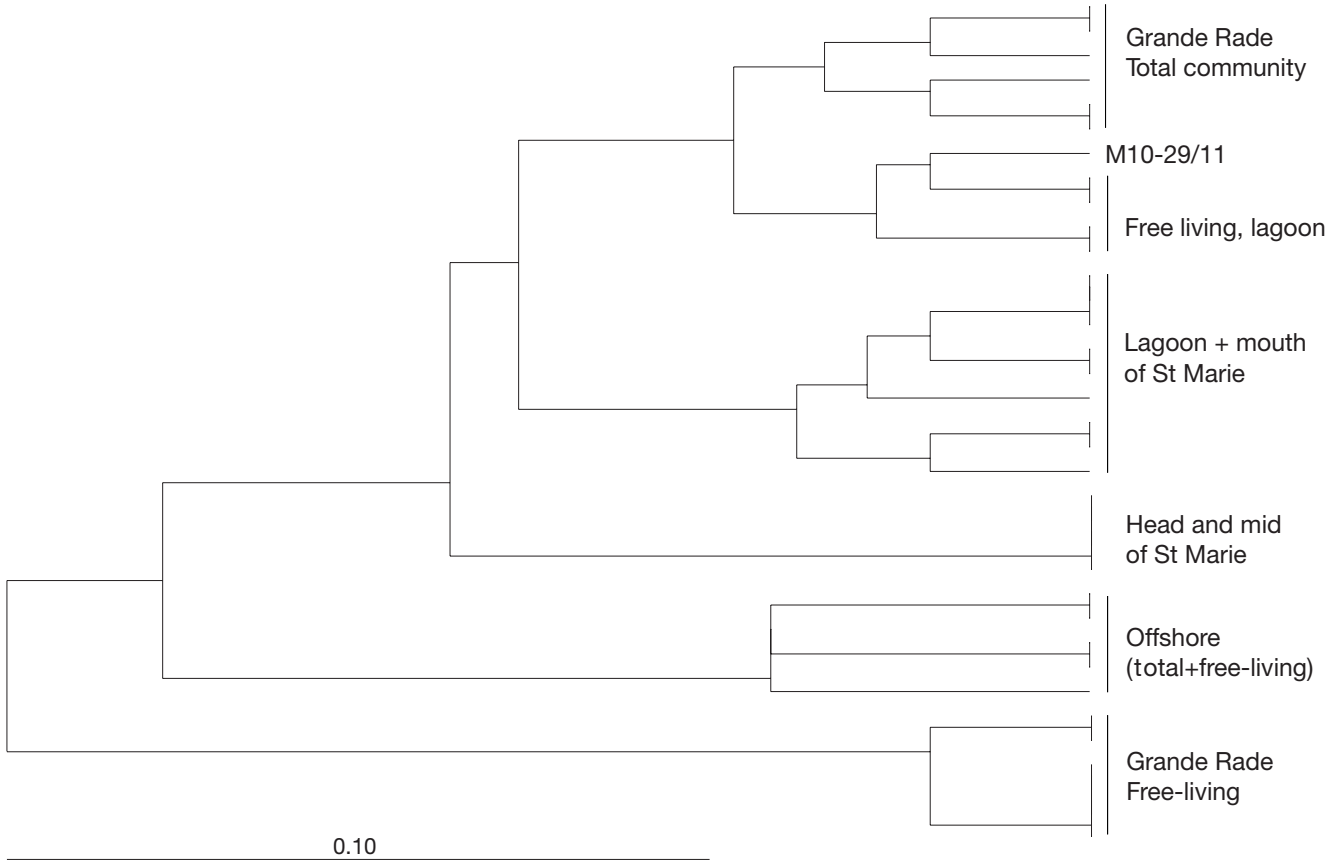

Fig. 3. Cluster analysis of denaturing gradient gel electrophoresis (DGGE) band patterns by the unweighted pair-group method. The distance matrix was calculated using a simple match similarity index

Grande Rade total communities and free-living communities from the lagoon. Specific clusters were found for communities from the middle and head of Sainte Marie Bay, from Grande Rade Bay, and from all lagoon stations (except Stn M10 sampled on 29 November) and the mouth of Sainte Marie Bay. Communities from the 2 bays were clearly different. Prefiltration did not affect the profiles at the open ocean station, whereas after prefiltration, i.e. when only the free-living communities were present, lagoon communities clustered together with non-prefiltered lagoon station communities (Stn M10 sampled on 29 November), and Grande Rade Bay communities clustered apart from all other communities.

Relationship between parameters

Regression and correlation analyses were performed with average values per station, since LeFT values were only available as average values per station, and not all stations were sampled at the same frequency (Table 1). Bacterial abundance increased strongly with chl *a* concentration and ciliate and viral abundance, but not with LeFT, whereas %HNA increased significantly with LeFT, chl *a* concentration, and viral abundance but not with ciliate abundance parameters (Table 5). Other significant relationships were those between LeFT and chl *a*, between viruses and chl *a* (although r was lower than for the relationship between viral and bacterial abundance), and between HNF or TNF abundance and ciliate abundance. The abundance of *Synechococcus* was strongly related to viruses, whereas the abundance of picoeukaryotes was strongly related to %HNA, HNF, and TNF.

Using correlation analysis, the number of bands of total communities was only related significantly to HNF, TNF, and eukaryote abundance (Table 5). However, we also found more complex relationships using regression analysis. For example, the number of bands was related to chl *a* concentration in a U-shape (Fig. 4). No relationship was detected between the number of bands of total communities and LeFT. The number of bands of the free-living community decreased linearly with LeFT, chl *a* concentration (Fig. 4), *Synechococcus*, %HNA, and bacterial, viral, and ciliate abundance.

Table 5. Spearman correlation coefficients among all parameters. Data are from Table 1–3 and were log or arcsine transformed. Temperature is not shown, as no significant correlation was found. R-TC: number of bands, total community; R-FLC: number of bands, free-living community; LeFT: local e-flushing time; chl *a*: chlorophyll *a*; Synecho: *Synechococcus*; PicoE: picoeukaryotes; HNA: high nucleic acid bacteria; HNF: heterotrophic nanoflagellates; TNF: total nanoflagellates. Values in **bold** are $p < 0.05$; $^{*}p < 0.01$; $^{**}p < 0.005$; $^{***}p < 0.001$; $^{****}p < 0.0005$

	R-TC	R-FLC	LeFT	Chl *a*	Synecho	PicoE	Bacteria	%HNA	HNF	TNF	Ciliates
R-FLC	0.297	1									
LeFT	0.155	**−0.840*** **	1								
Chl *a*	0.554	**−0.887****	**0.680**	1							
Synecho	0.021	**−0.875**	0.584	**0.608**	1						
PicoE	**0.676**	−0.174	0.226	**0.719***		1					
Bacteria	0.232	**−0.961****	0.309	**0.811****	**0.712***	0.576	1				
%HNA	0.419	**−0.651**	**0.826*****	**0.839*****	0.525	**0.623**	0.466	1			
HNF	**0.687***	0.146	−0.182	0.500	−0.083	**0.729***	0.548	0.213	1		
TNF	**0.614**	−0.066	−0.090	0.542	−0.023	**0.828*****	0.576	0.273	**0.975****	1	
Ciliates	0.466	**−0.689***	−0.006	0.577	0.376	0.451	**0.699***	0.191	**0.697***	**0.621**	1
Viruses	−0.034	**−0.910****	**0.618**	**0.779****	**0.907*****	0.464	**0.850*****	**0.676**	0.380	0.291	0.407

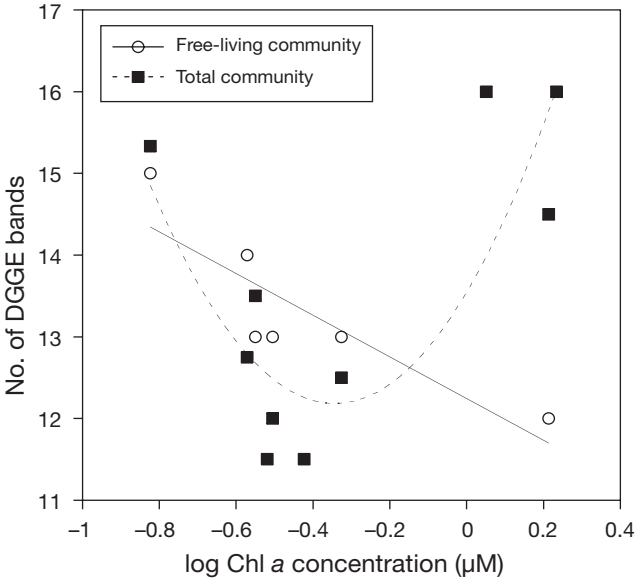

Fig. 4. Number of detected denaturing gradient gel electrophoresis (DGGE) bands of total and free-living communities in the southwest lagoon of New Caledonia as function of chl *a* concentration. For the free-living community, only data from Grande Rade Bay transects are available. Total community: $y = 10.95x^2 + 8.17x + 14.6$, $R^2 = 0.71$, $p < 0.001$; free-living community: $y = -2.35x + 13.47$, $R^2 = 0.81$, $p < 0.005$

Integrated view of data: PCA

Key parameters, namely richness, LeFT, bacteria, viral, HNF and ciliate abundance, and chl *a* concentrations were selected for PCA. Three significant components with an eigenvalue >1 were extracted, explaining 86.2% of the total variation (Table 6). The component matrix revealed 2 fundamental factors influencing the environment. Most of the variations in the barrier reef system were characterized by the first component (PCA-C1), where LeFT was strongly correlated with bacterial and viral abundance and chl *a* and was responsible for almost 50% of the variation in the system. The other 2 components, together explaining about 36% of the variation, revealed a relation between grazers and richness. The second component (PCA-C2) showed a positive correlation between the number of bands for the total community and HNF abundance, and the third component (PCA-C3) showed a negative correlation between the number of bands for the total community and ciliate abundance.

Table 6. Component matrix for the lagoon of Noumea. Extraction method: principal component analysis (PCA). Data set for PCA was reduced to 7 key parameters (no. of samples = 24). Three components were extracted. Correlations >0.4 are in **bold**. No. of bands: number of denaturing gradient gel electrophoresis (DGGE) bands for total community; LeFT: local e-flushing time; BA: bacterial abundance; VA: viral abundance; HNFA: heterotrophic nanoflagellate abundance; CA: ciliate abundance; chl *a*: chlorophyll *a*

	Lagoon of Noumea		
Parameter	C1	C2	C3
No. of bands	0.09	**0.56**	**0.67**
LeFT	**0.42**	−0.27	0.24
BA	**0.49**	−0.05	−0.14
VA	**0.47**	−0.30	−0.10
HNFA	0.24	**0.61**	−0.18
CA	0.20	0.39	**−0.61**
Chl *a*	**0.50**	0.02	0.24
% of variance	49.8	21.3	15.0
Cumulative	**86.2%**		

DISCUSSION

Potential control mechanisms of bacterial abundance

A lower bacterial abundance in the open ocean than in lagoon stations of coral reefs as shown in the present

study has been demonstrated previously (e.g. Torréton 1999). This difference was only moderate (Torréton 1999) and is supported by our study. The relationship between bacterial abundance and chl *a* concentrations (Table 5) suggests that bacterial production was linked to primary production as has been shown before for the barrier reef system of New Caledonia (Rochelle-Newall et al. 2008) and many other pelagic environments (e.g. Cole et al. 1988). Such a tight link is thought to be mainly the consequence of dissolved organic matter release from healthy phytoplankton cells (Baines & Pace 1991), which fuels bacterial production. Other links could be detritus formation and subsequent use by bacteria. Indeed, the concentration of transparent organic particles (TEP) was also well correlated with chl *a* (Mari et al. 2007b) and bacterial abundance (data not shown). It is known that %HNA often increases with bacterial production and that the HNA populations are often more active (e.g. Servais et al. 1999). We did not measure bacterial production in the present study, although previous work has shown that bacterial production increases along the trophic gradient in the lagoon in all seasons (Torréton et al. 2007, Conan et al. 2008, Rochelle-Newall et al. 2008). Thus, the strong correlation between %HNA (and total abundance) and chl *a* detected in the lagoon of Noumea and the fact that these parameters strongly increase along trophic gradients is a good indication of a link between bacterial and primary production as shown previously along the trophic gradients investigated (Rochelle-Newall et al. 2008). In the bays, the long water residence time and the positive buoyancy of TEP result in an efficient cycling loop of organic matter (Mari et al. 2007b), and this could be an additional mechanism explaining the high %HNA.

Our data support a previous report (Torréton et al. 2007) suggesting that water residence time has an influence on bacterioplankton biomass. Recalculating 2 data sets from a total of 6 cruises from the same stations as in the present study (Jacquet 2005, Torréton et al. 2007), a negative relationship was found between LeFT and bacterial turnover time (data were used as log-transformed averages per station from 3 cruises; Torréton data set: $r = -0.70$, $p < 0.01$, $n = 9$; Jacquet data set: $r = -0.74$, $p < 0.005$, $n = 10$). In the bays, water residence time was much higher than bacterial turnover time (Fig. 5). At Stn M05 (lagoon station closest to coast), LeFT and turnover times were similar, whereas for the other lagoon stations, bacterial turnover times were much lower than LeFT. Water residence time is a crucial ecological parameter in semi-confined environments, but it has not often been related to bacterial abundance and production or bacterial subpopulations (Crump et al. 2004). For example, it has been argued that specific communities can develop when the water residence time is higher than the bacterial turnover

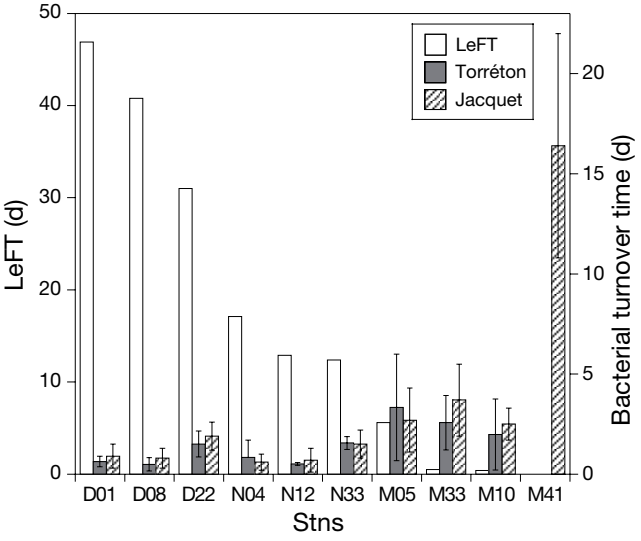

Fig. 5. Local e-flushing time (LeFT) and bacterial turnover times at the sampling stations. Water residence times were calculated for the most common trade wind conditions of 8 m s^{-1} (Mari et al. 2007a). Bacterial turnover times were calculated from 2 data sets available from the study stations (Jacquet 2005, Torréton et al. 2007). The Torréton data set was obtained from cruises in September 2000, June 2003, and October 2004, and the Jacquet data set was from April–May 2002, August 2002, and January 2003. Bacterial production was calculated from ^{3}H-thymidine incorporation rates (TdR) using a conversion factor of 2.85×10^8 cells mol^{-1} TdR obtained for the environment (Jacquet 2005)

time (Crump et al. 2004). PCA-C1 also suggests a positive link between water residence time and bacterial and viral abundance and chl *a* concentrations (Table 6), thus supporting the argument outlined here.

The abundances of bacteria and HNF often show a lack of coupling across trophic gradients, especially in more eutrophic environments (Gasol & Vaqué 1993, Gasol 1994). This is probably due to top-down control of HNF by larger protists such as ciliates. These findings have been used by Gasol and co-workers to develop a model predicting that grazing by flagellates controls bacterial production in oligotrophic but not in eutrophic environments (see also Pernthaler 2005). In support of these ideas, we did not find a significant relationship between bacterial and HNF or TNF abundance, whereas HNF and TNF abundance were highly correlated with ciliate abundance. This analysis could be influenced by mixotrophic flagellates, which are able to feed on bacteria and thus represent an additional mortality factor for bacteria. However, total or autotrophic flagellate abundance was also not coupled to bacterial abundance. The lack of correlation between HNF (or TNF) and bacterial abundance could also be due to flagellates grazing mainly on *Cyanobacteria* as shown for an atoll lagoon (Sakka et al. 2000). HNF (or TNF) abundance was not related significantly

to *Synechococcus, Prochlorococcus*, or *Cyanobacteria* but to autotrophic picoeukaryotes, suggesting that flagellates could have grazed on autotrophic picoeukaryotes. Sakka et al. (2000) also provided evidence that protistan grazing on bacteria is low in oligotrophic coral reef lagoons. Thus, the weak correlation between bacterial abundance and HNF abundance could not only be due to top-down control of HNF by ciliates but also due to selective feeding of HNF.

Using the approach of Gasol & Vaqué (1993), Weinbauer & Peduzzi (1995) found the same lack of coupling between bacterial and HNF abundance in the Adriatic Sea. In addition, they reported a strong correlation between bacterial and viral abundance. Since viral infection also increased with bacterial abundance and trophic conditions, they speculated that viral control was stronger in eutrophic than in oligotrophic environments. Compilations of literature data also suggest that on average, the viral impact can be greater in eutrophic than in oligotrophic environments (Weinbauer 2004, Parada et al. 2006). The reason for this could be the increased encounter rates with hosts and the lack of top-down control of viral abundance. HNF can ingest and digest viruses; however, since the removal rates are low (González & Suttle 1993), grazing is likely not an important mechanism controlling viral abundance. Viruses could also originate from sources other than heterotrophic bacteria, such as *Cyanobacteria*, which can be abundant at the study site; in addition, *Synechococcus* abundance was significantly related to viral abundance. However, the abundance of *Synechococcus* (and *Prochlorococcus* or C*yanobacteria*) was typically ca. 1 order of magnitude lower than the abundance of heterotrophic bacteria. Moreover, viral lysis rates seem to be lower for *Synechococcus* and *Prochlorococcus* than for heterotrophic bacteria (e.g. Garza & Suttle 1998, Baudoux et al. 2007). Thus, it is likely that the majority of viruses originated from the lysis of heterotrophic bacteria. In the bays, up to 30% of total viral abundance was attached to organic particles at the study site (Mari et al. 2007a). If this attachment is detrimental to viral infectivity (Simon et al. 2002, Weinbauer et al. 2009), attachment could be an important mechanism for viral decay (Suttle & Chen 1992). However, this potential loss was at maximum 30% of the total abundance and did not result in a disruption of the correlation between viral and bacterial abundance. Thus, this suggests either that attachment of viruses was not a major cause of loss of viruses or that losses were not substantial.

Solar radiation can destroy viral particles and cause losses of infectivity without losses of particles (Suttle & Chen 1992). Data from the study area suggest that DNA effective radiation attenuates more rapidly with depth in more coastal than in more offshore waters (Conan et al. 2008). Thus, a gradient in the losses of viral infectivity due to solar radiation can be expected, with the lowest effect in the bays. In addition, cell-specific bacterial production increased towards the bays (Torréton et al. 2007, Rochelle-Newall et al. 2008), and this could also result in an increase of viral infection (Wommack & Colwell 2000). However, these mechanisms did not disrupt the relationship between viral and bacterial abundance (Table 5). In the bays, it is possible that increased losses by attachment are balanced by reduced losses due to UV light and higher infection frequencies. Thus, assuming that the models of Gasol & Vaqué (1993) and Gasol (1994) are applicable, the data indicate that the relative impact on bacterioplankton mortality changed from viral lysis dominating in the bays to HNF dominating in the open ocean.

Bacterial community composition

Genetic fingerprints such as 16S rRNA gene-based DGGE have methodological constraints, which could influence our analysis. For example, multiple operons could be problematic, either resulting in an overestimation of a phylotype (in the case of sequence identity of multiple operons) or in suggesting higher diversity (in the case of sequence diversity of multiple operons). However, this is unlikely for the investigated types of water-column environments (Brown et al. 2005). Moreover, it is known that some primers select against *Gammaproteobacteria*. However, we used an improved primer set that also detects the SAR11 cluster and some *Gammaproteobacteria* (Sanchez et al. 2007). Indeed, the ubiquitous SAR11 cluster (Morris et al. 2002) was detected in our samples. In addition, plastids might be amplified. However, we did not obtain plastid sequences from the SW lagoon of New Caledonia. A high number of phylotypes belonging to *Rhodobacteriaceae* as in the present study was also found in the Mediterranean Sea using DGGE (Alonso-Saez et al. 2007, Malits & Weinbauer 2009), where it was also demonstrated by using fluorescence *in situ* hybridization (FISH) that *Roseobacter* can be numerically dominant (Alonso-Saez et al. 2007). Our detection of *Alteromonadaceae* sequences is more surprising, since the abundance of this group is thought to be low. However, bloom-like outbreaks have been documented recently for *Alteromonas* (Alonso-Saez et al. 2007), probably because they can react quickly to disturbances (Allers et al. 2007). At the study site, the *Alteromonas* bands were most typical for the bays. Assuming that *Alteromonas*-specific responses to disturbance exist, such disturbances could be mixing of oligotrophic offshore-water with mesotrophic particle-rich bay water.

It is well known from marine systems that bacterial community composition changes along open gradients such as estuaries (e.g. Bouvier & del Giorgio 2002, del Giorgio & Bouvier 2002, Crump et al. 2004). Using cluster analysis, we found that the gradients separated specific bacterial communities in the open ocean and the 2 bays. The system is characterized by gradients from oligotrophic open ocean waters to mesotrophic bays within a distance of ca. 20 km (Jacquet et al. 2006, Mari et al. 2007b, Rochelle-Newall et al. 2008). The gradients include water residence time, metal concentrations (Ni, Cr, and Zn), nutrient and chl *a* concentrations, particulate organic concentration and TEP contribution, and bacterial production (Mari et al. 2007b, Migon et al. 2007, Torréton et al. 2007). In addition, coral mucus harbors a large bacterial diversity (Rohwer et al. 2002), and mucus (and thus, probably also attached bacteria) is transported into the lagoon system (Wild et al. 2004). All of these parameters could have influenced bacterial community composition.

The community composition of attached and free-living bacteria can be different (DeLong et al. 1993, Acinas et al. 1999, Crump et al. 1999). Particle load increases from open ocean to bay stations (Mari et al. 2007b). Thus, the relative proportion of attached bacteria is likely much higher in the bays than in the open ocean. Fingerprints of total bacteria did not differ from free-living bacteria in the open ocean, whereas the strongest difference was found for Grande Rade Bay. Thus, our data suggest an abundant and phylogenetically specific attached bacterial community in the bays. The long water residence time as a consequence of constrained hydrodynamic circulation (Mari et al. 2007b) could provide enough time for the development of a specific or more pronounced community as has been previously suggested for another system (Crump et al. 2004). Bacterial turnover times were indeed much lower than the water residence times in the investigated bays (Fig. 5). In addition, the positively buoyant particles in the bays (Mari et al. 2007b), which keep the attached bacterial communities suspended, could have contributed to that. Moreover, this feedback system, where organic matter is not exported efficiently but is continuously degraded in the water column, results in refractory dissolved organic matter (Mari et al. 2007b), which could also have influenced the development of specific communities in the bays. Also, a longer water residence time could provide more opportunities for bacteria from other water masses or the atmosphere to become established and common. A similar mechanism could operate for organic matter compounds and metals, which could then also influence bacterial community composition.

Diversity hypotheses

It is noteworthy that we sampled a trophic gradient along which chl *a* concentration and bacterial production varied by more than an order of magnitude (Mari et al. 2007b, Torréton et al. 2007, Rochelle-Newall et al. 2008). Thus, the trophic gradient in the lagoon of Noumea allows for testing predictions from hypotheses describing the relationship between diversity and productivity. The number of bands detected using genetic fingerprinting is certainly an underestimation of species richness. Thus, as the number of DGGE bands is interpreted as an indicator of evenness, i.e. a low number of bands likely means a lower evenness and a higher dominance of detectable phylotypes (Bonilla-Findji et al. 2009). As such, DGGE bands can be used to assess hypotheses on mechanisms controlling diversity.

A general hypothesis assumes a unimodal distribution of diversity along a productivity gradient, with the highest diversity at intermediate productivity (e.g. Worm et al. 2002). Using chl *a* to represent productivity and the number of bands as a diversity parameter, the number of phylotypes of free-living communities decreased linearly with chl *a* concentration. Others have found a decrease in the number of phylotypes of free-living communities with increasing bacterial production and respiration (Reinthaler et al. 2005, Winter et al. 2005). This potentially suggests a common feature such as a change in evenness when trophic gradients are investigated (Worm et al. 2002). We found a unimodal distribution for total communities; however, this distribution showed a U-shape and not a hump form. It has been shown before that the relationship between chl *a* and the number of detected bands for bacterioplankton can be complex. Hump-shaped, U-shaped, and a lack of relationships between the richness of different large taxonomic groups of bacteria and chl *a* concentration have been demonstrated for freshwater systems (Horner-Devine et al. 2004a). Our findings (PCA, regression and cluster analysis) suggests that complex patterns can also originate from differences in community composition between free-living and attached bacteria and from varying contributions of the attached to total communities. The low dominance in offshore waters supports widely held but not thoroughly tested ideas that dominance is lower and species diversity is higher in oligotrophic than in eutrophic environments. The low dominance in the bays could then be due to a mixture of very different free-living and attached communities.

Mortality factors such as viral lysis and protistan grazing can also influence productivity-diversity relationships (Worm et al. 2002). For example, the number of detected phylotypes of the free-living community

decreased with viral abundance in the North Sea (Winter et al. 2005). A decrease in the number of bands of the free-living community with increasing viral abundance was also detected in the present study. PCA-C2 suggested a positive effect of HNF on the number of bands of the total community, and PCA-C3 indicated a negative effect of ciliates. HNF can sustain the number of detected phylotypes, especially when acting synergistically with viral lysis (Zhang et al. 2007, Bonilla-Findji et al. 2009). HNF and viruses can also modify the relationship between diversity (as the number of DGGE bands) and productivity (Bonilla-Findji et al. 2009). The PCA component matrix could thus suggest that, at high grazing pressure by HNF, bacterial evenness is sustained; at low grazing pressure, i.e. when HNF are top-down controlled by ciliates, only a few bacterial phylotypes may dominate.

CONCLUSIONS

This study is one of the few investigating bacterial community composition in tropical coral reef ecosystems. Bacterial community composition was clearly different between offshore waters and the semi-enclosed bays. Water residence time and mortality factors seemed to be a crucial parameter structuring bacterial communities. An unexpected potentially cascading effect of ciliate control on HNF, and thus on bacterial community composition, was detected. Our data also confirm the idea that it may be necessary to distinguish between attached and free-living bacterial community diversity and associated methods to assess productivity.

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