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REPORT

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Biomass, production and heterotrophic activity of bacterioplankton in the Great Astrolabe Reef lagoon (Fiji)

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Abstract Biomass, production and heterotrophic activity of bacterioplankton were determined for two weeks in the Great Astrolabe Reef lagoon, Fiji. Bacterial and Bacterial activities were distributed homogeneously throughout the water column (20 to 40 m deep) and varied little from site to site inside the lagoon. Bacterioplankton biomass and production also varied little over a diel period with coefficients of variation of 9 and 22%, respectively. On average, over the whole study, bacterial abundance was 0.77×10^9 cells l^{-1} and bacterial production averaged 0.36 µg-at. C l^{-1} d^{-1}. Bacterial abundance and production were greater in the lagoon than in oceanic waters. Attachment to particles seems to provide an advantage for bacterioplankton growth because specific growth rates for attached bacterioplankton were, on average, significantly greater than that of the free community. Growth efficiency, determined by correlating the net increase of bacterial biomass and the net decrease of dissolved organic carbon (DOC) in dilution cultures, was very low (average 6.6%). Using carbon growth efficiency and bacterial production rates, heterotrophic activity was estimated to average 5.4 µg-at. C l^{-1} d^{-1}. The turn-over rate of DOC (average 114 µg-at. C l^{-1}) due to bacterial consumption was estimated to be 0.048 d^{-1} during the period of study.

Key words Bacterioplankton · Bacterial production
Dissolved organic carbon · Coral reefs
Pacific ocean

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Introduction

Planktonic bacteria make important contributions to the biogeochemical cycles of marine pelagic ecosystems. In most oceanic environments, bacterial production represents a significant proportion of primary production (Ducklow and Carlson 1992) and in the most oligotrophic environments bacterial biomass may even exceed phytoplankton biomass (Fuhrman et al. 1989). In coral reefs environments, bacterioplankton have been studied mostly in the water column overlying coral reefs (e.g. Ducklow 1990; Ferrier-Pagès and Gattuso 1998), while atoll (Sorokin 1978; Yoshinaga et al. 1991; Torréton and Dufour 1996b) and island lagoons (Landry et al. 1984; Yoshinaga et al. 1991) have received less attention. Atoll and island lagoons may, however, represent large bodies of water where heterotrophic bacterioplankton with low C:N ratios relative to the phytoplankton (Lee and Fuhrman 1987), could be an important contributor to particulate nitrogen standing stocks and fluxes. Nutrient recycling is essential in coral reef areas, often characterized by low concentrations and inputs of new nutrients (Crossland and Barnes 1983). Hence, the understanding of bacterioplankton dynamics is essential to studies of carbon and nutrient cycling in coral reef environments.

This study describes heterotrophic bacterioplankton in the water column of the Great Astrolabe Reef lagoon in Fiji. The goal was to elucidate the importance of bacterial biomass, production and heterotrophic activity as compared to phytoplanktonic biomass and production, and their contribution to particulate organic carbon and nitrogen concentrations. An additional objective of this study was to estimate the contribution of attached bacteria to biomass and activity of the whole community and to determine if attachment was an advantage for bacterioplanktonic communities in the Great Astrolabe Reef lagoon.

Materials and methods

Study sites and sampling

The Great Astrolabe Lagoon is south of Viti Levu, the main island of Fiji. The general characteristics of the lagoon are summarized by Naqasima et al. (1992).

Vertical and spatial distributions of bacterioplankton abundance and production were investigated during a two week cruise on the ORSTOM R/V *Alis* from 18 to 29 May 1994. Water samples were collected every day with an acid-cleaned (2N HCl) 1.7-l Niskin bottle at 5 m depth intervals between 0 and maximum depth in the lagoon. A total of 10 sampling stations (20 to 40 m maximum depth, see Fig. 1) were investigated. Water samples were also collected every 3 h during a diel cycle at station 5 (at 10 m depth) to determine the validity of a single measurement at this scale, and to measure any diel trend of bacterial biomass and activity. For comparison with the lagoon, an oceanic station (OC) northeast of the lagoon (Fig. 1) was also sampled during the cruise. At this oceanic station, sampling was done between 0 and 100 m at 20 m depth intervals. Water samples were treated on board immediately after sampling. Every sample was processed using either disposable sterile plasticware or acid-washed polycarbonate bottles. Bacterial abundance and production were estimated for every sample. Free and attached bacterioplankton, production, and dissolved organic carbon were determined in selected samples.

Abundance, biovolume, and biomass of bacteria

Water samples were preserved with 0.2 µm filtered, buffered formalin (2% final concentration) and were stored at 2°C in the dark until filtration (within 6 h after sampling). Bacteria were collected onto 0.2 µm Nuclepore membranes after staining with DAPI (Porter and Feig 1980). Membranes were mounted on microscopic slides and stored at −20°C until counted using epifluorescence microscopy (magnification x 1000) within one month. Attached cells were also determined for the 10 m depth at every station. At least 400 cells (free + attached) in at least 20 fields were counted. Replicate filters of the same sample differed on average by 11.7% of the mean.

Mean cell volume was estimated by measuring length (l) and width (w) of bacteria on photographic slides with a digitizing tablet (final magnification of x 10 000). Cell volumes were calculated assuming cells to be cylindrical with hemisphere of diameter w at each end [volume $= \pi/4 \times w^2 (1 - w/3)$]. At least two slides were used per sample, counting a minimum of 150 cells. Mean cell volume estimation differed on average by less than 20% on the two slides of the same sample.

Bacterial biomass was computed from bacterial volumes. For cell volumes smaller than 0.070 µm^3, a constant value of carbon per cell was applied following Lee and Fuhrman (1987) who estimated cells to contain 1.67 fg-at. C, regardless of size in this range. For cells larger than 0.070 µm^3, the allometric model proposed by Simon and Azam (1989) was applied.

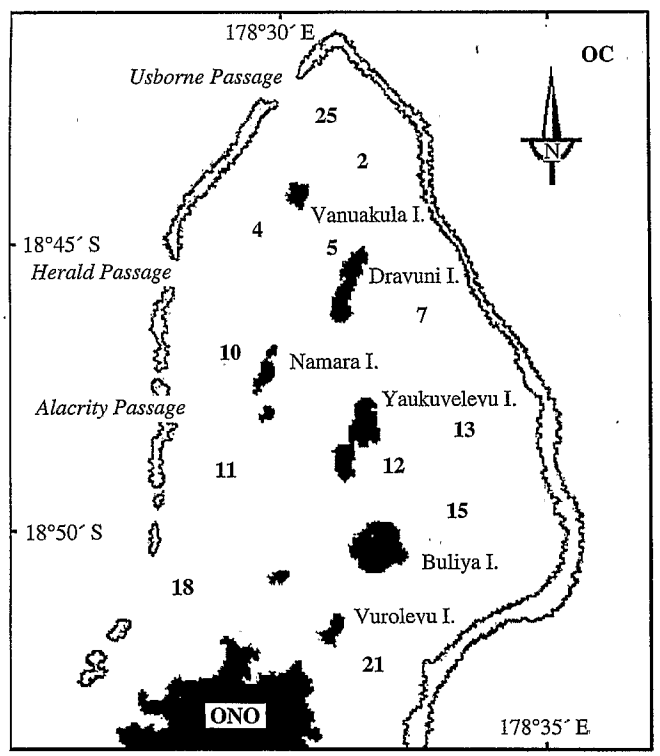

Fig. 1 Map of the Great Astrolabe Reef lagoon. Sampling locations are shown by station numbers

Bacterial production estimated by TdR incorporation

Production of bacterial biomass was determined by estimating the rate of [*methyl*-^{3}H]thymidine incorporation into DNA (TdR, Fuhrman and Azam 1982). Duplicate 10 ml water samples were incubated for 30 min with 10 nM [*methyl*-^{3}H]thymidine (Amersham, 1.74 TBq/mmol) at 28 °C (± 1 °C in situ temperature). Bacterial cells were collected by filtration onto 0.2 µm Nuclepore membranes. Macromolecules were precipitated with ice-cold 5% TCA and rinsed three times with ice-cold 5% TCA. Membranes were stored at −20 °C in scintillation vials. Before radioactivity determination (within two weeks), the filters were treated with 0.5 ml of 0.5N HCl (30 min at 100 °C) and a scintillation cocktail (Sigma-Fluor) was added to the vials. Incorporation was calculated after subtraction of a zero time blank. The proportion of TdR incorporation by the > 3 µm bacterioplankton size class was determined at every station on the 10 m depth samples using the procedure described, and by replacing the 0.2 µm membranes with 3 µm pore size ones.

Saturation kinetics

Saturation kinetics were assayed regularly in order to check if 10 nM labeled thymidine was sufficient to saturate the incorporation process and prevent isotope dilution. Five concentrations of the label were assayed in duplicate and in a zero time blank.

Isotope dilution

Isotope dilution assays (Pollard and Moriarty 1984) were performed at different stations in order to determine the contribution of labeled TdR to total incorporation. Eight replicate subsamples received 10 nM of labeled thymidine and different amounts of unlabeled thymidine (0, 20, 40 and 60 nM). A zero time blank was performed for every concentration and was subtracted from the signal. Maximum incorporation rates were computed from the regression of [L + A] versus $1/V_{obs}$: $[L + A] = V_{max} \times L/V_{obs} - P$, where L and A are the concentrations of labeled and unlabeled TdR, P is the "pool" of thymidine inducing intracellular and extracellular isotope dilution, and V_{obs} and V_{max} are observed and maximum TdR incorporation rates respectively.

Extraction of DNA

A total of 15 additional TdR incorporation assays were performed, in triplicate, as described previously. After the incubation, precipitation and filtration steps, membranes were stored at −20 °C until analysis. Labeled DNA was extracted enzymatically from these membranes following a modification of Wicks and Robarts' (1987) procedure as described in Torréton and Bouvy (1991). Recovery of the label into the DNA fraction was then compared with the label recovered using the standard TCA precipitation procedure on the same samples.

DOC determinations

Water samples were filtered through ashed (440 °C, 4 h) Whatman GF/F glass fiber filters to remove particulate carbon greater than 0.7 µm (Whatman GF/F nominal pore size) and 10 ml dispensed into replicate Teflon capped glass tubes (previously acid washed and ashed for 4 h at 550 °C). Each sample received $HgCl_2$ (10 ppm final concentration) to prevent bacterial growth. The tubes were then stored in the dark at 4 °C until analysis. After elimination of CO_2 by adding 0.1 ml 1N HCl and bubbling for 10 min with CO_2 free air, DOC was analyzed with a total organic carbon analyzer (Shimadzu TOC 5000). Blank values averaged 16 µg-at $C\,l^{-1}$; this value was subtracted from the sample values. The standard deviation for duplicate or triplicate analyses averaged 6 µg-at. $C\,l^{-1}$. Some of the DOC results reported in this study were also presented in Torréton et al. (1997).

Lagoon water cultures

A dilution method was used to prevent potential limitation of bacterial growth by nutrient availability and grazing by eukaryotes greater than 1 µm. Two dilution cultures were made by inoculating 10% and 30% of 1 µm filtrate of lagoon water (station 12, 04-23-95) into 0.2 µm filtered lagoon water. The absence of flagellates in the cultures was verified by microscopic examination. Bacteria were allowed to grow in the dark at 30 °C with gentle agitation. Periodically (every 1.5 to 2 h) water samples were removed and bacterial abundance, biovolume, DOC and ^{3}H-TdR incorporation rates were estimated. TdR was added at 20 nM in order to prevent isotope dilution and/or unsaturation of incorporation into fast growing populations in these cultures. Recovery of the label into the DNA and TCA fractions was estimated systematically in the cultures. The conversion factor of TdR incorporation into cell production was estimated by correlating increases in bacterial cells with TdR incorporation. Cell number increase and TdR incorporation were correlated using the cumulative method proposed by Bjornsen and Kuparinen (1991). Functional linear regressions (Ricker, 1973) were used to compute the thymidine conversion factor (TCF) assuming the relationship: cells = TCF (ΣTdR) + β, where cells represents the net increase of bacterial cells accumulated over the successive time intervals, ΣTdR is the integral of TdR incorporation accumulated over the successive time intervals and β is the intercept of the regression of cells versus ΣTdR.

Bacterioplankton carbon growth yield (CGY) was estimated for the same cultures by correlating DOC consumption with the increase of bacterial biomass. Functional linear regressions were used to compute the carbon growth yield (CGY). The relationship: $\delta BC = CGY\ (\delta\ DOC) + \beta$ was assumed, where, δBC is the net increase of bacterial carbon accumulated over the successive time intervals, δ DOC is the net decrease of DOC and β is the intercept of the regression. Production divided by CGY was used to assess bacterial DOC consumption in the lagoon.

Results

Validity of the thymidine method to estimate bacterioplankton production

Saturation kinetics

Five experiments performed at different stations in the lagoon showed that 10 nM ^{3}H-TdR was sufficient to saturate the incorporation process (Fig. 2). Bacterial production was therefore not underestimated by the routine use of 10 nM ^{3}H-TdR. In the cultures, the routine concentration of 20 nM was used. Occasional comparisons of rates with 40 nM TdR showed that maximum incorporation was achieved at 20 nM.

Isotope dilution

Dilution pools determined in 6 different experiments varied between −3.9 and 3.3 nM (Fig. 3) and none of them was significantly different from zero ($P > 0.05$, Student's t-test). Hence, the degree of participation of labeled TdR in DNA synthesis was maximal, i.e. labeled TdR constituted nearly 100% of TdR incorporated according to this procedure and it was not necessary to correct TdR incorporation for isotope dilution.

Incorporation into DNA

In the water column samples, label recovered in DNA averaged 75.0% of the total recovered in the cold-TCA precipitate with minor variation (SE = ± 1.3%, $n = 15$). This result shows that TdR is not extensively catabolized by the Great Astrolabe Reef assemblages,

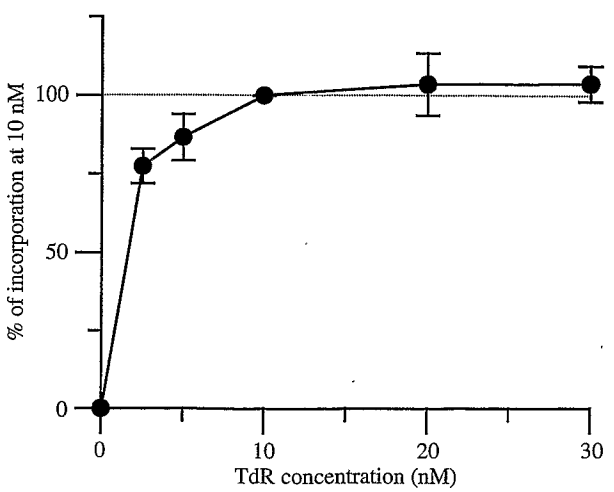

Fig. 2 Saturation kinetics of TdR incorporation rate with increasing concentration of labeled TdR. Rates were normalized by attributing 100% to the 10 nM value. Filled circles are mean values and error bars represent SE

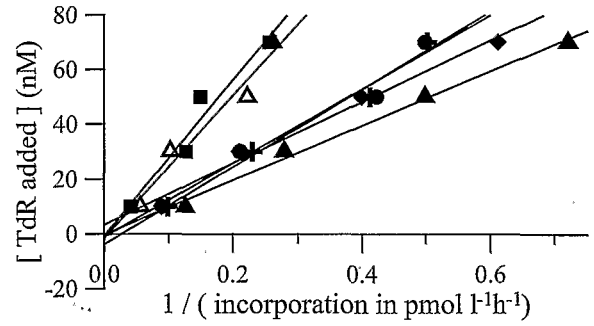

Fig. 3 Isotope dilution plots of TdR incorporation. Y-intercept represents the opposite of the dilution pool. Six experiments were performed on stations 2 (+), 5 (△), 7 (■), 10 (●), 11 (▲), and 13 (◆)

but rather that it is incorporated preferentially into DNA. For the remaining in situ samples, incorporation into DNA was calculated by multiplying incorporation into cold TCA precipitable material by 0.75. During bacterial growth in the cultures, recovery of the label in DNA and TCA fractions was stable (mean = 90.5%, SE = ± 3.1%, $n = 8$) and significantly higher than in situ ($P < 0.001$, Student's t-test).

Calibration of TdR incorporation against cell production

After a lag phase of less than 10 h, bacterial cells and TdR incorporation increased exponentially (Fig. 4). The two independent determinations of thymidine conversion factors led to similar results (see Fig. 5) with, on average, 0.7×10^{18} cells per mol of TdR incorporated into DNA. Incorporation of TdR into DNA (in mol l^{-1} h^{-1}) was therefore multiplied by 0.7×10^{18} to obtain bacterial production in cell l^{-1} h^{-1}. A carbon content of 1.67 fg-at. C $cell^{-1}$ (Lee and Fuhrman 1987) was used to obtain production values in carbon units.

Bacterial growth yield in the seawater cultures

DOC concentrations decreased significantly within the cultures ($P < 0.05$, see Fig. 4) while bacterial carbon increased. Cell multiplication and DOC consumption were correlated using the cumulative method described for TdR incorporation. The two determinations led to similar results (see Fig. 6) with an average CGY of 6.6%. Production values in µg-at. C l^{-1} h^{-1} were therefore divided by 0.066 in order to obtain heterotrophic activity of the bacterioplankton community.

Bacterioplankton variables in the Great Astrolabe Reef lagoon

Temporal variation

A diel cycle of abundance and activity was performed on 27 May at station 5. The observed variation were

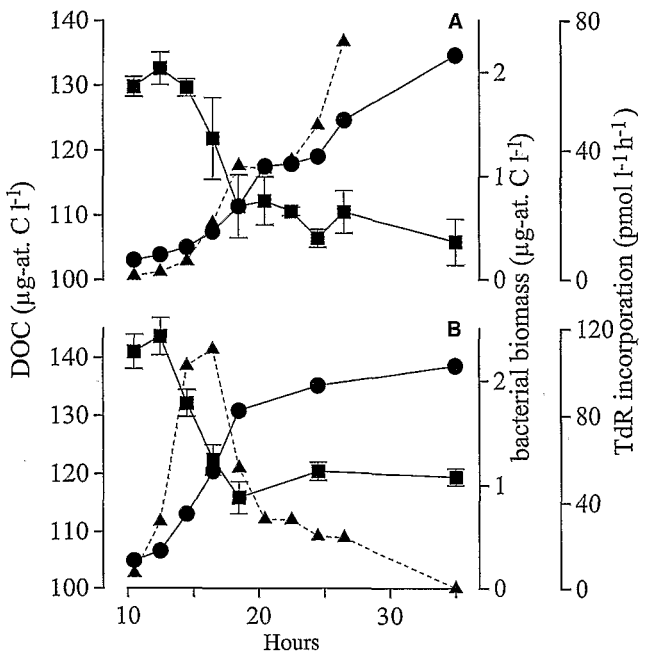

Fig. 4A,B Bacterial biomass (●), TdR incorporation into DNA (▲) and DOC concentration (■) in the seawater cultures. **A** 10% inoculum; **B** 30% inoculum

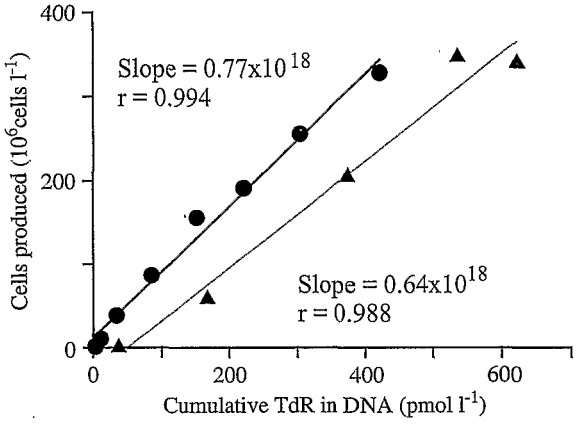

Fig. 5 Cells produced versus integral of TdR incorporation accumulated over successive time intervals. The slope of the regression lines represent the conversion factor (CF) for TdR incorporation into cell production. ●: 10% inoculum, ▲: 30% inoculum

moderate at this time scale with CV% of 9, 22 and 28 for bacterial abundance, production and growth rate, respectively (Fig. 7).

Vertical distribution

Bacterial variables showed little variation between depths within the water column with coefficients of variation (CV%) for the 10 stations averaging 11, 18 and 17% for bacterial abundance, production and

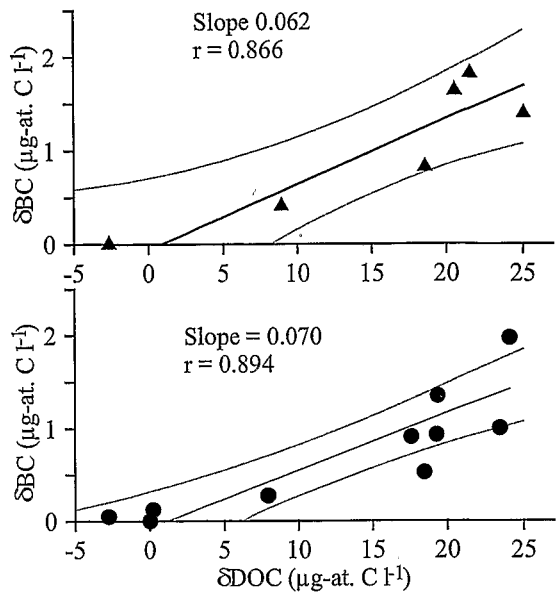

Fig. 6 Plot of bacterial carbon produced (δBC) versus DOC consumed (δDOC) over successive time intervals in the two cultures. The slope of the regression line represents the carbon growth yield (CGY). Dotted lines indicate 95% confidence limits. ●: 10% inoculum, ▲: 30% inoculum

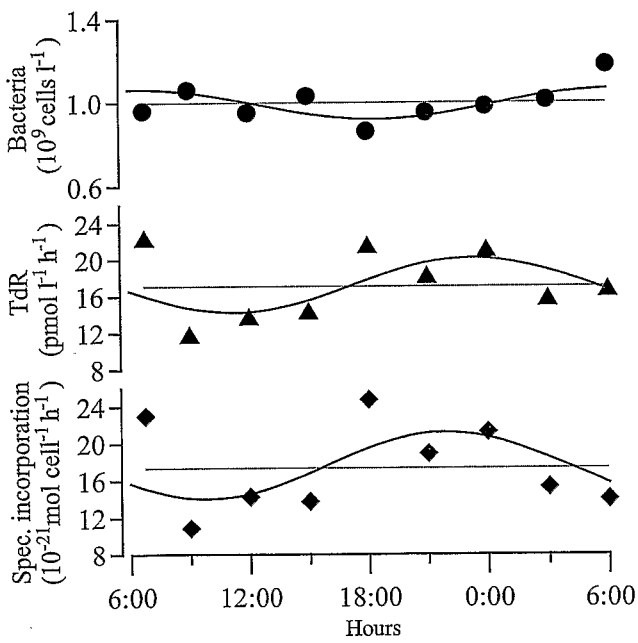

Fig. 7 Diel patterns of bacterioplankton parameters at station 5 at 10 m depth. Dotted horizontal lines represent average values. Plain curves represent the best fits and are not significant (see text)

growth rate, respectively (Table 1). Average values for vertical profiles did not differ from site to site in the lagoon (Table 1). Coefficients of variation for averages of the 10 profiles were 10, 19 and 21% for bacterial abundance, production and growth rate respectively.

Average values of bacterioplankton and related variables

Bacterial biomass, production and growth rate

Bacterial abundance averaged 0.77×10^9 cells l^{-1} (Table 2) over the whole study. Average cell volumes were small and stable in the lagoon, ranging from 0.042 to 0.065 µm^3 cell^{-1} (average = 0.055, Table 2). Based on 1.67 fg-at. C cell^{-1} (Lee and Fuhrman 1987, for cells <0.070 µm^3), average biomass was 1.28 µg-at. C l^{-1}. Based on an average incorporation rate of 12.8 pmol TdR $l^{-1} h^{-1}$, and using the TCF determined and 1.67 fg-at. C cell^{-1}, bacterial production averaged 15 ng-at. C $l^{-1} h^{-1}$ or 0.36 µg-at. C $l^{-1} d^{-1}$. With an average specific incorporation rate of 16.8×10^{-21} mol TdR cell$^{-1} h^{-1}$, the whole heterotrophic bacterioplankton community (free + attached) had an average specific growth rate of 0.282 d^{-1} and, therefore, an average generation time of 1/0.282 = 3.6 days.

Free and attached bacteria

Microscopy showed that a significant proportion of bacterioplankton cells, 10.4% of the total on average (at 10 m depth, see Table 3), were attached to particles greater than 3 µm. An even greater proportion of the total production could be attributed to attached bacteria. Indeed, TdR incorporation retained by 3 µm pore size membranes represented 14.1% of the total on average (Table 3). Therefore, when the abundance and activity of the free community were calculated (by subtracting attached from the total), the average growth rate (0.19 d^{-1}) was significantly lower ($P < 0.01$, non-parametric Wilcoxon paired test) than growth rates of the attached bacteria (0.30 d^{-1}).

DOC concentrations, heterotrophic activity and DOC turn-over

DOC concentrations determined in various areas of the lagoon ranged from 104 to 125 µg-at. C l^{-1} and showed very little variation among sampling sites or depths. A vertical profile determined at station 25 showed no significant vertical variation (average 117 µg-at. C l^{-1}, CV% = 3%, 4 levels). The coefficient of variation for all determinations inside the lagoon was only 6% around the mean value (114 µg-at. C l^{-1}, $n = 14$). Therefore, the average value for all the data seems to be representative of the average conditions in the lagoon during the period of study.

Total DOC turnover due to bacterioplankton consumption may be estimated from bacterial production (BP) and bacterioplankton carbon growth yield (CGY) determined in the two dilution cultures. Bacterial carbon consumption (BCC) would thus equal BP/CGY. With an average BP of 0.36 µg-at. C $l^{-1} d^{-1}$ and a CGY

Table 1 Averages (avg) and coefficients of variations (*cv*) for bacterial abundance, TdR incorporation and TdR specific incorporation per cell along vertical profiles at 10 stations. *Bact*: bacterial abundance (10^9 cells l^{-1}); *TTI*: tritiated thymidine incorporation into DNA (pmol l^{-1} h^{-1}); *TTI/cell*: thymidine specific incorporation (10^{-21} mol $cell^{-1}$ h^{-1}); *cv* in %

Stations		2	4	7	10	11	13	15	18	21	25
Bact	avg	0.72	0.79	0.70	0.89	0.73	0.72	0.70	0.69	0.75	0.61
	cv	*12*	*20*	*15*	*10*	*13*	*7*	*4*	*13*	*9*	*6*
TTI	avg	13.1	9.9	10.4	7.9	9.7	8.0	7.6	8.1	8.4	7.6
	cv	*10*	*23*	*11*	*32*	*23*	*17*	*16*	*22*	*11*	*15*
TTI/cell	avg	18.2	12.6	15.3	9.0	13.1	11.0	10.8	11.9	11.3	12.4
	cv	*7*	*19*	*23*	*34*	*12*	*15*	*16*	*28*	*12*	*12*

Table 2 Average bacterioplankton values recorded during this study. Lagoon data include 10 vertical profiles (47 samples) and a diel cycle at station 5 (9 samples). Biovolume, attached bacteria and TdR incorporation by attached bacteria were determined only on 12 samples (see Table 3), DOC was determined at 10 m depths at all stations and in addition at the surface at station 22 and at 0, 5 and 20 m at station 25. nd: not determined. Same symbols as in Table 1

	Abundance 10^9 cell l^{-1}	Biovolume μm^3 $cell^{-1}$	TTI pmol l^{-1} h^{-1}	TTI/cell 10^{-21} mol $cell^{-1}$ h^{-1}	Attached cells % of total	Attached TTI % of total	DOC µM
	lagoon						
Mean	0.77	0.055	12.8	16.8	10.4	14.1	114
SE	0.02	0.005	0.5	0.6	0.9	0.9	2
N	56	12	56	56	12	12	14
	ocean 0–40 m deep						
Mean	0.51	nd	1.0	2.0	nd	nd	110
SE	0.05		0.2	0.7			3
N	3		3	3			3

Table 3 Abundance, production and growth rate for free and attached bacteria. Bact: bacterial abundance 10^9 cells l^{-1}. Prod.: bacterial production (µg-at. C l^{-1} d^{-1}), µ: specific growth rate (d^{-1}). All stations except 25 were sampled at 10 m depth. Station 5 was sampled twice (on 27, 28-04-94); $n = 12$

	Total			Attached			Free			Attached Bacteria	Attached Production
	Bact	Prod.	µ	Bact	Prod.	µ	Bact	Prod.	µ	% of total	% of total
Minimum	0.550	0.195	0.14	0.045	0.024	0.11	0.505	0.158	0.14	6.0	9.0
Maximum	1.182	0.396	0.34	0.189	0.058	0.63	0.992	0.348	0.31	16.0	18.9
Mean	**0.818**	**0.267**	**0.20**	**0.088**	**0.037**	**0.30**	**0.730**	**0.230**	**0.19**	**10.4**	**14.1**
SE	**0.054**	**0.020**	**0.02**	**0.013**	**0.003**	**0.05**	**0.043**	**0.018**	**0.01**	**0.9**	**0.9**

of 6.6%, BCC equals 0.36/0.066 = 5.5 µg-at. C $l^{-1}d^{-1}$. Hence, DOC turn-over rate equals 5.5/114 = 0.048 d^{-1} and total DOC turn-over time is 1/0.048 = 21 days. Of course, the entire pool of DOC is unlikely to be available to bacteria. Therefore, this is an underestimation of the turnover time of the labile fraction.

Oceanic station

Bacterioplankton characteristics showed a classic vertical pattern with maximum abundance in surface water and maximum activity at 50 m (Fig. 8), just below the phytoplankton maximum (Charpy 1996). This vertical pattern was even more pronounced for specific incorporation rate per cell (an index of bacterioplankton growth rate). DOC decreased steadily from 116 µg-at. C l^{-1} in surface water to 91 µg-at. C l^{-1} at 100 m depth.

Discussion

Validity of the TdR method

As in other coral reef lagoons (Torréton and Dufour 1996a) small concentrations of TdR were sufficient to saturate the incorporation process and isotope dilution

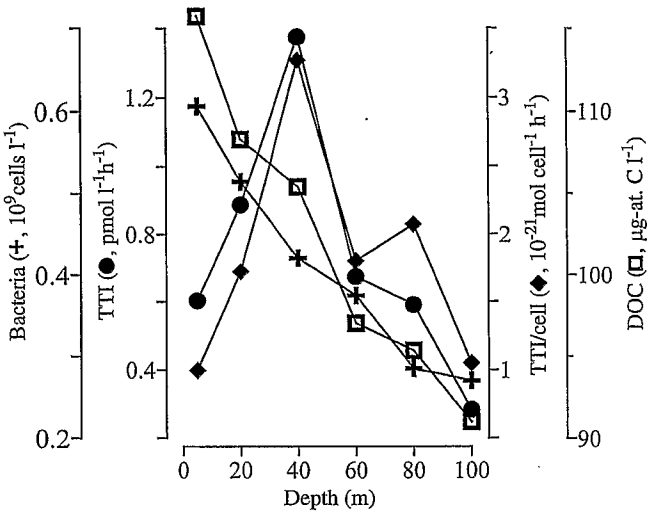

Fig. 8 Abundance, TdR incorporation, specific incorporation rate per cell and DOC in the water column of the oceanic station

was insignificant. Incorporation of the label into DNA represented 75.0 ± 1.3% of its incorporation into cold TCA precipitate in samples obtained directly from the lagoon, while it represented a significantly greater proportion ($P < 0.001$, Student's t-test) in actively growing assemblages of the dilution cultures (90.5 ± 3.1%). This difference has been observed in other coral reef lagoons (Torréton and Dufour 1996a) and, is of particular interest because TdR incorporation estimates DNA synthesis. DNA is not the only macromolecule recovered in the TCA precipitate labeled by ^{3}H-TdR, but usually for simplicity only tritiated thymidine incorporation into cold TCA precipitate (TTI_{TCA}) is determined. TCA derived production ($Prod_{TCA}$) is estimated by: $Prod_{TCA} = TTI_{TCA} \times TCF_{TCA}$ (1), where TCF_{TCA} is the "thymidine conversion factor" based on incorporation into TCA precipitate.

However, the real bacterial production value ($Prod_{DNA}$) should be given by incorporation into DNA (TTI_{DNA}), with: $Prod_{DNA} = TTI_{DNA} \times TCF_{DNA}$ (2), where TCF_{DNA} is the "thymidine conversion factor" based on incorporation into DNA. As the label in DNA constitutes on average 75.0% and 90.5% of the label in TCA precipitate on in situ samples and in lagoon water cultures, respectively, we can write: $TTI_{TCA} = TTI_{DNA}/0.75$ (3) and $TCF_{TCA} = 0.905$ TCF_{DNA} (4). Therefore, using Eqs. (3) and (4), (1) we obtain: $Prod_{TCA} = TTI_{DNA}/0.75 \times 0.905$ CF_{DNA} (1′). Using (2), (1′) we make: $Prod_{TCA} = Prod_{DNA} \times 0.905/0.75$

Cell production calibrated against TdR incorporation into cold TCA precipitate would therefore lead to a 1.2 fold overestimation of the real bacterioplankton production. The artifact is not very important in this ecosystem as the percent of label incorporated into DNA is rather elevated for in situ samples. However, in waters showing a stronger catabolism pathway for ^{3}H-TdR, this difference of incorporation pattern between in situ and cultured bacteria may lead to a much greater overestimation of bacterial production (Torréton and Bouvy 1991).

Bacterial growth yield in the seawater cultures

The two independent determinations allowed the estimation of an average CGY of 6.6%. Such low growth yields have been estimated in two Tuamotu atoll lagoons (Torréton et al. 1997) and are in the range (4–19%) of those determined in the most oligotrophic oceanic situations (Carlson and Ducklow 1996; Hansell et al. 1995). Like other studies relating consumption of DOC (or oxygen) and production of bacterial carbon determined from changes in bacterial abundance and biovolume, CGY values are highly dependent upon the carbon conversion factor (CCF) used. In this study, the allometric model proposed by Simon and Azam (1989) was employed. The use of extreme literature values of CCF would allow the calculation of CGY of 5.6% (with 10 fg-at. C μm^{-3}, Nagata and Watanabe 1990) and 26.2% (with 47 fg-at. C μm^{-3}, Bratbak 1985). In any case, CGY would be much lower than the 50% value most often cited in carbon budgets. The low CGY value determined in the present study could be interpreted as an index of severe bottom-up limitation of bacterioplankton.

Variation of bacterioplankton parameters in the Great Astrolabe Reef lagoon

Temporal variation

The unique diel experiment showed weak fluctuations of bacterial variability over a 24 h period (Fig. 7). The hypothesis of a diel trend was tested using non-linear regression (*Statistica* software) in the form $[variable] = a0 + (a1 \times \cos(2\pi/24 \times (T + a2)))$ where: $[variable]$ is bacterial abundance (Bact), incorporation rate of TdR (TTI) or specific incorporation rate per cell (TTI/cell); T is time of day; $a0$, $a1$ and $a2$ are coefficients to be calculated and 24 is the assumed periodicity in hours. None of the trends was significant ($P > 0.05$) since the regression coefficients were 0.64, 0.57 and 0.56 for Bact, TdR and TdR/cell, respectively. In addition, differences between day (6 h:00–18 h:00) and night averages were not significant ($P > 0.05$, non-parametric Mann-Whitney U-test). It is impossible to determine the absence or presence of a diel pattern of bacterioplankton processes from this limited data set. The important point from this study is that this weak trend, in contrast to the strong diel patterns observed by Moriarty et al. (1985) at Lizard Island, would not strongly bias an estimation of a diel average from discrete measurements. Bacterioplankton abundance, production and growth rate determined using discrete

sampling in the lagoon may thus be considered representative of the daily average values of 8, 21 and 26%, respectively. The day to day fluctuation of bacterial parameters during the two-week period should not have exceeded the spatial differences between stations since one or two stations were investigated every day.

Vertical distribution

Bacterial variables varied only slightly with depth. In order to test if bacterial biomass and activity varied with depth, values were ranked and a two-factor analysis of variance (ANOVA) was performed on the ranks. Applied to the data set of 10 profiles, ANOVA shows that bacterial biomass, production and growth rates were not significantly different among depths ($P > 0.05$, Table 4). This homogeneity for bacterial variables along vertical profiles was observed in Tikehau lagoon (Torréton and Dufour 1996b) and in other Tuamotu atoll lagoons (unpublished data) and is likely due to the strong vertical mixing driven by regular trade winds in these areas.

Spatial variation

Variability from site to site in the lagoon was also minimal (Table 1). ANOVA on ranked values showed, however, that these weak differences (1.4, 1.7 and 2.0 fold at most for abundance, production and growth rate, respectively) were significant between stations ($P < 0.01$, Table 4). The significant differences between stations should however not be interpreted as resulting from a significant spatial zonation because the different sites were sampled during successive days at different times of day. Variation between stations integrated, therefore, diel, day-to-day and spatial variation. The similar magnitude of variation between stations to that of diel variation suggests that spatial variation is moderate. Variation was greater for integrated values such as bacterial abundance and production, which varied between 2.1 and 2.4 fold at most, respectively. These weak spatial differences may have been due to depth differences between the stations.

Table 4 Two-factor ANOVA for bacterial abundance, TdR incorporation and TdR specific incorporation per cell. Values are rank transformed. Bact: bacterial abundance, TTI: TdR incorporation, TTI/cell: TdR specific incorporation per cell

Variable	Sources of variation	Df	Average of squares	F-ratio	*P*
Bact	Depth	4	277	1.93	>0.05
	Station	9	431	3.01	<0.01
	Error	36	143		
TTI	Depth	4	140	0.93	>0.05
	Station	9	464	3.09	<0.01
	Error	36	150		
TTI/cell	Depth	4	301	2.27	>0.05
	Station	9	462	3.48	<0.01
	Error	36	133		

Average values of bacterioplankton variables in the Great Astrolabe Reef lagoon

As short term fluctuations and spatial variability were low, it is possible to examine (1) the importance of average bacterial biomass, production and heterotrophic activity as compared to phytoplanktonic biomass and production, (2) their contribution to particulate organic carbon and nitrogen concentrations in the Great Astrolabe Reef lagoon, and (3) to compare bacterioplankton average values with those reported for other coral reef ecosystems.

Bacterial biomass

With 0.77×10^9 cells l^{-1} (Table 2), average bacterial abundance was very similar to the values reported by Yoshinaga et al. (1991) in Majero Atoll but about 1.5–2 times smaller than average values for Tikehau and Takapoto lagoons, Tuamotu, French Polynesia (Torréton and Dufour 1996a, b). Bacterial biomass in the Great Astrolabe lagoon was estimated at 1.28 µg-at. C l^{-1} and represents therefore 11% of the POC in this ecosystem (12.0 µg-at. C l^{-1}, Charpy et al. 1996). Based on a C:N molar ratio of 4.3 (Lee and Fuhrman 1987), bacteria would contain 0.39 fg-at. N $cell^{-1}$. A bacterial biomass of 0.3 µg-at. N l^{-1} would represent as much as 20% of the total PON in this lagoon (1.46 µg-at. N l^{-1}, Charpy et al. 1996). Undoubtedly, heterotrophic bacterioplankton constitutes an important part of nitrogen standing stocks of this lagoon. Phytoplankton was estimated by flow cytometry during the same campaign (Blanchot 1996). With 4.1×10^4, 8.5×10^4 and 2.2×10^3 cells ml^{-1} on average for *Prochlorococcus*, *Synechococcus* and pico-eukaryotic phytoplankton, respectively. Assuming that they contain 5.1, 8.7 and 259 fg-at. C per cell on average (Charpy and Blanchot 1998), mean phytoplankton biomass would be equivalent to 1.52 µg-at. C l^{-1}. Therefore, heterotrophic bacterioplankton represents a carbon biomass approximately equivalent to that of phytoplankton. With its lower C:N ratio, heterotrophic bacterioplankton nitrogen would likely exceed phytoplankton nitrogen.

Bacterial production

Bacterial production was on average 0.36 µg-at. C $l^{-1}d^{-1}$. During an investigation on bacteriobenthos production, Pollard and Kogure (1993) estimated an

average production of 0.55 µg-at. C $l^{-1}d^{-1}$ (SE = $\pm$ 0.1, $n = 6$, using a theoretical conversion factor of 0.5×10^{18} cells per mol of TdR incorporated into DNA and 2.5 fg-at. C $cell^{-1}$) in the 5 m deep water column overlying a seagrass bed 100 m from the shore of Dravuni island. Bacterioplankton activity was similar in these two studies. Bacterial production was also similar to bacterioplankton production determined for two Tuamotu atoll lagoons (Torréton and Dufour 1996a, 1996b). Integrated from the surface to the bottom of the lagoon, bacterial production averaged 8.3 mg-at. C $m^{-2}d^{-1}$ at the 10 stations investigated. This represents only 8% of the primary production determined during the same period (108 mg-at. C $m^{-2}d^{-1}$, Charpy 1996).

Generation time

The average generation time for the whole bacterioplanktonic community was 3.6 days. This is quite long compared to those estimated over coral reefs (Moriarty et al. 1985; Ferrier-Pagès and Gattuso 1998), but in agreement with those determined in atoll lagoons (Yoshinaga et al. 1991; Torréton and Dufour 1996b). This long generation time could be explained by a resource limitation of bacteria.

Free and attached bacteria

The distinction between free and attached bacterioplankton has ecological consequences because free and attached bacterial production are not exported in the same way, nor are they fueled by the same resources (Hoppe et al. 1988). Losses of attached bacteria may occur by sedimentation, negligible in free-living bacteria, or via grazing by higher trophic levels (mesozooplankton). Moriarty (1979), reported that up to 50% of bacteria were attached in coral reef waters at Lizard Island (Australia). In the Great Astrolabe Reef lagoon, attached bacteria represent on average only 10.4% of the total with moderate variation around this value (SE = $\pm$ 0.9, $n = 12$). This value is comparable to that reported in a Tuamotu atoll lagoon (Torréton and Dufour 1996b). The average growth rate of the free community (0.19 d^{-1}) was significantly lower ($P < 0.01$) than the growth rate of attached bacteria (0.30 d^{-1}), suggesting that attachment presents an advantage for the bacterioplankton community of the Great Astrolabe Reef lagoon. Particles present a favorable environment for microbial growth since bacterial exoenzyme activities release simple organic molecules which can be directly taken up by attached bacteria (Hoppe et al. 1988). In oligotrophic systems where bacteria are likely to be bottom-up controlled attachment to particles seems to lead to a greater growth rate for the microbial community.

DOC concentrations and heterotrophic activity

DOC concentrations reported for coral reef environments show a wide range of values (64–305 µg-at. C l^{-1}: Marshall et al. 1975; Hatcher 1983; Mackey et al. 1987; Yoshinaga et al. 1991; Suzuki et al. 1995; Pagès et al. 1997). With the exception of the high values measured by Hatcher (1983) at Houtman Abrolhos lagoon (up to 305 µg-at. C l^{-1}) and Yoshinaga et al. (1991) at Ponape island (225 µg-at. C l^{-1}), most of the values lie between 64–145 µg-at. C l^{-1}. Great Astrolabe Reef values (average 114 µg-at. C l^{-1}) fall within this lower range. Bacterioplankton demand for DOC was estimated to average 5.4 µg-at. C $l^{-1}d^{-1}$ for the period of study. Integrated from the surface to the bottom of the lagoon, the average demand for the 10 stations investigated was 126 mg-at. C $m^{-2}d^{-1}$ (8.3 mg-at. C $m^{-2}d^{-1}$/0.066) and was therefore nearly equal to primary production during the same period (108 mg-at. C $m^{-2}d^{-1}$, Charpy 1996). The net production of bacterial biomass was low compared to particulate primary production (8%), but the low estimated growth yield shows that the heterotrophic activity of bacteria was nearly equivalent to primary production. This confirms the importance of bacterioplankton to remineralization processes in the water column of coral reef lagoons.

Lagoon versus open ocean

Determining bacterioplankton characteristics in the oceanic water column was not the main objective of this study. However, considering the low variability observed in the lagoon, one may assume that the unique profile observed at the oceanic station was representative of average oceanic conditions during the period of study. Abundance values inside the lagoon were, on average, only 1.5 times higher than those of oceanic surface waters (average from 5 to 40 m, Table 2), but production values were about 13 fold greater in the lagoon than at the oceanic station. Therefore, specific incorporation rate per cell, an index of bacterial growth rate, was 8 times greater in lagoon water than at the oceanic station at the same depth (Table 2). If we assume the TCF determined in the lagoon to be applicable to oceanic waters, average generation time of oceanic bacterioplankton would be 30 days.

However, DOC values do not appear to be significantly different in the ocean (0 to 40 m). While bacterial growth rates have been frequently reported to be higher inside island or atoll lagoons than in surrounding oceanic waters, lagoon DOC concentrations may be similar, as in this work, or even lower than those of oceanic waters (Suzuki et al. 1995; Pagès et al. 1997). In the Great Astrolabe lagoon waters, the proportion of degradable DOC, estimated by the amount remaining after bacterial growth in the seawater cultures (Fig. 4), is low. This low proportion suggests that a smaller

fraction of the labile DOC pool in oceanic waters could be undetected using bulk DOC measurement, and could explain the large differences between oceanic water and lagoon bacterioplankton growth rates.

The main differences detected between ocean and lagoon waters are that (1) bacterioplankton variables present a strong vertical pattern in the ocean while vertical homogeneity seems to be the rule in the lagoon; and (2) bacteria are only slightly more abundant in lagoon waters but have a far greater activity than in the surrounding ocean. This later point has already been observed in Majuro (Yoshinaga et al. 1991) and in the Tuamotus (Torréton and Dufour 1996b).

In conclusion, in the Great Astrolabe Reef lagoon, variability of bacterioplanktonic processes was low both spatially and temporally over the 15 day period of the study. Bacterial biomass constituted a significant proportion of POC and was in the same range as phytoplankton biomass. Heterotrophic demand was of the same order as primary production. The low average growth rate for the bacterioplanktonic community (0.28 d^{-1}, $n = 56$), at an average temperature of about 28 °C, and the poor carbon growth yield (6.6%) both suggest bacterioplankton to be resource-limited in this lagoon. In this environment, attachment seems to provide an advantage for bacterial growth. Great Astrolabe Reef bacterioplankton had characteristics distinct from adjacent oceanic waters, and quite similar to Tuamotu lagoon assemblages, with a rather elevated biomass, a low growth rate and a poor carbon growth yield. All these features are consistent with a bottom-up limitation of bacterioplankton. Whether this limitation is due to poor DOC quality or inorganic nutrient limitation will be the object of another study.

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