Bacterioplankton biomass and production during destratification in a monomictic eutrophic bay of a tropical lagoon

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ABSTRACT: Vertical profiles of bacterial biomass (AODC method), production of biomass (thymidine method) and total ETS (Electron Transport System) activity were recorded on 14 dates during the destratification process in the monomictic eutrophic Biétri Bay (Ebrié Lagoon, Ivory Coast). Bacterial biomass and production were both very high, indicating the importance of heterotrophic processes in this system. Interpretation of ETS data suggests that bacteria or 'bacteria-associated organisms' are responsible for most of the oxygen consumed in the water column. Integrated bacterial production of biomass represented ca 80 % of carbon produced by primary producers, thus showing that autotrophic production does not meet heterotrophic requirements of bacteria. Destratification appears to be of less importance than suggested by the physical and chemical characteristics, resulting in a decrease of bacterial biomass and production by a factor of 2 in the epilimnion. In the hypolimnion, the 'anaerobic community' characterized by low specific incorporation rates (3.4×10^{-20} mol cell^{-1} h^{-1}) and high mean cellular volume (0.203 µm^3) during stratification is replaced by an 'aerobic community' with higher specific thymidine incorporation rates (6.6×10^{-20} mol cell^{-1} h^{-1}) and lower mean cellular volumes (0.151 µm^3). Interpretation of bacterial production of biomass in these newly oxygenated layers demonstrates that oxygen requirements in the aphotic zone are roughly equivalent to autotrophic production of oxygen in the euphotic zone, thus showing that oxygenation of the entire water column is necessarily transient and explaining the return to anoxic conditions before the establishment of a new density stratification.

INTRODUCTION

Planktonic bacteria are known to contribute significantly to carbon flow in the ocean (Azam et al. 1983). In mesotrophic and eutrophic systems such as lakes and estuaries, several authors have shown that bacterial production on a yearly basis can represent 20 to 63 % of primary production (Pedrós-Alió & Brock 1982, Bell et al. 1983, Lovell & Konopka 1985, Nagata 1987, Scavia & Laird 1987, Simon & Tilzer 1987) and occasionally more due to allochtonous organic inputs or phytoplankton senescence after a bloom (Lovell & Konopka 1985). In eutrophic system, bacteria are always more abundant and have higher mean cell volume and growth rates (Table 1) than they have in oceanic and coastal ecosystems, suggesting that heterotrophic activity represents a major pathway for carbon flux in these systems. The understanding of the ecology of these eutrophic ecosystems thus requires accurate determinations of biomass and production of heterotrophic bacteria.

Biétri Bay, in the Ebrié lagoon (Ivory Coast), is a monomictic bay near the permanent opening of the lagoon to the ocean (Fig. 1). Polluted by agro-industrial and domestic sewages, this bay is eutrophic (Arfi et al. 1981, Guiral 1984). The annual hydrological cycle of the bay has been recently described (Arfi et al. 1989). In the deepest part of the bay (10 to 14 m; average depth 3.8 m), vertical stratification of oxygen occurs for most of the year, defining an oxygenated epilimnion and an anoxic hypolimnion. The epilimnion is characterized by high microalgal biomass and relatively low nutrient concentrations. Within this layer the euphotic zone is limited to 2 m depth. The hypolimnion, from 3 to 4 m deep to the bottom, shows very high N-NH$_4^+$, P-PO$_4^{3-}$ and sulfide values (Carmouze & Caumette 1985, Arfi et al. 1989). Destratification is induced at the beginning of the year, during the dry season, when the density gradient is minimal (due to minimal continental flows and thus maximum marine influence), after a temperature decrease of surface waters due to coastal upwelling (Arfi et al. 1989). During the destratification process, N-NH$_4^+$, P-PO$_4^{3-}$ and sulfide loads decrease by

Table 1. Bacterial biomass and thymidine-based production estimates in some eutrophic and mesotrophic systems

Site[a]	Temp. °C	Bacterial abundance (10^9 cell l^{-1})	Average cell volume (μm^3)	Thym. incorp. rate (pmol l^{-1} h^{-1})	Conversion factor (10^{18} cell mol^{-1})	Bacterial production ($\mu gC\ l^{-1}$ h^{-1})	Thym. inc. per cell (10^{-20} mol $cell^{-1}$ h^{-1})	Specific growth rate (h^{-1})	Thym. conc. (nM)	Chl a ($\mu g\ l^{-1}$)	Integrated primary production (gC m^{-2} d^{-1})	Bacterial/ primary production	Source
Lake Norrvikken, Sweden (E) (epilimnion)	15–22	1–2.4	0.185	12–200	1.9–2.2	0.2–7.1	0.8–16	0.008–0.292	5	7–36	0.09–0.29	50 %	Bell et al. (1983)
Lake Biwa, Japan (M) (water column)	12–28	1–10	0.161	1–100	3–24	0.2–2.5	0.15–1.8		5	1–11		30 %	Nagata (1986, 1987)
Little Crooked Lake Indiana, USA (E) (water column)	8–30	1.7–6.3	0.120	<9–100	2.2	0.4–2.1	0.11–3.75	0.0024–0.082	2.22			45 %	Lovell & Konopka (1985)
Lake Oglethorpe, Georgia, USA (E) (water column)	10–25	5–16		1–40[b]					5		0.3–0.6		McDonough et al. (1986)
Head waters of the Rhode River estuary, Maryland, USA (surface)		0.3–54 (7.3)[c]	0.060	0.9–1600 (260)	0.2–8[d]	(0.26–10.8)		0.011–0.070	1	0.5–245 (42)			Rublee et al. (1984)
Frederiksborg Slotsso, Denmark (E)	6–7.5	8.6–17.9	0.042–0.048		2.1[e]	0.071–0.217	0.054–0.075	0.0008–0.0041	10–25	6–71	0.23[f]–0.923	0.6–7.3%	Riemann et al. (1982)
Lake Erken, Sweden (M) (1–5 m), spring bloom	2–5.7	0.5–1.52	0.083	(2.92)	1.6–2.9	0.05–0.071			10	4.9–22.6	0.034[f]–3.188	20%[g]	Bell & Kuparinen (1984)
Hartbeespoort Dam, South Africa (H)	21	5.2–14.8		15–78[h]							0.111[i]–1.605		Robarts et al. (1986)
York River Estuary, Virginia, USA (water column)	25	1–8	0.074	42–420	0.35	0.29–3.13	0.8–6.3	0.0083–0.0458	5				Ducklow (1982)
Biétri Bay, Ivory coast (E) (oxygenated epilimnion)	28–30	5.9–32.3 (18.2)	0.162	230–1940 (1233)	(0.746)	11–91 (58)	6–11.4 (7.78)	0.173–0.328 (0.242)	20	18–94 (55.8)	3.5–7.1 (5.26)	86 %	This study

[a] Trophic state: E, eutrophic; H, hypertrophic; M, mesotrophic; [b] Based on radioactivity recovered into DNA; [c] Parenthesis indicate mean values; [d] From Fuhrman & Azam (1982); [e] From Fuhrman & Azam (1982); [e] Corrected for isotope dilution and percent recovery into DNA; [f] gC m^{-3} d^{-1}; [g] Gross bacterial production (production + respiration/primary production); [h] <4 to 33 pmol l^{-1} h^{-1} DNA (both recalculated from figures); [i] in gC m^{-2} h^{-1}
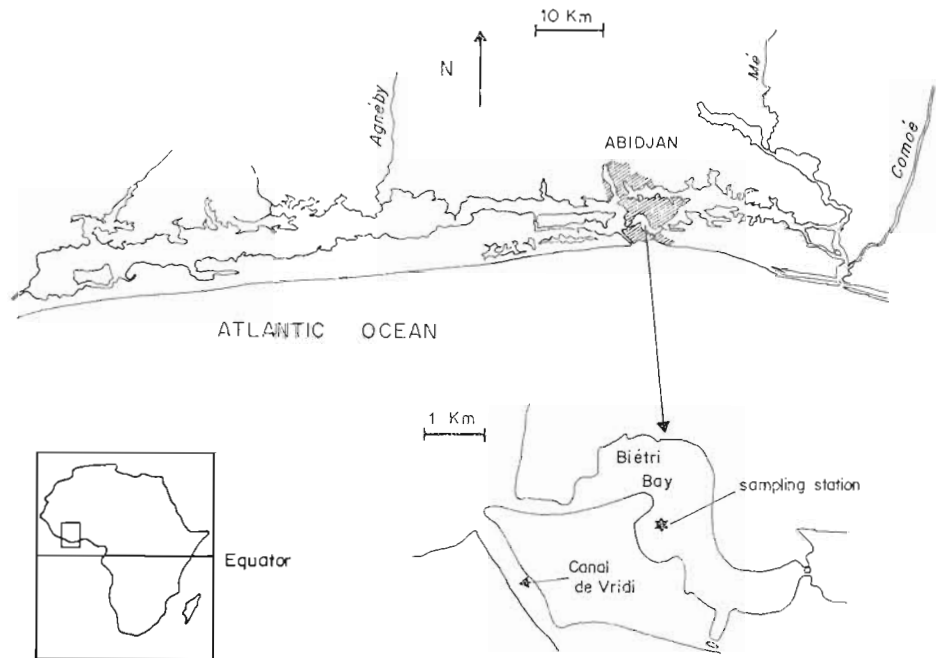
Fig. 1. Ebrié Lagoon system, Biétri Bay, and the sampling station

90, 60 and 100 % respectively (Guiral et al. 1989b). This period, with maximal penetration of oxygen into the deep layers, cannot be considered a true holomictic period, because the bottom topography of Biétri Bay does not allow vertical mixing (Guiral et al. 1989a).

The purpose of this study was to describe the time course of heterotrophic bacterial biomass and production during the destratification process, characterized by important variations in physical and chemical parameters in the water column and therefore expected to be the period of maximum change in bacteria-related parameters.

MATERIALS AND METHODS

Sampling, physical and chemical characteristics. Water samples were collected from one location near the center of the bay (Fig. 1), using a 10 l Niskin bottle mounted horizontally, at 0.5 m depth and at every meter from 1 to 9 or 10 m. Hydrological data were retrieved every day from 3 January to 5 February 1987, 1 h after low tide. Bacterial parameters were recorded at the same depths 14 times during this period, on samples maintained in the dark at 28°C. Water was stored in sterile acid-washed stoppered glass bottles to preserve in situ redox conditions until analysis at the laboratory (within 90 min).

Temperature and salinity were recorded using an YSI SCT meter, oxygen concentration with a YSI oxygen meter and redox potential with a Schott Geräte pH meter GG 819. Sulfide concentration was determined using the method described by Cline (1969).

Bacterioplankton biomass. Bacterial concentrations were determined by Acridine Orange Direct Counts (Hobbie et al. 1977) using an image analysis system (Van Wambeke 1988). A 6.7 × NFK photographic ocular allowed a final resolution of 0.186 µm per pixel. A total of 1000 to 2000 bacteria on 50 to 100 fields were counted under image analysis. Seven samples were counted both by eye and under image analysis. The average difference (in absolute values) between the 2 estimates was 7.4 % (data not shown).

Biovolumes were estimated by counting bacteria in 29 size classes from 1 to 45 pixel (0.035 µm^2 per pixel; 21 classes between 1 and 21 pixel, 8 classes between 22 and 45 pixel). Intercalibration between image analysis and photographic examination was based on projected surfaces. Conversion of surface (S) to volume (V) was achieved using the expression $V = 0.582 S^{1.14}$ for every cell, the best estimate for bacteria from Biétri Bay (unpubl.).

Converting bacterial biovolume estimates into bacterial carbon content requires a conversion factor. The carbon/volume ratio calculated by Watson et al. (1977) has been used by aquatic microbiologists for years. Recent estimations of this ratio, based on direct biovolume and carbon content determinations have provided a new set of conversion factors showing considerable variations. It has been shown that the carbon/volume ratio may vary with bacterial size (Lee & Fuhrman 1987, Norland et al. 1987). Thus, the minimum value of 1.06×10^{-13} gC µm^{-3} proposed by Nagata (1986) is used for Biétri Bay bacteria until direct estimates can be made, because the mean cell volume of the bacteria he used for carbon content determina-

tions (Whatman GF/C filtrate retained onto GF/F membrane) is very close to the mean cell volume of Biétri Bay bacteria (see 'Results').

Bacterial production of biomass. Bacterial production rates were estimated from the rates of methyl-(^{3}H)-thymidine incorporation into cold TCA precipitable material (Fuhrman & Azam 1982). Duplicate samples (3 to 5 ml) were incubated for 15 to 30 min in the dark at 28°C (± 1°C of in situ temperature) with methyl-(^{3}H)-thymidine (1.776 TBq mmol^{-1}; CEA, France) in sterile disposable polystyrene tubes. A concentration of 20 nM was found necessary to obtain maximal thymidine incorporation rate. Isotope dilution plots, performed using the procedure described by Pollard & Moriarty (1984), showed a non-significant participation of unlabelled thymidine to total incorporation. Lack of sufficient label at the time of the experiment prevented us from incubating samples from the anoxic hypolimnion in anaerobic conditions because of the volume of sample required. Incubations in both aerobic and anaerobic conditions (as described by McDonough et al. 1986) were performed on 2 profiles to examine the effect of artificial reoxygenation on the activity of anoxic samples.

Variability of the conversion factor for thymidine incorporation rate into bacterial production of biomass, determined by several authors (reviewed by Servais 1987) in different ecosystems, implies that this factor must be determined each time a new site is investigated. The empirical dilution technique of Kirchman et al. (1982) was used to estimate a conversion factor for bacterial assemblages in Biétri Bay. A sample (25 ml) of Biétri Bay water was inoculated into 500 ml of the same water filter-sterilized (0.22 μm Millipore membrane). Aliquots were removed every 45 min and parallel increases of thymidine incorporation rates and cell numbers were followed.

Finally, total population growth rates were calculated by dividing bacterial production in μg C l^{-1} h^{-1} by bacterial biomass in μg C l^{-1}.

Electron transport system (ETS) activity. ETS activity was determined using the INT 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-(phenyl-tetrazolium chloride) reduction technique. Water samples were pre-screened on 10 μm mesh to remove zooplankton (phytoplankton is not removed by this procedure; Arfi et al. 1981) and then filtered (< 15 cm Hg differential pressure) onto 47 mm Whatman GF/F filters within 1 h of collection. Filters were rinsed twice with ice-cold isotonic sterile NaCl solution to minimize reduction of INT by reduced compounds in anoxic layers, and stored in liquid nitrogen until analysis. ETS activity was assayed using the method described by Packard & Williams (1981) modified as follows: Homogenization with a Potter tissue grinder was achieved by sonication (2 × 1 min) in a

Bransonic 220 (50 W) ultrasonic bath. Sodium succinate was omitted from the assay as previous experiments had shown that no increase in activity was noticed. For each assay, a reduction blank with no substrate (NADH, NADPH) was made and substracted to account for reduction of INT by reduced compounds in anoxic layers and contribution of pigments to the 490 nm absorbance. For each set of assays, one assay was performed without enzyme extract and subtracted to account for non-enzymatic reduction of INT by the substrates.

All the assays were performed at 28°C in the dark, and expressed in micro-electrochemical equivalents (μeq.) l^{-1} h^{-1}. Recovery of bacteria on the glass fiber filters was estimated by incubating samples of Biétri Bay water with methyl-(^{3}H)-thymidine (1 nM final concentration) for 45 min. Incubation was stopped by adding formalin (3 % final concentration). For each sample a formalin pre-killed sample was used as a blank. Radioactivity in the TCA precipitate of the GF/F filtrate was compared to that of the unfiltered sample. Volumes filtered, depending on the sample depth, were the same as required for ETS filtrations. Recovery of the label on the GF/F filters ranged between 87 and 100 % (average 94.6 %).

Phytoplankton biomass. Chlorophyll a and phaeopigment concentrations were determined using a modification of Yentsch & Menzel's technique (1963). Pigments retained on GF/F filters were extracted in methanol and fluorescence was recorded before and after HCl addition, using a Turner 111 fluorometer. Chlorophyll concentrations were converted into phytoplankton biomass using a carbon/chlorophyll a ratio of 35 (w/w) determined by Lemasson et al. (1981) in a comparable biotope of the same lagoon.

Particulate carbon and nitrogen. Carbon and nitrogen retained on pre-combusted (450°C for 4 h), 25 mm size GF/F glass-fiber filters were determined using a Perkin-Elmer Micro-analyzer. Recovery of bacteria on these filters, checked on some samples, using the same procedure as for ETS filtration, was in the range 95 to 100 % (mean 96.1 %).

RESULTS

Samples were collected 14 times throughout the destratification process. Destratification occurred between 30 Jan and 3 Feb 1987, thus the water column was investigated 11 times during the stratified period (redoxcline between 3 and 4 m) and 3 times after destratification. Fig. 2 shows an example of the physical and biological parameters during stratification and during maximum oxygen penetration. Mean values
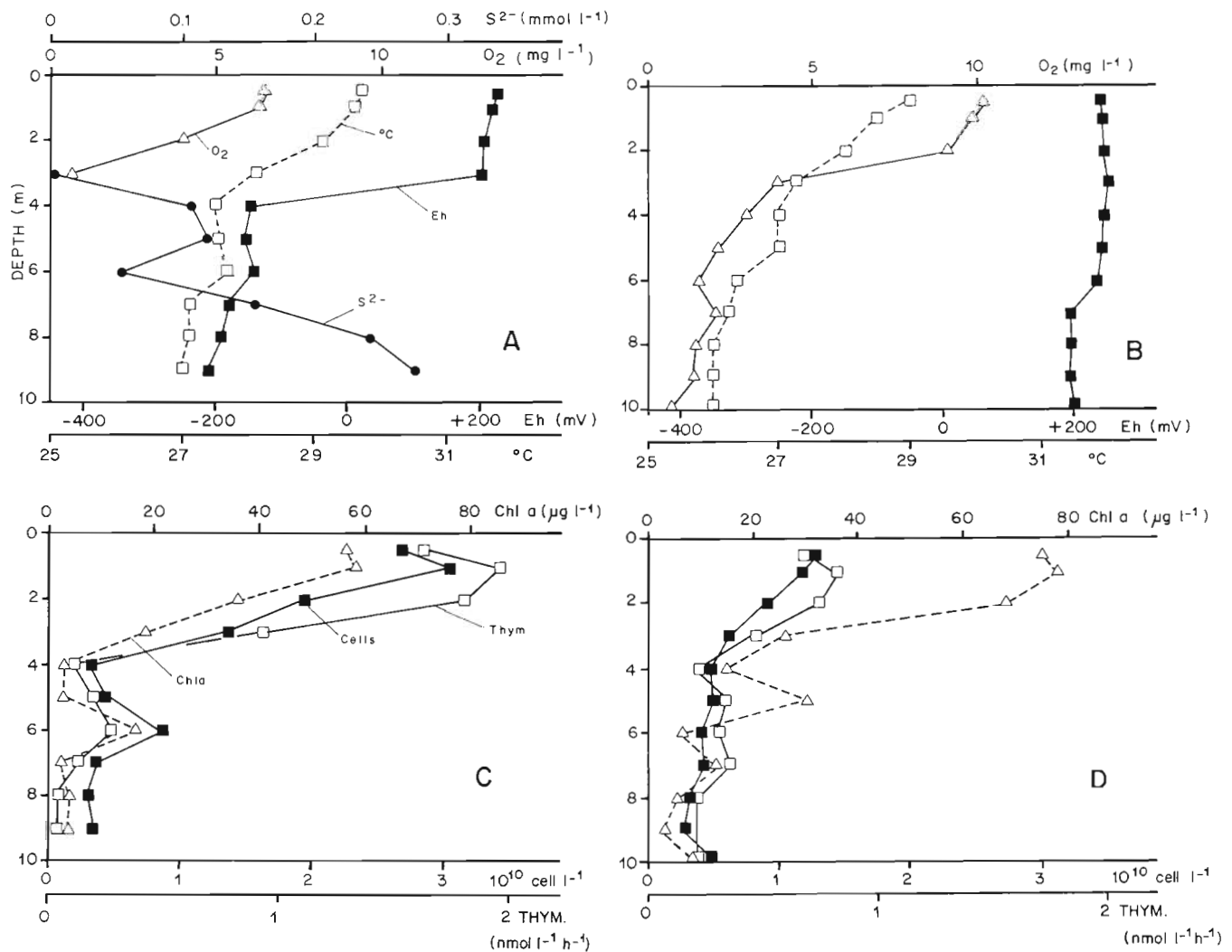
Fig. 2. Redox potential, oxygen and hydrogen sulfide concentration in (A) the stratified water column (27 Jan 1987), (B) the destratified water column (4 Feb 1987). Chlorophyll a, bacterial abundance and thymidine incorporation rate (C) during stratification, (D) and after destratification

and standard deviations for the parameters detailed below are listed in Table 2.

Bacterial numbers

During the stratified period bacterial numbers showed their maxima in the euphotic zone (0 to 2 m), ranging from 1.02 to 3.23×10^{10} cell l^{-1} (mean 2.01). In the aphotic zone (2 to 4 m) of the oxygenated epilimnion, bacterial numbers ranged from 0.47 to 1.84×10^{10} cell l^{-1} (mean 1.17). Minimum bacterial densities were obtained in the anoxic layers, ranging from 0.15 to 0.88×10^{10} cell l^{-1} (mean 0.51).

After destratification, bacterial numbers were 0.78 to 2.14×10^{10} cell l^{-1} (mean 1.14) in the euphotic zone (0 to 4 m) and 0.26 to 0.95×10^{10} cells l^{-1} (mean 0.48) in the aphotic zone.

Bacterial biovolume

Mean cell volumes in the entire water column before and after destratification are represented in Fig. 3. Of the 28 samples examined for mean bacterial volume, the only significant difference ($p < 0.001$, Mann-Whitney test) was found between anoxic and oxygenated samples: mean cell volume ($\pm$ SD) of 16 samples from oxygenated water was 0.153 ± 0.024 μm^3 and of 12 samples from anoxic layers was 0.203 ± 0.017 μm^3 (Table 3). As biovolume determination was based on projected surface, the difference between mean cell projected surface was also tested, and shown to be significantly different from zero ($p < 0.01$). Difference may be more important than absolute values, as microscopic examination of the anaerobic samples showed the occasional presence of very large S-shaped cells, not measured by the image analysis system (see 'Materials

Table 2. Mean values for some parameters recorded in Biétri Bay during this study

	Eh (mV)	O_2 (mg l^{-1})	Chl a (µg l^{-1})	Phaeo. (µg l^{-1})	Pigm. (µg l^{-1})	ETS (µeq. l^{-1} h^{-1})	Thym. (nmol l^{-1} h^{-1})	No. of bact. (10^{10} l^{-1})	Mean cell vol. (µm³)	Thym./bact. (10^{-20} mol cell^{-1} h^{-1})	POC (µg l^{-1})	PON (µg l^{-1})	Sulfide (µmol l^{-1})
Stratified euphotic zone	225[a] 15[b] 30[c]	6.77 2.55 30	64.2 16.6 30	33.9 22.0 30	98.1 30.2 30	73.8 18.8 30	1.339 0.417 30	2.01 0.64 18	0.167 0.027 5	8.33 1.73 12	5094 1253 11	944 221 11	0.00 0.00 30
Stratified aphotic epilimnion	199 25 8	2.38 1.64 8	24.2 11.4 8	21.5 13.5 8	45.7 20.6 8	30.9 8.3 8	0.836 0.379 8	1.17 0.47 5	0.136 – 1	6.46 2.79 5	2092 17 2	442 63 2	7.50 21.21 8
Total for epilimnion	220 20 38	5.84 2.98 38	55.8 22.7 38	31.3 21.0 38	87.1 35.6 38	64.8 24.6 38	1.233 0.455 38	1.82 0.69 23	0.162 0.028 6	7.78 2.19 17	4632 1606 13	867 277 13	1.58 9.73 38
Stratified anoxic hypolimnion	−175 58 63	0.09 0.38 63	7.7 7.2 63	13.3 12.2 63	21.0 17.7 63	16.1 5.7 61	0.201 0.176 63	0.51 0.27 37	0.203 0.017 12	3.41 1.91 37	700 461 27	115 64 27	178 248 63
Destratified euphotic zone	239 8 9	8.43 2.18 9	62.4 15.5 9	48.4 22.1 9	110.8 37.1 9	54.4 11.3 9	0.962 0.240 9	1.14 0.41 9	0.138 0.027 3	9.03 3.16 9	5052 615 3	736 60 3	0.00 0.00 9
Destratified aphotic layer	225 20 24	1.80 1.01 24	11.1 9.6 24	13.5 5.0 24	24.6 12.6 24	12.7 6.5 24	0.317 0.148 24	0.48 0.15 24	0.151 0.018 7	6.62 1.94 24	1002 480 8	163 94 8	0.00 0.00 24

[a] Mean
[b] Standard deviation
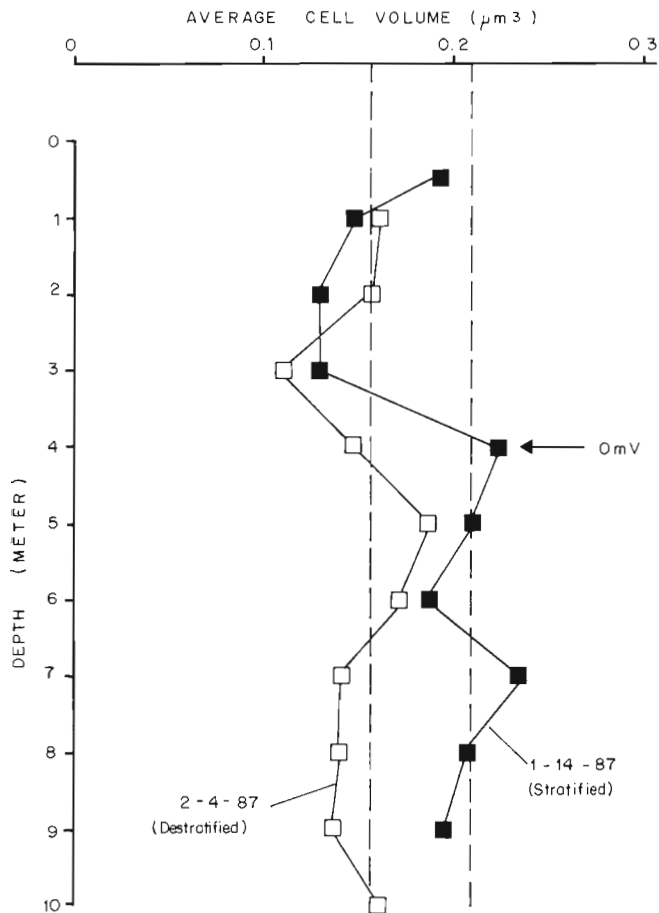[c] Number of observations

Fig 3. Average cell volume of bacteria in the stratified water column (14 Jan; ■) and after destratification (4 Feb; □). Dashed lines indicate mean values in oxygenated and anoxic samples

and methods'). Mean cell volumes therefore represent minimum estimates of the real mean cell volume (i.e. less than 1.41 μm spherical equivalents). These mean cell volumes of 0.153 and 0.203 μm^3 were used to convert bacterial abundances into total bacterial volumes in oxygenated and anoxic waters respectively.

Bacterial biomass

Bacterial carbon content, calculated using 1.06×10^{-13} g C μm^{-3}, resulted in an average of 295 μg C l^{-1} in oxygenated epilimnion and 110 μg C l^{-1} in anoxic hypolimnion in the stratified water column. After destratification, bacterial biomass averaged 185 μg C l^{-1} in the euphotic zone and 78 μg C l^{-1} in the aphotic layer.

^{3}H-thymidine incorporation

During the stratification period, incorporation of thymidine into cold TCA precipitate reached a maximum

in the euphotic zone of the epilimnion, ranging from 1.0 to 2.0 nmol l^{-1} h^{-1}, and decreased rapidly to between 0.1 and 0.5 nmol l^{-1} h^{-1} (corrected for overestimation – see below) in the anoxic hypolimnion (Table 2, Fig. 2). Comparison of anaerobic and aerobic incubations on 2 profiles showed that aerobic incubation resulted in a 15 % overestimation of the incorporation rate in the anoxic layers.

After destratification, maximum incorporation also occured in the euphotic zone, but was substantially lower than it was during stratification, ranging from 0.7 to 1.2 nmol l^{-1} h^{-1}. Below the euphotic zone, incorporation ranged from 0.26 to 0.95 nmol l^{-1} h^{-1}, higher than during the stratification period (Table 2, Fig. 2).

Specific incorporation rates were higher in the euphotic layer (6.0 to 11.4×10^{-20} mol cell^{-1} h^{-1}, mean 8.3×10^{-20}) and the aphotic zone of the oxygenated epilimnion (mean 6.5) than they were in the anoxic hypolimnion (1.6 to 5.7, mean 3.4×10^{-20}) in stratified waters. In the first 3 m of the destratified water column, specific incorporation rates were nearly the same as in the stratification period (5.3 to 14.3 – mean 9.0×10^{-20} mol cell^{-1}). At greater depths, activity per cell decreased (2.7 to 10.8 – mean 6.6), but was greater than during anoxia at the same depths (Fig. 4).

Conversion factor

Three dilution experiments gave similar results (Table 4, Fig. 5). Two fractions of the bacterial community could be distinguished by their size and growth rate: (1) A population of large rods (0.590 μm^3 mean cell volume), very actively growing with growth rates of 0.576 to 0.645 h^{-1}. (2) A population of smaller cells (0.130 μm^3 mean cell volume) with growth rates ranging from 0.147 to 0.200 h^{-1}.

Five hours after inoculation, the large rods represented 53 to 57 % of total biovolume (21 to 24 % in numbers) and 78 to 83 % of instantaneous production of biomass. Thymidine incorporation rate from 5 to 15 h was tightly coupled to production of these large rods (same growth rates), allowing conversion factors to be determined (Table 4). Based on the mean value of 0.746 $\times 10^{18}$ cells produced per mol of thymidine incorporated, 0.590 μm^3 mean cell volume, and 1.06×10^{-13} g C μm^{-3} (Nagata 1986), the conversion factor would be 46.7 μg C per nmol of thymidine incorporated into cold TCA precipitate for the aerobic community.

Production of biomass

Based on 46.7 μgC per nmol of thymidine incorporated, and assuming that production was constant throughout a

Table 3. Average projected surface and cell volumes for Biétri Bay bacteria

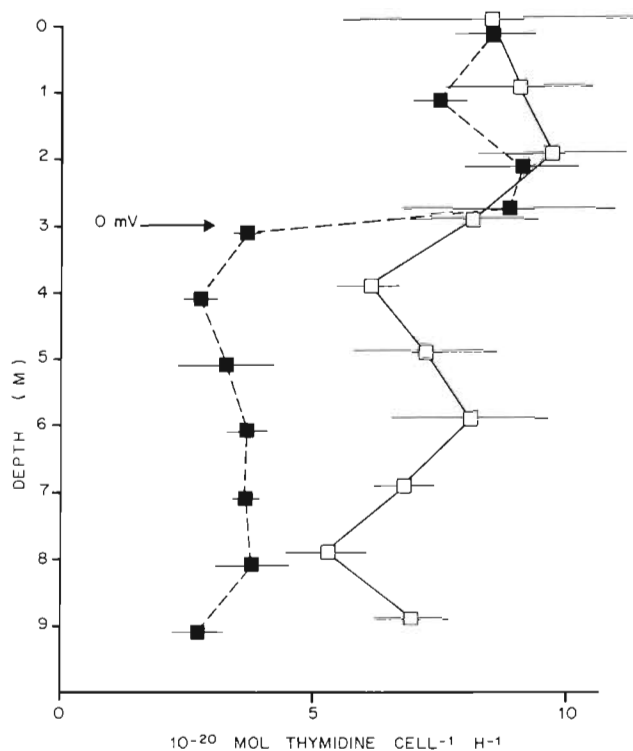| Oxygenated samples				Anoxic samples			
Sampling date 1987	Depth (m)	Average surface (μm²)	Average volume (μm³)	Sampling date 1987	Depth (m)	Average surface (μm²)	Average volume (μm³)
14 Jan	0	0.382	0.188	14 Jan	4	0.442	0.219
14 Jan	1	0.314	0.149	14 Jan	5	0.417	0.206
14 Jan	2	0.289	0.131	14 Jan	6	0.392	0.194
14 Jan	3	0.294	0.136	14 Jan	7	0.466	0.240
23 Jan	0	0.364	0.172	14 Jan	8	0.496	0.202
27 Jan	1	0.409	0.197	14 Jan	9	0.384	0.191
4 Feb	1	0.336	0.157	23 Jan	5	0.360	0.169
4 Feb	2	0.326	0.151	23 Jan	7	0.396	0.192
4 Feb	3	0.256	0.107	26 Jan	9	0.399	0.203
4 Feb	4	0.330	0.145	27 Jan	6	0.408	0.199
4 Feb	5	0.381	0.181	29 Jan	9	0.426	0.209
4 Feb	6	0.364	0.168	30 Jan	9	0.446	0.216
4 Feb	7	0.297	0.137				
4 Feb	8	0.304	0.137				
4 Feb	9	0.303	0.133				
4 Feb	10	0.345	0.156				
Average		0.331	0.153			0.412	0.203
SD		0.041	0.041			0.029	0.017

Fig. 4. Specific thymidine incorporation rate per cell. Average profiles before (■) and after (□) destratification. Horizontal bars represent standard error of the mean. Arrow indicates mean depth for Eh = O mV, thus, 3 m level was sometimes oxygenated, sometimes anoxic. The value for 0.5 m is plotted at 0 m for convenience

diurnal period, bacterial production of biomass would have ranged from 2.4 to 5.8 g C m^{-2} d^{-1} (mean 4.5) during the stratified period in the oxygenated epilimnion and from 4.5 to 6.2 g C m^{-2}d^{-1} (mean 5.5) in the whole water column after destratification. Thymidine incorporation rates in the anoxic hypolimnion were not converted into bacterial production of biomass because recent work (e.g. McDonough et al. 1986) has shown that percent recovery of the label into DNA is often quite different from that in oxygenated waters. No calibration between thymidine incorporation rate in cold TCA insoluble material and bacterial production of biomass was made in anoxic conditions during this study.

Growth rates of bacterioplankton

During the stratification period, growth rates ranged from 0.17 to 0.33 h^{-1} (mean 0.24 h^{-1}) in the oxygenated epilimnion.

After destratification, the same depths gave values ranging from 0.15 to 0.41 h^{-1} (mean 0.24), at lower levels growth rates ranged from 0.08 to 0.31 h^{-1} (mean 0.19).

ETS activity

During the stratification period, in the epilimnion, ETS activity (fraction < 10 μm) ranged from 40 to 100

Table 4. Regression parameters for 3 dilution cultures of Biétri Bay bacteria

Culture	Parameter[a]	Slope	(SE)	Intercept	(SE)	R	Difference to thym. slope[b]	Growth rate (h^{-1})	Intercept[c]	Conversion factor[d]
1	All cells	0.157	0.017	8.222	0.117	0.977	*	0.362	166.7	
1	Small cells	0.090	0.021	8.419	0.146	0.904	**	0.207	262.4	
1	Large cells	0.257	0.031	7.163	0.213	0.972	NS	0.592	14.55	
1	Thym. rate	0.228	0.024	−1.868	0.166	0.978		0.525	13.55	0.600
2	All cells	0.166	0.017	8.138	0.117	0.980	**	0.382	137.4	
2	Small cells	0.080	0.009	8.396	0.060	0.977	***	0.184	148.9	
2	Large cells	0.324	0.023	6.804	0.149	0.992	NS	0.746	6.37	
2	Thym. rate	0.287	0.020	−2.293	0.132	0.992		0.660	5.09	0.879
3	All cells	0.159	0.013	8.116	0.090	0.987	**	0.366	130.6	
3	Small cells	0.066	0.016	8.481	0.109	0.902	***	0.152	302.7	
3	Large cells	0.287	0.012	6.852	0.082	0.997	NS	0.661	7.11	
3	Thym. rate	0.253	0.021	−2.235	0.145	0.986		0.583	5.82	0.760

[a] Log (cells l^{-1}) and log nmol thym. l^{-1} h^{-1}
[b] *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; NS: not significantly different
[c] In 10^6 cell l^{-1} or pmol thym. l^{-1} h^{-1}
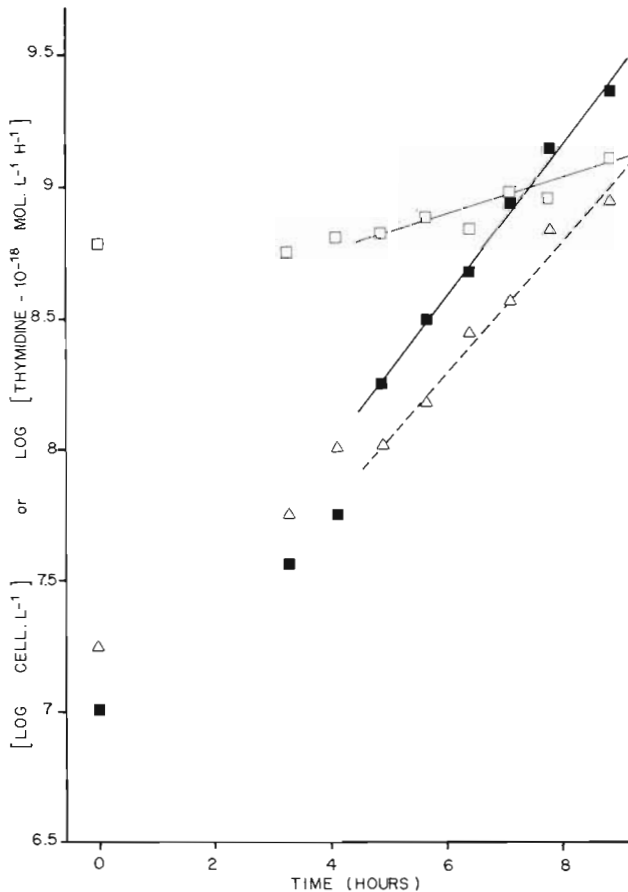[d] 10^{18} cells per mole of thymidine incorporated

Fig. 5. Time course of abundance of small (□) and large cells (■) and thymidine incorporation rate (△) in a dilution culture of Biétri Bay bacteria

μeq.l^{-1} h^{-1} in the euphotic zone (mean 74), and 22 to 46 in the aphotic oxygenated layer (mean 31). In the hypolimnion, ETS activity decreased with depth, with sometimes a higher value near the bottom. Within this layer, the activity ranged from 5.6 to 32 μeq. l^{-1} h^{-1} (mean 16.1).

In the destratified water column, activities were 31.6 to 66 μeq. l^{-1} h^{-1} (mean 54) in the euphotic zone decreasing to between 6 and 30 μeq. l^{-1} h^{-1} (mean 12.8) in the aphotic oxygenated zone.

Chlorophyll *a*, phytoplankton biomass

In the stratified water column, chlorophyll *a* showed a maximum in the euphotic part of the epilimnion and decreased sharply in the aphotic aerobic layer. Minimum values were obtained in the hypolimnion (Table 2). The same distribution was observed after destratification. Mean values were not significantly different in the euphotic zone during the 2 situations (Table 2).

Particulate organic carbon

Vertical profiles of POC determined on 5 occasions showed the same distribution as total pigment concentration and bacterial abundance (i.e. maximum in the epilimnion, drastic decrease at the redox discontinuity and minimum in the hypolimnion; Table 2).

DISCUSSION

In the deep parts of Biétri Bay, density stratification is observed most of the year, defining an oxygenated epilimnion and an anoxic hypolimnion. The period of investigation allowed us to describe some aspects of the structure and functioning of bacterioplankton during stratification, and to compare these observations with those obtained during the maximum of oxygen penetration.

Bacterial abundances reached a maximum in the euphotic zone of the epilimnion. Average cell volume estimates fall in the upper range of those reported in the literature and are probably typical of eutrophic biotopes (Table 1). The expected stratification of biomass and activity of the organisms, in response to physical and chemical stratification, was observed for bacterioplankton. Integrated over the oxygenated layer, bacterial biomass would represent an average of 10 % of total particulate carbon (phytoplankton corresponding to 60 % of total carbon). In the anoxic hypolimnion, using an underestimate of the true mean cell volume (see 'Materials and methods'), bacteria would represent at least 17 % of total particulate carbon.

Incorporation of thymidine also showed a pronounced maximum in the euphotic zone with very high values. The average conversion factor of 0.746×10^{-15} cells produced per mol of thymidine incorporated is rather low compared to literature data (Table 1), but the cells considered have a mean volume of $0.590 \ \mu m^3$. Using the same isotope concentration, Servais (1987) calculated a conversion factor of 0.5×10^{-18} cell mol^{-1} for Meuse River, Belgium, bacteria with mean cell volumes ranging from 0.3 to 1.8 μm^3, thus in close agreement with the value determined in this study.

Using a conversion factor derived from very large cells with a mean cell volume much higher than that currently determined in situ may be surprising. These large cells were counted separately on 2 profiles and were found to contribute 5 to 10 % of the total cell number (19 to 33 % in biovolume). If we assume that in situ growth rates of the 2 sub-populations are in the same proportions as in cultures (the large cells grow 3 to 4 times faster than the remaining population), then they would contribute 45 to 63 % of total biomass production.

Bacterial production of biomass is also among the highest cited in the literature, giving a very short turnover time of bacterial biomass (Table 1). This is also in strong agreement with the eutrophy of Biétri Bay and the high temperatures in the lagoon (Fig. 3).

The measured ETS activity, sum of the activities of all microorganisms less than 10 μm, followed the same general pattern as the other biomass parameters. A correlation matrix showed that correlations between ETS activity and both bacteria and chlorophyll a were highly significant (Table 5). A stepwise linear regression was used to estimate respective contributions of bacteria or 'bacteria-associated organisms' to total ETS activity. Finally, up to 89 % of ETS variance may be explained by chlorophyll a concentration and bacterial abundance (Table 6). As the constant term was not significantly different from zero, a multiple regression was derived assuming that the constant term was zero. The final model is:

$$ETS = 0.576 \ Chl \ a + 18.6 \ Bact.$$

(n = 56, ETS in μeq. l^{-1} h^{-1} at 28 °C, Chl a in μg l^{-1}, Bact. in 10^{10} cells l^{-1})

ETS/chlorophyll a ratios fall in the range reported by Romano et al. (1987a) for phytoplankton from the Mediterranean Sea.

Few data on the ETS/bacterium ratio are available in the literature. Relexans et al. (1984) gave 176 and 188 $\times \ 10^{-10}$ μeq.cell^{-1} h^{-1} for 2 species of freshwater bacterioplankton in vitro. Christensen & Packard (1979) gave values for 5 species of marine bacteria ranging from 4 to 112×10^{-10} μeq.cell^{-1} h^{-1}. However, in these studies, bacteria were growing exponentially and counted as colony forming units (CFU). Even if we assume that 1 CFU = 1 bacterial cell in an actively growing culture, cultured bacteria are known to be much larger than natural bacterioplankton. Romano & Navarro (1985) found an ETS/bacterial cell ratio of 25×10^{-10} μeq.cell^{-1} h^{-1} (counted by epifluorescent microscopy) in surface seawater near the sewage outlet of Marseilles, France, where bacterial biomass was likely to exceed phytoplankton biomass as suggested by bacterial abundance (1.4×10^{10} cells l^{-1}) and chlorophyll concentration (1.66 μg l^{-1}). Romano et al. (1987b) found 10 to 142×10^{-10} μeq.cell^{-1} h^{-1} for exponentially growing bacteria from natural communities. This value is near the range 18 to 74×10^{-10} μeq.cell^{-1} h^{-1} at 20 °C (overestimated by the presence of cyanobacteria) recalculated from Bell & Ahlgren's (1987) data. These values are close to the ratio determined in this study.

Based on these coefficients, bacteria or 'bacteria-associated biomass' would represent 46 to 87 % of total ETS activity in the oxygenated epilimnion (mean 60 %). In the unstratified water column, 'bacterial ETS' would represent an average of 53 % of total ETS in the euphotic zone and 96 % in the aphotic layer (average 67 % in the whole water column).

Thus 'bacteria-associated organisms' appear to be a major constituent of total measured ETS activity (less than 10 μm) in this system. Several authors (see review by Packard 1985), have shown that respiration/ETS ratios are different for bacteria, protozoans and phytoplankton (1.1, 0.25 and 0.17, respectively; ETS and

Table 5. Spearman's Rank Correlation Matrix for biomass parameters in Biétri Bay

	Chlorophyll a	Phaeopigments	Bacteria	ETS	Particulate carbon	Particulate nitrogen
			Aerobic layers			
Chlorophyll a		0.5146 (71) •••	0.7355 (56) •••	0.8397 (71) •••	0.8304 (24) •••	0.8765 (24) •••
Phaeopigments	0.5573 (62) •••		0.4098 (56) ••	0.4767 (71) •••	0.1365 (24)	0.0583 (24)
Bacteria	0.3491 (36) •	−0.2544 (36)		0.8768 (56) •••	0.7544 (18) ••	0.8369 (18) •••
ETS	0.2911 (59) •	0.0024 (59)	0.8030 (34) •••		0.7823 (24) •••	0.8137 (24) •••
Particulate carbon	0.7147 (26) •••	0.5590 (26) ••	0.4336 (12)	0.1654 (25)		0.9426 (24) •••
Particulate nitrogen	0.7657 (26) •••	0.6039 (26) ••	0.2797 (12)	0.3228 (25)	0.8829 (26) •••	
			Anoxic hypolimnion			
Coefficients (sample size) significance level: ••• $p < 0.001$; •• $p < 0.01$; • $p < 0.05$						

respiration expressed in the same units). These data suggest that, even in the euphotic zone, the contribution of phytoplankton to total respiration would be of minor importance in Biétri Bay. In fact, in the oxygenated epilimnion of the stratified water column, total community respiration averaged 5.2 g C m^{-2} d^{-1} (Bambara 1989), thus in strong agreement with the average bacterial production of biomass of 4.47 g C m^{-2} d^{-1}, if we assume a bacterial growth yield around 50 %.

During the stratification period, bacterial production represented nearly 80 % of integrated primary production (mean 5.26 g C m^{-2} d^{-1}), determined by the oxygen production method, in agreement with a virtually zero net production of oxygen on a 24 h basis (Bambara 1989). Using a 50 % growth yield, heterotrophic requirements would represent 160 % of autotrophic production in Biétri Bay. Such high bacterial production values compared to primary production are rarely found in literature data. Lovell & Konopka (1985) showed that bacterial production may occasionally represent up to 190 % of primary production in the Little Crooked Lake (Indiana, USA) in response to algal senescence and allochtonous carbon inputs due to spring rains. No annual cycle of bacterial production was derived for Biétri Bay. However, previous work has shown that bacterial production is likely to be of the same order throughout the year (Torréton unpubl.), and that primary production was near its maximum at

the period of investigation (Carmouze & Caumette 1985). Therefore, it seems that bacterial heterotrophic activity relies largely on allochtonous organic inputs on a yearly basis.

The anoxic hypolimnion was characterized by a lower bacterial abundance and a higher mean cell volume than the upper layer. Thymidine incorporation rates were substantially lower in anoxic conditions in Biétri Bay. Some authors have shown a maximum of bacterial production at the interface between the oxygenated epilimnion and the anoxic hypolimnion (McDonough et al. 1986, Zehr et al. 1987). Eleven profiles were performed in stratified waters with an anoxic hypolimnion and no maximum of activity was noticed at the interface. However, sampling every meter may have been insufficient in the gradient layer.

Lower thymidine incorporation rates in anoxic conditions are related to both lower bacterial numbers and lower specific incorporation per cell. During the period of investigation, thymidine incorporation rate integrated in the anoxic water column was found to be inversely related to the sulfide concentration in the hypolimnion (Fig. 6). Some obligate anaerobic prokaryotes such as sulfate-reducing bacteria are known to lack the thymidine kinase necessary to incorporate the label (Pollard & Moriarty 1984). We do not know if this incorporation is due to facultative anaerobic prokary-

Table 6. Stepwise selection for ETS activity. ETS in µeq. l^{-1} h^{-1}, chlorophyll a and phaeopigments in µg l^{-1}, bacteria in 10^{10} l^{-1}

| | | (a) Aerobic layers | | | |
Step	Variables not in model	Partial correlation	Variables in model	Coefficient	R^2
1	Chl a	0.876			0.000
	Phaeo.	0.614			
	Bacteria	0.866			
2	Phaeo.	0.100	Chl a	0.967	0.766
	Bacteria	0.750			
3	Phaeo.	0.202	Chl a	0.588	0.898
			Bacteria	19.41	

| Final model | $R^2 = 0.898$ | n = 56 | | |
Variable		Coefficient	95 % Confidence limits	
Constant		− 2.04	− 6.88	2.76
Chl a		0.588	0.452	0.720
Bacteria		19.4	14.7	24.1

| Final model with constant = 0 | | | |
Variables	Coefficient	95 % Confidence limits	
Chl a	0.576	0.444	0.704
Bacteria	18.6	14.3	22.8

| | | (b) Anoxic hypolimnion | | | |
Step	Variables not in model	Partial correlation	Variables in model	Coefficient	R^2
1	Chl a	0.429			0.000
	Phaeo.	0.162			
	Bacteria	0.787			
2	Chl a	0.203	Bacteria	23.0	0.619
	Phaeo.	0.177			

| Final model | $R^2 = 0.619$ | n = 34 | | |
Variables		Coefficient	95 % Confidence limits	
Constant		5.96	2.64	9.32
Bacteria		23.0	16.5	29.5

otes developing in the hypolimnion and less active with increasing amounts of sulfide, or to aerobic bacteria sedimenting from the upper layers, with mortality rates increasing with sulfide. A third possibility might be a mortality rate of sedimenting heterotrophs independent of sulfide and an increasing velocity of sedimentation due to the density gradient decrease during the last few days of the stratification period. However, the absence of evidence for higher sedimentation rates (chlorophyll a and phaeopigments were not increasing) does not support this hypothesis.

The origin and significance of thymidine incorporation rate into cold TCA insoluble material in the anoxic hypolimnion of Biétri Bay remain to be explained. Several authors have shown that thymidine incorporation in the anoxic layers of stratified lakes was partially inhibited by oxygen (McDonough et al. 1986). A possible reason for the stimulation of incorporation in aerobic conditions might be the reactivation of sedimenting aerobic heterotrophs from the upper layers, or the stimulation of facultative anaerobic bacteria by oxygen. This would agree with the inverse correlation between thymidine incorporation rate and sulfide concentration. Further work will need incubation in anoxic conditions, determination of percent recovery of the label in the DNA and empirical calibration with bacterial growth in anoxic conditions.

An attempt was made to attribute ETS activity in the anoxic layer as done above for oxygenated waters. Chlorophyll a was eliminated by stepwise regression
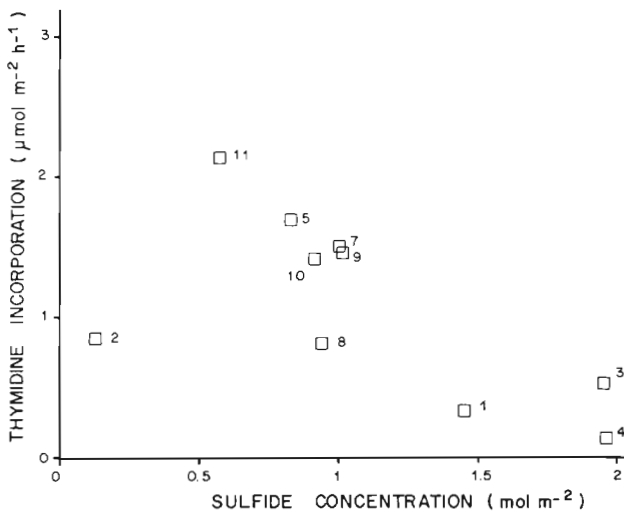
Fig. 6. Plot of integrated thymidine incorporation rate versus integrated hydrogen sulfide in the anoxic hypolimnion before destratification

and 61 % of ETS variation could be explained by the following equation:

$$ETS = 23.0 \text{ Bact.} + 6.0$$

(same units as above).

The ETS/bacteria ratio was not significantly different from that determined in oxygenated layers, although thymidine incorporation rate per cell was significantly lower. It is known that the same organic compound will generate less energy at -250 mV than at $+250$ mV according to the available electron acceptors. Moreover, organic matter sedimenting from upper levels comprises presumably a higher fraction of refractory material, yielding less energy than labile molecules. High ETS activities per volume in the anoxic layers suggest, however, significant mineralizing activities.

During maximum penetration of oxygen, bacterial abundance decreased by a factor of ca 2 in the surface waters. The simultaneous decrease of thymidine incorporation rates was due to a decrease in bacterial densities, as it was shown that specific incorporation per cell was constant during the 2 periods.

On the other hand, chlorophyll a remained constant in the euphotic zone, averaging 188 mg m^{-2} (SE = 10) in stratified waters and 180 mg m^{-2} (SE = 13) in the destratified water column. Primary production was not determined during the mixing period. However, the lower extinction coefficient (not shown), associated to the same phytoplankton biomass, suggests that primary production was roughly equal or a little more than in the stratified water column (productivity is limited by light in Biétri Bay and not by nutrient availability; Dufour & Durand 1982).

In the newly oxygenated layer (3 to 10 m), bacterial abundance was roughly the same as during anoxia. However, the anaerobic community, with high cell volume and low specific incorporation rate, was replaced by a conglomerate more typical of oxygenated layers, with lower mean cell volume and higher specific incorporation rate.

In the entire water column, bacterial abundance decreased from the average 9.1×10^{13} cell m^{-2} (SE = 0.9) in the stratified water column to 6.1×10^{13} cell m^{-2} (SE = 0.6) in destratified waters, excluding vertical distribution as the cause of disappearance of bacteria in the surface waters. In contrast Ducklow (1982) found an increase of bacterial biomass and productivity in response to the stimulation of primary production during destratification in the York River (Virginia, USA). Phytoplankton is assumed not to be stimulated by destratification in Biétri Bay, therefore we could not expect a stimulation of bacterial activity and biomass like in the York River. The decrease of bacterial biomass remains, however, to be explained. Export of that biomass by sedimentation is not supported by CHN and chlorophyll data, which show no significant difference in the deep layers, and thus suggest no increase of sedimentation rates during the destratification process. Other possibilities are an increase of grazing or a bactericidal effect of marine waters (Moebus 1973). We have no indications to confirm these hypotheses.

Thymidine incorporation rates, integrated over the entire water column, were not different in the stratified water column (5.3 $\pm$ 0.4 µmol m^{-2} h^{-1}) and after destratification (4.9 $\pm$ 0.4). But we are not sure that incorporation rates in anoxic conditions can be directy compared with those in oxygenated layers. Incorporation in the oxygenated waters averaged 4.0 $\pm$ 0.4 µmol m^{-2} h^{-1} during the stratification period, i.e. a little less than after destratification. This similarity is due to the vertical extension of the aerobic water column after destratification.

Bacterial production averaged 2.04 g C m^{-2} d^{-1} in the aphotic zone after destratification. If we assume a growth yield of 0.5, the respired carbon would have the same value, equivalent to a consumption of 5.44 g O$_2$ m^{-2} d^{-1}, with a respiratory quotient of 1. This rough estimation clearly shows that oxygen demand in the aphotic zone is too high to allow oxygenation for a long time. Oxygen diffusion from the upper levels cannot supply the aphotic zone, and anoxic conditions return rapidly (around 15 d). This situation is held stable by the density gradient created by the inflows in the rainy season.

CONCLUSION

The purpose of this work was to study the effect of annual destratification on bacterial biomass and pro-

duction, which was expected to be the period of the most important changes in the bacterial community. A recent study (Arfi et al. 1989) has shown that changes in the physical and chemical characteristics (PO_4^{3-}, NH_4^+, etc.) are very important in the hypolimnion during destratification. It seems, however, that these changes should be attributed to adsorption-release mechanisms related to the redox potential of the hypolimnion rather than to microbial activity (Guiral et al 1989b). Results of this study suggest that the destratification process in Biétri Bay is of less quantitative importance to microbial productivity and, in any case, that the stimulation observed in other ecosystems does not occur in this system.

Biétri Bay, like other eutrophic systems, is characterized by high bacterial biomass and production. The relative stability of bacterial biomass associated to a very short turnover time requires high rates of export of bacteria by predation, autolysis (Servais 1987) and/or sedimentation. Grazing by microzooplankton is known to be a major factor in the control of bacterial biomass in aquatic systems. Dilution culture experiments have shown that an important fraction of bacterial production may be due to a population of large rods representing only 5 to 15 % of bacterial numbers, and thus highly controlled in situ. Size-selective grazing on bacteria has been demonstrated recently by Andersson et al. (1986). If this is the case in Biétri Bay, these large cells would thus contribute significantly to the recycling of organic matter and its transfer to higher levels of the trophic chain, in spite of their low numbers in situ.

However, losses of attached bacteria by sedimentation as suggested by Pedrós-Alió & Brock (1982) and Ducklow et al. (1982) may be an important factor in microbial dynamics in eutrophic systems. Preliminary experiments have shown that, in Biétri Bay during the stratification period, up to 30 % of bacterial production may be due to attached bacteria, suggesting the possibly high contribution of sedimentation to biomass losses.

The understanding of the importance of bacteria in the food chain in this system requires estimation of the respective contributions of these export. This will be the object of a further study.

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