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Physical, chemical, and microbiological characteristics of microbial mats (KOPARA) in the South Pacific atolls of French Polynesia

L. Mao Che, S. Andréfouët, V. Bothorel, M. Guezennec, H. Rougeaux, J. Guezennec, E. Deslandes, J. Trichet, R. Matheron, T. Le Campion, C. Payri, and P. Caumette

Abstract: Microbial mats that develop in shallow brackish and hyposaline ponds in the rims of two French polynesian atolls (Rangiroa and Tetiaroa) were intensively investigated during the past three years. Comparative assessment of these mats (called kopara in polynesian language) showed remarkable similarities in their composition and structure. Due to the lack of iron, the color of the cyanobacterial pigments produced remained visible through the entire depth of the mats (20–40 cm depth), with alternate green, purple, and pink layers. Profiles of oxygen, sulfide, pH, and redox showed the anoxia of all mats from a depth of 2–3 mm. Analyses of bacterial pigments and bacterial lipids showed that all mats consisted of stratified layers of cyanobacteria (mainly *Phormidium*, *Schizothrix*, *Scytonema*) and purple and green phototrophic bacteria. The purple and green phototrophic bacteria cohabit with sulfate reducers (*Desulfovibrio* and *Desulfobacter*) and other heterotrophic bacteria. The microscopic bacterial determination emphasized the influence of salinity on the bacterial diversity, with higher diversity at low salinity, mainly for purple nonsulfur bacteria. Analyses of organic material and of exopolymers were also undertaken. Difference and similarities between mats from geomorphological, microbiological, and chemical points of view are discussed to provide multicriteria of classification of mats.

Key words: microbial mats, cyanobacteria, bacterial pigments, French Polynesia atolls, exopolymers.

Résumé : Les tapis microbiens qui se développent dans des mares saumâtres et hyosalines en pourtour de deux atolls de la Polynésie Française (Rangiroa et Tetiaroa) ont été étudiés intensivement au cours des trois dernières années. Un suivi comparatif de ces tapis (appelés kopara en Polynésien) a montré des similitudes remarquables dans leur composition et leur structure. À cause de l'absence de fer, la couleur des pigments reste visible dans toute la profondeur des tapis (20 à 40 cm de profondeur), avec des alternances de vert, pourpre et rose. Les profils d'oxygène, de sulfure et de redox montrèrent l'anoxie des tapis dès 2 à 3 mm de profondeur. Les analyses des pigments et des lipides bactériens ont montré que tous les tapis consistaient en des couches stratifiées de cyanobactéries (principalement *Phormidium*, *Schizothrix* et *Scytonema*) et de bactéries phototrophes pourpres et vertes. Ces dernières cohabitent avec des bactéries sulfato-réductrices (*Desulfovibrio* et *Desulfobacter*) ainsi que d'autres bactéries hétérotrophes. Les observations microscopiques ont montré l'influence de la salinité sur la diversité bactérienne des différents tapis principalement pour les bactéries pourpres non sulfureuses. Des analyses de matériaux organiques et d'exopolymères ont également été réalisées. Les différences et les similitudes entre les tapis sont discutées du point de vue géomorphologique, chimique et microbiologique de façon à fournir des critères de classification des tapis microbiens de Polynésie.

Mots clés : tapis microbiens, cyanobactéries, pigments bactériens, atolls de la Polynésie Française, exopolymères.

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Introduction

Marine microbial mats are encountered in a large variety of sites found in coastal areas, such as sandy beaches (Van Gernerden et al. 1989), marshes (Nicholson et al. 1987; Pierson et al. 1987), deltas and estuaries (Guerrero et al. 1993), hypersaline ponds (Krumbein et al. 1977; D'Amelio et al. 1989; Caumette et al. 1994), salterns, and lagoons, either in cold, temperate, or warm climates. These ecosystems are generally constituted of vertically laminated, stratified, cohesive, viscous layers, a few millimetres to several centimetres thick, produced by the development of different microorganisms that coexist through steep vertical gradients of oxygen, sulfide, and light. Most of these mats are composed of oxygenic phototrophic prokaryotes forming green layers on top and anoxygenic phototrophs forming purple or green-brown layers below in the anoxic zone, where they cohabit with anaerobic heterotrophs.

Microbial mats may also be found in atolls (Defarge et al. 1994a, 1994b, 1996), one of the main types of coral reef formations with banks, fringing, and barrier reefs. Mats are encountered in shallow ponds in the elevated sections (motu or islet) of the rims of the atolls, in residual spillways, in dry lagoons, and on flat areas that may be temporarily submerged. A few French Polynesian mats, locally called "kopara", have been previously described by Trichet (1967) and investigated from a geochemical point of view (Defarge et al. 1994a, 1996). These mats dominated by cyanobacteria (Rougerie et al. 1997) were remarkable for their production of a large number of viscous laminated layers due to the excretion of exopolysaccharides (EPS), each layer a few millimetres thick forming an entire mat of about 20–50 cm thick. In contrast to most of the coastal microbial mats, kopara is mainly constituted of pure bacterial material, free of other organic deposits, such as plant material, clay, iron compounds, or metal-organic chelates. Therefore, the color of the microbial pigments remains visible in the core samples down to the bottom of the mat. Previous geochemical analyses (Trichet 1967; Defarge et al. 1994a, 1994b) have shown the important role of this pure material in the geochemical cycle of carbon, in carbonate precipitation, forming modern stromatolites also called microbialites (Burnes and Moore 1987), and in the formation of phosphorite deposits (Rougerie et al. 1997; Trichet and Fikri 1997).

During the past 20 years, most of the studies on microbial mats in coastal zones, including atoll mats, focused on their biogeochemical role, mainly in the biogeochemical cycles of carbon and sulfur (Van Gernerden 1989; Jørgensen and Cohen 1977), with the helpful use of microsensors for environmental parameters (Kühl et al. 1994; Revsbech et al. 1983). Only a few studies have reported on the microbial biodiversity within the mats (Ward et al. 1994; Nübel et al. 1999), and only a few microorganisms have been isolated and studied. The latter are restricted to a few physiological groups of microbes, such as cyanobacteria (Cohen et al. 1975), purple anoxygenic bacteria (Caumette 1993; Stal et al. 1985; Glaeser and Overmann 1999), fermentative bacteria (Mouné et al. 1999, 2000), sulfate reducers (Caumette et al. 1991), or methanogens (Oremland and King 1989).

In this study, atoll kopara mats of French Polynesia were intensively studied using a multidisciplinary comparative approach to describe their physico-chemical and microbiologi-

cal features. The aim of this study was (i) to check the influence of the geomorphological environment of the mats on their physico-chemical and microbiological features and (ii) to describe the bacterial communities, pigment compounds, and EPS from the most interesting mats. Two French Polynesian atolls, Rangiroa and Tetiaroa, have been investigated for this purpose.

Materials and methods

Sampling sites and procedure

Rangiroa atoll (15°14'23'S, 147°60'58'W) (Fig. 1) was selected because its rim (202 km long) is one of the most diversified in the Tuamotu Archipelago (Andréfouët et al. 2001), including most of the known types of rim. It is also the second largest atoll in the world (1763 km²). In October 1997, we surveyed the ponds that could be inventoried and mapped according to a mosaic of three multispectral SPOT-HRV images at a 20-m resolution. The algorithm used to locate the ponds is described in Andréfouët and Roux (1998). In a second stage, a large pond (IT) in the south of the Tetiaroa atoll (16°90'S, 149°35'W, Society Archipelago) was investigated in October 1997 (Fig. 1). Tetiaroa, an atoll easily accessible, allows for checking to see if the information derived from Rangiroa is valid for other mats in other atolls. In the Rangiroa atoll, for each pond identified by remote sensing, a quick survey was made to verify the degree of stratification of the mats. Salinity and temperature were systematically measured. Only a few mats presented nice nondisturbed laminations. Therefore, only two representative laminated mats were investigated in detail (located in motu Pavete, R2, and Tivaru, R59, in the north-western part). In Tetiaroa, the mat located in IT was sampled for comparisons with Rangiroa mats.

Samples were collected by means of a plexiglas core-sampler, 12 cm in diameter and 60 cm long. After collection, the cores were sliced and subsamples were frozen for further analyses described below. The three microbial mat systems were studied for both their microbial community structure and EPS contents. The different sections of R2 (Table 1) were the uppermost green layer (layer R2-1; 0–0.05 cm), a purple–red layer (layer R2-2; 1–3 cm), a red layer (layer R2-3; 3–5 cm), and two lower sections of beige sand: layers R2-4 and R2-5 (5–10 cm and 10–14 cm, respectively) and layers R2-6 to R2-8 (14–21 cm and 28–50 cm, respectively). Samples from R59 (Table 1) were divided into R59 G (layers 1b and 1v; 0–1 cm), R59 P (layers 1' and c; 1–2 cm depth), and R59-2 (below 2 cm depth), corresponding to the green layer, the purple layer, and the deeper sediment (white–pink sandy material), respectively. The different sections of IT (Table 1) comprise an uppermost green layer (layer IT-1; 0–0.5 cm), a purple–red layer (layer IT-1' + IT-2; 0.5–6 cm), a beige layer (IT-3 + IT-4; 6–13 cm), a red layer (IT-5; 13–19 cm), and the sandy sediment (layers IT-6-7; 19–26 cm depth).

Physical and chemical analyses

Water temperature and dissolved oxygen were directly measured *in situ* with a YSI 55 oxymeter. Water salinity was estimated *in situ* with an ATAGO S-10 refractometer.

Oxygen profiles along the core were estimated by using micro-electrodes connected to a picoammperemeter Keithley 480, according to the method described by Van Gernerden et al. (1989).

pH and redox were measured in the sliced sections of the laminated mats with mini-electrodes of pH or redox connected to a CRISON 507 pH/redox meter.

Total acid-volatile sulfide (AVS) was analyzed by using the methylene blue method after acid extraction, according to Cline (1969) and Jørgensen and Cohen (1977). Samples of the mats were collected in the sliced sections of the cores and fixed in 10 mL of

10% Zn-acetate solution. The samples were then distilled in the laboratory through N₂ after addition of pure HCl. The H₂S–Zn-acetate distillates were analyzed after methylene blue formation with a spectrophotometer KONTRON UVIKON 737. The method has a detection limit of about 10 µM with a standard error of about 5%.

Analyses of organic and mineral carbon

Analyses of organic carbon were done by coulometric method (applied to samples from stations IT and R2), after decarbonation of sample by 1N HCl at room temperature. The total organic carbon content was deduced from titration of 0.25 N NaOH by the CO₂ produced during the oxidation of the residual organic carbon. R59 samples were analyzed by pyrolytic method (Espitalié et al. 1985).

Inorganic carbon has been determined by difference between total carbon and organic carbon on a LECO CNS autoanalyzer (IT and R2 samples) or specifically by pyrolysis (R59 samples).

Microorganism observations and pigments analyses

From the subsamples collected in the sliced sections of the cores, the bacterial communities were directly observed under phase-contrast microscopy, by using a Olympus microscope OM2. Both oxygenic and anoxygenic phototrophic bacteria were identified on morphological features according to the descriptions given in the *Bergey's Manual for Systematic Bacteriology* (1993). A subsample of each sliced section of the core samples was then fixed in 3% formaldehyde and sea-water solution supplemented with borax buffer, for further observations of cyanobacteria.

Subsamples for scanning electron microscopy (SEM) were glued to aluminium stubs, gold-sputtered, and examined using a Philips XL 30 operating at 30 kV. EDAX analysis (EDAX Edx 4i) was carried out on nongold-coated specimens, according to Costerton and Irvin (1981) and Vandevivere and Baveye (1992).

Pigments were analyzed by high-pressure liquid chromatography (HPLC) on freeze-dried sliced sections, as mentioned in Table 1. An exact amount comprised between 0.3 and 4 g of freeze-dried material was ground and extracted in methanol–acetone (6:4, v/v) in the dark. HPLC was performed with a Hewlett Packard system (two 110 pumps and a 1040A detector) with a reverse-phase column Zorbax ODS (4.6 mm diam., Du Pont de Nemours Instruments). A linear gradient from 0.3 M ammonium acetate in methanol–acetonitrile–water (5.1:3.6:1.3, by vol.) to acetonitrile–ethyl–acetone (3:7, v/v) was used with a flow rate of 2 mL·min⁻¹. The isolated pigments were detected with a spectrophotometer equipped to run the spectrum of each pigment at each peak in the chromatogram. The pigments were identified according to their retention time and their spectra between 300 and 800 nm by comparison with quantitative spectra of standard pigments obtained with the same method. According to the amount of freeze-dried material analyzed (Table 1), we could estimate the amount of each pigment by the mass of freeze-dried sample material. A standard error of 5–10% was applied to each calculation.

Exopolysaccharide (EPS) and lipids analyses

Freeze-dried samples (1 g) were extracted for lipid analysis following a modified Bligh and Dyer method (Bligh and Dyer 1959; White et al. 1979). The extracted lipids were fractionated into neutral lipids, glycolipids, and polar lipids by silicic acid column chromatography, using appropriate volumes of dichloromethane, acetone, and methanol, respectively.

Analyses of lipids were done by gas chromatography (GC) and gas chromatography – mass spectrometry (GC–MS). Nonadecanoic acid (C19:0) was added to the polar phospholipid fractions as an internal standard. The samples were subjected to a mild acid methanolysis, and the resulting fatty acid methyl esters were sepa-

rated on a Fisons 8000 GC equipped with a flame ionization detector. A fused silica capillary column coated with a nonpolar phase was used (60 m × 0.20 mm i.d.; film thickness 0.25 µm). Hydrogen was the carrier gas. The temperature program was as follows: initial temperature 50°C for 1 min, increased to 140°C at 15°C·min⁻¹ for 6 min, then to 280°C at 2°C·min⁻¹ for 55 min, and finally to 300°C at 4°C·min⁻¹ for 5 min.

GC–MS analyses were carried out using a Carlo Erba (Rodano, Italy) model 5160 HRGC chromatograph coupled to a quadrupole Nermag R10–10H mass spectrometer. The GC–MS analyses were performed under the same conditions as those described above, but helium was used instead as the carrier gas (1.5 bar). Identification of fatty acid methyl esters was based on comparison of relative retention times against internal standards and GC spectra. Electron energy in electron impact was 70 eV. The position of double bonds was determined by preparing dimethyldisulfide adducts (Nichols et al. 1986).

For EPS analyses, dry samples (10 g) were suspended in 200 mL of 20 g·L⁻¹ NaCl and heated at 80°C for 4 h. Solutions were then centrifugated at 12 000 × g for 15 min to remove sediments and cells. Exopolymers were precipitated from the supernatant with ethanol at 50% (v/v) and washed with ethanol–water mixtures (70–100%). They were dialyzed against distilled water and lyophilized. EPS were stored at room temperature. In the EPS, the protein content was determined by the method of Lowry et al. (1951), with bovine serum albumin as standard. Uronic acid contents were measured by the *meta*-hydroxyphenyl method (Filisetti-Cozzi and Carpita 1991), and neutral sugars were analyzed by the orcinol-sulfuric acid method (Rimington 1931).

For fourier transform infra-red (FTIR) spectroscopy, pellets were obtained by grinding a mixture of 2 mg of exopolysaccharides with 200 mg of dry KBr and then pressing into a 16 mm diam. mold. FTIR spectra were recorded on a BOMEM MB100 instrument with a resolution of 4 cm⁻¹. Spectra were obtained in the 4000–400 cm⁻¹ region, and sulfate contents were determined by the method of Lijour et al. (1994).

Sugar determination was done by GC analyses. Methanolysis was performed in methanolic 2 M HCl at 100°C for 4 h, and the methylglycosides were converted to the corresponding trimethylsilyl derivatives, as described by Montreuil et al. (1986). Hydrolysis was performed in 2 M trifluoroacetic acid at 120°C for 2 h, and the monosaccharides were reduced and converted in the corresponding alditol acetates, as described by Blakeney et al. (1983).

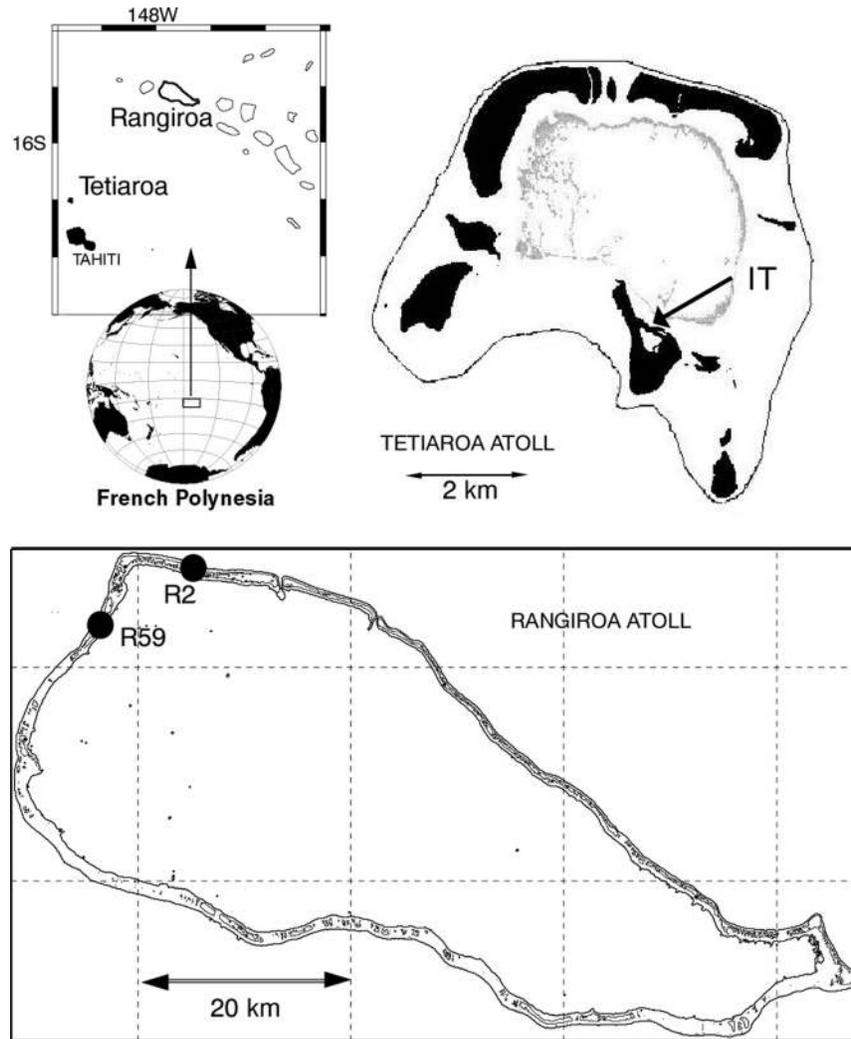
GC was carried on a GC-8000 system, using a CP-Sil-5CB column (0.25 mm × 60 m), operated from 50 to 140°C at 20°C·min⁻¹ and from 140 to 240°C at 2°C·min⁻¹ for the *per-O*-trimethylsilyl methylglycosides. A temperature programme of 50–150°C at 20°C·min⁻¹ and 150–230°C at 1.5°C·min⁻¹ was used for alditol acetates.

Results

Inventory and macroscopic description of the mats in Rangiroa and Tetiaroa

In Rangiroa, 66 regions were classified as Kopara, using SPOT HRV imagery (Andréfouët et al. 2001, 2002). The survey performed in October 1997 confirmed that all these regions included microbial mats, but with various types of deposits. Most of them were not laminated, mainly because of animal or anthropogenic activities inside the pond. Others may have been flushed in the recent past after big swell events and may have lost their laminated stratified structure. Finally, only four ponds were well laminated over a large area. Two of these ponds (R2 and R59) were investigated in

Fig. 1. Location of the studied mats in Rangiroa (R2 and R59 mats) and Tetiaroa (IT) atolls. In Tetiaroa map, motu (emerged land) appears black, the inner slope of the lagoon appears light gray, mats and residual spillways inside motus appear white. Similar details cannot be shown for Rangiroa because of a different scale.



October 1997 during the preliminary survey and again in April 1999. They are located in the north-western part of the atoll (Fig. 1), in the motus Pavete (R2) and Tivaru (R59).

R2 has already been described by Defarge et al. (1994a). The 30-cm-thick microbial mat covered a surface of about 10 000 m², under 20 cm of water depth. The water salinity ranged from 5 to 7 ppt, and the water temperature was 34°C at the time of sampling. In the R59 pond, the orange mat had a surface of 7000 m² and was 10–15 cm thick. This mat was partly covered with a 10 cm water depth and was partly open to air. Water salinity and temperature ranged, respectively, between 20 and 38 ppt and 31 and 36°C. A high-resolution map of this mat is available in Andréfouët et al. 2002. R2 and R59 represent hyposaline and saline mats, respectively.

In Tetiaroa (Fig. 1), the pond IT had an area of 70 000 m² and presented two environmental types of mats. The eastern part was strongly bioturbated by fish activities and water entrance. Conversely, the western part, in central position in the motu, presented the expected stratified type of mat. Sampling took place on this part of the mat. The mat was about

30 cm thick. The water salinity was almost constant at 5 ppt. The temperature of the water ranged from 30 to 34°C.

The three marmelade-like mats (R2 and R59 in Rangiroa and IT in Tetiaroa) were cohesive, forming gelatinous or viscous layers, were soft to the touch, and were free of granular hard material. The red, pink, or grey colors were preserved in the entire depth of the mats, as revealed by the core samples in Plexiglas tubes. The mats occurring in the oligohaline ponds IT and R2 were similar in their colors. The surfaces were mostly dark green. Below the surface layer, a green layer occurred and was generally 4–10 mm thick. Then, a purple layer of 4–5 mm in thickness was generally present. Deeper, below these three thin layers, red, pink, or grey thicker layers (20–60 mm thick) alternated to the bottom of the mat. By contrast, in the brackish to marine pond R59, the microbial mat had an orange surface layer, 5 mm thick with an irregular surface due to the formation of vertical small cones. Below this orange layer, an olive-green layer of 5–10 mm in thickness was present above a red to purple layer of about 10–15 mm in thickness. Below this, a

Table 1. Amount of material analyzed by HPLC.

Sliced section	Depth (cm)	Amount of freeze-dried material (g)	Layer color
IT-1	0–0.5	0.3	Green
IT-1'	0.5–1	0.3	Purple
IT-2	1–6	0.3	Red
IT-3	6–10	0	Beige
IT-4	10–13	2	Beige
IT-5	13–19	0.7	Red
IT-6-7	19–26	4	Pink sand
R2-1	0–0.5	0.3	Green
R2-2	1–3	0.3	Purple
R2-3	3–5	0.3	Red
R2-4	5–10	0.3	Beige
R2-6	14–21	0.5	Brown
R2-8	28–50	1	Pink
R59-1b (R59 G)	0–0.5	0.5	Dark green
R59-1v (R59 G)	0.5–1	1	Green
R59-1' (R59 P)	1–1.3	0.5	Purple
R59-c (R59 P)	1.3–2	0.5	White
R59-2	>22	0.5	Sandy pink

Table 2. Identification of pigments from the mats analyzed by HPLC according to their elution order.

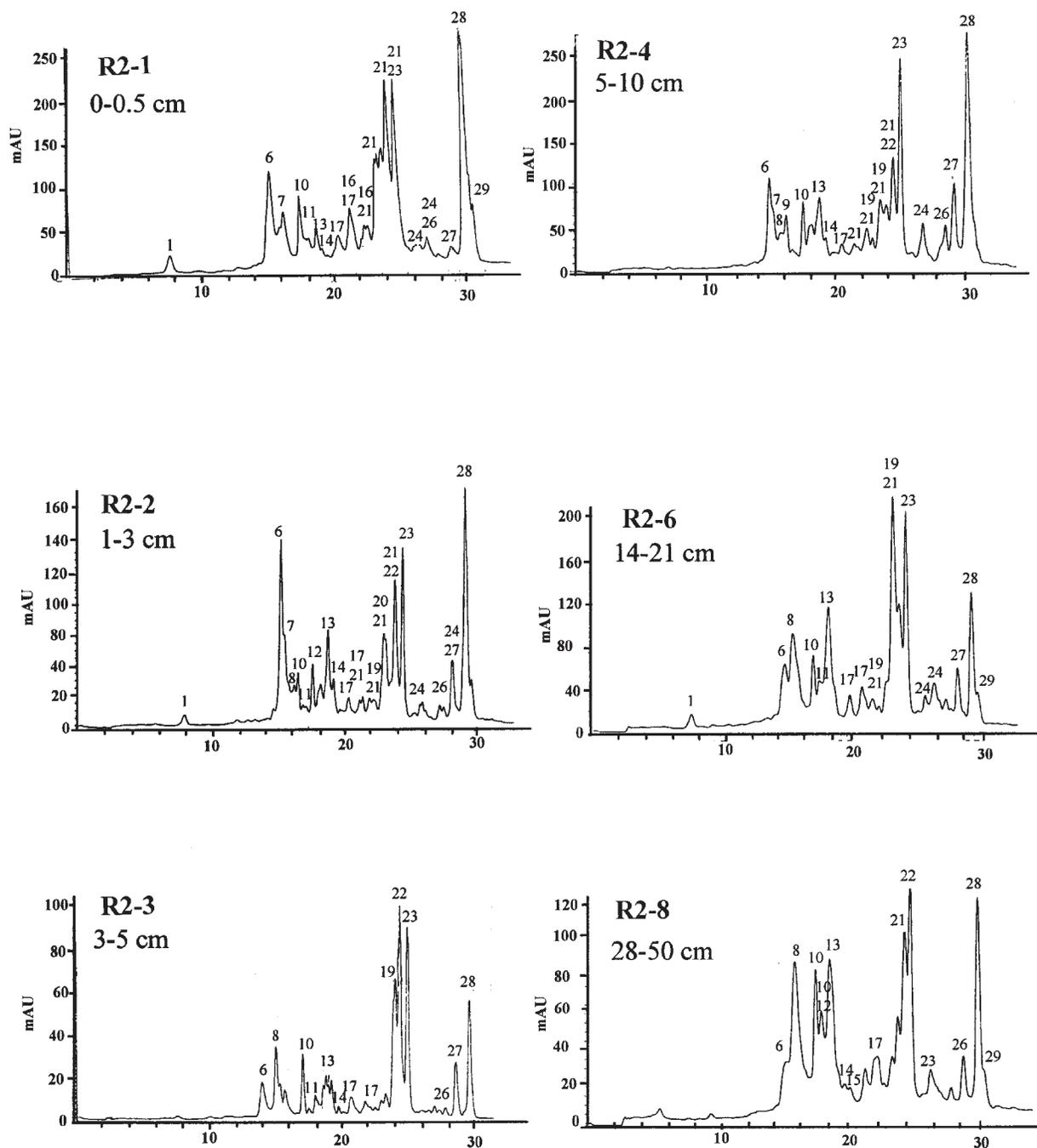
Pigment	Elution order
Scytonemin	1
Fucoxanthin	2
Astaxanthin	3
Aphanizophyll	4
Bacteriopheophorbide <i>a</i>	5
Myxoxanthophyll	6
<i>cis</i> -Myxoxanthophyll	7
Unknown carotenoid 477	8
Caloxanthin	9
Zeaxanthin	10
<i>cis</i> -Zeaxanthin	11
Unknown rhodopin-like	12
Canthaxanthin	13
<i>cis</i> -Canthaxanthin	14
<i>cis</i> -Canthaxanthin	15
Okenone	16
Bacteriochlorophyll <i>a</i>	17
Bacteriochlorophyll <i>c</i>	18
Unknown pigment (4oxo g.carotenoid?)	19
Spirilloxanthin	20
Chlorophyll <i>a</i> (409)	21
Rhodopin	22
Echinenone	23
Bacteriopheophytin <i>a</i>	24
Lycopene	25
Phaeophytin <i>a</i>	26
γ -Carotene	27
β -Carotene	28
<i>cis</i> β -Carotene	29

red to pink layer of 50–70 mm was observed. Within this latter layer, very thin whitish grey layers could occur. Below, the sediment consisted of white to pink sandy material with grains of an average diameter of 0.5–1 mm.

Physical and chemical characteristics of the three mats studied

The pond IT was similar to R2 with respect to its salinity and geomorphological environment. In the three mats, oxygen was present in the upper few millimetres of the mats, as revealed by the microprofiles (Figs. 8, 9, and 10). In the oligohaline mats (IT and R2), oxygen was maximum (200 μM) at the surface of the mat and decreased down to 0 μM at 0.5 and 1.5 mm depth, respectively. In contrast, in the R59 mat, the maximum oxygen concentration was found at 1 mm depth reaching 200 μM under $425 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. It decreased below this depth to be only 25 μM at 2 mm depth and down to 0 μM at 4 mm depth. These observations are in agreement with the pH and redox profiles. In the two oligohaline mats (IT and R2), the highest pHs were detected in the surface of the mat (pH 7.4 and 9.4, respectively). Below in the IT mat, the pH decreased regularly down to pH 6.6 at 150 mm depth in the red-brown lowest layer. In the R2 mat, it decreased strongly in the first 20 mm depth down to pH 6.65 and then was gradually stabilized at around pH 7.2 down to 300 mm depth in the brown sand below the mat. The redox potential in both mats had negative values decreasing regularly from the surface of the mats (–150 or –60 mV for IT or R2, respectively) to the deepest layers of the two mats (–351 and –350 mV, respectively). In contrast, in the brackish to marine R59 mat the highest pH value corresponded to the oxygen maximum at 1 mm depth (pH 9.28). Below this depth, the pH decreased down to 7.3 at 70 mm depth. The redox potential with positive values in the surface of the mat (+80 mV) decreased rapidly to be negative at 5 mm depth (–162 mV). Below this, it decreased regularly down to –420 mV at 70 mm depth.

Fig. 2. HPLC analyses of pigments extracted from samples collected in a core of the Rangiroa (R2) mat at different depth horizons. The numbers above the HPLC peaks correspond to the pigments listed in Table 2. The chromatograms present the peaks of the eluted pigments at only one wavelength (550 nm).



AVS were measured in the three mats. In the oligohaline mats R2 and IT, AVS values were very close, increasing from 0 mM at the surface of the mat up to 0.7–1 mM at 100 mm depth. In the brackish to marine mat, the amount of AVS was higher, up to 2.5 mM at 70 mm depth (Fig. 9) showing a most important sulfide production in this marine mat.

Organic and mineral carbon were analyzed in the three mats (Figs. 8, 9, and 10). The resulting values were still higher for the organic carbon than for the mineral carbon. In

the upper 30 mm of the three mats, these values ranged from 15 to 22% of organic carbon weight per freeze-dried mat weight. This percentage decreased with depth to be only 1–10% w/w at the bottom of the mats. In contrast, mineral carbon increased slightly with depth, from 1 to 9% in the upper layers to 6–11% in the deepest layers. X-ray diffractometry revealed that mineral carbon deposits consisted mainly of magnesium carbonate precipitates (magnesite, $MgCO_3$), which are typical of microbial bioprecipitation or organo-mineralization processes (Defarge et al. 1994a, 1994b).

Fig. 3. Distribution of the main pigments of cyanobacteria and anoxygenic phototrophic bacteria detected in the sampled core of the Rangiroa (R2), through the depth of the mat. The concentration of each pigment was calculated by comparison of the spectrum of each eluted pigment detected by its peak in the chromatogram with the spectrum between 300 and 800 nm of a standard solution of each pigment. A standard error of 5–10% should be applied to each calculation.

Microbiological and pigment characteristics

The major structure and building of the mats were due to the large development of filamentous cyanobacteria. In R2 and IT oligohaline mats, the upper 5 mm green layers were dominated by living cyanobacteria, microscopically related to the genus *Phormidium* and the family *Chroococcaceae*, according to morphological observation. Some *Scytonema*-like filaments could also be observed in the surface layers of the emerging parts of both mats. Below this, the entire mats were constituted of the organic sheaths of nonliving cyanobacteria that form the organic structure of the mats. In the brackish to marine mat R59, the surface orange layer was composed of a dominant population of *Schizothrix*-like cyanobacteria that form small cones at the surface. In addition, some filaments of *Chloroflexus*-like bacteria were observed in this upper orange layer. Below this, the olive-green layer contained *Phormidium* and *Chroococcaceae* as dominant morphotypes. Similar, to the previous two mats, the entire R59 mat was also composed of *Phormidium*-like sheaths.

Anoxygenic phototrophic bacteria were observed in several purple and red layers of the three mats and were tentatively identified on a morphological basis. The dominant morphological type corresponded to purple phototrophs containing intracellular sulfur globules, like *Chromatium* and *Thiocapsa*, or those without sulfur globules, like *Rhodospirillum* or *Rhodopseudomonas*, *Rhodobium*, and *Blastochloris* (Figs. 8, 9, and 10). Several strains of these different anoxygenic phototrophs were isolated and maintained in pure culture for further physiological analyses. These strains will be presented and discussed in a further paper. In the thin purple layer occurring below the cyanobacterial green layer, *Chromatium*-like and *Thiocapsa*-like cells were dominant in the three microbial mats. These bacteria were still present, but less abundant in the deepest red layers where they cohabited with the other types of phototrophic purple nonsulfur-like bacteria (*Rhodospirillum*-like or *Rhodopseudomonas*-like bacteria).

The pigments produced by the oxygenic and anoxygenic phototrophs were analyzed in the different layers of the mats. The analyzed pigments identified by HPLC are listed in Table 2. Twenty-nine different pigments were identified according to standard characteristics. Figures 2–5 present the HPLC chromatograms obtained at 550 nm and the estimated pigment composition obtained from the samples of the oligohaline ponds R2 in Rangiroa and IT in Tetiaroa. Pigments were present and preserved in the entire mats; however, their concentrations decreased with depth. Thus, the chromatograms of the deeper layers were obtained with 20 times more concentrated samples than chromatograms of the upper layers.

Scytonemin is only present in the upper green layer of the two oligohaline mats R2 and IT as a photoprotectant pigment. In this layer, the dominant pigments were typical of cyanobacteria with Chlorophyll (Chl) *a*, echinenone, β -carotene, and myxoxanthophyll as the major pigments, with less

abundant other carotenoid pigments, such as zeaxanthin. Chl *a* was found very abundant in R2 (Fig. 3) in the surface layer. Most of the cyanobacterial pigments were abundant from the surface layer down to 6 cm depth, and some of them were found dominant at this depth (zeaxanthin, canthaxanthin, β -carotene, myxoxanthophyll, and echinenone). Concentrations decreased in the lower layers, but still could be detected down to 20 cm depth (Fig. 3). Concentrations measured in micrograms per gram of dry weight were lower in IT (Figs. 4 and 5). In addition to these cyanobacterial pigments, purple bacterial pigments could be detected mainly in the purple and red layers between 0.5 and 6 cm depth. The most abundant pigments were bacteriochlorophyll (Bchl) *a*, bacteriopheophytin, and rhodopin, typical of purple bacteria, and γ -carotene, typical of the *Chloroflexaceae* group (green filamentous bacteria). All anoxygenic phototrophic pigments could be detected in very low concentrations below 10 cm depth (Fig. 3). The pigments of anoxygenic phototrophs were in lower concentrations than the cyanobacterial pigments, particularly γ -carotene, typical of green phototrophs, which were often detected in very low amounts at the lowest limit of all the different pigments.

Results obtained for the R59 mat are presented in Figs. 6 and 7. In this mat, only the upper 2 cm was analyzed. As for the two previous mats, the cyanobacterial pigments, Chl *a* and γ -carotene, were the most abundant. The other cyanobacterial pigments were less abundant. Bchl *a* is also very abundant in the purple and red layers below the orange layer. γ -Carotene is abundant at 2 cm depth.

Distribution of microbial lipids and EPS in the three mats

The phospholipid-like fatty acid (PLFA) profiles from the different mats are listed in Table 3. They showed remarkable similarities among the three different sites. The microbial biomass expressed as total PLFA ranged from 154 to 542 nmol·g⁻¹, with a maximum for all mats occurring in the subsurface sediments.

All sampling sites were characterized by the presence of mono-unsaturated fatty acids C16:1 ω 7, C18:1 ω 9, and C18:1 ω 7. These were the predominate mono-unsaturates in the upper layer of the microbial mats. The deeper layers of IT and R2 mats were characterized by a decrease in these mono-unsaturated acids. This is clearly associated with an increase in saturated acids, with 10Me C16:0 and cyclo C19:0 as the major acids. These acids were present in low concentrations in the R59 samples.

SEM images revealed the presence of mineral constituents sometimes surrounded by a fibrous film, identified as an exopolymer based on both physical aspects and C and O content, as determined by EDAX. Yields of EPS extracted from the bacterial mats are listed in Table 4. In the oligohaline mats IT and R2, the highest amounts of EPS were found in the uppermost layers, 26.8 and 46.8 mg·g⁻¹ of dry sample, respectively. For these mats, the exopolymer proportion decreased markedly with increasing depth and

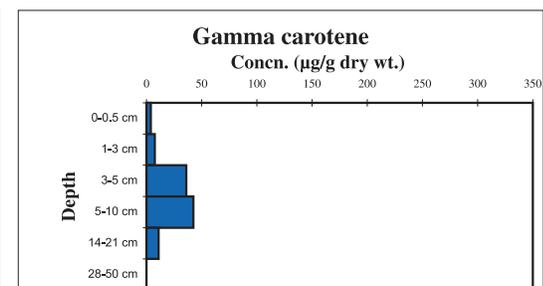
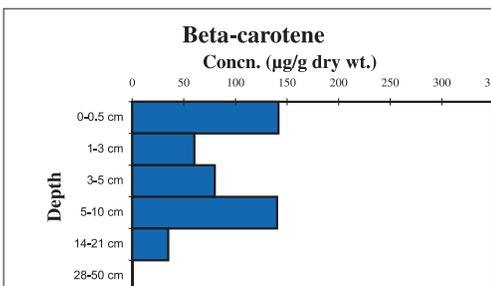
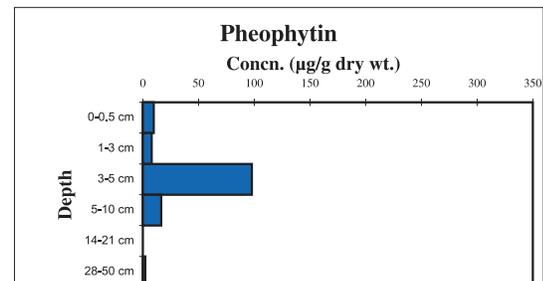
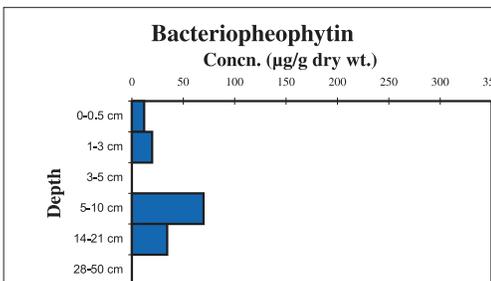
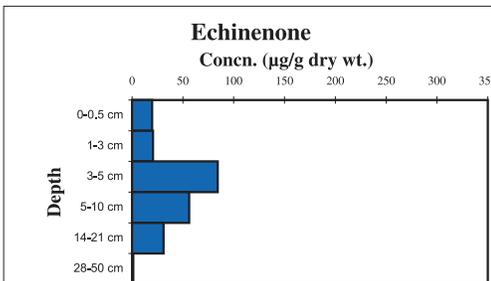
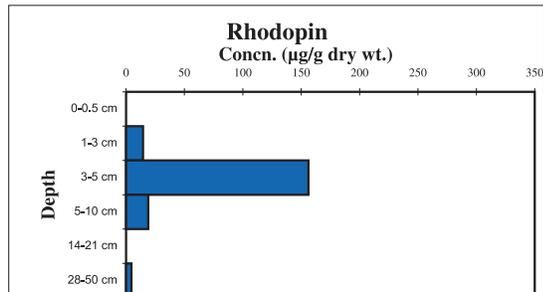
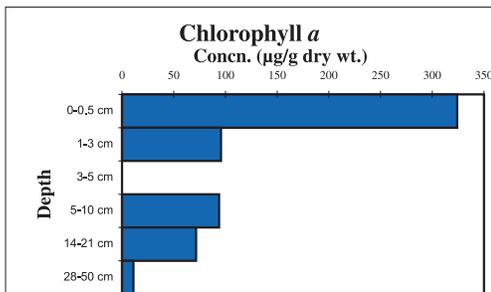
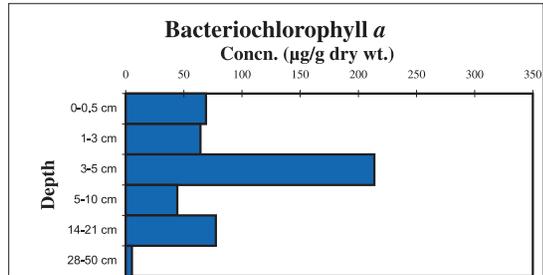
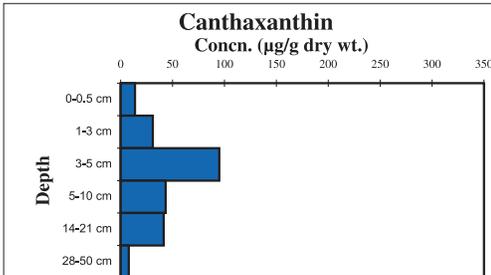
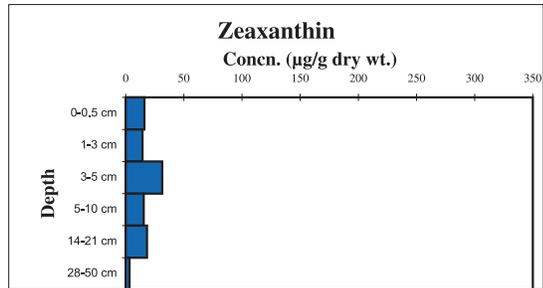
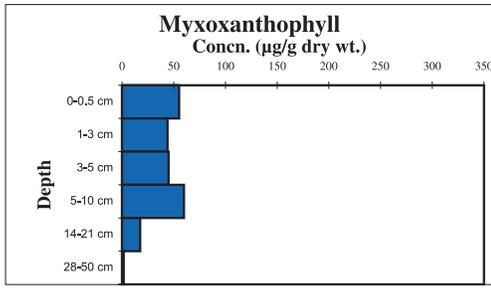
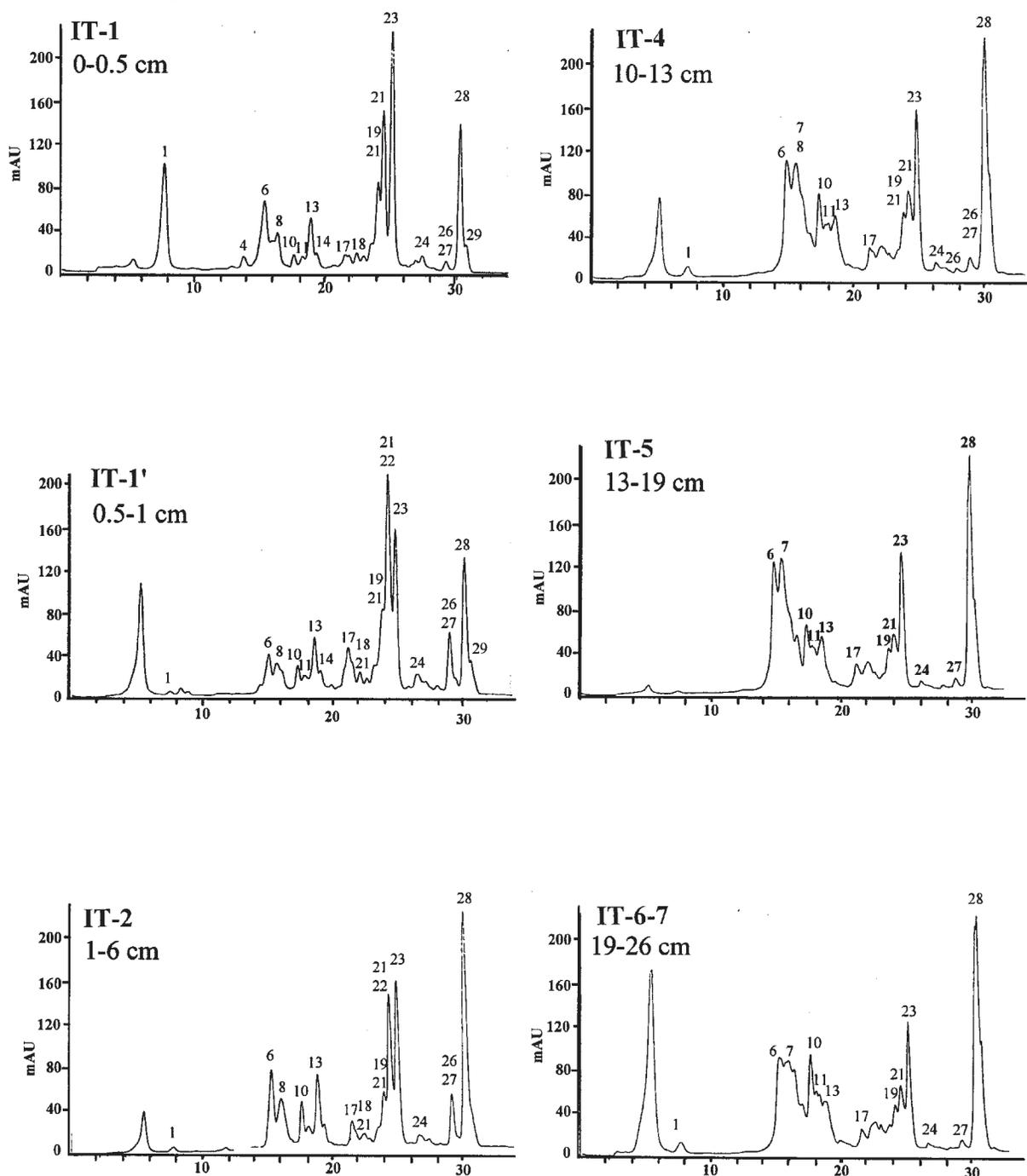


Fig. 4. HPLC analyses of pigments extracted from samples collected in a core of the Tetiaroa (IT) mat at different depth horizons. The numbers above the HPLC peaks correspond to the pigments listed in Table 2. The chromatograms present the peaks of the eluted pigments at only one wavelength (550 nm).



was negligible beyond 8 cm in the R2 mat. Conversely, in the R59 mat, the highest amount was detected in the purple layer (48.8 mg·g⁻¹ dry sample). The whole mat had a very high EPS content along its entire depth.

The gross chemical composition of the crude EPS fractions is listed in Table 5. Protein concentrations ranged from 4 to 13%. Neutral sugars predominated for all samples, but all EPS were polyanionic in nature due to the presence of uronic acids, with concentrations ranging from 11% to 18%.

Infrared spectroscopy was performed on all EPS fractions. FTIR exhibited a broad band beyond 3000 cm⁻¹, resulting from O-H and C-H stretching bands at 3420 cm⁻¹ and 2900 cm⁻¹, respectively, and also exhibited an intense absorption band at 1630–1650 cm⁻¹ with a shoulder at 1730 cm⁻¹ due to the presence of carboxylic groups of the uronic acids. Sulfate contents, as determined by the presence of a doublet at 1250 cm⁻¹, ranged from 6 to 10%.

GC analysis of monosaccharides, such as *per-O*-

Fig. 5. Distribution of the main pigments of cyanobacteria and anoxygenic phototrophic bacteria detected in the sampled core of the Tetiaroa (IT) mat, through the depth of the mat. The concentration of each pigment was calculated by comparison of the spectrum of each eluted pigment detected by its peak in the chromatogram with the spectrum between 300 and 800 nm of a standard solution of each pigment. A standard error of 5–10% should be applied to each calculation.

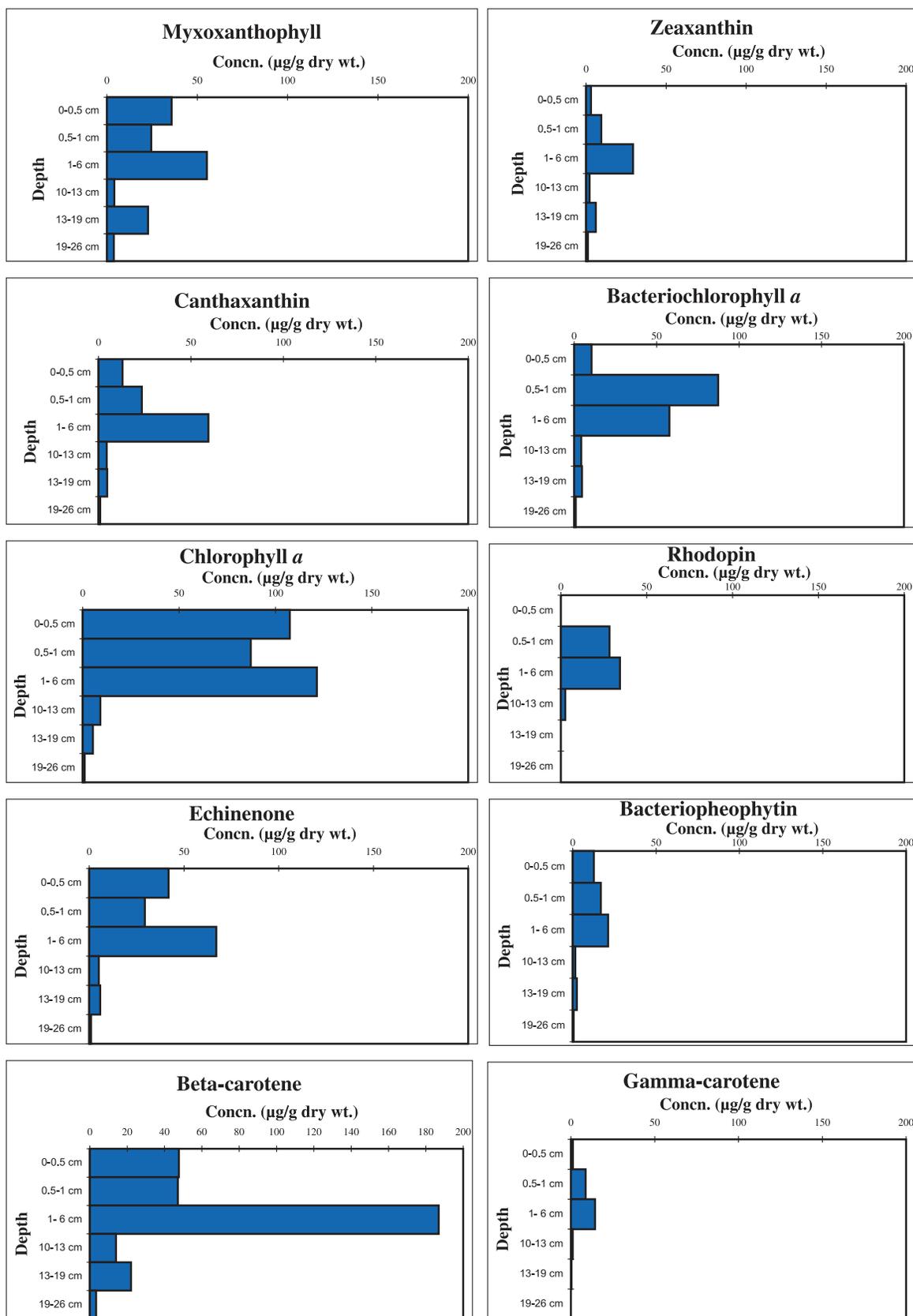
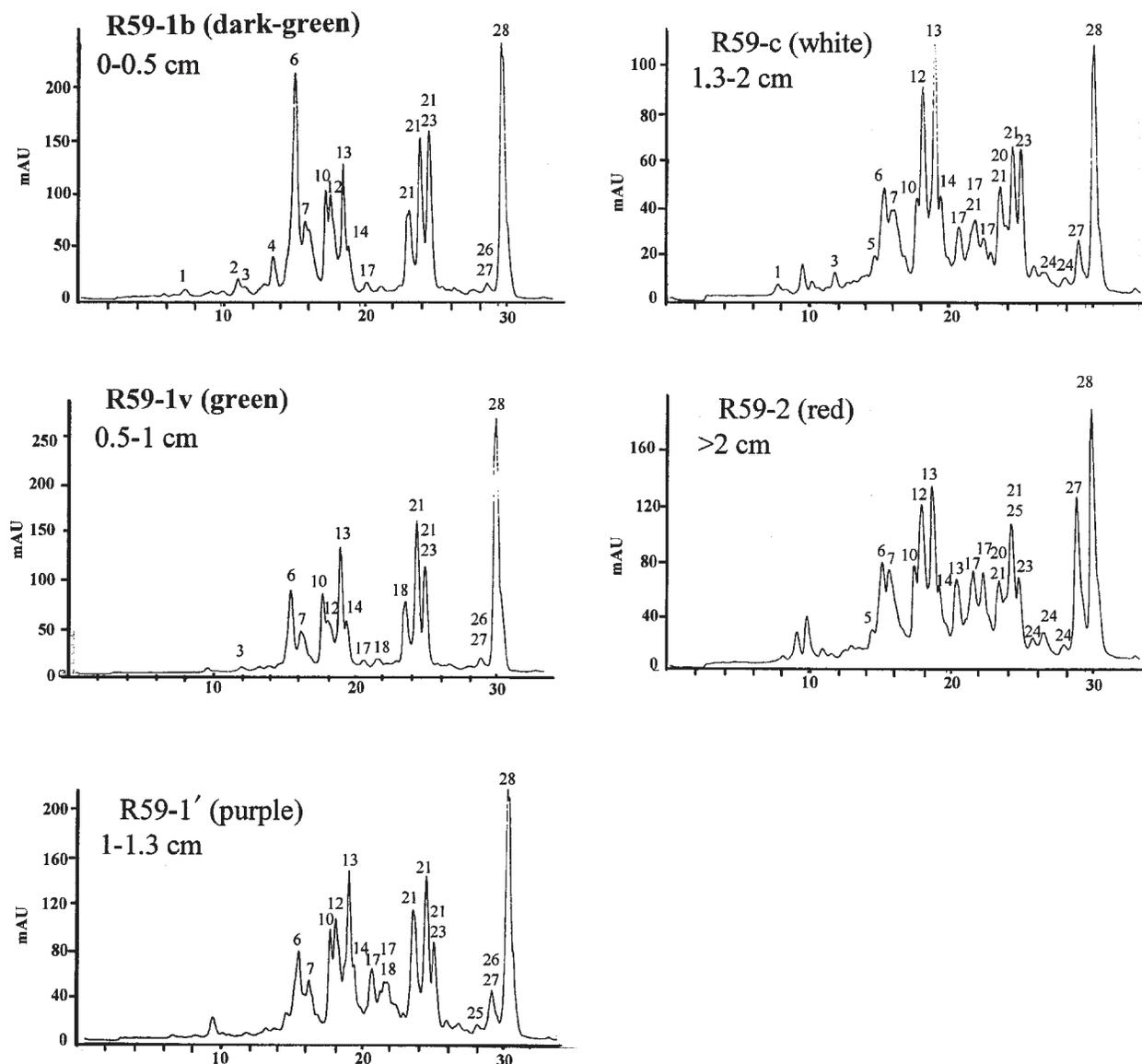


Fig. 6. HPLC analyses of pigments extracted from samples collected in a core of the Rangiroa (R59) mat at different depth horizons. The numbers above the HPLC peaks correspond to the pigments listed in Table 2. The chromatograms present the peaks of the eluted pigments at only one wavelength (550 nm).



trimethylsilyl methylglycosides or alditol acetates, is provided in Table 6. For both sampling sites, the EPS composition showed no marked changes with depth. Glucose, galactose, and mannose predominated as neutral sugars with equal amounts in all samples. 6-Deoxy hexoses, such as rhamnose and fucose, were found in lower concentrations, as was xylose. Only traces of arabinose were determined. Glucuronic and galacturonic acids were the only uronic acids identified in all fractions, with the first one predominating.

Discussion

Geomorphological environments of the mats

According to Andréfouët et al. (2001, 2002), kopara mats in Rangiroa atoll were encountered in three types

of ponds within different geomorphological configurations.

Mats of the eastern part of the atoll, lagoon-side, and at the periphery of motus

These areas are frequently flushed by lagoonal waters and may actually belong to the intertidal domain. These grey to black mats are a few centimetres in thickness.

Mats of the northern and western side of the atoll, in residual spillways

Residual spillways make narrow basins orient transversally through the rim. Water seldom enters the spillways via the ocean, only when very high northern swells hit the atoll. On the other hand, lagoonal water can frequently flush the spillway when strong southeastern tradewinds blow in austral winter. Small tongues of kopara mats a few centimetres thick may exist only in these shallower and protected

Fig. 7. Distribution of the main pigments of cyanobacteria and anoxygenic phototrophic bacteria detected in the sampled core of the Rangiroa (R59) mat, through the depth of the mat. The concentration of each pigment was calculated by comparison of the spectrum of each eluted pigment detected by its peak in the chromatogram with the spectrum between 300 and 800 nm of a standard solution of each pigment. A standard error of 5–10% should be applied to each calculation.

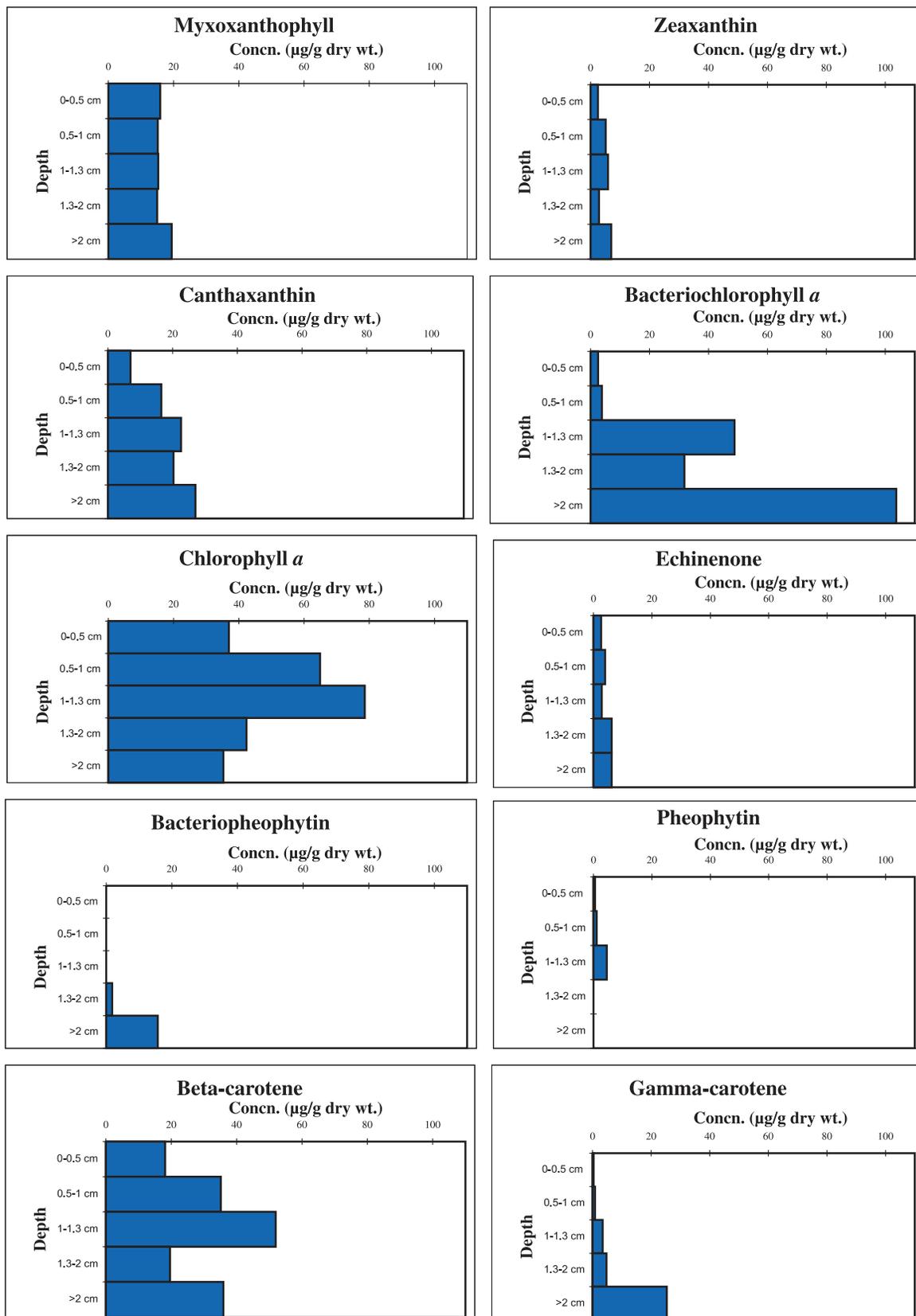


Fig. 8. Description and physico-chemical characteristics of the Rangiroa mat R2.

Depth (mm)	Layer color	Dominant photosynthetic microorganisms	Major pigments $\mu\text{g/g}$ dry wt.	Oxygen profile % saturation			pH	Redox (mV)	AVS (mM)	% w/w organic c	% w/w mineral c
				0	50	100					
0	Dark green	<i>Scytonema</i> , <i>Phormidium</i> + <i>Chroococcales</i>	Chla (300) β -Carotene (130)				9.40	-60	0.00		
	Purple	<i>Thiocapsa</i> , <i>Chromatium</i>					8.53	-170	0.10		
10	Red	<i>Rhodospirillum</i> <i>Blastochloris</i> , <i>Rhodobium</i>	β -Carotene (60) Bchla (60)				7.97	-330			
20							6.65	-360			
30							7.25	-350	22.9		
40	Red-	<i>Phormidium</i> sheaths <i>Thiocapsa</i>	Bchla (200) Rhodopin (150) Phaeophytin (100)				7.11	-401	0.03		
50	brown	<i>Chromatium</i> <i>Rhodospirillum</i>									
60											
70											
80							7.32	-340			
90		<i>Phormidium</i> sheaths some <i>Thiocapsa</i>	Echinonone (60) Bacteriopheophytin (70)	7.20	-350	0.70	13.7				
100	Pink										
150						2.50	1.0				
200	Brown sand			7.60	-350		7.3				
300				7.24	-320		4.3				

Fig. 9. Description and physico-chemical characteristics of the Rangiroa mat R59.

Depth (mm)	Layer color	Dominant photosynthetic microorganisms	Major pigments $\mu\text{g/g}$ dry wt.	Oxygen profile % saturation			pH	Redox (mV)	AVS (mM)	% w/w organic c	% w/w mineral c
				0	50	100					
0	Light orange	<i>Chloroflexus</i> , <i>Phormidium</i>	Chla (40)				8.50	+80	0	11.4	6.4
	Green	<i>Phormidium</i> , <i>Schizothrix</i> , <i>Spirulina</i>	Chla (60)				9.28	-162	0.015	11.4	6.4
10	Purple	<i>Chromatium</i> <i>Thiocapsa</i>	Bchla (40)				8.10	-370		21.3	2.0
20	Grey	<i>Phormidium</i> sheaths few <i>Chromatium</i> , <i>Thiocapsa</i>	Bchla (80)				7.42	-390		17.3	1.6
30	Red	<i>Phormidium</i> sheaths <i>Chromatium</i> , <i>Thiocapsa</i>	Bchla (600)				7.33	-404	1.8		
40	White-grey		β -Carotene (50)				7.59	-411			
50	Red	<i>Phormidium</i> sheaths					7.36	-414	2.2	8.5	6.4
60	Pink						7.34	-417			
70							γ -Carotene (25)	7.32	-420	2.5	14.8
80	Sand										
90											
100											

parts, but they are frequently invaded by vegetation. However, despite their protection, these mats are very narrow and their thickness averages only a few centimetres.

Mats inside the large motus

All the large ponds with thick stratified mats are encountered in large motus. The studied mats were found in ponds

Fig. 10. Description and physico-chemical characteristics of the Tetiaroa mat IT.

Depth (mm)	Layer color	Dominant photosynthetic microorganisms	Major pigments $\mu\text{g/g}$ dry wt.	Oxygen profile		pH	Redox (mV)	AVS (mM)	% w/w organic c	% w/w mineral c
				% saturation						
0	Dark green	<i>Phormidium</i> and <i>Chroococcales</i>	Chla (90) β -Carotene (40)			7.40	-150			
10	Purple	<i>Thiocapsa</i> , <i>Chromatium</i> , <i>Chloroflexus</i>	Chla (90)			7.30	-100		15.8	
20	Red	<i>Phormidium</i> sheaths, <i>Thiocapsa</i> , <i>Rhodospirillum</i>	Bchl a 90			6.90	-300			
30									20.3	
40	Pink	<i>Phormidium</i> sheaths <i>Thiocapsa</i>	Bchl a (60) Chla (120)			6.70	-355			
50										
60						6.60	-335			
70	Grey	<i>Phormidium</i> sheaths	β -Carotene (180) Echinenone (60)						10.5	
80										
90						6.70	-345			
150	Red-brown	<i>Phormidium</i> sheaths	Chla (10) Bchl a (5)							
200										
250	White sand and pink sand					6.60	-351			

belonging to this third geomorphological configuration. The oligohaline R2 pond as well as the IT pond in Tetiaroa are ellipsoidal, far from the coastline of the motu, while the saline R59 pond is narrow and elongated, very close and parallel to the coastline of the motu.

During another survey (Andréfouët et al. 2001), we found many ponds inside large motus, but most of them were disturbed by human (fishing activities, sewages) and animal (crabs, fishes) activities as well as by vegetation overgrowth. In these ponds, the mats were well developed, reaching 20–50 cm in thickness, thus showing that this third morphological configuration is the best configuration for the development of interesting stratified mats in atoll environments. The degree of protection from flushing sources (lagoon or ocean) appears to be the most important factor, after human and animal disturbances. The pond IT, in a large motu of Tetiaroa, confirmed this diagnostic obtained from Rangiroa survey.

Microbial characteristics of the kopara mats

The microbial mats that develop in ponds R2 and R59 in Rangiroa and in pond IT in Tetiaroa were very impressive, by both their beautiful colors and their thicknesses. Most of the microbial mats so far described in oligohaline to marine environments are much thinner, a few millimetres to a few centimetres in thickness (Stal et al. 1985; Nicholson et al. 1987; Vincent et al. 1994; Van Gernerden et al. 1989). The most developed mats similar in thickness to those described in this study were observed mainly in hypersaline environments, reaching 20 cm to more than 50 cm in thickness

(Krumbein et al. 1977; Potts 1980; Thomas 1984). The most remarkable observation of the Rangiroa and Tetiaroa mats was their nice pink to red colors down to the bottom, suggesting that the kopara have been constituted of pure bacterial material free of other organic deposits or iron precipitates. The deeper colored layers have shown that the bacterial pigments were maintained and preserved during a long period within the mat environment. According to Trichet (2001), the deepest layers were formed about 1000–1500 years ago. The preservation of the pigments could be favored by the anoxic environment due to the presence of free sulfide. The lack of iron in these special environments prevented sulfide from trapping in the form of black FeS; subsequently, the free sulfide is present in the exopolymeric matrix of the entire mat, up to the top layer, below the oxic zone (1–3 mm depth).

The major pigments detected in the three mats showed the dominance of cyanobacteria and their specific carotenoids (β -carotene, echinenone, and myxoxanthophyll). The surface layer of all mats was enriched with sheath pigments (scytonemin) absorbing at 350–450 nm, thus reducing substantially the penetration of short wavelength radiations. Detailed analyses have shown that both photosynthetic pigments (chlorophylls and bacteriochlorophylls) and carotenoids pigments of oxygenic and anoxygenic phototrophs were very well preserved in their structures and well distributed along the mat depth. So far, the long-term preservation of carotenoid pigments in deep layers has been reported only for very dense hypersaline mats (Villanueva et al. 1994),

Table 3. PLFA profiles in the section samples from the Tetiaroa (IT) and Rangiroa (R2 and R59) mats.

Fatty acids (%)	IT-1	IT-1' + IT-2	IT-3 + IT-4	IT-5	R2-1	R2-2	R2-3	R59 G + R59 P
iC14:0	0.33	0.77	0.39	0.29	0.81	1.35	0.89	0–4.7
C14:0	0.73	1.03	0.90	0.84	1.09	1.58	1.38	0.68–1.74
iC15:0	4.05	5.45	5.66	6.42	6.13	6.70	4.19	2.7–5.9
aC15:0	1.75	3.42	2.87	3.24	2.73	3.98	3.27	1.18–3.17
C15:0	0.85	1.11	1.87	1.76	1.10	1.10	0.83	0.97–2.23
iC16:0	2.57	4.13	4.24	4.91	4.49	5.77	4.50	1.80–3.86
C16:1 ω 9	3.38	1.10	0.43	0.76	1.61	0.93	0	0.32–1.72
C16:1 ω 7	7.76	5.36	1.64	0.25	4.48	3.02	2.30	3.78–5.81
C16:1 ω 5	1.70	1.73	1.60	0.21	3.18	0.63	0	0.50–1.82
C16:0	17.99	16.19	18.71	21.97	18.80	14.10	28.62	20.58–26.83
iC17:1 ω 7c	0.38	1.78	1.38	0.00	0.95	1.61	1.59	0–0.32
10Me C16:0	2.55	8.20	17.65	20.43	3.98	3.66	8.87	0–0.67
iC17:0	1.27	1.91	2.02	2.30	1.51	1.88	1.28	0.92–1.96
aC17:0 + C17:1 ω 8	1.82	3.31	3.41	3.84	2.57	3.29	2.15	1.25–3.56
C17:1 ω 6	0.45	0.68	3.15	3.53	1.55	0.26	0	0–0.77
Cyclo C17:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0–0.99
C17:0	0.95	1.46	1.74	1.87	1.32	1.49	1.78	1.07–2.01
C18:3	0.66	0.90	0.57	0.00	0.45	0.52	0	0.52–2.22
C18:2 ω 6,9	0.32	1.21	2.83	0.74	0.72	0.89	0	0.18–0.61
C18:1 ω 9	10.01	3.30	1.81	1.28	9.93	3.68	3.89	0–6.65
C18:1 ω 7c	25.94	16.01	2.46	2.52	9.52	20.67	10.13	13.30–27.52
C18:1 ω 7t	1.44	0.23	1.41	1.49	0.48	0.29	0	0–2.4
C18:1 ω 5	0.57	0.24	0.39	0.00	0.34	0.18	0	0–0.62
C18:0	4.34	6.78	10.42	11.48	7.77	7.12	15.12	3.19–15.24
brC19:1	4.54	3.57	2.56	3.48	3.43	3.37	2.30	0–3.80
10Me C18:0	1.37	3.02	1.16	1.34	2.05	2.00	1.22	0–0.56
Cyclo C19:0	0.41	3.31	4.47	5.07	4.29	4.33	4.17	2.43–8.82
C20:1 ω 9	0.00	1.74	2.37	0.00	2.18	2.78	0	0.92–2.02
C20:1 ω 7	0.00	0.52	1.42	0.00	1.76	1.14	0	0.75–1.46
C20:0	1.12	0.70	0.47	0.00	0.76	1.51	1.52	0.46–4.26
Total PFLA (nmol·g ⁻¹)	323.54	422.97	179.42	154.38	414.62	474.12	168.40	158.3–521.7

Table 4. Total amount of the exopolysaccharides (EPS) in the section samples of Tetiaroa (IT) and Rangiroa (R2 and R59) mats (means of five analyses \pm standard deviation).

Section of the mats	Depth (cm)	Amount of EPS (mg·g ⁻¹ dry material)
IT-1	0–1	26.8 \pm 0.8
IT-1' + IT-2	1–6	11.2 \pm 0.7
IT-3 + IT-4	6–13	4.3 \pm 0.1
IT-5	13–19	4.5 \pm 0.1
R2-1	0–2.7	46.8 \pm 1.1
R2-2	2.7–4.5	9.4 \pm 0.7
R2-3	4.5–8	1 \pm 0.5
R2-4	8–13.7	0
R2-5	13.7–18.5	0
R59-2		25.1 \pm 1.1
R59 P		48.2 \pm 1.7
R59 G		37.1 \pm 1.2

such as for the Solar Lake (Sinai) hypersaline mat during a period estimated at 2500 years (Boon and De Leeuw 1987). Here, the present study has shown that good preservation of

pigments is also possible in oligohaline mats. In the top layers, the ratio of carotenoids:Chl *a* was similar for the three mats (Table 7): between 0.3 and 0.4 for myxoxanthophyll:Chl *a*, 0.5 and 0.8 for β -carotene:Chl *a*, and 0.1 and 0.4 for echinenone:Chl *a*. In the deeper layers, these ratios increased to reach 1–6 according to the pigments and the mats. Such an increase was generally observed for β -carotene in several mats (Vincent et al. 1994; Villanueva et al. 1994), but not for myxoxanthophyll:Chl *a* or echinenone:Chl *a* ratios, which were found to decrease with depth (Vincent et al. 1994).

The typical pigments of purple phototrophic bacteria (Bchl *a* and rhodopin) were abundant at 1 cm, 2 cm, and 3 cm depth in IT, R59, and R2 mats, respectively. These pigments proved the massive development of anoxygenic phototrophic bacteria with carotenoids of the spirilloxanthin series. In addition, in the same layer, the abundance of γ -carotene was typical of the presence of anoxygenic green bacteria, such as *Chloroflexus*. However, Bchl *c*, which is characteristic of some green bacteria and typical of *Chloroflexus*, was not detected in the mats or was in very low amounts. These carotenoids and Bchl pigments of purple and green bacteria were detected in many mats (Palmisano

Table 5. Colorimetric analysis of the exopolysaccharide fractions in the section samples of the Tetiaroa (IT) and Rangiroa (R2 and R59) mats. Values are expressed in grams per 100 grams of mat (means of five analyses \pm standard deviation, $n = 5$).

Section	Neutral sugar	Uronic acid	Sulfate	Protein
IT1	39 \pm 2.3	15 \pm 1.3	10 \pm 2	5 \pm 1.1
IT-1' + IT-2	33 \pm 1.8	18 \pm 1.7	6 \pm 2	8 \pm 1.1
IT-3 + IT-4	33 \pm 1.8	15 \pm 2.4	4 \pm 2	4 \pm 1.1
IT-5	40 \pm 2.8	11 \pm 1.1	6 \pm 2	4 \pm 1.1
R2-1	31 \pm 1.7	16 \pm 2.4	8 \pm 2	12 \pm 1.2
R2-2	25 \pm 2.2	13 \pm 1.1	8 \pm 2	13 \pm 0.3
R2-3	28 \pm 1.7	14 \pm 0.8	6 \pm 2	4 \pm 0.1
R59-2	25.5 \pm 1.1 – 45.9 \pm 1.6	11.8 \pm 0.5 – 16.5 \pm 1.1	7 \pm 2	12.5 \pm 0.2
R59 P	30.5 \pm 1.4	17.9 \pm 2.2	9 \pm 2	10.8 \pm 0.2
R59 G	30.2 \pm 1.4	17.5 \pm 1.6	6 \pm 2	12.1 \pm 0.4

et al. 1989; Villanueva et al. 1994). They were still abundant in the top layers below the living layer of cyanobacteria. In general, Bchl *a* is the major detected pigment of anoxygenic phototrophs. Rhodopin has also been detected in hypersaline mats (Villanueva et al. 1994) and γ -carotene was associated with Bchl *c*, which is typical of *Chloroflexus*-like bacteria (Villanueva et al. 1994).

Regarding the identified microorganisms, the studied mats were not very diversified. Concerning the phototrophic organisms, only a few morphotypes of filamentous cyanobacteria formed the structure of the mat, and only a few morphotypes of purple or green bacteria could be observed within these mats. Pigment and lipid analyses confirmed this observation. In addition, lipid analyses revealed the presence of sulfate reducers (*Desulfovibrio* and *Desulfobacter*). The dominant purple bacteria were nonsulfur bacteria within the genera *Rhodobium*, *Rhodospirillum*, and *Rhodobacter*, according to phenotypic features of the isolated species (Bothorel 2000). Some purple sulfur bacteria could be related to the genera *Marinochromatium* and *Thiocapsa*. Purple sulfur bacteria were apparently dominant when observed under microscopic observation, thus forming purple layers as is frequently observed in microbial mats (Caumette et al. 1994). The purple nonsulfur bacteria are considered ubiquitous organisms, but not really abundant in microbial mats. In the kopara mats, these bacteria were found to be very abundant, thus showing a good adaptation to the environment of the mats and particularly to free sulfide. These interesting features may be characteristic of Polynesian mats. It is, however, difficult to generalize, since no other bacteriological studies were reported for Pacific mats. In R59, some *Chloroflexus*-like filaments were observed with the cyanobacterium *Schizothrix*. These green bacteria filaments should be present in the three mats, as detected by their specific pigments (Bchl *c* and γ -carotene).

Mineral and organic structure of the mats

The precipitated mineral carbon forming white layers within the mats was composed of pure magnesium carbonate (magnesite), which is typical of organomineralization processes (Defarge et al. 1994a, 1994b, 1996; Trichet and Defarge 1995). Indeed, aragonite, which is derived from detritic origin or from precipitation, was not very important in these mats.

All mats in this study contained a relatively high percentage of organic carbon than did mats in previous studies (Defarge 1983), thus corresponding to relatively pure bacterial material. The organic material corresponded mainly to the exopolymers excreted by the cyanobacteria and some bacteria. The highest amounts of EPS are clearly related to the highest biomass. These exopolymers can have different physiological functions to prevent desiccation (De Winder 1990), to preserve the integrity of the mat, and to maintain cyanobacterial hydrophobicity (Bar-Or and Shilo 1987; Shilo 1989). They also have a very important role as the site of the precipitation of carbonates (Defarge et al. 1994a, 1994b, 1996). They are also involved in the formation of atoll phosphorites (Trichet and Fikri 1997).

Regarding the high variability of sugar types in the polysaccharides of most cyanobacteria species (Bertocchi et al. 1990; Huang et al. 1998), no significant differences within the EPS fractions of the three sampling sites can be pointed out. Adjusting glucose to a molar ratio of 2 as reference, molar ratios for EPS extracted from the Tetiaroa mat, for example, are Glc:Gal:Man:Rha:Fuc:Ara:Xyl:GlcA:GalA (2.0:1.8:1.6:0.5:0.5:0.3:1.1:0.8:0.6). Both the uronic acid content (up to 18%) and the sulfate content point to these polymers as potential molecules for biotechnological applications in many industrial sectors. Further studies will involve the purification and chemical analysis of the different EPS constituting the mats, along with the identification and characterization of the EPS producers.

Conclusion

Though a small number of mats have been sampled, our stratified sampling scheme based on remote sensing observations at atoll scale allows us to draw conclusions valid for most of the atolls of French Polynesia (Andréfouët et al. 2001). Atypical atolls (raised or tilted) may show different geomorphological configuration that may justify further investigations; however, this comparative study has provided multicriteria to classify and characterize mats. Differences among mats from other coastal environments have been highlighted as well as differences within atoll mats.

The mats encountered in protected hyposaline and brackish to marine ponds in two different atolls allow us to point to the most remarkable features of atoll kopara mats. It is

Table 6. Monosaccharide composition of the exopolysaccharide fractions in the sampled sections of the Tetiaroa (IT) and Rangiroa (R2) mats, by using two methods for analyses. Values are expressed in grams per 100 grams of mat.

Section	Method	Glc	Gal	Man	Rha	Fuc	Ara	Xyl	GlcA	GalA
IT-1	TMS	9	7	6	2	2	1	7	5	3
	AA	10	11	8	2	2	1	4	–	–
IT-1' + IT-2	TMS	8	6	5	2	1	1	4	4	2
	AA	6	6	6	1	1	1	2	–	–
IT-3 + IT-4	TMS	9	6	5	1	2	1	3	4	3
	AA	10	9	7	2	2	1	2	–	–
IT-5	TMS	9	7	5	2	2	1	3	4	3
	AA	7	7	5	1	2	1	2	–	–
R2-1	TMS	9	6	5	1	1	1	2	3	2
	AA	12	10	10	3	2	2	2	–	–
R2-2	TMS	11	7	7	1	1	0	2	2	2
	AA	10	8	7	2	2	2	3	–	–
R2-3	TMS	7	5	3	1	0	0	1	2	2
	AA	7	5	4	2	2	2	3	–	–

Note: TMS, GC analysis of the *per-O*-trimethylsilyl methylglycosides; AA, GC analysis of the alditol acetates; Glc, glucose; Gal, galactose; Man, mannose; Rha, rhamnose; Fuc, fucose; Ara, arabinose; Xyl, xylose; GlcA, glucuronic acid; GalA, galacturonic acid.

Table 7. Carotenoid:Chl *a* ratios in the core depth of the Tetiaroa (IT) and Rangiroa (R2 and R59) mats (a standard deviation of 5–10% should be applied to each calculation).

Core depth (cm)	Myxoxanthophyll:Chl <i>a</i>			β -Carotene:Chl <i>a</i>			Echinenone:Chl <i>a</i>		
	IT	R2	R59	IT	R2	R59	IT	R2	R59
0	0.35	0.05	0.4	0.5	0.5	0.8	0.4	0.07	0.1
0.5	0.25	0.45	0.25	0.6	0.6	0.7	0.4	0.2	0.07
1	0.42	0.6	0.2	15	1.4	0.7	0.5	1	0.07
5	0.42	0.6	0.4	15	1.4	0.5			0.15
10	0.2	0.6		3	0.5		1	0.6	
15	4	0.3		6			2	0.5	
20	5								

known that mats in poorly protected geomorphological environments did not show any interesting stratified layers (Andréfouët et al. 2001, 2002). Conversely, undisturbed mats in naturally protected environments, like the mats investigated in this study, are characterized by their structure, stability, and purity. Despite a relatively low diversity, analyses of bacterial pigments and bacterial lipids pointed out the dominance of some specific groups of cyanobacteria, anoxygenic purple bacteria, and sulfate reducers. The morphological bacterial determination emphasized the influence of salinity on the bacterial diversity, with higher morphological (morphotype) diversity at low salinity, mainly for purple nonsulfur bacteria. Differences occur also in the surface layers, a noteworthy feature for remote sensing of mats and large-scale inventory. Low salinity mats with green surface layers were dominated by *Phormidium*-like cyanobacteria, and brackish to marine mats with orange surface layer were dominated by *Schizothrix*-like cyanobacteria. In all the sampled mats, photosynthetic and carotenoid pigments were very well distributed and well preserved in their structures along the mat depth. Though comparable pigmentation was encountered, the concentrations of pigments and the carotenoid:Chl *a* ratios may vary between mats along depth. Regardless of salinity, all mats showed a high percentage of

organic material from bacterial origin, without significant differences within the EPS fractions and with high biotechnological potential. Conversely, EPS concentrations vary with depth in hyposaline mats, but not in brackish ones.

The kopara mats presented very remarkable structures, but further investigations are still required. For instance, several phototrophic bacteria isolated and maintained in pure strains will be described in further papers. However, this study represented the first microbiological investigation in Pacific microbial mats. Other studies in different mats in other regions of the Pacific archipelagos will be required for comparison.

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