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Emission spectroscopy and kinetic fluorometry studies of phototrophic microbial communities along a salinity gradient in solar saltern evaporation ponds of Eilat, Israel

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ABSTRACT: The planktonic and benthic microbial communities in 8 hypersaline evaporation ponds of the Israel Salt Company in Eilat, Israel, with salinities ranging from 58 to 329 g l^{-1} (total dissolved salt), were studied using fluorescence emission spectroscopy and kinetic fluorometry. With increasing salinity, the anoxygenic phototrophic bacteria (containing bacteriochlorophyll *a*, bchl *a*) formed a significant and increasing fraction of the planktonic phototrophic biomass. While the bchl *a*/chl *a* molar ratio was 0.01 at the lowest salinity, it reached almost 1 at the higher salinities. In the benthic communities, emission spectroscopy revealed depth-dependent changes in the photophysiology of benthic oxygenic phototrophs, and spatial variability in the abundance of several groups of anoxygenic photosynthetic bacteria (green bacteria containing chlorosomes and purple bacteria containing LH1). In general, the emission signal of the benthic oxygenic phototrophs (diatoms and Cyanobacteria) was dominated by photosystem I (detected in some cases down to 5 cm of sediment depth). The signal of photosystem II and phycobilisomes was several times weaker and was observed mostly in the surface layers. The spectroscopic data of microbial communities were complemented by microscopic characterization.

KEY WORDS: Phototrophic microbial communities · Hypersaline · Cyanobacteria · Plankton · Emission spectroscopy · Kinetic fluorometry

INTRODUCTION

Multi-pond solar salterns, used worldwide for salt production along the coasts in tropical and subtropical areas, present us with an environment with increasing salt concentrations, from seawater to NaCl saturation. Rich and varied communities of photosynthetic microorganisms develop along the salt gradient, both in the water of the ponds and in benthic mats. Among the organisms encountered are eukaryotic microalgae such as the green alga *Dunaliella*, which is typically found in the hypersaline brines of the crystallizer ponds, diatoms that are present in the benthic and planktonic communities at lower salinities, different types of unicellular and filamentous Cyanobacteria that often display colorful layers in the gypsum crusts covering the bottom of ponds with

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salinities of ~150 to 200 g l^{-1}, and anoxygenic phototrophs such as purple sulfur bacteria, which are often found below the benthic cyanobacterial communities (Cornée 1982, Caumette et al. 1994, Sørensen et al. 2004, 2005, Ionescu et al. 2007, Oren 2009, this Special Issue).

In view of the presence of dense communities of phototrophs as well as the great diversity of photosynthetic microorganisms found along the salt gradient, salterns are ideal systems for the study of primary production and other photosynthesis-related processes in the natural environment (Oren 2009). A multidisciplinary study was therefore conducted on the microbiology of the solar salterns of Eilat, Israel. Aspects investigated included the characterization of the phototrophic microbial communties in the ponds and measurements of primary production using oxygen microelectrodes (Sørensen et al. &, this Special Issue; Woelfel et al. 2009, this Special Issue) and planar optodes (Woelfel et al. 2009). To complement the classical taxonomic approach, we applied emission spectroscopy and kinetic fluorometry techniques that allowed us to obtain semi-quantitative and qualitative information on the presence of individual groups of phototrophic microorganisms, based on their specific pigment–protein complexes.

MATERIALS AND METHODS

Site description and sample collection. The sampling sites are situated in the saltern evaporation ponds of the Israel Salt Company at Eilat near the Red Sea coast of Israel and Ein Evrona, which is 10 km north of the city of Eilat. The plant is a network of connected ponds and channels with a salinity gradient of 58 to 329 g l^{-1} (total dissolved salt). The ponds are labeled with company numerical codes 61, 62, 63, 64, 103, 200, 201 and 304. The corresponding salinities of the ponds were 62, 88, 58, 114, 157, 206, 303 and 329 g l^{-1}, respectively. Ponds were sampled in March 2008, using a 20 µm mesh plankton net for plankton, and sterile spatulas and hammers for benthic mats and crusts. The samples of individual layers were placed in plastic tubes and stored at 4°C until spectroscopic analysis or fixed with 4% formaldehyde for species identification. Table 1 shows the chemical parameters and salinity of individual ponds. All samples were collected around midday to limit possible variability due to migration of benthic organisms between the layers, which can be caused by diel vertical oscillation in oxygen and sulfide gradients. The thickness of the benthic communities in the studied ponds varied from a 0.3 cm mud layer in Pond 61 to a 10 cm thick stratified gypsum crust with layers of various colors in Ponds 200 and 201. Where possible, the layers of the community were sampled separately for the identification of species and spectroscopic analysis.

Emission spectroscopy. The method utilizes the fact that many of the characteristic pigment–protein complexes of phototrophic organisms emit fluorescence at different and characteristic wavelengths when cooled to low temperature. For example, phycobilisomes (PBS) typically emit in the range of 600–660 nm, photosystem II (PSII) at 685 and 695 nm, photosystem I (PSI) at 700–730 nm, bacterial LH I complex at ~880–920 nm, etc. (see Govindjee 2004 for a review). In addition, measurement of the emission spectra under different excitations provides information about the major absorbing pigments in the samples. Here, we used the novel portable instrument for measuring low temperature (77K) emission spectra described by Suggett et al. (2009, this Special Issue). Each of the collected samples was transferred to a GF/F filter in the laboratory and placed into the sample holder, which was cooled to the temperature of liquid nitrogen (77K). Then, the sample was illuminated by narrow-band excitation provided by light-emitting diodes (LEDs). We used LEDs with maximum emission at 390, 455, 470, 505, 530 and 590 nm. The emitted fluorescence signal was then collected, spectrally dispersed and detected in the spectral range of 190 to 1000 nm. Proper care was taken to correct for instrument sensitivity and to subtract the instrument background signal. For sample preparation, we used a protocol similar to that of Suggett et al. (2009) with the following modifications: for plankton samples, we filtered 50 ml of the water samples; the benthos samples of individual layers were stored in Eppendorf tubes that were vortexed before measurement and 250 µl of the well-mixed suspension were poured and spread on GF/F filters. If the suspension contained large mineral particles, care was taken to evenly spread these across the filter.

Kinetic spectroscopy. We also used the room-temperature kinetic infrared fluorometric technique described by Koblížek et al. (2005) for quantification of oxygenic and anoxygenic phototrophs in the plankton samples. Here, plankton samples were directly analyzed in the measuring chamber of the instrument. The absolute concentrations of chl *a* and bchl *a* were estimated from the amplitudes of the variable fluorescence (chl *a* variable fluorescence was measured in the 680–720 nm range, bchl *a* in the spectral range >820 nm). The instrument was calibrated regularly against standard laboratory cultures with known pigment concentrations.

Microscopy and statistical analyses. A light microscope (Olympus BX51) with a digital camera (Olympus DP 70) was used to identify species and document species composition. Subsamples for diatom identification were prepared following methods described by Beneš et al. (2002) and mounted into the artificial resin pleurax. Frustules were observed using the Olympus BX51

light microscope with differential interference contrast (DIC) and a scanning electron microscope (SEM JEOL 6380). Sample preparation for the SEM followed the protocol of Nebesářová (2002). Diatom taxa were identified according to the identification key used by Veselá (2006).

Species composition of microbial communities in the sampled ponds were compared using principal component analysis (PCA). The species data for the analysis were entered as presence/absence data. The analysis was performed using the program CANOCO (Ter Braak & Šmilauer 1998) and an ordination diagram was created using CanoDraw software (Šmilauer 1992).

RESULTS

Microscopic characterization of the planktonic and benthic phototrophic communities

The samples were analyzed in parallel using optical microscopy and the fluorescence techniques. The planktonic communities invariably showed low species diversity. Phytoplankton of Ponds 61, 62, 63, 64, 103 and 200 was dominated by *Nitzschia* aff. *N. lorenziana.* In Ponds 63 and 200, this diatom was rather rare and heterotrophic flagellates accompanied it. Numerous single-celled rod-like bacteria dominated the plankton of Pond 201. At the highest salinity (Pond 304), *Dunaliella salina* was the sole phototroph encountered. Benthic communities were more diverse, with 9 to 32 different morphotypes of phototrophs. The oxygenic phototrophic microbial community of the salterns consisted of 26 species of diatoms, 14 species of Cyanobacteria, 2 green algae and 1 species of Chrysophyceae. An overall comparison of microbial community compositions of the ponds studied is provided as a PCA ordination diagram (Fig. 1).

Spectroscopic analysis of planktonic microbial communities

The low temperature emission spectra provide information about the presence and relative abundance of phototrophic microorganisms with distinct pigment–protein complexes. Using blue (455 nm) excitation, the emission spectra of plankton from Ponds 61 to 64 were dominated by emission from PSII, with maxima at 685 and 695 nm (Fig. 2A), indicating that the community is dominated by oxygenic phototrophs. PSI emission (band with maximum at ~715 nm) characteristic of Cyanobacteria was observed only in plankton from Pond 63. Similarly, PBS emission (usually observed in the spectral region of 550–660 nm) was observed only in Pond 63 when appropriate excitation was used (530 nm, data not shown). The emission spectra of plankton from ponds with higher salinity confirmed the presence of oxygenic photosynthetic organisms in all studied ponds (Fig. 2B), with a maximum in the PSII region (681–687 nm) and a shoulder at longer wavelengths that indicates the presence of some PSI (710 nm) emission. Spectroscopy detected oxygenic phototrophs even in Pond 103, where no organisms could be detected by microscopy. However, in these high-salinity ponds, the photosynthetic emission bands were always superimposed on a strong background emission of an unknown fluorophore. This background emission was excited only in the 450–510 nm spectral regions, i.e. within the absorption band of carotenoids. We noted that the intensity of this background emission increased with salinity, and in some cases, clear maxima could be observed at 575 and 620 nm. The presence of a strongly absorbing carotenoid pigment was confirmed by absorption spectroscopy of particles in the high-salinity samples (S. Gehnke, unpubl. data).

In addition to oxygenic photosynthetic organisms, emission spectroscopy revealed the presence of photosynthetic bacteria containing bacteriochlorophylls. They were present in the plankton of all studied ponds, except for the crystallizer pond with the highest salinity (Pond 304). In emission spectra, 2 major bands were present, with maxima at 885 and 910 nm (Fig. 3A). These bands were preferentially excited by irradiance >500 nm and are typical for bchl *a*-containing phototrophic bacteria. We assume that the different emission bands are characteristic of different bacterial species with different bacterial light-harvesting complexes. In each pond, one of the bands was dominant: the 885 nm band in Ponds 61, 63, 64 and 103, and the 915 nm emission band for bacteria in Ponds 62 and 201.

The fluorescence kinetics technique allowed us to estimate the absolute abundance of photosynthetic planktonic microorganisms in the studied ponds (Fig. 4A). The biomass of both the oxygenic and bacterial anoxygenic phototrophs was highest in ponds with a salinity of 60–120 g l^{-1} (Ponds 61, 62, 64). The maximum chl *a* value was detected in Pond 62 (chl *a* = 11.3 nM, salinity of 88 g l^{-1}), while the highest bchl *a* concentration was observed in Pond 64 (bchl *a* = 0.3 nM, salinity of 114 g l^{-1}). At higher salinities, the biomass of oxygenic phototrophs declined steeply with increasing salinity, but bchl *a* concentration remained high up to a salinity of ~200 g l^{-1}. Only at the highest salt concentration (Pond 304, salinity of 329 g l^{-1}) no bchl *a*-containing phototrophs were detected. As a result, the relative molar ratio of bchl *a*/chl *a* was almost 1 at higher salinities of up to ~200 g l^{-1} (Fig. 4B). This is significantly higher than the usual ratios of bchl *a*/chl *a* in oligotrophic seawater as determined by this

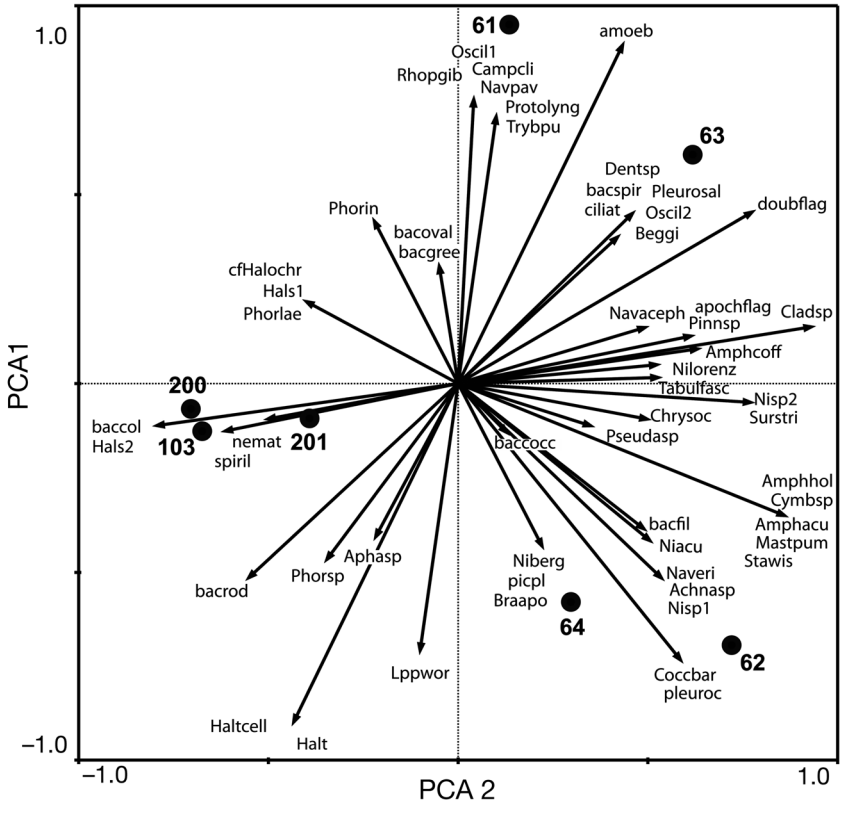

Fig. 1. Principal component analysis (PCA) describing the species composition of microbial communities in different saltern ponds. (●) Sampling ponds. The first and second canonical axes together explain 46.7% of the total variability. Abbreviations are given below

Abbreviation	Species or taxon description
Achnasp	*Achnanthes* sp.
amoeb	amoebas
Amphacu	*Amphora acutiuscula*
Amphcoff	*Amphora coffeaeformis*
Amphhol	*Amphora holsatica*
Aphasp	*Aphanocapsa* sp.
baccocc	single-celled coccoid bacteria
baccol	colorless colonial coccoid bacteria
bacfil	long filamentous bacteria
bacgree	green colonial coccoid bacteria
bacoval	big oval colorless single-celled bacteria
bacrod	rod-like bacteria
bacspir	long motile spiral bacteria
Beggi	*Beggiatoa* sp.
Braapo	*Brachysira aponina*
Campcli	*Campylodiscus clipeus*
cfHalochr	*Halochromatium*-like(big oval purple singlecelled bacterium with granulation)
Chrysoc	cf. *Chrysococcus*
ciliat	ciliates
Cladsp	*Cladophora* sp.
Coccbar	*Cocconeis bardawilensis*
Cymbsp	*Cymbella* sp.
Dentsp	*Denticula* sp.
doubflag	flagellates with 2 flagella
Hals1	*Halospirulina tapeticola* type 1 (thicker)
Hals2	*Halospirulina tapeticola* type 2 (thinner)
Halt	colonial *Halothece* sp.
Haltcell	single-celled *Halothece* sp.
apochflag	apochromatic flagellates
Lppwor	*Leptolyngbya* cf. *woronichinii*
Mastpum	*Mastogloia pumilla*
Navaceph	*Navicula acephala*
Naveri	*Navicula* cf. *erifuga*
Navpav	*Navicula pavillardii*
cfNavic	cf. *Navicula*
nemat	nematodes
Niacu	*Nitzschia acula*
Niberg	*Nitzschia* cf. *bergii*
Nilorenz	*Nitzschia* aff. *N. lorenziana*
Nisp1	*Nitzschia* sp. 1
Nisp2	*Nitzschia* sp. 2
Oscil1	*Oscillatoria margaritifera* type 1 (thicker)
Oscil2	*Oscillatoria margaritifera* type 1 (thinner)
Phorin	*Phormidium* cf. *inundatum*
Phorlae	*Phormidium laetevirens*
Phorsp	*Phormidium* sp. (thicker, bent tip)
picpl	single-celled spherical cyanobacterial picoplankton
pleuroc	unidentified pleurocapsacean cyanobacterium
Pleurosal	*Pleurosigma salinarum*
Protolyng	*Leptolyngbya* subg. *Protolyngbya* sp.
Pseudasp	*Pseudanabaena* sp.
Rhopgib	*Rhopalodia gibberula*
spiril	*Spirillum*-like bacteria
Stawis	*Stauroneis wislouchii*
Surstri	*Surirella striatula*
Tabulfasc	*Tabularia fasciculata*
Trybpu	*Tryblionella punctata*

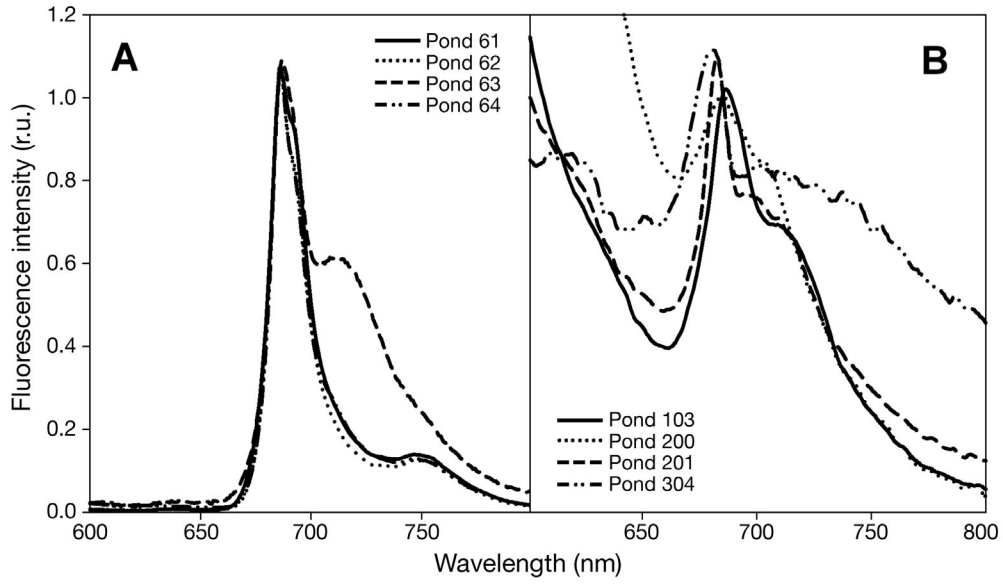

Fig. 2. Low temperature (77K) fluorescence emission spectra (intensity in relative units, r.u.) of oxygenic plankton in ponds with (A) low salinity (58–114 g l^{-1}) and (B) high salinity. Samples were excited by blue (455 nm) excitation. Spectra were normalized to emission at 685 nm. Note the increasing background signal in the high-salinity ponds

technique (bchl *a*/chl *a* ~ 0.01 in the Gulf of Aqaba, salinity of 40 g l^{-1}; H. Medová, unpubl. data). All of the ponds studied here can be classified as eutrophic based on phosphate concentrations (Table 1). Thus, the differences in bchl *a*/chl *a* ratios here as compared with that in the Gulf of Aqaba are likely due to differences in trophic conditions.

Spectroscopic analysis of benthic microbial communities

Spectroscopic studies were performed on the benthic communities from Ponds 62, 63, 64, 103, 200 and 201. The studied ponds differed from each other in the character of their sediments. Sediments of Ponds 62 to

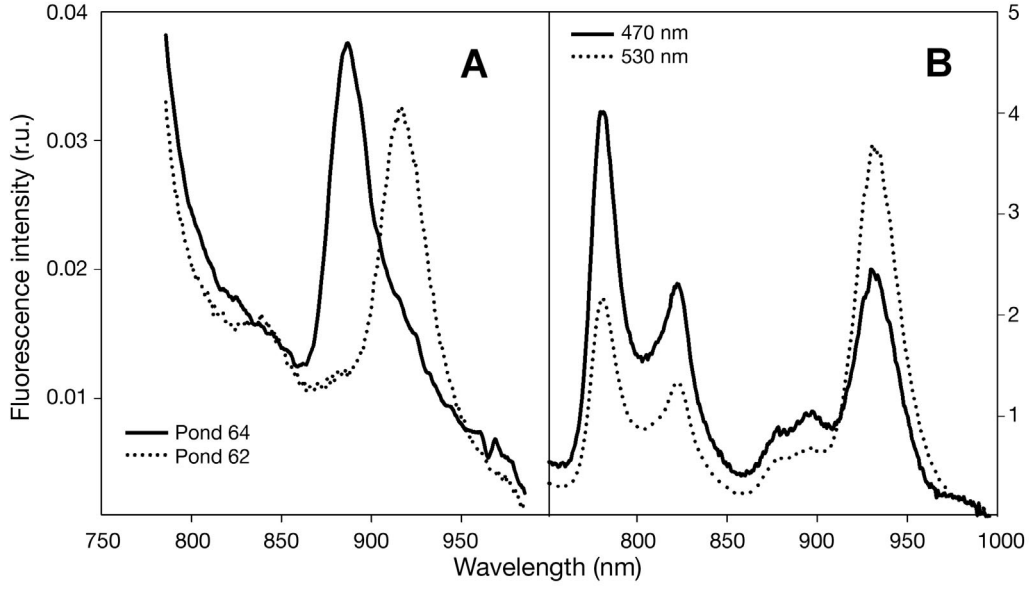

Fig. 3. Examples of 77K fluorescence emission spectra of anoxygenic phototrophic bacteria. In the plankton of Ponds 61, 63, 64 and 103, the spectra were dominated by a single emission band with a peak at ~885 nm; in Ponds 62 and 201, the bacteria emitted with a maximum at 915 nm. (A) shows representative spectra of Ponds 64 and 62, both of which were excited at 530 nm. (B) Example of the complex spectra of samples from the benthos, obtained with the pink layer of sediment from Pond 200. Excitation dependence of the spectra reveals the presence of different light-harvesting pigments. Data in (A) and (B) are normalized to emission at 685 nm

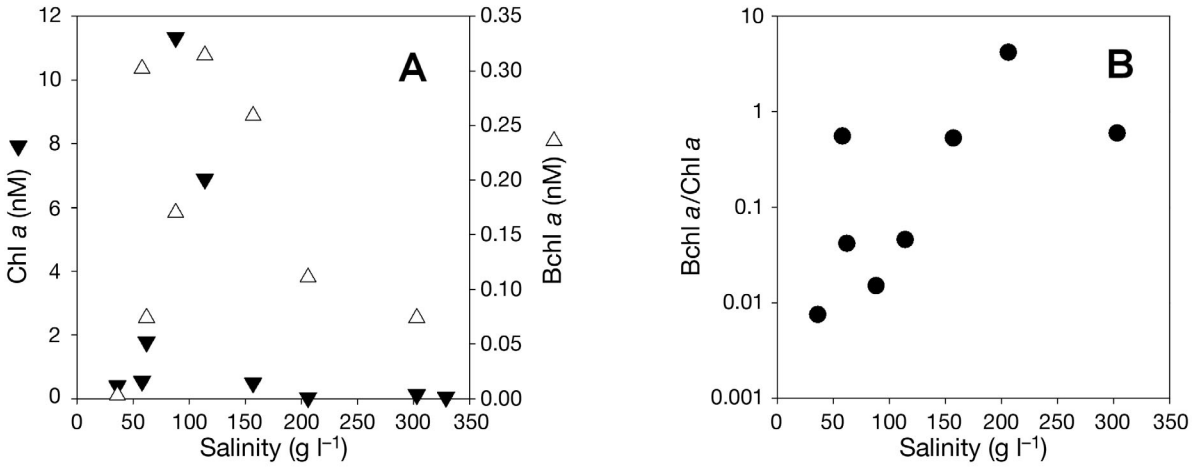

Fig. 4. (A) Concentration of oxygenic plankton (estimated as chl *a*, ▼) and anoxygenic phototrophic bacteria (estimated as bchl *a*, Δ) as revealed by fluorescence kinetic spectroscopy. Also included in the graph are data for the oligotrophic Stn A in the Gulf of Aqaba, which was sampled during the same period. (B) Estimated ratio of anoxygenic to oxygenic pigment biomass as calculated from data in (A). Note the logarithmic scale

64 were muddy and their surfaces were covered with dense cyanobacterial mats. Gypsum crusts of various consistencies formed the bottoms of Ponds 103, 200 and 201. The spectroscopic data collected during analysis of the different sediment layers are summarized in Table 2.

The sediment from Pond 62 (salinity of 88 g l^{-1}) had an upper layer containing Cyanobacteria (*Halothece* sp., *Phormidium* sp.), diatoms (*Amphora acutiuscula*, *Navicula* cf. *vandamii*, *Nitzschia* aff. *N. lorenziana*) and the green filamentous alga *Cladophora* sp. The communities of several lower layers (0.25–3 cm) consisted of the cyanobacterium *Phormidium* cf. *inundatum*, the diatoms *Cymbella* sp., *Nitzschia* aff. *N. acula*, cf. *Navicula*, and others. The 3–4 cm layer was characterized by the cyanobacterium *Leptolyngbya* cf. *woronichinii* and the diatoms *Achnathes* sp., *Nitzschia* sp. and *Navicula* cf. *erifuga*. Spectroscopy revealed the presence of benthic oxygenic and anoxygenic phototrophs in the upper 2 cm. The dominant pigment complex of oxygenic benthic phototrophs was PSI with an emission maximum at 705 nm. PSII formed only a shoulder band at 685 nm, with an estimated PSI intensity of 20%. No emission signals from PBS were detected, despite the fact that morphological observations revealed the presence of Cyanobacteria. The emission from anoxygenic phototrophic bacteria showed a major emission band with maxima at ~917 nm (at 77K, 890 nm at room temperature) and a smaller shoulder at ~840 nm. Additionally, we detected anoxygenic phototrophs with emission maxima at longer wavelengths (>975 nm) in the deeper parts of the sediment (from 1–4 cm depth).

The benthic phototrophic community from Pond 63 (salinity of 58 g l^{-1}) was characterized by *Oscillatoria margaritifera* as the dominant species in the top 2.5 cm, accompanied by *Phormidium* cf. *inundatum*, *Phormidium laetevirens*, and diatoms (cf. *Navicula, Pleurosigma salinarum*, *Amphora acutiuscula*). *Halochromatium*-like purple sulfur bacteria were observed at 1.5–2.5 cm. Spectroscopy confirmed the taxonomic observations. Oxygenic phototrophs were detected down to a depth of 6.5 cm in the sediment. The PSI band was always dominant, with a maximum at 715–725 nm (depending on the wavelength of excitation). Only in the surface layers (0.1–1.5 cm) was the PSI signal accompanied by emission for PBS (maxima at 645 and 660 nm). In the deeper layers, the PBS signal disappeared and PSI was accompanied by a new band with a maximum at ~670 nm. The signal of anoxygenic bacteria reflected the taxonomic diversity. In the surface layer, it was dominated by bands of purple bacteria at 910–920 nm, while addi-

Table 1. Chemical parameters of water in the evaporation ponds

Pond	NO_2^- (µmol l^{-1})	NO_3^- (µmol l^{-1})	NH_4^+ (µmol l^{-1})	PO_4^{3-} (µmol l^{-1})	Salinity (g l^{-1})
61	0.33	3.65	1.13	4.26	62
62	0.14	3.76	0.10	2.00	88
63	0.36	3.14	46.70	2.02	58
64	0.19	3.22	0.13	1.98	114
103	0.18	3.75	0.23	3.54	157
200	0.19	4.52	0.50	4.08	206
201	0.47	7.16	0.30	6.58	303
304	0.45	9.59	3.63	7.33	329

Table 2. Intensities (heights of the bands in arbitrary units after background subtraction) of individual emission bands of benthos samples of Ponds 62, 63, 64, 103, 200 and 201. Based on their characteristic wavelengths, individual emission bands observed in the spectral range of 550–1000 nm (Band) were tentatively assigned to respective groups of phototrophic organisms (Assignment). Each of the assigned emission bands was excited by specific wavelengths (Excitation). The emission intensities were corrected for differences in the intensities of individual excitation light-emitting diodes (LEDs). Cyano: cyanobacteria; Oxygenic: oxygen evolving phototrophs; Chlorosomes: green bacteria; Purple: purple bacteria. Missing values: no peaks detected

Band:	Background	PBS 600–650	B670	PSI 705–725	PSII 680–695	B780–800	B820–850	B880–890	B910–920	B930–950	B975
Assignment:	Carotenoids	Cyano	PSII?	Oxygenic		Chlorosomes		Purple			
Excitation (nm):	470	530	530	455	455	530	530	530	530	530	470
Sediment depth (cm)											
Pond 62											
0.0–0.25				5600					917		
0.25–1.0				1600					150		50
1.0–1.5				1500					130		150
1.5–2.0				1200	120				80		150
2.0–3.0				150	10				90		
3.0–4.0				140							90
4.0–5.0											25
Pond 63											
0.1–0.5		1500		650					60		
0.5–1.5		400		1700					10		70
1.5–2.5				1400		100			60		120
2.5–3.5			60	700		40			60		30
3.5–4.5			30	180					20		
4.5–5.5			40	140					15		
5.5–6.5			30	100							
5.0–6.0											
Pond 64											
0.0–0.2		180		850	1600		30		100		
0.2–0.5		220		2700	600		30		160		40
0.5–1.0		180		800	200		20		120		
1.0–2.0		200		900	150				120		
2.0–3.0		120		230	90		10		80		
3.0–4.0		100		120	30		5		30		70
4.0–5.0		90		70	10				15		80
Pond 103											
0.0–0.2	200			1400	780	50		50		80	
0.2–0.3	203	1400		7500	1200	150		80		60	
0.3–0.4	205	400		760	320	150		760		1000	
0.4–0.5	300	3000		2500	900		100			560	
0.5–1.0	200	700		2600	800	200	100	100		540	
1.0–2.0	200	180	400	480		70		100		250	
2.0–3.0	300		110	300		50		120		250	
3.0–4.5	220		120	250		60		50		80	
Pond 200											
0.0–0.5	400	400		4200	1200	80		120			60
0.5–1.0	100	50		1350	470	50		20			30
1.0–1.5	250			1000	470			20			40
1.5–2.0	210	350		510	210	40		30		160	20
2.0–2.5	120	350	1650	750		1000	600	300		1710	
2.5–3.0	450	300	300	570		220	120	150		440	
3.0–3.5	350		120	160		60	35	40		90	
3.5–4.0	370		170	150		100		50		40	
4.0–4.5	250		90	150		50		50		70	
4.5–5.0	250		60	60		80		60		90	
Pond 201											
0.0–0.5	250			600	850				25		
0.5–1.0	270	250		800	420				30		
1.0–1.5	280	230		350	175				400		
1.5–2.0	300	1800		2300	400				200		
2.0–2.5	400			270	30				3100		
2.5–3.0	470	280		350	100				315		
3.0–3.5	300	70		120					45		

tional bands with maxima at ~800 and >975 nm were observed in deeper layers.

In Pond 64 (salinity of 114 g l^{-1}), the upper sediment layers were characterized by the Cyanobacteria *Leptolyngbya* cf. *woronichinii* and *Halothece* sp. and the diatoms cf. *Navicula*, *Mastogloia pumila*, *Achnanthes* sp., *Amphora* spp., *Cocconeis bardawilensis*, *Nitzschia* aff. *N. lorenziana*, and *Nitzschia* aff. *N. acula*. The deeper layers (2–5 cm) contained a *Gomphosphaeria*-like cyanobacterium, an unidentified pleurocapsacean cyanobacterium and dying filaments of *Cladophora* sp. In the surface layer (0–0.2 cm), the emission signal was dominated by PSII bands at 685 and 695 nm. PSI emission formed only a shoulder band with a maximum at 720 nm. In the deeper layers (down to 2 cm), PSI dominated the signal and the ratio of PSI/PSII band intensities gradually increased from 2 to 4. PBS had only a minor contribution that decreased with depth. In the deepest layers where oxygenic photosynthesis could still be observed (4–5 cm), the remaining oxygenic phototrophs only had a chl *a*-containing antenna. Interestingly, in deeper layers, a strong emission band from PBS was observed at ~650 nm. The anoxygenic bacteria showed mostly a single dominant emission band at 920 nm and a shoulder at 850 nm. In addition, some bacteria with long-wavelength emission maxima were present in the subsurface layer and in the deepest layers (>3 cm).

Pond 103 (salinity of 157 g l^{-1}) had colonial and single-celled *Halothece* sp., *Halospirulina tapeticola*, *Leptolyngbya* cf. *woronichinii*, and *Phormidium* sp. in the upper layer (0–1 cm). The middle layers (1–3 cm) were colonized mainly by Cyanobacteria (cf. *Leptolyngbya*, *Phormidium* cf. *inundatum*) and by the diatom *Nitzschia* aff. *N. lorenziana*. The deepest layer (3–4.5 cm) showed the presence of Cyanobacteria (*Aphanocapsa* sp., *Pseudanabaena* sp., *Phormidium laetevirens*), together with the diatoms *Amphora coffeaeformis* and *Nitzschia* aff. *N. acula*. The emission spectra of samples from the crust (top 0.4 cm of this gypsum sediment) showed the presence of oxygenic phototrophs with pronounced PSI emission at ~720 nm and only a minor signal for PBS (650 and 665 nm). The PSI emission was almost 7× higher than that of PSII. There were abundant anoxygenic phototrophs in the crust, with bands at 930, 886 and 780 nm. In the pink layer (0.3–0.4 cm) of the crust, the 886 nm emission was twice as intense as the PSI band (when both were excited at 455 nm; data not shown).

Spectra revealed that samples from 0.4–1 cm in the sediment were dominated by the PSI signal at 720 nm, and the PBS signal at 650 nm was most pronounced only in the thin 0.4–0.5 cm layer. Anoxygenic bacteria showed a peak at 945 nm with minor bands at ~850 and 810 nm. Chlorosome emission at 770 nm was observed in the green layer at 0.5–1 cm depth. In the deeper layers (1–4.5 cm), the emission from oxygenic PSI was less pronounced because of a strong background emission signal similar to that described above for planktonic samples; usually, this signal was structured into bands with maxima at ~575 and 620 nm. The anoxygenic bacteria emitted at 2 bands: 885 and 935 nm.

In Pond 200 (salinity of 206 g l^{-1}), the upper layers (0–2 cm) were colonized mainly by the Cyanobacteria *Halothece* sp., *Leptolynbya* cf. *woronichinii* and *Phormidium laetevirens*. The deeper layers (1.5–2.5 cm) contained the Cyanobacteria *Halospirlina tapeticola* (both thin and thick types), cf. *Leptolyngbya*, *Phormidium* cf. *inundatum*, and *Phormidium* sp. Below was a layer colonized by the single-celled cyanobacterium *Halothece* sp. together with the purple sulfur bacterium *Halochromatium* sp. Spectroscopy confirmed the presence of oxygenic phototrophs in all layers. Cyanobacteria with a characteristic PSI band were dominant in the top layers down to 2 cm. While PSII emitted at 685 nm in these top layers, we observed a gradual shift of this emission band to 670 nm in the deeper layers. PBS were clearly present only in the top layer. In all layers, the emission spectra contained the above described background signal with maxima at 575 and 620 nm. According to the spectral signatures, several classes of anoxygenic bacteria were present. In the top 2 layers (0–1 cm), there were bacteria with emission maxima at ~890 nm and some with a long-wavelength maximum at >975 nm. The composition of the anoxygenic biomass changed significantly at layer 4 (1.5–2.0 cm), where a strong emission band at 930 nm was observed (Fig. 3B). This band was accompanied by a broad shoulder down to 870 nm. Further analysis of it by deconvolution into Gaussian curves suggested that it consisted of 2 bands peaking at ~875 and 895 nm. The layer at 2.0–2.5 cm was dominated by green bacteria containing chlorosomes (emission at 780 and 820 nm). Microscopic observation revealed the presence of rather big ovoid *Halochromatium*-like purple sulfur bacteria in this layer. At all deeper layers, bacteria emitting at 930 nm with a shoulder at 890 nm were still detected. Chlorosome-containing green bacteria were also present in the deeper layers.

Generally, main bacterial emission bands from the sediments obtained from Ponds 103 (salinity of 157 g l^{-1}) and 200 (salinity of 206 g l^{-1}) could be divided into 6 spectral groups. These comprised bands centered approximately at 875, 885, 895, 905 and 930 nm and a putative emission band peaking at >970 nm (Fig. 5). While these bands were not resolved equally well in different layers, their presence was often revealed by deconvolution of the spectrum into a sum of Gaussian curves. In most layers, at least 2 of the bands were detected.

The sediment layers from Pond 201 (salinity of 303 g l^{-1}) could be divided into 4 groups according to their species composition. The uppermost layer (0–0.5 cm) was characterized by the cyanobacterium *Leptolyngbya* cf. *woronichinii* and single-celled *Halothece* sp. Layers 2 and 3 (0.5–1.5 cm) contained the cyanobacteria *Phormidium* cf. *inundatum* and single-celled *Halothece* sp., the diatom *Nitzschia* aff. *N. lorenziana*, and the chrysophycean alga cf. *Chrysococcus*. Layers 4 and 5 (1.5–2.5 cm) mainly contained the colonial cyanobacterium *Halothece* sp., together with *Phormidium* cf. *inundatum*. Unlike the oxygenic phototrophs in the deeper layers (0.5–2.0 cm) with pronounced emission maxima for PSI, the organisms in the top, orange-colored layer had maximum emission at 685 nm (PSII). The strongest PSI signal was observed in the green-colored layer (1.5–2.0 cm). The diversity of pigment–protein complexes of the anoxygenic bacteria was minimal. Throughout all layers, we detected only bacteria with an emission maximum between 905 and 910 nm.

DISCUSSION

The evaporation ponds represent a wide variety of salinities, ranging from 58 to 329g l^{-1}. A high diversity of Cyanobacteria, diatoms, green algae and anoxygenic phototrophic bacteria was observed.

With the exception of recent works of Bachar et al. (2008) and Kühl & Polerecky (2008), no studies were performed in which spectroscopic techniques such as emission spectroscopy and kinetic fluorometry were applied to natural communities of halophilic and halotolerant benthic and planktonic phototrophs. Spectroscopic data in the low-oxygen layers showed the presence of both green and purple photosynthetic bacteria. The former were identified by the simultaneous presence of emission bands at ~780 nm (bchl *c*) and 820 nm (bchl *a*), which originate from chlorosomes (see van de Meene et al. 2007 for spectra of both green sulfur and filamentous bacteria). Two groups of anoxygenic phototrophs are known to use chlorosomes for light harvesting: green sulfur bacteria (*Chlorobi*) and green filamentous bacteria (*Chloroflexi*). As shown by Schmidt & Trissl (1998a,b) and recently by van de Meene et al. (2007), the chlorosome emission of both groups is very similar; however, they differ by the presence of an emission band at ~900 nm, which is present only in *Chloroflexi* and originates from the inner LH1-type of light-harvesting complex similar to the inner antenna of purple bacteria. Members of this group have recently been found in hypersaline environments (Klappenbach & Pierson 2004, Bachar et al. 2008). However, we were unable to find a convincing correlation between the intensity of chlorosome emission and the intensity of any of the emission bands located in the >870 nm region; thus, we were unable to unambiguously decide on the type of green bacteria present in our samples.

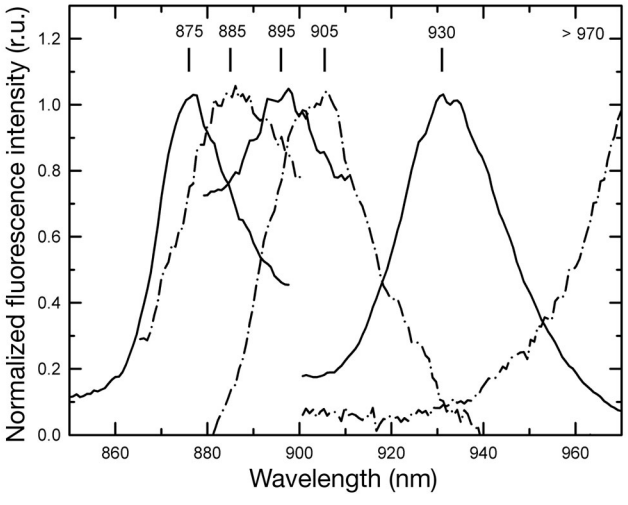

Fig. 5. Main emission bands of purple bacteria from Ponds 103 and 200. Peaks are labelled with their respective emission spectra. Note that the peak with a maximum at ~930 nm was also present in the spectra labeled 875, 885 and 895. This band was deleted for clarity of presentation, as was the 875 nm shoulder in the spectrum labeled 895. Spectra labeled 875 and 885 were detected in Pond 103, while the rest were from Pond 200. Spectra were normalized to the intensity (in relative units, r.u.) of the labeled bands

As shown in Fig. 5, at least 6 spectral bands can be distinguished in the 870–1000 nm region of the emission spectra. Since emission of the bchl *a*-containing pigment–protein complexes can reach well above 1020 nm under cryogenic temperatures (Permentier et al. 2001), one can assume that the bands lying above 870 nm pertain to bchl *a*. because the high efficiency of LH2 to LH1 energy transfer prevents the external antenna from contributing significantly to the emission spectrum even in species that contain external (LH2/LH3) antenna complexes, we suggest that all bands in Fig. 5 belong to inner antenna, LH1. This suggests the presence of at least 6 different bacterial species, although an attempt at more detailed taxonomic assignment based solely on spectroscopic data is not tenable.

The division of the phototrophic microorganisms into 3 functional groups according to their emission spectra (oxygenic phototrophs containing PSI and PSII, green bacteria containing chlorosomes and purple bacteria containing LH1) allowed us to semi-quantitatively compare the vertical distribution of phototrophs in the benthos of the studied ponds (Fig. 6). In general, the quantity of oxygenic phototrophs in all ponds decreased exponentially with depth, confirming the dependence of their abundance on irradiance levels

and oxygen availability. Oxygenic phototrophs with a dominant PSI signal were observed as deep as 5 cm below the surface. In Ponds 62, 63 and 64, no green anoxygenic bacteria were observed; only purple bacteria were seen, and their abundance also decreased with depth down to 5 cm (with the exception of Pond 63, where maximum abundances were observed 2 cm below the surface). Significantly higher abundances of anoxygenic bacterial phototrophs (both green and purple) were observed in the well-structured benthic layers in high-salinity Ponds 103, 200 and 201. Green bacteria formed a significant fraction of the emission signal (and thus of the community) only in Ponds 103 and 200. The data shown in Table 2 and plotted in Fig. 6 also allowed us to roughly estimate the relative contribution of the oxygenic and anoxygenic phototrophs. While anoxygenic bacteria formed only a minor part of the community in the surface layers of Ponds 62 and 63, they clearly dominated over oxygenic phototrophs (~30× in the 2.0–2.5 cm layer of Pond 201) in the deeper layers of the high-salinity crusts.

In some of the deeper (>1cm) layers, we observed an emission band with a maximum at ~670 nm. Since this band substituted the classical emission band of PSII at

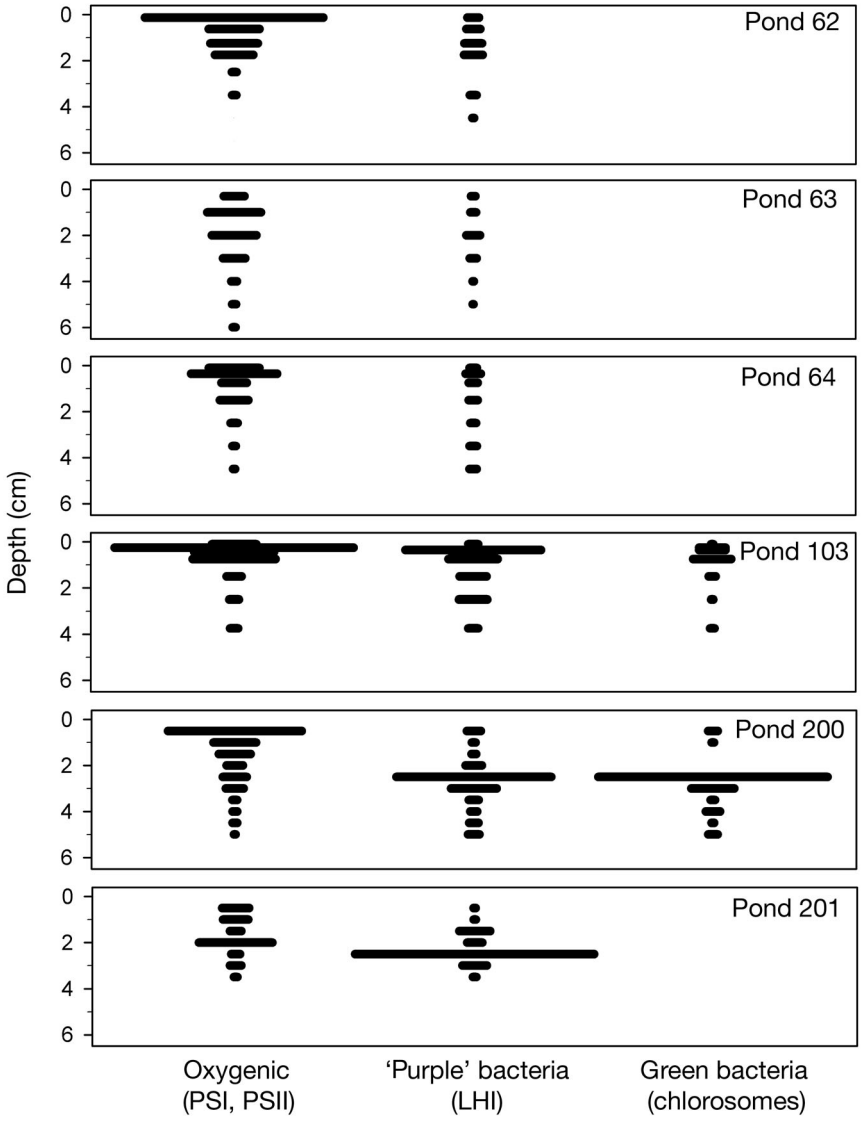

Fig. 6. Graphic presentation of spectroscopic data from Table 2, showing the vertical distribution of the main types of photosynthetic organisms within the benthos. The emission bands were grouped according to their pertinence to the main groups of phototrophic organisms: oxygenic phototrophs (PSI and PSII), purple bacteria (all groups containing LH1 antenna, including aerobic anoxygenic bacteria) and green bacteria (containing chlorosomes). The widths of the bars denote normalized intensities of respective emission bands. For each of the 3 spectral groups, the longest bar denotes the layer with the highest abundance of a given type of phototrophs among all ponds (corresponds to 1 after normalization)

685 nm (we never observed both bands simultaneously in the same spectrum), we speculate that this represents some form of PSII that is modified under the microanaerobic and dark conditions in the deeper benthos. A band at a similar position has been observed in etiolated higher plants and was assigned to the presence of protochlorophyll etioplasts (Boddi et al. 1993). A second peculiarity was the background signal that was always observed in samples from higher salinities, both in the benthos and in the plankton (see e.g. Fig 2B and Table 2, column 'Background'). This signal often had discernible maxima at 575 and 620 nm. It is of unknown origin and we can only speculate that it might come from some forms of carotenoids (retinal, salinixanthin, β-carotene) that are known to accumulate at higher salinities. This would be supported by the fact that the band is preferably excited by wavelengths 450–500 nm. Alternatively, it might be emitted by some inorganic fluorophore that is contained in the highly concentrated brines. Further experiments are necessary to understand this phenomenon.

Planktonic communities of the investigated ponds were far less diverse than the benthic mats. Diatoms were most frequently encountered in our microscopic survey. The spectroscopy technique revealed the presence of anoxygenic purple phototrophic bacteria in all ponds studied (with the exception of the most saline pond, 304). These planktonic anoxygenic bacteria were aerobic, since we detected levels of oxygen that were at or above saturation (data not shown) in all ponds (except for Pond 304), indicating that the observed phototrophs belonged to aerobic anoxygenic phototrophic (AAP) bacteria. AAP bacteria are a group of phototrophic organisms that depend mostly on heterotrophic metabolism, but are capable of utilizing light as an auxiliary source of energy (Koblížek et al. 2003, Yurkov & Csotonyi 2009). The AAP, as judged by their emission spectra, showed only a limited diversity, but formed a progressively increasing fraction of the community as the salinity of the environment increased, confirming their tolerance to extreme environments (Yurkov & Csotonyi 2003, 2009). The very high bacterial abundances in Ponds 63 and 61 (with salinity of ~60 g l^{-1}) is probably related to the high organic and nutrient content of these shallow and well-mixed ponds, which are optimal conditions for photoheterotrophic metabolism.

The present study shows that classical microscopy and modern spectroscopic techniques complement each other well and that their combination can provide new information on the structure and photophysiology of both planktonic and benthic communities of phototrophs in natural ecosystems over a wide range of salt concentrations. Application of spectroscopy allowed the first documentation of increasing amounts of bchl *a* with increasing salinity in the plankton of the salterns. The massive occurrence of chlorosome-containing green anoxygenic phototrophic bacteria in the benthic mats at salinities exceeding 150 g l^{-1}, as detected here using emission spectroscopy, has also not been reported before. The organisms that harbor these pigments now deserve further in-depth study. Emission spectroscopy also supported the microscopic observation of viable autophototrophs in deeper sediment layers, which might be considered an artifact without this proof. Spectroscopy also revealed the presence of an unknown fluorophore that is present at high salt concentrations.

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LITERATURE CITED

Bachar A, Polerecky L, Fischer JP, Vamvakopoulos K, de Beer D, Jonkers HM (2008) Two-dimensional mapping of photopigment distribution and activity of *Chloroflexus*-like bacteria in a hypersaline microbial mat. FEMS Microbiol Ecol 65:434–448

Beneš J, Kaštovský J, Kočárová R, Kočár P, Kubečková K, Pokorný P, Starec P (2002) Archaeobotany of the Old Prague Town defence system, Czech Republic: archaeology, macro-remains, pollen, and diatoms. Veg Hist Archaeobot 11:107–119

Boddi B, Ryberg M, Sundqvist C (1993) Analysis of the 77-K fluorescence emission and excitation spectra of isolated etioplast inner membranes. J Photochem Photobiol B 21: 125–133

Caumette P, Matheron R, Raymond N, Relexans JC (1994) Microbial mats in the hypersaline ponds of Mediterranean salterns (Salins-de-Giraud, France). FEMS Microbiol Ecol 13:273–286

Cornée A (1982) Bactéries des saumures et des sédiments des marais salants de Salin-de-Giraud (Sud de la France). Géol Mediterr 9:369–389

Govindjee (2004) Chlorophyll *a* fluorescence: a bit of basics and history. In: Papageorgiou GC, Govindjee (eds) Chlorophyll fluorescence: a signature of photosynthesis Kluwer Academic Publishers, Dordrecht, p 1–42

Ionescu D, Lipski A, Altendorf K, Oren A (2007) Characterization of the endoevaporitic microbial communities in a hypersaline gypsum crust by fatty acid analysis. Hydrobiologia 576:15–26

Klappenbach JA, Pierson BK (2004) Phylogenetic and physiological characterization of a filamentous anoxygenic photoautotrophic bacterium 'Candidatus *Chlorothrix halophila*' gen. nov., sp. nov., recovered from hypersaline microbial mats. Arch Microbiol 181:17–25

Koblížek M, Béjà O, Bidigare RR, Christensen S and others (2003) Isolation and characterization of *Erythrobacter* sp. strains from the upper ocean. Arch Microbiol 180:327–338

Koblížek M, Ston-Egiert J, Sagan S, Kolber Z (2005) Diel changes in bacteriochlorophyll *a* concentration suggest rapid bacterioplankton cycling in the Baltic Sea. FEMS Microbiol Ecol 51:353–361

Kühl M, Polerecky L (2008) Functional and structural imaging of phototrophic microbial communities and symbioses. Aquat Microb Ecol 53:99–118

Nebesářová J (2002) Elektronová mikroskopie pro biology. Multimediální učební texty (Multimedia learning text), www.paru.cas.cz/lem/book/index.html

Oren A (2009) Saltern evaporation ponds as model systems for the study of primary production processes under hypersaline conditions. Aquat Microb Ecol 56:193–204

Oren A, Sørensen KB, Canfield DE, Teske AP, Ionescu D, Lipski A, Altendorf K (2009) Microbial communities and processes within a hypersaline gypsum crust in a saltern evaporation pond (Eilat, Israel). Hydrobiologia 626:15–26

Permentier HP, Neerken S, Overmann J, Amesz J (2001) A bacteriochlorophyll *a* antenna complex from purple bacteria absorbing at 963 nm. Biochemistry 40:5573–5578

Schmidt KA, Trissl HW (1998a) Combined fluorescence and photovoltage studies on chlorosome containing bacteria — I. Whole cells of *Chloroflexus aurantiacus.* Photosynth Res 58:43–55

Schmidt KA, Trissl HW (1998b) Combined fluorescence and photovoltage studies on chlorosome containing bacteria — II. Whole cells and chlorosome-depleted membranes of *Chlorobium limicola.* Photosynth Res 58:57–70

Šmilauer P (1992) CANODRAW Users guide v. 3.0. Microcomputer Power, Ithaca, New York

Sørensen KB, Canfield DE, Oren A (2004) Salt responses of benthic microbial communities in a solar saltern (Eilat, Israel). Appl Environ Microbiol 70:1608–1616

Sørensen KB, Canfield DE, Teske AP, Oren A (2005) Community composition of a hypersaline endoevaporitic microbial mat. Appl Environ Microbiol 71:7352–7365

Sørensen K, Řeháková K, Zapomělová E, Oren A (2009) Distribution of benthic phototrophs, sulfate reducers, and methanogens in two adjacent saltern evaporation ponds in Eilat, Israel. Aquat Microb Ecol56:275–284

Suggett DS, Stambler N, Prášil O, Kolber Z and others (2009) Nitrogen and phosphorus limitation of oceanic microbial growth during spring in the Gulf of Aqaba. Aquat Microb Ecol 56:227–239

Ter Braak CJF, Šmilauer P (1998) CANOCO reference manual. Microcomputer Power, Ithaca, New York

van de Meene AML, Le Olson T, Collins AM, Blankenship RE (2007) Initial characterization of the photosynthetic apparatus of 'Candidatus *Chlorothrix halophila*', a filamentous, anoxygenic photoautotroph. J Bacteriol 189:4196–4203

Veselá J (2006) Benthic algal communities and their ecology in sandstone periodically desiccated brook in National Park Bohemian Switzerland (Czech Republic). Czech Phycology 6:99–110

Woelfel J, Sørensen K, Warkentin M, Forster S, Oren A, Schumann R (2009) Oxygen evolution in a hypersaline crust: *in situ* photosynthesis quantification by microelectrode profiling and use of planar optode spots in incubation chambers. Aquat Microb Ecol 56:263–273

Yurkov VV, Csotonyi JT (2003) Aerobic anoxygenic phototrophs and heavy metalloid reducers from extreme environments. In: Pandalai SG (ed) Recent research developments in bacteriology, Vol 1. Transworld Research Network, Trivandrum, p 247–300

Yurkov VV, Csotonyi JT (2009) New light on aerobic anoxygenic phototrophs. In: Hunter CN, Daldal F, Thurnauer MC, Beatty JTh (eds) The purple phototrophic bacteria. Springer, Dordrecht, p 31–55

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