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ORIGINAL PAPER

Spatial distribution of diatom and cyanobacterial mats in the Dead Sea is determined by response to rapid salinity fluctuations

Stefan Häusler · Miriam Weber · Dirk de Beer · Danny Ionescu

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Abstract Cyanobacteria and diatom mats are ubiquitous in hypersaline environments but have never been observed in the Dead Sea, one of the most hypersaline lakes on Earth. Here we report the discovery of phototrophic microbial mats at underwater freshwater seeps in the Dead Sea. These mats are either dominated by diatoms or unicellular cyanobacteria and are spatially separated. Using in situ and ex situ O_2 microsensor measurements we show that these organisms are photosynthetically active in their natural habitat. The diatoms, which are phylogenetically associated to the *Navicula* genus, grew in culture at salinities up to 40 % Dead Sea water (DSW) (14 % total dissolved salts, TDS). The unicellular cyanobacteria belong to the extremely halotolerant *Euhalothece* genus and grew at salinities up to 70 % DSW (24.5 % TDS). As suggested by a variable O_2 penetration depth measured in situ, the organisms are exposed to drastic salinity fluctuations ranging from brackish to DSW salinity within minutes to hours. We could demonstrate that both phototrophs are able to withstand such extreme short-term fluctuations. Nevertheless, while the diatoms recover better from rapid fluctuations, the cyanobacteria cope better with long-term exposure to DSW. We conclude that the main reason for the development of these microbial mats is a local dilution of the hypersaline Dead Sea to levels allowing growth. Their spatial distribution in the seeping areas is a result of different recovery rates from short or long-term fluctuation in salinity.

Keywords Cyanobacteria · Diatoms · Hypersaline · Dead Sea · Salinity fluctuations · Spatial distribution

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S. Häusler · M. Weber · D. de Beer · D. Ionescu
Max Planck Institute for Marine Microbiology, Celsiusstrasse 1, 28211 Bremen, Germany

M. Weber
HYDRA Institute for Marine Sciences, Elba Field Station, Campo Nel Elba, LI, Italy

D. Ionescu (✉)
Leibniz Institute for Freshwater Ecology and Inland Fisheries, Alte Fischerhütte, Neuglobsow, 16775 Stechlin, Germany
e-mail: ionescu@igb-berlin.de

Introduction

The Dead Sea is a hypersaline desert lake which is characterized by 347 g L^{-1} total dissolved solids (TDS) and an unusual ionic salt composition consisting of 1.98 M Mg^{2+}, 1.54 M Na^{+}, 0.42 M Ca^{2+} and 0.21 M K^{+} and about 6 M Cl^{-}(Oren 2010). Since the beginning of the 20th century the water budget of the lake has been negative leading to a drop in lake level of about 1 m per year and an increasing salinity (Oren 2010). Due to the high concentration of divalent cations which have a chaotropic (destabilizing) potential on biological macromolecules, only highly adapted organisms can grow in this environment (Oren 2010; Oren 2013). Since the early 1940s a number of halophilic Bacteria, Achaea, unicellular algae of the genus *Dunaliella* and amoeboid and ciliate protozoa have been described and partly isolated from the lake's water column and sediment (Elazari-Volcani 1940; Elazari-Volcani 1943a; Elazari-Volcani 1943b; Elazari-Volcani 1944; Oren 2010). Nowadays, the resident microbial community which is able to cope up with the extreme conditions of the Dead

Sea is mostly composed of *Archaea* (Bodaker et al. 2010). The algae *Dunaliella* is the only primary producer in the Dead Sea (Oren 2010); however, it is not able to grow in pure Dead Sea water (DSW). Blooms only develop after heavy rainfall events and a resulting dilution of the upper water layer of the Dead Sea as was only observed twice in the recent past, once in 1980 and between 1992 and 1994 (Oren et al. 1995).

Cyanobacteria and diatoms are phototrophic primary producers and are commonly found in a variety of hypersaline environments including lagoons, salt lakes, solar salt ponds or hypersaline sulphur springs (Nübel et al. 1999; Nübel et al. 2000; Wieland and Kühl 2000a; Ionescu et al. 2007; Oren 2012; Farías et al. 2013). Although some cyanobacterial and diatom strains from the Dead Sea were obtained in enrichment cultures at a time when the salinity of the lake was lower than today (Elazari-Volcani, 1940; 1944), cyanobacteria or diatoms have never been observed directly in the Dead Sea neither in the water nor in sediment samples.

Recently, a system of subsurface freshwater springs was discovered in the Dead Sea (Ionescu et al. 2012). These springs are located on the west coast of the lake and emerge at depths between 2 and 30 m and possibly even deeper. The salinity of the pure spring water (4–88 g L^{-1} TDS) is significantly lower than the surrounding DSW. At certain locations where the spring water slowly seeps out of the sediment we discovered dense microbial mats which are visually dominated by either diatoms or unicellular cyanobacteria. We suggested earlier that one reason for their development is probably the formation of reduced salinity microenvironments on sediments (Häusler et al. 2014). However, variations in spring water flow velocity suggested that the organisms are exposed to substantial salinity fluctuations in the time range of minutes to hours (Häusler et al. 2014). So far we were not able to demonstrate under which condition these organisms are active and if they are indeed able to survive the proposed substantial short-term salinity fluctuations. Thus, using in situ and ex situ microsensor measurements we now show that these phototrophs are active in their natural environment and are able to survive substantial, fast salinity fluctuations. We partially describe their phylogenetic affiliation and speculate on the reasons for the spatial separation.

Materials and methods

Location, spring water sampling and chemistry

Measurements and sampling of diatom and cyanbacterial microbial mats were performed in November 2012. Water sampling procedure and determination of the physicochemical parameters of the spring water were done as described in Ionescu et al. (2012), where also a detailed description of the system can be found.

In situ measurements

In situ O_2 measurements in a diatom mat were conducted using a Clark-type oxygen microelectrode (tip diameter 20 µm) with a guard cathode (Revsbech and Jørgensen 1986) connected to the diver-operated microsensor system, DOMS (Weber et al. 2007). A set of three profiles were measured every 45 min over a period of 23 h. The signal obtained in air saturated DSW represented the O_2 concentration corresponding to 100 % air saturation and was determined using a modified Winkler protocol for Dead Sea water (Nishri and Ben-Yaakov 1990) at in situ water temperature. The reading in anoxic Dead Sea water and anoxic fresh water (prepared by dissolution of 0.1 g Sodiumdisulfite in 50 ml water) as well as in the anoxic layers of the mat was the same, and taken as zero O_2 (Wieland and Kühl 2000b). Each set of profiles obtained was visualized and interpolated using the sigma plot software (Systat software inc). Light intensity during the measurement was monitored with a photosynthetic active radiation light logger (Odyssey, Data flow systems, Christchurch, New Zealand) located at the site of measurement. Spectral light availability was measured with a USB 4000 spectrometer (Ocean Optics, Duiven, The Netherlands) connected to the DOMS (Weber et al. 2007). A depth profile of spectral light was measured by recording spectra every meter according to the dive computer Elite T3 (Aeris, San Leonardo, USA). Depth was corrected for density related pressure differences.

Ex situ microsensor measurements

Ex situ O_2 microsensor measurements were also conducted with the DOMS in the same way as described above on retrieved cores of a cyanobacterial and diatom mat. The pore- and overlying water salinity corresponded in both cores to 80 % of DSW (determined by weighing a known volume and comparison to a calibration curve). During sampling the diatom mat got pushed approximately 1 cm into the sediment, to the core wall. Nevertheless, illumination could be applied through the transparent core wall using a Schott lamp (KL 2500 LCD, SCHOTT) at an intensity of 30 µmol photons m^2 s^{-1}.

Culture conditions and partial characterization

The same cores used for ex situ microsensor measurements served as inoculum for enrichment cultures. The culture medium was prepared on a basis of sterile filtered (0.2 µm filter, Millipore) DSW diluted with deionized sterile water

to a final concentration of 30 % DSW. The cyanobacterial and diatom media were supplemented with all components of the BG 11 medium (Rippka et al. 1979) except $MgSO_4$ and $CaCl_2$ or of the f/2 medium (Guillard and Ryther 1962), respectively. Cultures were grown at 28 °C under white fluorescent light with an intensity of 30 µmol photons m^2 s^{-1} (corresponding to in situ light intensity) with a light dark cycle of 12 h. Growth occurred within 3–4 weeks and each culture contained either unicellular cyanobacteria or diatoms with the same morphology as observed from in situ samples. To test growth in different salinities, the obtained enrichment cultures served as inoculum for media containing increasing concentrations of DSW between 5 and 100 % DSW (tested in duplicates with a resolution of 10 % between 10 and 100 % DSW media). pH ranged from 7.12 in 5 % DSW to 5.95 in 100 % DSW media. Artificial biofilms were obtained by placing sterile GFF filters (4 h at 400 °C) in the cultures until an algal microbial mat developed after 5–6 weeks. The ability to survive prolonged exposures to pure DSW was tested by incubation of the artificial biofilms in pure DSW for 1 week and afterwards transferring them to low saline media. Growth was always determined by visual observation after 4–6 weeks.

Light microscopy and scanning electron microscopy (SEM)

Environmental and culture samples were examined using a Zeiss Axioplan light microscope with a 100 W Hg lamp and filter sets for detecting chlorophyll auto fluorescence. SEM images of the diatoms were obtained by filtration of the samples on Ag/Pd pre-coated filters (0.2 µm, Millipore) and examination in a Quanta 250 FEG scanning electron microscope (FEI, Hillsboro, Oregon, USA).

16S rRNA gene clone libraries and phylogenetic analysis

DNA was extracted from a 1.5 ml sample from the initial 30 % DSW cultures of the cyanobacteria and diatoms using a phenol chloroform protocol identical to the one described in detail in Ionescu et al. (2012). Cyanobacterial 16S rRNA and diatom 18S rRNA genes were amplified by polymerase chain reaction (PCR) using the primer pairs CYA106F/CYA781R (Nübel et al. 1997) and EUK A/B (Medlin et al. 1988), respectively. The 50 µl PCR reaction contained 100 ng of template DNA, 0.5 µM of each primer, 0.2 mM of dNTPs, 0.5 units of DreamTaq DNA polymerase and the respective buffers (Thermo Scientific, Germany). PCR's were performed in a thermo cycler (Mastercycler, Eppendorf, Germany) using the following programs. For Cyanobacteria: 5 min at 94 °C, followed by 35 cycles of 45 s at 94 °C, 45 s at 60 °C and 45 s at 72 °C; followed by 10 min final extension at 72 °C. For diatoms: 5 min at 94 °C, followed by 35 cycles of 1 min at 94 °C, 1 min at 55 °C and 2 min at 72 °C; followed by 10 min final extension at 72 °C and 1 cycle 10 min at 15 °C. A clone library was prepared from the obtained PCR amplicons using the TOPO® TA Cloning® Kit (pCR4-TOPO, Invitrogen, Karlsruhe, Germany) followed by sequencing of the inserts using the BigDye Terminator v 3.1 Cycle sequencing Kit (Applied Biosystems, Foster City, USA) according to the manufactures manual. The obtained sequences were analyzed using the program Sequencher 4.6 (Gene Codes Corp.) and afterwards the sequences were compared to the NCBI database using BLAST (Altschul et al. 1990).

Short-term salinity response on oxygenic photosynthesis

To evaluate the short-term response of the organisms to changing DSW concentrations, O_2 production was taken as proxy for cell damage while manipulating the DSW concentrations in the media. Specifically, artificial biofilms were grown on GFF filters as described above and then transferred to a small flow chamber (1 cm × 0.5 cm) to which medium was continuously supplied using a peristaltic pump (MINIPULS® 3, Gilson). Light was provided in all experiments from a Schott lamp (KL 2500 LCD, SCHOTT) at an intensity of 30 µmol photons m^2 s^{-1} (corresponding to maximum in situ light irradiance and growth conditions). A Clark-type oxygen microelectrode (tip diameter 20 µm) with a guard cathode (Revsbech and Jørgensen 1986) was placed inside the biofilm at the O_2 production peak and O_2 production was determined by the light–dark shift method (Revsbech et al. 1981) in triplicates. The microsensor was calibrated for O_2 at each salinity using the reading obtained in the oxygenated water column of the flow chamber as the 100 % value. The 0 % value was determined using media bubbled with N_2 gas. The corresponding O_2 concentration for the different media was calculated using empirical values determined from Winkler titration as was done for the in situ microsensor measurements.

Two experiments were conducted simulating short-term spring water flow scenarios: (1) A gradual decrease in spring water flow and thus an increase in DSW concentration followed by a gradual increase in spring water flow to the initial salinity. (2) A sudden stop of spring water flow and thus an immediate exposure to pure DSW followed by an instant reestablishment of the original flow. The first scenario was simulated by increasing the concentration of DSW in the medium gradually over time in 10 % DSW steps every 15 min up to 100 % DSW and

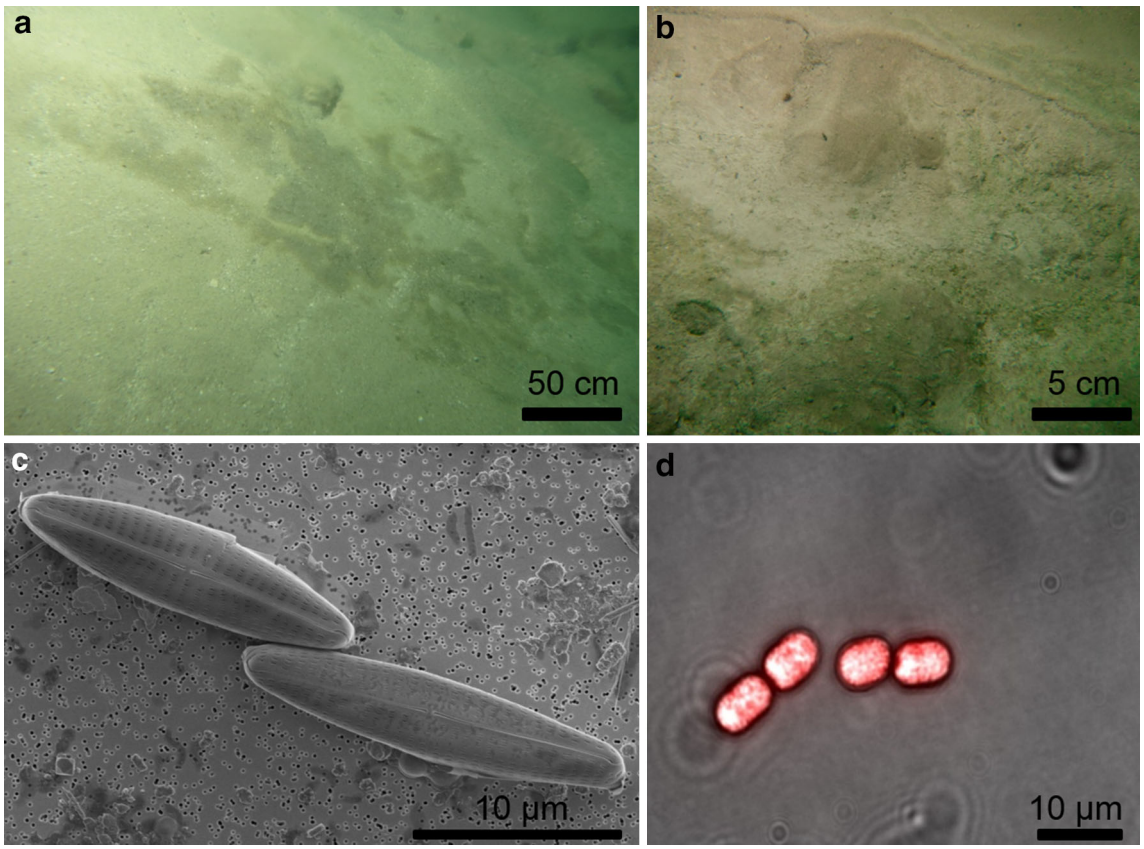

Fig. 1 **a**, **b** In situ images of the two types of microbial mats observed in the Dead Sea where spring water seeps out of the sediment. The brownish microbial mats spreading over several square meters (**a**) are dominated by pennate diatoms with the main morphotype shown in a SEM image (**c**). The greenish mats only cover small patches of sediment (~5 cm in diameter) and are dominated by unicellular cyanobacteria as shown in a light microscope image (**d** autofluorescence image)

then gradually decreased again to the starting salinity or lower. This was done using a diatom or cyanobacterial artificial biofilm grown at either a high (40 % DSW) or low salininty (5 % DSW) The second scenario was simulated by exposing biofilms of either cyanobacteria or diatoms which were grown at high salinity (40 % DSW), to a sudden exposure to 100 % DSW for 15 min following an immediate downshift in salinity back to 40 % DSW. The recovery was monitored over time. Although the fluctuations might be even more rapidly than 15 min, this was the minimum time required for medium exchange and microsensor signal stabilization. When the salinity was changed in both experiments, the old medium was removed by a syringe until only little medium was left. Then the flow cell was flushed at least 3 times with the new medium and gross photosynthesis was determined at the end of each shift to allow for microsensor signal stabilization. To ensure comparable rates the sensor was not moved during the experiment. All experiments were done in duplicates with each replicate experiment conducted with a new biofilm which was previously not exposed to salinity shifts.

Results

The diatom mat investigated in situ was roughly 0.5–1 mm thick and could be clearly distinguished from the sediment by a brownish color (Fig. 1a). It spreads over several square meters on the sediment surface. In contrast, the cyanobacterial mats were greenish in appearance and their distribution was limited to patches of a few square centimeters (Fig. 1b). These mats were either dominated by pennate diatoms (Fig. 1c) or unicellular cyanobacteria (Fig. 1d), respectively. Both types of microbial mats could be found at similar water depths between 3 and 10 m and only occurred where slow spring water seepage was observed.

The physical and chemical water properties of the pure spring water sampled from the diatom mat are summarized and compared to the Dead Sea in Table 1. The spring water is warmer and significantly less saline and less acidic than the DSW. Silica and dissolved inorganic carbon (DIC) concentration in the spring water were four to five times higher than in DSW whereas nitrate and phosphate were in the same range. Dissolved organic matter (DOM) could be

Table 1 Physico-chemical parameters of the Dead Sea and the spring water seep where the diatom mat was observed

Sample	Temp (°C)	pH	TDS (g l^{1})	K^+ (mM)	Na^+ (mM)	Ca^+ (mM)	Mg^{2+} (mM)	Si(II,IIII) (mM)	DOC (µM)	DIC (mM)	NO_3^- (µM)	PO_4^{3-} (µM)
Dead Sea	24.9	6.16	338	201	1460	508	1952	0.08	1366	1.05	3–8[a]	0.2[a]
Diatom seep	29.1	6.93	59	38	259	83	329	0.34	330	5.56	1.29	0.23

[a] Values taken from Stiller and Nissenbaum (1999)

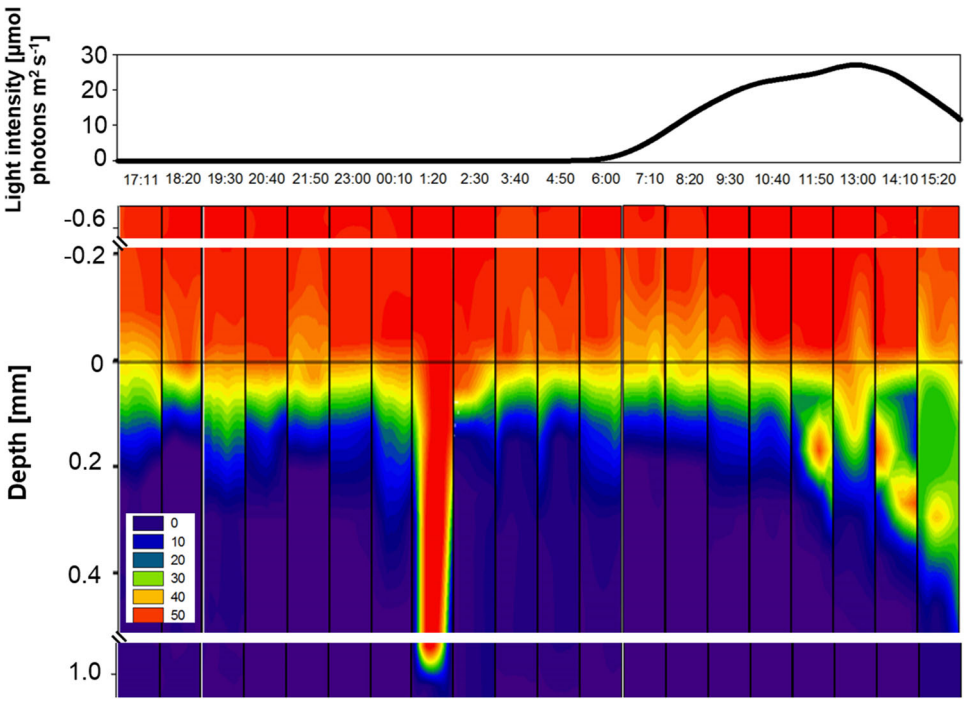

Fig. 2 In situ O_2 profiles measured in the diatom mat. *Each panel* represents a set of three subsequent profiles measured each hour. *X* and *Y*-axis corresponds to time and depth, respectively. O_2 concentrations are *color-coded* in µM (see *color bar* on the *lower left*). Fluctuating O_2 penetration depth indicates fluctuating spring water flow. Oxygen evolution is detected at high light in the *upper* mat surface as indicated by the incident light intensities shown in the *top graph*

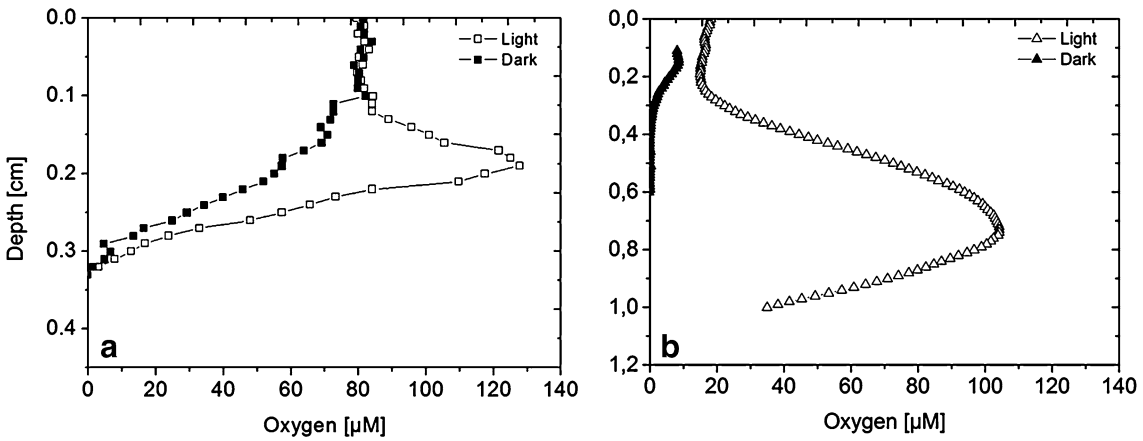

Fig. 3 Dark and light (30 µmol photons m^{-2} s^{-1}) O_2 profiles measured ex situ in a cyanobacterial dominated microbial mat (**a**) and diatom dominated microbial mat (**b**). The salinity in the overlying and porewater corresponded to 80 % of Dead Sea water salinity. Extensive O_2 production could be detected in both microbial mats

detected in higher concentrations in the Dead Sea. Due to technical complexities, no pure spring water from the cyanobacterial mats could be obtained. Nevertheless, we expect no large differences between the pure spring waters in the diatom and cyanobacterial site since most springs sampled previously in the system had similar physico-chemical properties (Ionescu et al. 2012).

Long-term in situ O_2 profiling in the diatom mat, revealed a highly dynamic system (Fig. 2). Oxygen concentration decreased sharply at the mat surface from 49 µM in the overlying water to anoxic conditions inside the sediment. Oxygen penetration depth fluctuated between 0.1 and 0.2 mm with a single exception of 0.9 mm in the night. When incident light was above 25 µmol photons m^{-2} s^{-1}, O_2 was gradually detected up to 0.5 mm in the sediment. Ex situ measurements conducted less than 3 h after sampling on both mats showed extensive O_2 production upon illumination (Fig. 3) at a salinity corresponding to 80 % of DSW.

Both organisms could be enriched in culture. Although more diatom morphotypes were observed in the in situ sample, the dominating one had the same morphology as those in our enrichment cultures (Fig. S1). Only one cyanobacterial morphotype was present in the in situ sample and corresponded to the enrichment cultures. Limited growth was observed in diluted non-amended DSW and growth was strongly stimulated by the addition of nutrients. Diatoms grew between 5 and 40 % DSW whereas cyanobacteria between 5 and 70 % DSW. When high saline grown (40 % DSW) artificial biofilms of both organisms were exposed for 1 week to 100 % DSW and subsequently transferred back to the original growth medium only the cyanobacterial culture re-grew.

Partial 16S rRNA gene sequences obtained from the cyanobacterial culture were 97 to 98 % identical to *Euhalothece* sp., strain MPI 96N304. The partial 18S rRNA gene sequences obtained from the diatom culture were 96–98 % identical to *Navicula salinicola.* Morphological identification using the SEM images of the diatoms confirmed the classification to the *Navicula* genus (M. Edlund, personal communication).

The in situ bacterial diversity from the diatom mat is presented in Fig. S2 and will not be further discussed. Similar data from the cyanobacterial mats is not available.

Oxygen evolution was linearly reduced in all artificial biofilms with increasing DSW concentrations in the media (Fig. 4a, b). Biofilms grown at high salinity (40 % DSW) were able to perform photosynthesis over the complete salinity spectra; however, in pure DSW the cyanobacterial mat and the diatom mat reached only up to 3 and 0.5 % of the initial value, respectively. Upon consequent exposures to lower DSW concentrations, O_2 evolution immediately recovered. When the starting salinity was reached, 90 min after the exposure to 100 % DSW, the cyanobacterial photosynthetic activity recovered completely whereas the diatoms only reached between 40 and 60 % of their initial value. When the salinity was further decreased down to 5 % DSW in the medium, cyanobacterial activity decreased once more, whereas that of the diatoms stayed relatively constant between 60 and 80 % of the initial value. In contrast to the high saline grown biofilms O_2 production in the low saline adapted biofilms (5 % DSW) could only be detected until 60 % of DSW in the media. Recovery of the photosynthetic potential was delayed in the diatom biofilm and no recovery was observed in the cyanobacterial biofilm (Fig. 3b) not even after 12 h.

A rapid salinity shift applied to both high saline adapted cultures resulted in an immediate inhibition of O_2 production in both organisms (Fig. 4c). Initial recovery of photosynthetic activity was equal in both cultures. However, within 90 min after the exposure to pure DSW the diatoms completely recovered their initial photosynthetic activity, whereas the cyanobacterial activity only recovered to about 40 % of the initial value in the same time frame and did not increase further within the following 12 h.

Discussion

In this study we provide evidence for active cyanobacteria and diatoms in the Dead Sea. In addition to in situ measurements of O_2 production in the diatom mat under ambient light (Fig. 2), photosynthetic activity could be demonstrated for both mat forming organisms in freshly collected samples upon illumination (Fig. 3). We discuss here the possible reasons for the development of these dense phototrophic microbial mats in the Dead Sea and the ecological implications of the stress exerted on the organisms by the salinity fluctuations in this extreme environment.

The microbial mats were only found in areas where spring water seepage was observed. This strongly suggests that one reason for the development of these mats is a mean, local, salinity reduction of the Dead Sea water by the significantly less saline spring water (Table 1). The formation of such reduced salinity environments was recently demonstrated from flume experiments mimicking the spring water flow (Häusler et al. 2014). This is further supported by the fact that neither diatoms nor cyanobacteria could grow in pure DSW and only grew in diluted media. The salinity growth range observed for the diatoms between 5 and 40 % DSW (1.75–14 %, weight of TDS per volume) is in the range of the salinity tolerance observed for other halotolerant diatoms between 0.5 and 15 % TDS (Clavero et al. 2000). In contrast to the diatoms, the cyanobacterial enrichments were more salt tolerant and

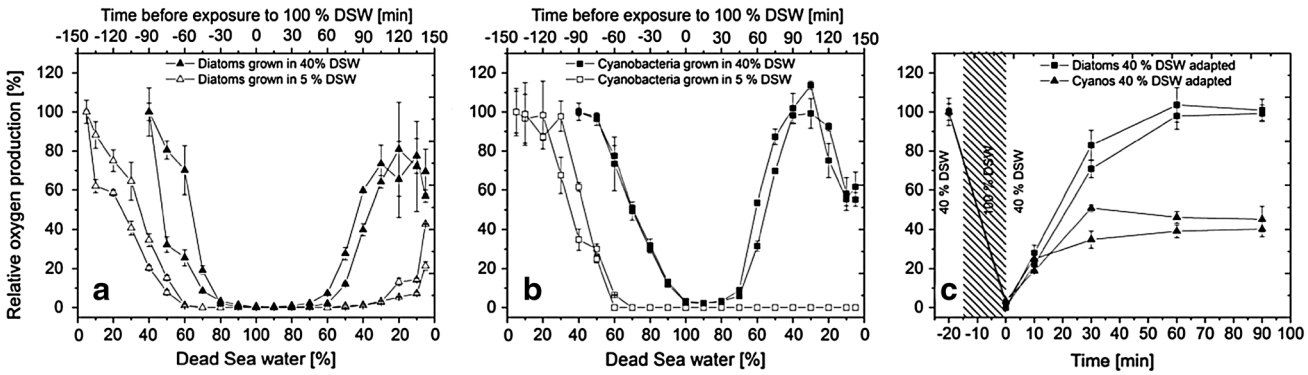

Fig. 4 Gross photosynthetic activity response of diatom (**a**) and cyanobacterial (**b**) artificial biofilms grown in 5 % DSW (Dead Sea water) media and 40 % DSW media to stepwise increase in salinity to 100 % DSW and subsequent decrease to starting salinity. **c** Recovery of photosynthetic activity in 40 % DSW media of 40 % DSW grown artificial biofilms of cyanobacteria and diatoms exposed to 15 min pure DSW

grew in media containing 5 and 70 % DSW (1.75–24.5 % TDS). Garcia-Pichel et al. (1998) previously showed that cyanobacteria which are closely related to the *Euhalothece* cluster are able to grow at salinities between 1.5 % to NaCl saturation (approximately 28–34 % TDS). Elevated concentration of Mg^{2+}, which is the main cation in pure DSW (Oren 2010), is likely to be the reason why growth of the cyanobacteria is not observed at higher concentrations of DSW. $MgCl_2$ is a highly chaotropic salt, known to denature cellular macromolecules at concentrations already below 1 M (Hallsworth et al. 2007).

Local salinity reduction is the main reason for the growth of the biofilms but the supply of nutrients by the spring water is beneficial as well. Only limited growth of both organisms could be observed in non-supplemented diluted DSW. The addition of nutrients to the same media increased the growth of both the diatoms and the cyanobacteria. The spring water is enriched in silica (Table 1) which is necessary for the synthesis of the diatom's frustules and is considered a major limiting nutrient for diatom growth (Martin-Jézéquel et al. Martin-Jézéquel et al. 2000). Phosphate and nitrate are present in similar concentrations in the spring water and in the Dead Sea and thus extra supply by spring water does not play a role for the presence of mats at the springs. Many phototrophic organisms possess a carbon concentrating mechanism (CCM) to achieve high intracellular CO_2 concentrations and prevent oxygenase activity of the RubisCo and thus can also thrive at low DIC concentrations (Kaplan and Reinhold 1999; Giordano et al. 2005). However, the supply of high concentrations of DIC (5.5 mM, Table 1) from the spring water might also be beneficial, leading to energy saving by the down regulation of the CCM as suggested for diatoms (Hopkinson et al. 2011). The effects of the different nutrients supplied by the spring water were not investigated separately. Nevertheless, the reasons for the development of the phototrophic microbial mats in the underwater springs of the Dead Sea may not be substantially different from those leading to *Dunaliella* blooms in the water column, namely a reduction in salinity and the input of nutrients (Oren et al. 1995).

The proposed exposure of the microbial mats to fluctuating salinity, as indicated by previous variable flow measurements (Häusler et al. 2014) and observations by scuba divers (Ionescu et al. 2012), is also evident in the in situ microsensor measurements (Fig. 2). Oxygen penetration depth measured every hour in the diatom mat varied and even displayed a sudden deepening at night. Since the spring water is anoxic and photosynthesis is absent at night, the only explanation for the increased O_2 penetration into the sediment is a reduced spring water input and subsequent sinking of heavier, oxygenated Dead Sea brine into the sediment. Thus the organisms inhabiting the Dead Sea underwater springs have to deal with increasing and decreasing salinities in a matter of minutes to hours. From these measurements the degree of salinity fluctuations cannot be inferred but under the most extreme case the salinity could occasionally increase to pure DSW as simulated in our experiment (Fig. 4).

Considering the different tolerance and response to changes in ambient salinity of the diatoms and cyanobacteria, a first picture emerges about their microenvironments allowing us to hypothesize about the observed distribution of the microbial mats in the Dead Sea. The average salinity, the organisms are exposed to in situ, is likely to be at the upper level that allows for growth, as only cells adapted to high salinity are acclimated to cope up with extensive salinity fluctuations. This conclusion is further supported by the observation that both organisms could perform photosynthesis at 80 % DSW shortly after collection (Fig. 2). This was only observed in artificial biofilms grown at high salinity (Fig. 4a, b). The absence of

diatoms in the cyanobacterial microbial mats could result from a mean higher salinity which is too high to allow for diatom growth (50–70 % DSW) or from long exposures (days to weeks) to DSW which can only be tolerated by the cyanobacteria. As will be discussed in the following paragraphs, one reason for the absence of cyanobacteria in the diatom mats could result from frequent, and sudden short-term fluctuations which are better tolerated by the diatoms and thus may allow them to outcompete the cyanobacteria in such areas.

The salinity fluctuations caused by the varying flow regime of the springs exert an extreme stress on the organisms in the mat as can be deduced from the decreasing photosynthetic activity measured in the artificial biofilms exposed to increasing DSW concentrations (Fig. 3). This is in accordance to the general stress effect observed for cyanobacteria and algae exposed to substantial increase in external salt concentrations using NaCl (Kirst 1990; Sudhir and Murthy 2004; Allakhverdiev and Murata 2008). Osmotic water loss and an increase of intracellular ion concentration impair cellular processes like photosynthesis by the disturbance of the well-balanced intracellular water activity and ion homeostasis. This effect can be reversible upon exposures to low salinity whereas if the exposure to high salinity is too long, Na^+ ions can irreversibly damage photosystems (PS) I and II due to the dissociation of extrinsic proteins (Allakhverdiev et al. 2000; Allakhverdiev and Murata 2008). The effect of the unique salt composition of DSW with its high concentrations of Mg^{2+} and Ca^{2+} on PS I and II is unknown and warrants further investigations. Nevertheless, the observed reversibility of the photosynthetic inhibition in high saline adapted cells (Fig. 3a, b) implies that protective measures against ionic damage were already present in these cells due to their growth salinity. Among these protective measures are likely an increased concentration of compatible organic osmolytes which are known to protect the photosystems from ionic damage (Murata et al. 1992; Papageorgiou and Murata 1995; Ohnishi and Murata 2006), unsaturation of fatty acids in membrane lipids, which may lead to e.g. enhanced Na^+/H^+ antiporter activity (Allakhverdiev and Murata 2008), as well as changes in the proteome (Bhargava and Srivastava 2013). Hence, high saline adapted cells are better adapted to withstand salinity fluctuations.

Comparing the different response of the high saline grown cyanobacteria and diatoms to a gradual or rapid salinity change suggests that the cyanobacteria are able to withstand longer exposures to high DSW concentrations without damage (at least 165 min in DSW concentrations higher than the adaptation salinity; Fig. 4a, b). This is further evident from the cyanobacterial biofilm which can re-grow in low saline media after being exposed for 1 week to pure DSW, whereas the diatoms do not. In contrast, the diatoms can recover faster than the cyanobacteria after a sudden exposure to DSW (Fig. 4c). When the cyanobacteria were exposed to a sudden increase in external salt concentration it probably led to a high intracellular ion concentration which inhibited ion export systems (e.g. Na^+/H^+ antiporters; Allakhverdiev and Murata 2008). The export systems may have been still functioning in the gradual salinity shift, thereby keeping the intracellular ionic concentration relatively low and thus preventing photosystem damage. This may explain why the cyanobacteria could recover completely their photosynthetic potential within 90 min after the exposure to pure DSW in the gradual salinity shift, but not in the same timeframe after the sudden DSW shock. In the case of diatoms, the exposure time of 15 min to pure DSW was too short to damage the photosynthestic machinery as occurred in the longer exposure to higher salinities resulting from the gradual shifts (Fig. 4b, c). The compartmentalization of eukaryotic cells (chloroplasts, nucleus, mitochondria, etc.) and different permeability of ions of the distinct membranes, could lead to a better protection against short-term exposures to high salinities. Vacuoles, known to be present in *Navicula* species (Round et al. 1990), could also have a role in the recovery from a short-term, extreme salinity shock by compartmentalizing Na^+ from the cytoplasm as shown for red algae and plants (Mostaert et al. 1996; Parks et al. 2002). Thus, due to the more complex structure diatoms could have an advantage over the cyanobacteria in a spring environment exposed to sudden extreme short-term salinity shifts. In addition, the diatoms could have an advantage in hypo-osmotic conditions where ionic damage does not occur (e.g. when the spring water flow is higher than average). No apparent additional loss of O_2 production was observed when the high saline grown diatoms were subjected to hypo-osmotic treatment (below 40 % DSW in this case; Fig. 4a, b), whereas the cyanobacterial O_2 production activity was again impaired during these conditions. A similar resistance of photosynthetic activity to hypo-osmotic conditions was observed in microalgae with strong cell walls (Hellebust 1985). Strong cell walls are likely to prevent bursting of the cells under hypo-osmotic conditions (Bisson and Kirst 1995).

Growth of both organisms is limited to a water depth <10 m, although springs are observed down to at least 30 m (Ionescu et al. 2012). Light is strongly attenuated with depth in the spring's area (Fig. S3) reaching an intensity of about 30 μmol m^2 s^{-1} at 10 m (Fig. 2); however, benthic diatoms were reported to thrive at much lower light levels at continental slopes (McGee et al. 2008). Thus, the light level or quality (Fig. S3) in deeper areas might not be sufficient to balance the high energy required for osmoregulation (e.g. osmolyte synthesis and energy

consuming cation efflux systems) and/or protein synthesis (Allakhverdiev et al. 2005). Indeed, the diatoms seem to be light limited as in situ O_2 evolution was only detected close to the maximum in situ light intensity of 30 µmol photons m^{-2} s^{-1} (Fig. 2). However, the role of light with respect to the salinity tolerance needs to be determined separately.

We here demonstrate the existence of active, extreme halotolerant phototrophic organisms inhabiting freshwater springs in the Dead Sea which can tolerate short-term exposures to an unusual salt composition beyond their growth tolerance and rapidly recover their photosynthetic potential. We presented evidence that the local regime of salinity fluctuations determines which of the two types of organisms dominates. As compared to other microbial environments exhibiting salinity fluctuations in the orders of hours to days like intertidal microbial mats (Kohls et al. 2010; Stal 2012), the fast and extreme salinity fluctuations in the spring system discovered in the Dead Sea are unique. Furthermore, the ability of these organisms to recover their photosynthetic potential after the exposure to pure DSW is remarkable. In addition to 1.5 M Na^+, pure DSW contains high concentrations of divalent cations (2 M Mg^{2+} and 0.5 M Ca^{2+}) which have a more chaotropic (destabilising) potential on biological macromolecules than monovalent ions (Cacace et al. 1997; Hallsworth et al. 2007; Oren 2013). Thus, the exposure to pure DSW must be even more damaging to cellular macromolecules than pure NaCl solutions which are used for all other studies mostly in moderate concentrations (Kirst 1990; Sudhir and Murthy 2004; Bhargava and Srivastava 2013). It will be fascinating to explore in detail the molecular response of these organisms to these extreme salinity shifts.

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