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The Dead Sea – alive again

A. Oren

Division of Microbial and Molecular Ecology, The Alexander Silberman Institute of Life Sciences, and the Moshe Shilo Center for Marine Biogeochemistry, The Hebrew University of Jerusalem, 91904 Jersualem (Israel)

Abstract. In the thirteen years of quantitative studies on the microbiology of the Dead Sea from 1980 onwards three distinct periods can be discerned. Mass development of the green unicellular alga *Dunaliella parva* (up to 8,800 cells/ml) and red archaeobacteria (2×10^7 cells/ml) was observed in 1980, following a dilution of the upper water layers by rain floods. This bloom disappeared at the end of 1982 as a result of a complete mixing of the water column. During the period 1983–1991 the lake was holomictic, and no *Dunaliella* cells were observed. Viable bacteria were present during this period in very low numbers. Heavy rain floods during the winter of 1991–1992 caused a new stratification as the upper five meters of the water column became diluted to 70% of their normal salinity. In this upper water layer *Dunaliella* reappeared (up to 3×10^4 cells/ml at the beginning of May, rapidly declining to less than 40 cells/ml at the end of July), and a bloom of red archaeobacteria (3×10^7 cells/ml) once more imparted a red coloration to the lake.
Key words. Dead Sea; Halobacteriaceae; *Dunaliella.*

Since the pioneering work of Elazari-Volcani in the late 1930's/early 1940's[19], a wealth of information has accumulated on the microbiology of the Dead Sea. A considerable number of halophilic and halotolerant microorganisms have been isolated from the lake, and many of these have been extensively studied[9]. However, no quantitative studies on the microbial ecology of the lake have been performed prior to 1980, with the exception of a study of bacterial and algal community sizes during the years 1963–1964[4]. This lack of data is to be regretted in view of the fact that the properties of the Dead Sea as an ecosystem have changed considerably during the years. The water balance has been negative since the beginning of this century, and this has caused profound changes in salt concentrations, ionic composition, and the physical structure of the water column.

In all quantitative studies on the aerobic water column of the Dead Sea the dominant organisms were found to be unicellular green algae (*Dunaliella parva*), and several types of red halophilic archaeobacteria, microscopically seen as flat irregular cells[4,9].

In its present state the water of the Dead Sea, because of its high salinity and the peculiar ionic composition (dominance of divalent cations), does not support actively growing communities of microorganisms, and these develop only in those rare cases in which large amounts of rain water cause a substantial dilution of the upper water layer. Such an event was witnessed twice during the thirteen years – from 1980 onwards – in which systematic quantitative studies on the microbial ecology of the Dead Sea have been performed. Thus three distinct periods can be discerned: 1) the microbial bloom that developed in the summer of 1980, and remained present till the end of 1982, 2) the period 1983–1991, in which conditions suitable for the development of microbial blooms did not occur, and 3) a new bloom in the spring-summer of 1992.

The bloom of 1980

Massive rain-induced floods in the winter of 1979–1980 caused the formation of a relatively diluted surface water layer, separated from the more saline brines by a pycnocline at a depth of 10–25 m. This stratification remained for 3 years. A mass development of the green unicellular alga *Dunaliella* (up to 8,800 cells/ml)[15] and bacteria (2×10^7 cells/ml)[6] was observed in the summer of 1980, restricted to the upper water layer above the pycnocline. In this mixed layer algal and bacterial densities were quite uniform. The dense community of red archaeobacteria imparted a reddish color to the lake. The *Dunaliella* community declined rapidly at the end of the year, bacterial numbers also decreased at the end of 1980, but a stable community of about 5×10^6 cells/ml subsisted in the upper water layer for more than 2 years[6].

No similar blooms developed in the years 1981–1991. The reason for this can be understood from the results of simulation experiments in the laboratory and in outdoor tanks[16]. It was found that two conditions are required for the development of *Dunaliella* in the Dead Sea water: phosphate has to be available, and the salinity of the brine has to drop to values below 1.21–1.22 g/ml (compared with a value of about 1.235 in undiluted Dead Sea water). During the years 1981–1991 the specific gravity of the upper water layers of the Dead Sea never reached values below 1.22 g/ml, and *Dunaliella* did not grow. As the growth of the heterotrophic bacte-

rial community depends on organic matter produced by *Dunaliella*, the only primary producer in the lake, no new bacterial development was observed either.

The period 1983–1991

In late 1982 we witnessed a sudden decline in bacterial numbers in the upper water layers of the Dead Sea, from around 3–4 × 10^6 cells/ml to numbers much below 10^6 cells/ml[8]. This decline was a result of the final disappearance of the stratification of the lake that originated from the winter floods in 1979–1980, and the elimination of the separated shallow layer to which the bacterial community was formerly restricted.

During the period 1983–1991 the lake was holomictic, and overturn events in autumn occurred annually[1]. Not only did conditions never become favorable for the development of *Dunaliella* (and thus no new organic material became available from primary production processes), the conditions for the small bacterial community that remained present in the lake became ever more extreme. As a result of the continuing drop in water level halite precipitated from the lake, and the relative concentration of divalent cations steadily increased. The water activity of the brine during this period was estimated at about 0.67 and even lower[11]. In spite of these apparently highly unfavorable conditions, viable bacteria were present throughout this period in very low numbers, as was shown by measurements of incorporation of labeled organic compounds, such as glycerol and amino acids. Inhibitor studies showed that this bacterial community consisted of archaeobacteria[10,11]. Thus, for example, in December 1988 the number of particles microscopically resembling bacteria was about 8.4 × 10^5/ml, a number probably overestimating the real bacterial numbers. A low (but significant) rate of incorporation of amino acids and other radioactively labeled substrates subsisted, which was abolished by low concentrations of bile salts (which cause lysis of halophilic archaeobacteria). Amino acids incorporation was also completely inhibited by the inhibitor of halobacterial protein synthesis anisomycin[10].

The bloom of 1992

Unprecedented heavy rain floods during the winter of 1991–1992 caused a rise in the water level of the Dead Sea by almost 2 m. The lake became stratified again, with a pycnocline at a depth of 4–5 m, isolating an upper layer of water with a specific gravity of 1.17–1.18 g/ml during the months April–June. As expected from previous field observations[15] and simulation experiments[16], *Dunaliella* developed again in the Dead Sea, with peak population densities even higher than those attained in 1980 (fig. 1): values between 1.2 and 1.5 × 10^4 cells/ml were found throughout the upper water layer on May 20, 1992. In a single sample collected from the shore on May 4, 3.3 × 10^4 cells/ml were

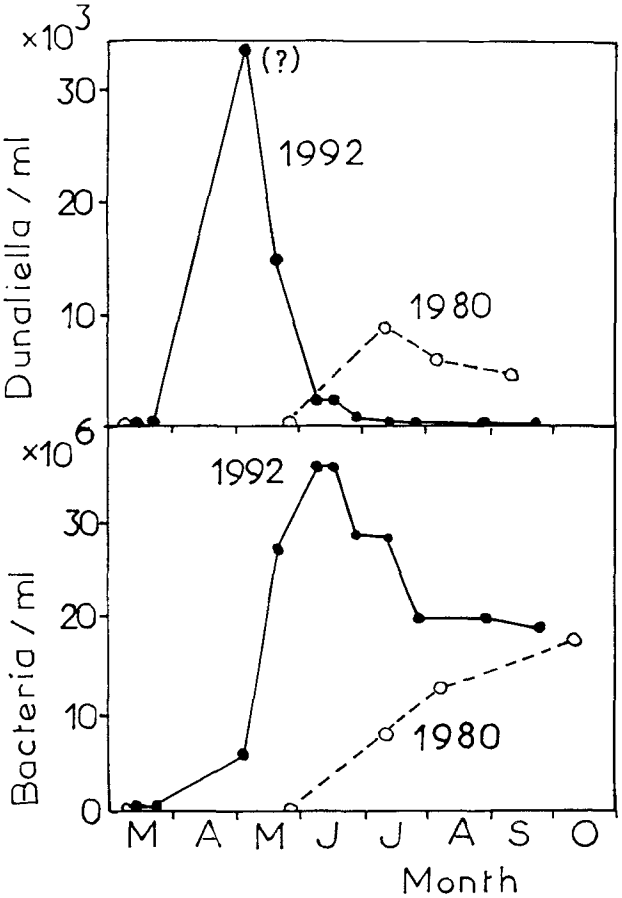

Figure 1. Average numbers of *Dunaliella* (upper panel) and bacteria (lower panel) in the upper 4–5 m of the Dead Sea water column in 1980 and in 1992. The peak in *Dunaliella* numbers of 3.3 × 10^4 cells/ml on May 4, 1992 was based on a single sample collected from the shore, and it is unknown to what extent this sample was representative for the surface water layer of the entire lake. For the enumeration of *Dunaliella* cells water samples were stained with iodine, cells were collected on Millipore filters, and counted microscopically on the filters. Bacteria were enumerated microscopically using a Petroff-Hauser counting chamber, if necessary after prior concentration by centrifugation.

counted, but this sample is not necessarily representative for the surface water layer of the entire lake. The 1992 *Dunaliella* bloom developed much earlier in the season than the 1980 bloom, reached higher peak values, but also declined much faster: at the end of June 1992 less than 40 cells/ml were left in the upper water mass (fig. 2). Concomitantly with the *Dunaliella* bloom red pleomorphic halophilic archaeobacteria multiplied to peak values of about 3 × 10^7 cells/ml (fig. 3), once more imparting a red coloration to the lake.

An unusual phenomenon, never reported before in the Dead Sea, was observed in late summer of 1992: after the decline of the *Dunaliella* population in the surface layer above the pycnocline, a renewed development of *Dunaliella* occurred, but this time at a depth of 6–10 m, at the lower end of the pycnocline (figs. 2, 4). The nature of this deep algal maximum is still far from understood: at these depths the light intensity is

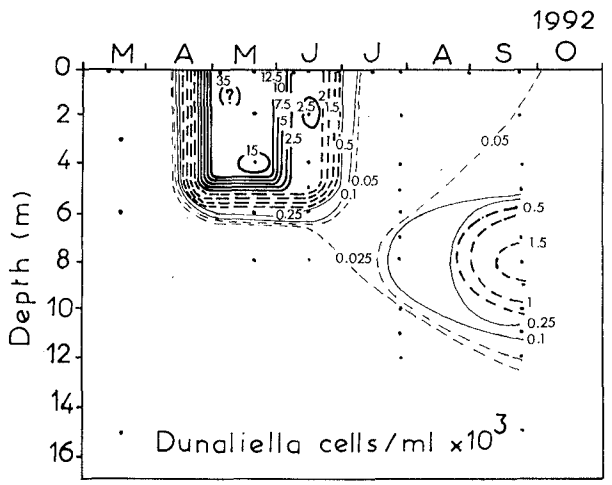

Figure 2. Seasonal and vertical distribution of *Dunaliella* in the Dead Sea, March–September 1992.

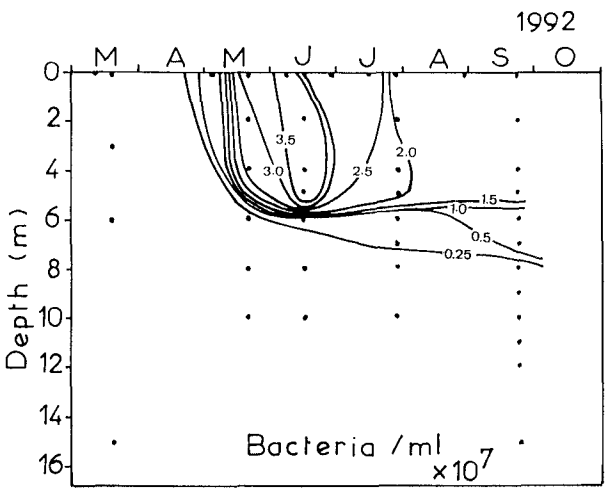

Figure 3. Seasonal and vertical distribution of bacteria in the Dead Sea, March–September 1992.

very low, due to the turbidity caused by the dense bacterial community present; measurements in 1980 showed a tenfold decrease in downwelling light intensity for every 2 m depth[15]. Moreover, simulation studies have shown that at the salinities present (specific gravity 1.22–1.23 g/ml and higher) growth of *Dunaliella* is negligible[16]. One hypothesis is that the development of the algae in the deeper layers may be related to the availability of nutrients – possibly phosphate. The possibility can also not be excluded that the motile *Dunaliella* cells maintain a diel migration, and may at times be found closer to the surface, where the salinity is lower and more light is available for photosynthesis. However, for logistic reasons sampling was performed only around noon.

Unanswered questions

The field observations summarized above lead to many questions, most of which remain unanswered at the

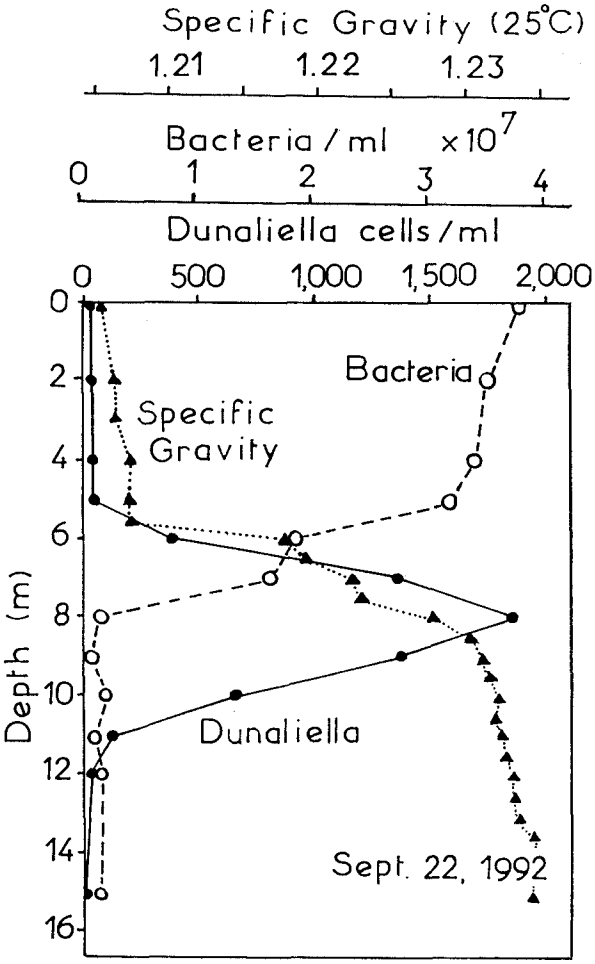

Figure 4. Vertical distribution of *Dunaliella* cells and bacteria relative to the pycnocline in the Dead Sea water column, sampled September 22, 1992, 8 km east of Ein Gedi.

present time, but which are amenable to experimental approaches. The following are only a few of the questions to be asked:

1) *Dunaliella* is the only primary producer in the Dead Sea, and the heterotrophic bacteria apparently develop at the expense of organic compounds released either by healthy and/or by degenerating *Dunaliella* cells. It is tempting to postulate that glycerol may be the main compound involved, as glycerol is synthesized and accumulated by *Dunaliella* cells as an osmotic stabilizer, and as glycerol is a substrate readily used by halophilic archaeobacteria. However, hardly any quantitative information exists on the availability and turnover of glycerol in the Dead Sea and other hypersaline environments.

2) Reliable estimates of primary production in the Dead Sea are still lacking. This is both due to logistic reasons, but also to more fundamental questions with respect to the methodology to be used. The commonly used methods to measure primary production are based on incorporation of $^{14}CO_2$, followed by filtration and

determination of the amount of label retained in the particulate fraction. These methods may lead to a gross underestimation of the primary production. *Dunaliella* cells are very fragile, and burst upon filtration and drying of the filters[3], thereby releasing the intracellular glycerol which may represent a substantial fraction of the newly produced organic carbon. The few attempts that have been reported in the literature to measure primary production in other hypersaline lakes dominated by *Dunaliella*[17] do not take this problem into account.

3) What are the factors that led to the formation of the secondary development of *Dunaliella* in the deeper water layers in the summer of 1992, and how do the algae there survive unfavorable conditions of exceedingly high salinities and suboptimal light intensities?

4) The Dead Sea has been an inoculum source for the isolation of representatives of all four recognized genera of neutrophilic aerobic halophilic archaeobacteria: *Halobacterium*[7], *Haloferax*[5], *Haloarcula*[13], and *Halococcus*[19]. Hardly anything is known on the contribution of the different genera and species to the bacterial community in the lake. Direct microscopic examination of Dead Sea water samples shows a dominance of pleomorphic flat cells (characteristic of the genera *Haloferax* and *Haloarcula*). It should be remembered, however, that *Halobacterium* isolates, when grown at suboptimal conditions, often lose their normal rod shape. Enumeration of colony-forming bacteria on plates, followed by characterization of the organisms developing, is of little use, as plating efficiency has been low in all cases examined. The highest viable counts obtained were bacteria of the type *Halobacterium sodomense*: two or more orders of magnitude lower than the microscopically determined bacterial numbers. The question of the nature of the dominant bacterium in the Dead Sea (presently under investigation) can possibly be solved using such techniques as lipid analysis (based on the differences in polar lipid composition of the different genera of halophilic archaeobacteria[18]), and/or ribosomal RNA sequence analysis.

5) The metabolism of monovalent ions (Na^+, K^+) in both *Dunaliella* and in halophilic archaeobacteria is well known. However, the mechanisms which enable them to cope with extremely high concentrations of divalent cations (Mg^{2+}, Ca^{2+}, about 1.8 M and 0.4 M, respectively), such as found in the Dead Sea brines, have hardly been investigated.

6) After the height of the microbial blooms in 1980 and 1992 a phase of decline was observed, in which bacterial and algal numbers in the lake decreased, often rapidly. What is the fate of the cells that disappear? Among the possibilities we can name autolysis, sedimentation to the bottom of the lake, and predation by protozoa. The last option is rather unlikely, as protozoa have rarely, if at all, been seen in Dead Sea water samples[9].

7) During the years 1983–1991 a small, but viable and active, community of halophilic archaeobacteria maintained itself in the Dead Sea in the absence of the primary producer *Dunaliella*. Hardly anything is known on the mechanisms of survival of these organisms during years of apparent nutrient starvation. One possibility is the use of the light-driven proton pump bacteriorhodopsin as a means to derive energy from sunlight. It was indeed shown that the bacterial community at the end of the 1980 bloom had a large content of bacteriorhodopsin[14].

Haloarcula marismortui, a Dead Sea isolate which was never shown to produce bacteriorhodopsin, was suggested to possess an inherent low need for maintenance energy, and its ion gradients across the membrane were reported to remain unchanged upon starvation[2].

Conclusions

Because of the paucity of field studies of hypersaline lakes, our understanding of the ecology of extremely halophilic microorganisms lags behind our knowledge on their physiology, biochemistry, and molecular biology. In most cases hardly anything is known on the natural abundance and the in situ physiology of the different halophilic organisms, whose properties we often know well in pure culture. In most cases the proper questions relevant to the existence of the organisms in their ecosystems have not yet been asked[12].

Life in the Dead Sea in its present state seems to depend primarily on unusual, 'catastrophic' events of abundant rainfall in its catchment area. Though it may be expected that a simple ecosystem like the Dead Sea, with only one primary producer, and one or a few – physiologically very similar – heterotrophic bacteria, should be relatively easy to understand, comparison of the two bloom events of 1980 and of 1992 shows that our real understanding of the factors underlying the development of the algal and bacterial communities is still extremely limited, in spite of the wealth of field and laboratory data that have been collected since indigeneous life was first discovered in the Dead Sea in 1936[20].

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1 Anati, D. A., and Shasha, S., The stability of the Dead Sea stratification. Israel J. Earth Sci. *38* (1989) 33–35.

2 Ginzburg, M., Ion metabolism in whole cells of *Halobacterium halobium* and *H. marismortui*, in: Energetics and Structure of Halophilic Microorganisms, pp. 561–577. Eds S. R. Caplan and M. Ginzburg. Elsevier/North-Holland Biomedical Press, Amsterdam 1978.

3 Goldman, J. C., and Dennett, M. R., Susceptibility of some marine phytoplankton species to cell breakage during filtration and post-filtration rinsing. J. exp. mar. Biol. Ecol. *86* (1985) 47–58.

4 Kaplan, I. R., and Friedmann, A., Biological productivity in the Dead Sea. Part I. Microorganisms in the water column. Israel J. Chem. *8* (1970) 513–528.
5 Mullakhanbhai, M. F., and Larsen, H., *Halobacterium volcanii* spec. nov., a Dead Sea halobacterium with a moderate salt requirement. Arch. Microbiol. *104* (1975) 207–214.
6 Oren, A., Population dynamics of halobacteria in the Dead Sea water column. Limnol. Oceanogr. *28* (1983) 1094–1103.
7 Oren, A., *Halobacterium sodomense* sp. nov., a Dead Sea halobacterium with an extremely high magnesium requirement. Int. J. syst. Bact. *33* (1983) 381–386.
8 Oren, A., The rise and decline of a bloom of halobacteria in the Dead Sea. Limnol. Oceanogr. *30* (1985) 911–915.
9 Oren, A., The microbiology of the Dead Sea, in: Advances in Microbial Ecology, Vol. 10, pp. 193–229. Ed. K. C. Marshall. Plenum Press, New York 1988.
10 Oren, A., Estimation of the contribution of archaebacteria and eubacteria to the bacterial biomass and activity in hypersaline ecosystems: novel approaches, in: General and Applied Aspects of Halophilic Microorganisms, pp. 25–31. Ed. F. Rodriguez-Valera. Plenum Press, New York 1991.
11 Oren, A., Bacterial activities in the Dead Sea, 1980–1991: survival at the upper limit of salinity. Int. J. Salt Lake Res. *1* (1992) 7–20.
12 Oren, A., Ecology of extremely halophilic microorganisms, in: The Biology of Halophilic Bacteria, pp. 25–53. Eds R. H. Vreeland and L. I. Hochstein. CRC Press, Boca Raton 1992.
13 Oren, A., Ginzburg, M., Ginzburg, B. Z., Hochstein, L. I., and Volcani, B. E., *Haloarcula marismortui* (Volcani) sp. nov., nom. rev., an extremely halophilic bacterium from the Dead Sea. Int. J. syst. Bact. *40* (1990) 209–210.
14 Oren, A., and Shilo, M., Bacteriorhodopsin in a bloom of halobacteria in the Dead Sea. Arch. Microbiol. *130* (1981) 185–187.
15 Oren, A., and Shilo, M., Population dynamics of *Dunaliella parva* in the Dead Sea. Limnol. Oceanogr. *27* (1982) 201–211.
16 Oren, A., and Shilo, M., Factors determining the development of algal and bacterial blooms in the Dead Sea; a study of simulation experiments in outdoor ponds. FEMS Microbiol. Ecol. *31* (1985) 229–237.
17 Stephens, D. W., and Gillespie, D. M., Phytoplankton production in the Great Salt Lake, Utah, and a laboratory study of algal response to enrichment. Limnol. Oceanogr. *21* (1976) 74–87.
18 Torreblanca, M., Rodriguez-Valera, F., Juez, G., Ventosa, A., Kamekura, M., and Kates, M., Classification of non-alkaliphilic halobacteria based on numerical taxonomy and polar lipid composition, and description of *Haloarcula* gen. nov., and *Haloferax* gen. nov. Syst. appl. Microbiol. *8* (1986) 89–99.
19 Volcani, B. E., The microorganisms of the Dead Sea, in: Papers Collected to Commemorate the 70th Anniversary of Dr. Chaim Weizmann, pp. 71–85. Collective Volume, Daniel Sieff Research Institute, Rehovoth 1944.
20 Wilkansky, B., Life in the Dead Sea. Nature *138* (1936) 467.