Hydrobiologia **405:** 1–9, 1999.

Opinion

This is a series of contributions, expressing explicit opinions with regard to contemporary topics in limnology. These texts will be based mostly on new books with a large impact, but can also refer to other currently debated topics. Suggested topics for opinion articles or documented reactions to published contributions should be sent to the editor-in-chief; they will be considered for publication in subsequent issues.

Microbiological studies in the Dead Sea: future challenges toward the understanding of life at the limit of salt concentrations*

Aharon Oren
Division of Microbial and Molecular Ecology, The Institute of Life Sciences, and The Moshe Shilo Minerva Center for Marine Biogeochemistry, The Hebrew University of Jerusalem, Jerusalem 91904, Israel
E-mail: orena@shum.cc.huji.ac.il

Key words: Dead Sea, *Dunaliella*, halophilic Archaea, bacteriorhodopsin, bacteriophages, fungi

Introduction

On February 6 1999, Benjamin Elazari Volcani, the pioneer of microbiological research of the Dead Sea, passed away. It was Ben Volcani (Wilkansky) who more than 60 years ago, demonstrated that the Dead Sea is inhabited by a variety of microorganisms (Wilkansky, 1936; Elazari-Volcani, 1940; Volcani, 1944). At the end of his long and prolific scientific career, devoted mainly to the study of silica metabolism in diatoms, Volcani returned to his studies of the Dead Sea biota. His last research papers on the subject were published in the year of his death at the age of 84 (Arahal et al., 1999; Oren & Ventosa, 1999; Ventosa et al., 1999).

Since Volcani's early studies our understanding of the Dead Sea as a habitat for halophilic microalgae and prokaryotic microorganisms (Archaea as well as Bacteria) has increased dramatically. The first quantitative determinations of the algal and bacterial community sizes in the lake were performed in 1963–1964 (Kaplan & Friedmann, 1970), but only from 1980 onwards has a systematic monitoring of the spatial and temporal distribution of the microbial communities in the lake been performed. We now have a reasonable understanding of the biological processes in the lake, the main components of its biota and the dynamics of their appearance and decline as influenced by abiotic factors. Our knowledge of the microbial ecology of the Dead Sea has been summarized in a number of review papers (Nissenbaum, 1975; Oren, 1988, 1997, 1999a).

The Dead Sea is a very harsh environment even for those microorganisms best adapted to life at high salt concentrations. Thus the lake presents fascinating challenges to the biologist who attempts to understand the biological processes in its water and sediments. Not only does the Dead Sea contain the highest salt concentration of all natural lakes inhabited by living organisms, but the peculiar ionic composition of its water, with its high concentrations of divalent cations magnesium and calcium, is highly inhibitory even to those microorganisms best adapted to life in the lake. These organisms thus live at or near the upper limit of their tolerance toward high divalent cation concentrations.

In the late 1930s when the first microbiological studies of the Dead Sea were performed the salinity of the lake's water was much lower than at present. Volcani states that at the time the total salt concentration at the lake surface was 269 g l^{-1} – being 80% of the present-day value of 340 g l^{-1}, increasing to 327 g l^{-1} at 50 m depth (Volcani, 1944). A survey performed in 1959–1960 showed the lake to be meromictic, with

* This paper is dedicated to the memory of Benjamin Elazari Volcani, the pioneer of microbiological research of the Dead Sea, who passed away on February 6, 1999.

a ‘permanent’ pycnocline at a depth of about 40 m (Neev & Emery, 1967).

Since the beginning of this century the water balance of the Dead Sea has been negative (Gavrieli et al., 1999). The decrease in water level, due in part to climatic changes and intensified by the diversion of fresh water from the Sea of Galilee and the Jordan river, has resulted in an increase in salinity of the upper water layers. This led to the disappearance of the pycnocline and to an overturn of the water column in the beginning of 1979. The mean values for the ionic concentrations in 1996 were (in mol l^{-1}): Mg^{2+}, 1.887; Na^{+}, 1.594; Ca^{2+}, 0.436; K^{+}, 0.199; Cl^{-}, 6.335; Br^{-}, 0.068, and SO_4^{2-}, 0.005. The lake is now holomictic, but meromictic regimes have occurred from 1979 to 1982 and from 1992 to 1995 as a result of massive inflow of fresh water during unusually rainy winters, with the formation of a pycnocline at depths varying between 5 and about 15 m (Gavrieli et al., 1999). The overall decrease in water level continues: from the beginning of 1981 to the end of 1998 the lake surface level has dropped from -402 m to -412 m.

The chemical properties of the lake have changed beyond a mere increase in the total ionic concentrations. Massive amounts of NaCl have precipitated from the water column to the lake bottom as halite crystals. The weight of halite that has precipitated between 1976 and 1991 was estimated at 2.2×10^6 ton (Gavrieli, 1997). The precipitation of halite has caused an additional increase in the already extremely high ratio of divalent to monovalent cations of the Dead Sea water.

The systematic survey of microbial life in the Dead Sea from 1980 onwards has yielded the following overall picture:

1. *Dunaliella* sp. (designated in the past as *Dunaliella viridis* or *Dunaliella parva*) is the only primary producer in the lake. Its appears as blooms in certain years only and algal blooms are not an annually recurring phenomenon. Mass development of the alga were observed in the summer of 1980 (Oren & Shilo, 1982) and in the spring of 1992 (Oren, 1993a, 1999a,b; Oren et al., 1995a). Laboratory simulations have shown that two factors must be fulfilled for a *Dunaliella* bloom to develop in the lake: the upper water layers must become diluted to a significant extent (10% at least) with fresh water from rain floods, the Jordan river, etc. and phosphate, being the limiting nutrient, must be available (Oren & Shilo, 1985). Such conditions were found only after the exceptionally rainy winters of 1979–1980 and 1991–1992.

2. Development of *Dunaliella* blooms is followed by massive growth of red halophilic Archaea of the family *Halobacteriaceae*. These Archaea were found in numbers of up to 1.9×10^7 ml^{-1} in the summer of 1980 and up to 3.5×10^7 ml^{-1} in 1992 (Oren, 1983a, 1993a, 1999b; Oren & Gurevich, 1995). As a result of their high carotenoid content (mainly derivatives of the C_{50} carotenoid α-bacterioruberin) these Archaea imparted a reddish color to the waters of the Dead Sea at the time of the blooms.

3. When conditions become unfavorable for the biota as a result of an increase in the salinity of the upper water layers due to excess evaporation, *Dunaliella* rapidly disappears from the water column and archaeal communities slowly decline.

4. When stratification ends and a new holomictic episode starts, the remainder of the Archaeal community that was previously restricted to the upper water layers above the pycnocline and/or thermocline becomes distributed evenly over the entire water column (Oren, 1985, 1999a,b; Oren & Anati, 1996). Cell densities and heterotrophic activities during the holomictic periods are extremely low (Oren, 1992).

The present trend of decreasing water level and increasing salinity may be reversed in the future, when plans to connect the Dead Sea with the Mediterranean or with the Gulf of Aqaba (Red Sea) will be implemented. Such plans have been discussed at several times in the past. The difference in elevation of more than 410 m between the Dead Sea surface and mean sea level will enable the generation of hydroelectric energy, at the same time counteracting the continuing drop in the level of the Dead Sea (Ne’eman & Schul, 1983). Since the peace treaty between the State of Israel and the Hashemite Kingdom of Jordan was signed in 1994, the idea has been revived. Part of the treaty includes a pre-feasibility study for the construction of a Red Sea – Dead Sea canal along the Arava valley. The energy produced is then to be used for the production of 800 million m^3 of desalinated seawater annually (Gavrieli et al., 1999).

A thorough understanding of the biological phenomena in the Dead Sea is essential to enable us to predict the possible effects of the inflow of large amounts of seawater into the lake. In spite of our increasing understanding of the biology of the Dead Sea, a number of key questions remain. In the present essay, I will define a number of open questions and future challenges that should keep researchers of the Dead Sea biota busy in the years to come.

Longevity of *Dunaliella* cysts in Dead Sea sediments

Blooms of *Dunaliella* rapidly develop in the upper meters of Dead Sea water column as soon as conditions become favorable, i.e. as soon as massive winter floods and water flow from the Jordan river have caused the formation of a sufficiently diluted epilimnion. No *Dunaliella* cells were observed in the water column in the spring of 1980, but in the months July–August the alga rapidly grew to a population density of up to 8.8×10^3 cells ml^{-1} (Oren & Shilo, 1982). From the end of 1991 onwards, to the beginning of 1992 no algae were found in the lake. Salinity values were too high and the algae appeared unable to adapt to life at the continuously increasing salinity and more specifically the increase in divalent cation concentration of the brine. However, at the onset of a new meromictic period in the beginning of 1992 with the formation of an epilimnion diluted to 70% of the salinity of the lower water mass, a new bloom of *Dunaliella* rapidly appeared, with up to 1.5×10^4 cells ml^{-1} and maybe even higher in April 1992 (Oren, 1993a, 1999a,b; Oren et al., 1995a).

The question of what the source of the inoculum may have been that gave rise to mass development of *Dunaliella* as soon as conditions become favorable has been addressed before and two ideas have been brought forward. One possibility is that the inoculum is to be found in hypersaline springs that occur at several sites on the shore of the lake. Thus, *Dunaliella* was found in the outflow of a sulfur spring (Hamei Mazor) with a salinity of 169 g l^{-1}, being about half of that of the Dead Sea itself (Oren, 1989). The second option is that the blooms developed from resting stages that survived in the bottom sediments of the lake. When the 1992 *Dunaliella* bloom declined, formation of thick-walled cysts was observed, that sank to the bottom (Oren et al., 1995a). It is also noteworthy that intact chlorophyll *a* was found in deep sediments in the Dead Sea, probably derived from algal blooms in the past (Nissenbaum et al., 1972).

Remote sensing studies, using multispectral analysis of LANDSAT images, led us to decide in favor of the second hypothesis. The bloom in 1992 started simultaneously in the shallow areas all around the lake. Such a spatial pattern can only be explained by the germination of *Dunaliella* cysts present in the shallow sediments that became exposed to the reduced salinities above the pycnocline (Oren & Ben-Yosef, 1997).

No quantitative information is available on the occurrence of *Dunaliella* cysts in the sediments of the Dead Sea, nor do we know how long these cysts may remain viable in the brines that are too saline to support growth of the alga. Another important question that should be addressed is what factors may initiate germination of *Dunaliella* cysts present in the sediment. A study of the recovery of living algae from Dead Sea sediments of different depths (both shallow sediments that have been exposed to lowered salinities in 1992 and deep sediments that have not) after different treatments such as suspension at lowered salt concentrations and addition of nutrients, can be expected to shed more light on the question of the source of the inoculum and the factors that trigger the onset of the algal blooms in the Dead Sea.

The formation of a deep chlorophyll maximum following a *Dunaliella* surface bloom

As stated above, *Dunaliella* blooms have been observed twice in the Dead Sea in recent years: in the summer of 1980 and in the spring of 1992. Both blooms started with the algal population being evenly distributed at all depths above the pycnocline that separated the diluted upper water layer from the heavy undiluted brines. The 1980 bloom then slowly declined, to disappear altogether toward the end of 1981 (Oren & Shilo, 1982). However, the 1992 bloom rapidly declined in May, hardly a month after having reached its peak density. Then in August–October a secondary bloom developed and this time the cells were found concentrated as a ‘deep chlorophyll maximum’ at a depth of 7–10 m near the pycnocline (Oren et al., 1995a). The cells appeared healthy and active, in spite of the low light intensity (less than 1% of that available at the surface) and in spite of the exceedingly high salinity of the water near the bottom of the pycnocline. During the subsequent months the chlorophyll maximum deepened even more, closely following the changes in depth of the pycnocline. In August 1993 the *Dunaliella* maximum (3000 cells ml^{-1} in August 1993) was found at 14 m depth.

Photosynthetic planktonic microorganisms are often found as a deep chlorophyll maximum in many seas and lakes. The phenomenon is generally related to the quest of the phytoplankton for inorganic nutrients that are in short supply near the surface (Parsons et al., 1977). In the Dead Sea phosphate has been shown to be the limiting inorganic nutrient (Oren,

1983a; Oren & Shilo, 1985). However, few reliable data have been published on the phosphate concentrations available and this is to a large extent due to the difficulty in performing high-precision chemical analyses in the presence of molar concentrations of other interfering salts. The value of 0.04 mg l^{-1} of inorganic phosphorus in the water column, obtained by colorimetric determination as molybdophosphate following coprecipitation of the phosphate with aluminium hydroxide (Nissenbaum et al., 1990), may be considered to represent the range of concentrations that may be encountered. More extensive data, including on the vertical distribution of phosphate at the time of the *Dunaliella* blooms in the past, are lacking.

The occurrence of the deep chlorophyll maximum at the end of 1992 – beginning of 1993 is especially intriguing in view of the high salinity, not greatly different of that of undiluted Dead Sea water, at which the cells were found. This finding is incompatible with the observation that a significant dilution is essential for *Dunaliella* to grow in Dead Sea water (Oren & Shilo, 1985). It can only be concluded that the ecophysiology of *Dunaliella* in the Dead Sea is still incompletely understood. It is also far from clear why such a deep chlorophyll maximum did not develop during the earlier bloom in 1982 (Oren & Shilo, 1982).

The identity of the dominant halophilic Archaea in the Dead Sea

When *Dunaliella* is present as primary producer, halophilic Archaea are also found in high numbers in the Dead Sea. Between 2.3×10^6 and 8.9×10^6 cells ml^{-1} were counted in 1963–1964 (Kaplan & Friedmann, 1970), up to 1.9×10^7 cells ml^{-1} were found in the surface layers in the summer of 1980 (Oren, 1983a) and in the spring of 1992 a maximum community density of 3.5×10^7 cells ml^{-1} was reached (Oren & Gurevich, 1995; Oren, 1999a,b). The dense communities of the Archaea rich in carotenoid pigments imparted a reddish color to the Dead Sea water during these periods. Glycerol, produced by *Dunaliella* as osmotic stabilizer, is probably one of the main sources of organic nutrients on which the Archaea thrive (Oren, 1995).

Several species of halophilic Archaea have been isolated from the Dead Sea in the past. Early isolates include *Haloarcula marismortui* (Volcani, 1944; Oren et al., 1990) and *Haloferax volcanii*, named after Ben Volcani who first documented the presence of bacterial life in the lake (Mullakhanbhai & Larsen, 1975). During the 1980–1981 bloom we isolated *Halorubrum sodomense* (Oren, 1983b) and the bloom of 1992 yielded a culture of a novel species described as *Halobaculum gomorrense* (Oren et al., 1995b).

We still know very little on the contribution of the above-mentioned species of halophilic Archaea (and possibly other, yet to be described species as well) to the archaeal community in the Dead Sea. In an attempt to obtain information on the community structure of the halophilic Archaea in the lake during the 1992 bloom, a study was made of the polar lipids present in the community biomass, in view of the fact that the different genera of halophilic Archaea can often be identified according to their specific glycolipids. The bloom contained one major glycolipid – the sulfated diglycosyl diether lipid S-DGD-1, while the diether derivative of phosphatidylglycerosulfate (PGS) was absent (Oren & Gurevich, 1993). Such a composition is characteristic of representatives of the genera *Haloferax* and *Halobaculum*. *Halorubrum*, while possessing a sulfated diglycosyl diether lipid, contains PGS and *Haloarcula* is characterized by a triglycosyl diether lipid and presence of PGS as well.

Nowadays the characterization of 16S rRNA genes directly amplified from the biomass collected is one of the most popular approaches toward the characterization of natural communities of Bacteria and Archaea. Application of this approach to saltern crystallizer ponds in Spain and in Israel has shown that the dominant Archaeon in that environment belongs to a new taxonomic group of which no representatives have yet been brought into culture (Benlloch et al., 1995; Rodríguez-Valera et al., 1999). This approach has yet to be applied to the Dead Sea biomass samples from the 1992 bloom that have been preserved.

The role of bacteriophages in the regulation of archaeal community densities in the Dead Sea

Little is known on the processes responsible for the decrease in the archaeal communities in the Dead Sea following the decline of the *Dunaliella* blooms. Salt-saturated environments such as the Dead Sea are unique among microbial ecosystems in the absence of protozoa and other bacteriovorous planktonic organisms that regulate prokaryotic community size in most ecosystems.

In the late 1930s – early 1940s Volcani succeeded to grow in enrichment cultures a ciliate and a di-

mastigamoeba from Dead Sea water and sediment samples after very long incubation periods (months–years) (Elazari-Volcani, 1943, 1944; Volcani, 1944). These were isolated at a time in which the salinity of the upper water layers of the lake was much lower than at present. Later studies have never ascertained the presence of protozoa in the Dead Sea ecosystem and their importance in regulating community densities of microorganisms in the lake is probably negligible. Ciliate and amoeboid protozoa were abundantly found in the hypersaline (169 g/l total salt) sulfur spring of Hamei Mazor (at the time of Volcani's studies submerged below the lake surface, now exposed on the shore) (Oren, 1989). It is thus well possible that the protozoa cultured by Volcani may have been derived from such less saline ecosystems in the Dead Sea area.

However, bacteriophages may play an important role in the community dynamics of halophilic Archaea in the Dead Sea. Electron microscopic examination of water samples collected during the decline of the archaeal bloom in 1994–1995 showed large numbers of virus-like particles, many of those spindle-shaped similar to some other viruses described to attack Archaea. Numbers of virus-like particles exceeded those of the prokaryotic cells ten-fold on the average (Oren et al., 1997). Aggregates of virus-like particles were occasionally observed, resembling recent burst events of a bacterium with the release of mature bacteriophages. Viruses were also implicated as one of the causes of the decline in bacterial numbers in hypersaline solar saltern ponds in Spain (Guixa-Boixareu et al., 1996).

No attempts have yet been made to isolate from the Dead Sea bacteriophages that may cause lysis of such Dead Sea Archaea as *Haloarcula marismortui*, *Haloferax volcanii*, *Halorubrum sodomense* and *Halobaculum gomorrense*. It may even be more relevant to search for those viruses that attack the – yet unknown – type of Archaea that dominates the community during blooms such as those observed in 1980 and in 1992 and to investigate what types of microorganisms are lysed by the dominant types of viruses encountered in the brine.

The role of retinal pigments in the energy household of archaeal communities in the Dead Sea

Halophilic Archaea of the family *Halobacteriaceae* are heterotrophic microorganisms that use simple organic compounds such as amino acids and certain sugars as energy source. Glycerol, produced by *Dunaliella* as osmotic solute, is probably one of the main sources of carbon and energy for the archaeal community in the Dead Sea (Oren, 1993b, 1995). However, some species have in addition the potential of using light as energy source via the light-driven proton pump bacteriorhodopsin located in the cell membrane, often as patches of 'purple membrane'. Those species that have the ability of synthesizing bacteriorhodopsin (e.g. *Halobacterium salinarum*) produce the pigment only under specific conditions: light should be available and oxygen concentrations should be low.

Although the possession of the bacteriorhodopsin light-driven proton pump may be expected to be of great ecological advantage to the halophilic Archaea, very little is known on the occurrence of the pigment in hypersaline environments (Oren, 1994). Most species of the *Halobacteriaceae* are unable to produce bacteriorhodopsin and the color displayed by archaeal blooms such as occur in saltern crystallizer ponds is the red color of bacterioruberin carotenoids (peak absorbance near 498 nm with a minor peak around 530 nm and a shoulder at about 470 nm) and not the purple one of bacteriorhodopsin (maximum absorbance at 570 nm).

Of the halophilic Archaea isolated from the Dead Sea only *Halorubrum sodomense* has thus far been shown to synthesize purple membrane with bacteriorhodopsin (Oren, 1983b). No bacteriorhodopsin synthesis has been demonstrated as yet in as *Haloarcula marismortui*, *Haloferax volcanii* and *Halobaculum gomorrense*.

The biomass collected from the Dead Sea in 1981 had a prominent purple color and contained large amounts of bacteriorhodopsin. This finding represented the first report of the occurrence of the pigment in a natural community of halophilic Archaea (Oren & Shilo, 1981). It was subsequently shown that bacteriorhodopsin was the pigment responsible for at least part of the light-dependent CO_2 incorporation in the lake at the end of 1981 – beginning of 1982, when no or at best very few *Dunaliella* cells were present. Involvement of bacteriorhodopsin was inferred from the action spectrum of the CO_2 incorporation process and the lack of inhibition by inhibitors of algal photosynthesis (Oren, 1983c). The true nature of the bacteriorhodopsin-driven light-dependent CO_2 incorporation is still unknown, but it does not appear to involve ribulose bisphosphate carboxylase as the key enzyme.

The question to what extent light energy absorbed by bacteriorhodopsin may contribute to the development of archaeal communities in the Dead Sea remains to be answered. In this respect it is interesting to note that no such purple color as the one that had appeared in 1980–1981 was observed in the archaeal bloom in the lake in 1992. Moreover, *Halobaculum gomorrense*, which may have been the main component of the biomass at the time as judged by the polar lipid pattern (see above), seems to be unable to produce bacteriorhodopsin (Oren et al., 1995b). These observations may also indicate that the species composition of the 1980–1981 bloom and the 1992–1993 archaeal blooms may have been different.

The importance of halophilic and halotolerant fungi as degraders in the Dead Sea

Until recently prokaryotic microorganisms (mainly Archaea, to a limited extent also Bacteria) were considered to be the only decomposers in the Dead Sea (Oren, 1988, 1997). However, it has recently been suggested that fungi, long neglected as a component of the food web in the Dead Sea and in other hypersaline environments as well, may also play a role.

During the years 1995–1997, a variety of fungi were isolated from the Dead Sea, both from surface water at the shore and in the center of the lake and from deep water samples. At least 22 species were found, most of them belonging to the Ascomycotina, but mitosporic fungi and Zygomycotina were encountered as well (Buchalo et al., 1999). Most species identified were common soil bacteria, not well adapted to life at high salt concentrations. However, one of the isolates, described as a new species *Gymnascella marismortui* (Ascomycotina), is a true halophile that grows well in media containing 50% Dead Sea water and even higher (Buchalo et al., 1998, 1999).

Many fungi are known to function well at very low water activities. In addition, fungi generally prefer a slightly acidic pH. Therefore the properties of the Dead Sea – hypersaline, with a pH of about 6, and organic material being available at least during certain periods – would appear suitable for fungal life. To what extent the types of fungi isolated from the Dead Sea are present in the lake as vegetative hyphae and may contribute to the heterotrophic activity in the lake remains to be determined.

Anaerobic processes in the Dead Sea sediments

From the bottom sediments of the Dead Sea Volcani isolated a number of halophilic anaerobic bacteria (Volcani, 1944). These include bacteria that fermented glucose or lactose and grew at 25% salt and also denitrifiers. Several aerobic halophilic Archaea, including *Haloarcula marismortui*, are able to grow by denitrification. Unfortunately the fermentative isolates were not preserved.

Only in the early 1980s were new isolates of halophilic fermentative Bacteria obtained from the Dead Sea and from other hypersaline lakes. Dead Sea isolates include *Halobacteroides halobius* (Oren et al., 1984), *Orenia marismortui* (Oren et al., 1987; Rainey et al., 1995), and *Sporohalobacter lortetii* (Oren, 1983d; Oren et al., 1987). These all belong to the order Haloanaerobiales (low G+C branch of the Gram-positive bacteria) (Rainey et al., 1995). The fermentative obligatory anaerobic halophilic Bacteria proved to form a very interesting group, apparently unique among the Bacteria in their mode of osmotic adaptation as they use KCl rather than organic osmotic solutes to balance the high intracellular salt concentrations in their environment. The occurrence of these fermentative Bacteria in the Dead Sea sediments is well established, but much remains to be learned on their distribution and activities *in situ*.

Much less is known on the process of dissimilatory sulfate reduction in the bottom sediments of the Dead Sea. Prior to the 1979 overturn of the water column the hypolimnion of the meromictic Dead Sea was anaerobic and contained sulfide (Neev & Emery, 1967). Stable isotope analyses showed that the sulfide was enriched in light sulfur isotopes relative to the sulfate, which points to bacterial sulfate reduction as the source of the sulfide (Nissenbaum & Kaplan, 1976). The microorganisms responsible for the formation of the sulfide have never yet been isolated and we still do not know any bacterium that is capable of performing dissimilatory sulfate reduction at the level of salinity encountered in the Dead Sea. Attempts to quantify sulfate reduction in Dead Sea sediments by following the formation of $H_2^{35}S$ from $^{35}SO_4^{2-}$ never gave conclusive evidence for the occurrence of bacterial sulfate reduction (A. Oren, unpublished results).

The potential for methanogenesis in the Dead Sea sediments was demonstrated only recently, when production of $^{14}CH_4$ was shown in sediment slurries incubated with ^{14}C-labeled methanol (Marvin Di-

Pasquale et al., 1999). No methanogenesis was found with other potential substrates such as acetate, trimethylamine, dimethylsulfide or methionine. Methanol cannot be expected to be available in the lake at high concentrations, but the extent of methanogenesis in the sediments surely deserves a thorough examination.

Due to the massive precipitation of halite from the water column in recent years (Gavrieli, 1997) large parts of the bottom sediments of the Dead Sea have now become covered with a thick salt crust. This crust makes sampling of the sediments below very difficult, if not altogether impossible. The shallow sediments, however, remain accessible to conventional sampling equipment.

Epilogue

The rapidly changing Dead Sea presents us with an interesting large-scale experiment in microbial evolution and adaptation with respect to high and ever increasing divalent cation concentrations.

Many of the microorganisms isolated from the Dead Sea proved not only extremely tolerant toward high magnesium concentrations, but also dependent on high concentrations of divalent cations for structural integrity and growth (Cohen et al., 1983; Oren, 1983b, 1986, 1998, 1999c; Oren et al., 1995b). However, the microbial communities in the lake proved unable to adapt to the extremely rapid increase in salinity and more specifically in the concentrations of divalent cation concentrations in recent years (Gavrieli et al., 1999). At the time of writing (June 1999), archaeal community densities in the lake were very low and no *Dunaliella* cells were observed at all.

Life in the Dead Sea in its present state thus primarily depends on those rare events of abundant rainfall in the catchment area that lead to the formation of a sufficiently diluted epilimnion. The Archaea and the resting stages of the algae in the bottom sediments are waiting for their opportunity to multiply the moment conditions will become suitable again. In view of the ever increasing salinity of the Dead Sea water and the concomitantly increasing relative concentrations of inhibitory divalent cations and also in view of the increasing extent in which excess rainwater in the catchment area is diverted for agricultural use, such microbial bloom events may be expected to become rarer and rarer.

Implementation of plans to connect the Dead Sea with the Gulf of Aqaba or with the Mediterranean will undoubtedly cause drastic changes in the biology of the lake. Far-reaching chemical changes in the water chemistry are also expected to occur. Presently the lake is saturated or even slightly oversaturated with respect to gypsum (Katz et al., 1981). Mixing of seawater (28 mM SO_4^{2-} in the Gulf of Aqaba) with Dead Sea water containing over 0.43 M Ca^{2+} may be expected to lead to massive formation of gypsum crystals. To what extent massive blooms of algae and Archaea will color the lake green or red will depend to a large extent on the amounts of less-saline water that will enter the lake and on the mode of mixing of the light seawater with the heavy Dead Sea brines. Establishment of a meromictic state with an epilimnion with less than 300 g l^{-1} total salts may easily lead to the development of microbial blooms. The extent of these blooms will probably be determined by the amounts of phosphate that will enter the lake. A thorough understanding of the biological phenomena in the Dead Sea in its present state, supplemented with experiments simulating the effects of a reduction in salinity by addition of seawater (see e.g. Oren & Shilo, 1985) are thus necessary to allow reliable predictions how the biological properties of the lake may change in the future.

Acknowledgements

This study was supported in part by grant no. 95-00027 from the United States - Israel Binational Science Foundation (BSF, Jerusalem).

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