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On February 6, 1999, Benjamin Elazari Volcani died in La Jolla, CA, USA, from kidney failure at the age of 84 (Fig. 1). Volcani (born Wilkansky) will be remembered as the pioneer of the microbiological studies of the Dead Sea. In a short article published in *Nature* in 1936 [39], Volcani gave the first description of an indigenous microbial community adapted to the extremely harsh conditions of the Dead Sea with its high salt concentration and the unique ionic composition of its salts. His findings were further documented in his Ph.D. thesis submitted to The Hebrew University of Jerusalem in 1940 [5] and in a review article [38].

In 1939, Volcani became a member of the Sieff Institute in Rehovot, later renamed the Weizmann Institute of Science; he headed its laboratory of microbiology until 1959, when he joined the faculty of the Scripps Institution of Oceanography, University of San Diego, La Jolla, USA, where he remained as professor of marine biology until he retired in 1985. In his retirement, Volcani resumed his investigations of the Dead Sea biota. Bottles with old enrichment cultures, dating from the 1930s and stored in his laboratory at Scripps for more than 50 years, were opened, and these appeared still to contain a variety of viable microorganisms [1, 2, 37]. Volcani's last publications on the forms of life in the Dead Sea appeared in the year of his death [2, 37].

The Dead Sea presents fascinating challenges to the biologist who attempts to understand the biological processes in the lake. Not only does the lake contain the highest salt concentration of all natural lakes inhabited by living organisms, but the peculiar ionic composition of the Dead Sea water, with 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 magnesium concentrations.

Since the days of Volcani's pioneering research on the biology of the Dead Sea our knowledge of the lake's biota has increased tremendously (for reviews see e.g. [18, 21, 24, 35]). In the present essay we will attempt to assess the importance of Volcani's work in the light of our present understanding of the biological processes in the Dead Sea.

## Benjamin Elazari Volcani (1915–1999): Sixty-three years of studies of the microbiology of the Dead Sea

### Physical and chemical properties of the Dead Sea and their long-time changes

In the late 1930s, when Volcani performed the first microbiological studies of the Dead Sea, the salinity of the lake's water was lower than at present. Volcani stated that at the time the total salt concentration at the lake surface was 269 g/l, increasing to 327 g/l at 50 m depth [38]. A survey performed in 1959–1960 showed the lake to be meromictic, with a "permanent" pycnocline at a depth of about 40 m [17].

Since the beginning of this century the water balance of the Dead Sea has been negative [12]. The decrease in water level, due in part to climatic changes and intensified by the diversion of fresh water from the Jordan river, has led to an increase in the salinity of the upper water layers. This resulted in the disappearance of the pycnocline, followed by an overturn of the water column at the beginning of 1979. Presently the salt



**Fig. 1** Benjamin Elazari Volcani (1915–1999). Photograph taken by A. Ventosa in Alicante, Spain, in September 1989

content of the Dead Sea water is around 340 g/l at all depths. The mean values for the ionic concentrations in 1996 were (in mol/l):  $Mg^{2+}$ , 1.887;  $Na^+$ , 1.594;  $Ca_2^{2+}$ , 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 1979–1982 and from 1992–1995 as a result of massive inflow of fresh water during unusually rainy winters [12].

The chemical properties of the lake have changed beyond a simple increase in the total ionic concentrations. At the time of Volcani's early studies precipitation of  $CaCO_3$  were occasionally observed, causing whitening of the water [3, 38]. No such events have been documented in recent years; however, due to the negative water balance, massive amounts of NaCl now precipitate from the water column to the lake bottom as halite crystals. The weight of halite that has precipitated between 1976 and 1992 was estimated to be over about  $2.550 \times 10^6$  ton [11]. 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.

## Photosynthetic microorganisms in the Dead Sea

From enrichment cultures set up with Dead Sea water and sediment samples, Volcani succeeded in growing a variety of photosynthetic microorganisms. These include eukaryotic flagellate algae (*Dunaliella* sp.), well adapted to the high salinities prevailing at the time in the upper water layers, and an unidentified green flagellate with a single flagellum that grew at 1–27% salt, with slight growth at 30% [5, 6, 38]. Different types of cyanobacteria were also found that grow at lower salt concentrations (up to about 18%) [38].

Although indigenous life was discovered in the Dead Sea as early as 1936, the first quantitative assessments of the microbial community densities in the lake were made only as late as 1963–1964 [14]. It is now clear that a small *Dunaliella* species (designated in the past as *Dunaliella viridis* or *Dunaliella parva*) is the main or only primary producer in the lake. The green flagellate with a single flagellum isolated by Volcani have never been observed in the lake since, nor have been cyanobacteria reported to occur in significant numbers. Cyanobacteria, however, can nowadays be found in abundance in hypersaline springs on the lake's shore, such as Hamei Mazor (a spring with a salinity of about 169 g/l that was submerged at the time of Volcani's studies, when the water level was higher than at present) [22].

*Dunaliella* was recorded to occur in surface water in high numbers in 1964 (up to  $4 \times 10^4$  cells/ml). From 1980 onwards a comprehensive sampling and monitoring program has been carried out, resulting in an extensive data set covering algal densities over the seasons at different depths. Blooms of *Dunaliella* cells were observed during the period 1980–1981 and again in 1992–1994: up to  $8.8 \times 10^3$  cells/ml were counted in 1980 [27] and  $1.5 \times 10^4$  cells/ml and maybe even higher densities in 1992 [23, 30]. The

appearance of *Dunaliella* in the Dead Sea was triggered by the massive rain floods in the winters of 1979–1980 and 1991–1992 that caused a significant dilution of the upper water layers, and initiated a meromictic state of the lake. *Dunaliella* cells were found only in the mixed epilimnion above the pycnocline. In most samples examined, the distribution of the algae was uniform from the water surface down to the pycnocline. However, during the period August–October 1992 cells were found concentrated as a “deep chlorophyll maximum” at a depth of 7–10 m near the pycnocline [30].

Laboratory simulations have shown that a dilution of the upper water layers with more than 10% fresh water is required to initiate a mass development of the alga, provided that all essential nutrients are available. Input of sufficient phosphate is also necessary, as phosphate is the limiting nutrient in the Dead Sea.

When the salinity becomes too high for the *Dunaliella* cells, they rapidly disappear from the water column. During the decline of the dense algal bloom in the summer of 1992, *Dunaliella* cells were observed to form cysts that sank to the bottom of the lake [30].

## Halophilic Archaea

Among the microorganisms isolated by Volcani that are best adapted to the extremely high salt concentrations of the Dead Sea are a number of red halophilic Archaea belonging to the family Halobacteriaceae. *Haloarcularia marismortui*, first described by Volcani as *Flavobacterium* (or *Halobacterium*) *maris-mortui*, was reported to grow at NaCl concentrations from 18% up to saturation [5, 10, 38]. The generic name *Halobacterium* was first used by Volcani in his Ph.D. thesis, and he consolidated the nomenclature of the genus in his contribution to the 7th edition of *Bergey's Manual of Determinative Bacteriology* in 1957 [10]. The original culture of *Halobacterium marismortui* has not been preserved, but a similar strain now described as *Haloarcularia marismortui* was isolated from the lake in the late 1960s [13], and a formal description of the species was based on this isolate [29]. In addition, Volcani also isolated a “red micrococcus”, probably being a *Halococcus* strain [5, 38].

Additional novel halophilic Archaea have been isolated from the Dead Sea by other investigators; these include *Haloferax volcanii*, named after Volcani [16], *Halorubrum sodomense* [20], and *Halobaculum gomorrhense* [21].

Recently a number of bottles containing enrichment cultures, dating from the 1930s and stored in Volcani's laboratory at the Scripps Institution of Oceanography in La Jolla for more than 50 years, were opened, and the microorganisms they contained were analyzed. Molecular analysis showed that these cultures contained a variety of archaeal species, some of them novel [1, 37]. This suggests that our understanding of the biodiversity of the lake is still far from complete.

Quantitative assessments of the archaeal community in the Dead Sea showed that halophilic Archaea are often present in

very high concentrations: between  $2.3 \times 10^6$  and  $8.9 \times 10^6$  cells/ml were counted in 1963–1964 [14]; up to  $1.9 \times 10^7$  cells/ml were found in the lake's surface layers in the summer of 1980 [19], and in the spring of 1992 a maximum community density of  $3.5 \times 10^7$  cells/ml was reached [26]. The dense communities of the Archaea rich in carotenoid pigments imparted a reddish color to the Dead Sea water both in 1980 and in 1992 [19, 23, 26], and a reddish hue had been also observed in 1964 [14]. Development of archaeal blooms is correlated with the occurrence of large numbers of *Dunaliella*, the primary producer in the lake, which supplies the necessary organic material. Glycerol, produced and accumulated by *Dunaliella* as an osmotic stabilizer, is probably one of the main sources of carbon that support the development of the archaeal community.

Little is known on the contribution of the different genera and species of halophilic Archaea to the community in the Dead Sea. During the 1992 bloom, polar lipid analyses were performed to obtain information on the types of Archaea present. The lake's biomass contained one major glycolipid (the sulfated diglycosyl diether lipid S-DGD-1), whereas the diether derivative of phosphatidylglycerosulfate (PGS) was absent [25]. These observations may suggest that the dominant Archaeon in the Dead Sea at the time belonged to the genus *Halorubrum* or to *Halobaculum*. No significant amounts of those specific glycolipids were found that would have indicated a massive presence of *Haloarcula marismortui* in the biomass at the time.

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## Halophilic and halotolerant aerobic Bacteria

In addition to halophilic Archaea, Volcani isolated a number of halophilic or halotolerant Bacteria from the Dead Sea. Two of these have been preserved and described as new species: *Chromohalobacter marismortui* [36] (originally described as *Chromobacterium maris-mortui* [5, 38]), and *Halomonas halmophila* [4] (originally *Flavobacterium halmophilum* [5, 38]). Another interesting isolate, which unfortunately was not preserved, is *Pseudomonas halestorgus* [5, 38]. From the old enrichment cultures, a new halophilic *Bacillus* species, *Bacillus marismortui*, was recently isolated [2]. Non-halophilic representatives of the genus *Bacillus* were also found in the Dead Sea [5, 38]. These were termed "haloresistant" by Volcani. These organisms probably survived exposure to the hypersaline brines of the Dead Sea in the form of resistant endospores, and cannot be considered to belong to the indigenous microbiota of the lake. To honor Volcani's achievements in the field of halophilic Bacteria, the bacterial genus *Volcaniella* was named after him [33].

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## Anaerobic microorganisms in the sediments

From the bottom sediments of the Dead Sea, Volcani isolated

a number of halophilic anaerobic bacteria [7, 38]. These include fermentative bacteria—they ferment glucose or lactose and grow at 25% salt—and denitrifiers. Several aerobic halophilic Archaea, including *Haloarcula marismortui*, can grow by denitrification. Unfortunately, the fermentative isolates obtained by Volcani 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, and the group (order Haloanaerobiales) appeared to be an extremely interesting one [34]. Dead Sea isolates belonging to this order are *Halobacteroides halobius* [32], *Orenia marismortui*, and *Sporohalobacter lortetii* [34]. *Sporohalobacter lortetii* was named after the French microbiologist M. L. Lortet, who in the last decade of the 19th century isolated a number of pathogenic non-halophilic representatives of the genus *Clostridium* from Dead Sea mud [15]. These probably had survived as resistant endospores, and should thus be designated haloresistant bacteria according to Volcani's classification.

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## Protozoa

In enrichment cultures incubated for very long periods (months to years), Volcani found two types of protozoa: a ciliate and a dimastigamoeba [8, 9, 38]. Later studies have never ascertained the presence of protozoa in the Dead Sea ecosystem or their possible importance in regulating community densities of microorganisms in the lake. Viruses, however, may be implicated in the decline of archaeal and algal blooms: many virus-like particles were observed upon examination of water samples in the electron microscope [28].

Ciliate and amoeboid protozoa were abundant in the hypersaline (169 g/l total salt) sulfur spring of Hamei Mazor, which, at the time of Volcani's studies, was submerged below the lake surface, and now is exposed on the shore [22]. Thus, the protozoa cultured by Volcani might have derived from less saline ecosystems in the Dead Sea area.

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## Epilogue

The rapidly changing Dead Sea can be considered an interesting large-scale experiment in microbial evolution and adaptation with respect to higher and higher divalent cation concentrations. At the time of writing, archaeal community densities in the lake were very low, and no *Dunaliella* cells were observed at all. The Archaea and the resting stages of the algae in the bottom sediments were waiting for their opportunity to multiply when conditions would become suitable again. Life in the Dead Sea in its present state thus depends primarily on those rare events of abundant rainfall in the catchment area that lead to the formation of an epilimnion sufficiently diluted. The ever increasing salinity of the Dead Sea water and the concomitantly increasing

relative concentrations of inhibitory divalent cations, along with the increasing extent in which excess rainwater in the catchment area is diverted for agricultural use, suggest that such microbial bloom events may become rarer and rarer.

Volcani's studies in the late 1930s provide us with valuable information on the types of microorganisms found in the Dead Sea during a period in which the overall salinity was much lower than today, and the upper water layer had less than 80% of its present-day salt concentration. Unfortunately, no quantitative information was collected by Volcani, and almost 30 years had to pass until the first enumerations of microorganisms in the lake were performed [14]. Nevertheless, the information to be found in Ben Volcani's legacy, consisting both of his publications and of those bottles of enrichment cultures preserved from the 1930s, is of the utmost importance to understand the microbial ecology of the Dead Sea, not only in the past but also in the present.

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