

Chapter 3

ECOLOGY OF HYPERSALINE ENVIRONMENTS

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INTRODUCTION

About 30 years ago ZoBell (1946) reported some observations, parts of which are quoted in Table 3-1 and which might aptly serve as a starting point for the present discussion. ZoBell observed the ability of bacteria from habitats of different salinity to grow on nutrient media of different salinity. By salinity of the nutrient medium was meant the content of NaCl. Using sewage (low or no salt) as inoculum into the nutrient media, he found the highest number of bacteria developing in the media with the lowest salt concentrations. Increasing salt concentrations prevented the growth of an increasing proportion of the bacteria. These findings were not surprising: NaCl is well-known as an agent against bacterial growth; this is the basis for its widespread use as a preservative against bacterial spoilage. When using sea water as the source of bacteria, the highest number developed in the nutrient media at sea water salinity. Much lower numbers developed both at higher and lower salinities. At saturating salt concentration none of the bacteria from sea water developed. Samples from Great Salt Lake gave the highest viable count of bacteria on media saturated with NaCl. Lowering the NaCl concentration gave decreasing viable counts. At the lowest NaCl concentration tested (0.5%) was found 1/50 of the count at saturation. The findings when using marine saltern as the source of bacteria, were strikingly similar to those from Great Salt Lake.

TABLE 3-1.

Comparative bacterial counts obtained by ZoBell (1946) by plating from habitats of different salinity on nutrient media of different salinity.

Source of bacteria	Salinity of nutrient media (%)					
	30	15	7	3.5	2	0.5
	Average growth index					
Sewage	0	3	6	14	45	100
Sea water	0	7	38	100	62	19
Great Salt Lake	100	72	26	14	8	2
Marine Saltern	100	65	30	19	13	4

The figures of Table 3-1 deal with bacteria only, and even limited to those developing on a specified nutrient medium. The findings lead, nevertheless, to a postulate for which a good deal of additional evidence has accumulated since ZoBell made his observations more than 30 years ago: The indigenous population of hypersaline environments are, as a rule, rather special organisms adapted to live in the strong brine, and they even prefer, or require, the high salinity in their environment for growth and reproduction.

NaCl is the dominating salt component of the hypersaline environments, ponds and lakes, throughout the world. There are, however, some exceptions to this rule. The Dead Sea contains, in addition to Na^+ , a high concentration of Mg^{++} . In some cases Na_2CO_3 may be a dominating component; so has also been found for CaCl_2 . The hypersaline environments are mostly aerobic, but also anoxic situations are encountered. The acidity may differ considerably from one environment to another.

Through the years many observations have been reported on life in hypersaline environments. We are, however, still far from a thorough understanding of the ecology of such environments. The observations are sparse and scattered and often irrelative so that they are difficult to compare. Still a rough picture is emerging from somewhat more detailed studies of a few geographically well-known hypersaline environments, namely Great Salt Lake (Utah), the Dead Sea (Israel), and the alkaline lakes of Wadi Natrun (Egypt). A survey of the known ecological relations of these environments is given in the following, together with those of the marine salterns which are situated in coastal areas various places around the world. These environments have some major ecological properties in common, but there are also characteristic differences.

GREAT SALT LAKE

Great Salt Lake is predominantly a NaCl lake. The saline water also contains a relatively high, but not dominating, amount of SO_4 . Twenty years ago Great Salt Lake contained about 20% solids throughout the lake, the only dominating chemical component being NaCl, as mentioned. In 1957 a rock-filled railroad causeway was completed across the lake, dividing it into a northern and a southern basin. The only connection between the two basins are two small culverts, each about 5 m wide and 3 m deep. About 95% of the water from watershed streams flow into the southern part of the lake. During the past 20 years the salt concentration has decreased to about 12–13% in the southern part, and is estimated to reach about sea water salinity in another 20 years. On the other hand, in the northern basin evaporation has caused the salt concentration to reach saturation (Table 3-2), and thus created an interesting ecological situation which has recently been studied in some detail by Post (1977).

In the southern part of the lake there is at present a fairly rich population of organisms comprising a wide variety of types, as compared to the northern part where only a few but characteristic types of organisms occur in conspicuous numbers.

In the northern basin the hypersaline water column is not more than 10 m deep. Most of the brine is aerobic although the concentration of O_2 in the brine is modest. At the bottom, however, and certainly in the sediment, the condition is for the most part anoxic

TABLE 3-2.

Major ions of hypersaline lakes and brines, in g/l.

	Great Salt Lake, northern basin ¹	Dead Sea ²	Wadi Natrun lakes ³		Marine saltern Puerto Rico ⁴
			Zugm	Gaar	
Na ⁺	105.4	39.2	142.0	137.0	65.4
K ⁺	6.7	7.3	2.3	1.4	5.2
Mg ⁺⁺	11.1	40.7	0	0	20.1
Ca ⁺⁺	0.3	16.9	0	0	0.2
Cl ⁻	181.0	212.4	154.6	173.7	144.0
Br ⁻	0.2	5.1	—	—	—
SO ₄ ⁼	27.0	0.5	22.6	48.0	19.0
HCO ₃ ⁻ /CO ₃ ⁼	0.72	0.2	67.2	6.6	—
Total salinity	332.5	322.6	393.9	374.2	253.9
pH	7.7	5.9–6.3	11.0	10.9	—

1 Post (1977)

2 Nissenbaum (1975)

3 Imhoff *et al.* (1978)

4 Nixon (1970)

and H₂S can be shown to be present. The temperature varies from -5°C during the winter to +40°C during the summer. pH is about 7.7.

The dominating organisms of the northern basin, their numbers and biomass, are listed in Table 3-3; the data are taken from the interesting article of Post (1977).

The typical brine algae, *Dunaliella salina* and *D. viridis*, are the dominating primary producers. Although they seem to thrive better at lower salt concentrations (Brock, 1975) they apparently grow and reproduce quite well in the hypersaline brine of the northern basin. *D. salina* occurs as a planktonic form. 0.2 – 1 x 10³/ml are common numbers observed, and peak blooms of 10⁴/ml which corresponds to a maximum estimated biomass of 24 g/m³. *D. viridis* is not common in the plankton but mostly occurs on the underside of wood and rocks where the cells are not exposed to direct sunlight, and altogether in relatively modest numbers. This is interesting in view of the fact that in the southern and less saline basin of Great Salt Lake *D. viridis* is found to be the principal planktonic alga, reaching levels as high as 2 x 10⁵/ml, *i.e.* a biomass of about 150 g/m³ (Stephens and Gillespie, 1976).

Post (1977) found that in the northern basin the chemoorganotrophic bacteria are by far the most conspicuous members of the biota. The dominating types are the extremely halophilic bacteria of the genera *Halobacterium* and *Halococcus*. Their numbers do not show much fluctuation through the year. An average of 7 x 10⁷/ml was observed. This corresponds to a biomass of about 300 g/m³, the figures being somewhat lower during the winter and somewhat higher during the summer. A biomass of 300 g/m³ is indeed an impressive figure. The halobacteria and the halococci, being red in color due to carotenoids

TABLE 3-3.

Characteristic organisms of Great Salt Lake, northern basin (Post, 1977). Less conspicuous or less known types are shown in brackets.

	no./ml	g/m ³
Algae		
<i>Dunaliella salina</i> (max.)	10 ⁴	24
<i>Dunaliella viridis</i> (max.)	0.2 x 10 ⁴	1.4
Chemotrophic bacteria		
<i>Halobacterium</i> (av..)	7 x 10 ⁷	300
<i>Halococcus</i> (Sulfate reducing bacteria) (Others)		
(Cyanobacteria)		
(Protozoa)		
Brine shrimp	1	0.1
Brine fly		

in the cells, are present in the hypersaline brine in such quantities that they impress a red color upon the brine. Their biomass exceeds by at least a 10-fold that of the maximum observed algal bloom.

Brock (personal communication) has observed that also other chemotrophic bacteria may be present in high numbers, but these bacteria have not yet been closer identified. Sulfate reducing bacteria and methane producing bacteria are suspected to be present since hydrogen sulfide and methane production have been observed, but such bacteria have not been isolated. Bacteriophage specific for halobacteria have been shown to occur, and may play an ecological role by their ability to lyse the bacteria.

Cyanobacteria and protozoa have been encountered in the hypersaline water of the northern basin, but in modest numbers and may not be very active. Some of them are suspected to be brought in by the water flowing through the culverts.

The brine shrimp (*Artemia salina*) and the brine fly (*Ephydra* spp.) are characteristic organisms encountered during the summer. Both the brine shrimp and the brine fly larvae seem to do well in the extremely salty environment of the northern basin. The eggs of the brine fly are hatched in the strong brine, and the larvae apparently feed well on the microorganisms. The eggs of the brine shrimps have not been demonstrated to hatch in the strong brine of the northern basin. It is therefore possible that the larvae (nauplia) and the shrimp are carried into the northern basin with the water from the south (Post, 1977).

Turning now to the ecological aspects of the northern basin of Great Salt Lake it should first be pointed out that the temperature is quite low during the winter ($<5^{\circ}\text{C}$ in the period December–March), and not much metabolism seems to go on. The bacteria are probably resting, and the algae too. No brine shrimp or brine fly is there. Only when the temperature reaches above 10°C in April/May is there an increase in bacteria, but not much. The algae (*D. salina*) increase significantly in number, and eventually (June/July) the brine fly and the brine shrimp appear. The temperature keeps in the range $20\text{--}35^{\circ}\text{C}$ most of the time from June through September.

The algae are, of course, the primary producers, and they provide food for the organotrophic community, both the brine shrimp, the brine fly larvae and the bacteria. The brine shrimp is known to feed directly on the algae, especially *D. viridis*, and this is also likely to be the case, but not proved, for the brine fly larvae. The bacteria are envisioned to feed on excretion products of the algae, possibly glycerol to a significant extent and autolysates of the algae and the small animals. That is the simple food chain of this almost closed ecosystem.

A point of interest is that the content of dead organic material is rather high in the strong brine of the northern basin. There is a good deal of insoluble organic material in the form of pupal cases, dead brine shrimp and egg masses. This insoluble material seems to be very slowly converted by the bacteria. The content of soluble organic material has not been determined directly, but has been estimated to be of the order 100 mg/l which is a high figure compared to other bodies of water. The chemical nature of the soluble organic material has not been determined (Post, 1977).

According to Post (1977) nitrogen seems to be a key element in the ecosystem of the northern basin. Nitrate and nitrite could not be detected in the brine. Ammonia seems to be the main source of inorganic nitrogen, being utilized as such by the algae, and may at times be a limiting factor since it is present in long periods in very small (undetectable) amounts. No nitrogen fixation, nitrification or denitrification have been detected. The N cycle may thus be very simple: NH_3 is converted by the algae to organic N which in turn is converted back to NH_3 by the metabolism of the organotrophs, the latter step possibly in significant part through uric acid, an excretion product of the brine fly larvae.

As mentioned above sulfate is an abundant, but not dominating, component in the brine. At the bottom, *i.e.* at the lower $1\text{--}2\text{ m}$ of the not more than 10 m deep water column, and in the sediment, anoxic conditions prevail, and H_2S can be shown to be present. This is a strong indication of the presence of halophilic or halotolerant sulfate reducing bacteria, but they have not been studied.

Post (1977) reported that when simply keeping lake water from the northern basin in the laboratory at 28°C for 6 months, the bacteria and algae counts rose by one or more orders of magnitude above the highest levels observed in the lake. Post suggested that this observation might give some clue to which might be limiting factors for the biota under natural conditions. One important factor may be the temperature; the summer period available for growth is short. In the laboratory experiment the grazing invertebrates were not present. It is just possible that under natural conditions these invertebrates put a constraint on the population size of the algae and thus limit their organic output which provides food for the bacteria. A third factor which at times probably limits the biota under natural conditions is ammonia.

THE DEAD SEA

The Dead Sea differs hydrologically from Great Salt Lake in a number of respects. The total salinity (*i.e.* the sum of salts in g/l) is about the same, but the salt composition differs (Table 3-2). Concerning the cations the major differences are a much lower content of Na^+ and much higher contents of Mg^{++} and Ca^{++} in the Dead Sea. The very high content of Mg^{++} is indeed a unique phenomenon.

The dominating anion in the Dead Sea is Cl^- , such as is generally encountered in hypersaline, natural brines. However, SO_4^{--} is very low compared to other brines, as *f.ex.* Great Salt Lake.

Another characteristic of the Dead Sea is its great depth, with a maximum of 320 m. According to Nissenbaum (1975) one should from an ecological point of view distinguish between the upper water mass, from the surface to about 80 m, which is aerobic and has temperatures in the range 23–36°C, and the lower water mass below 80 m which is anoxic, contains H_2S (0.5–1 mg/l), and has a fairly constant temperature in the range 21–23°C. pH is in the range 5.8–6.4 throughout the water column; this is noticeably lower than in Great Salt Lake.

The Dead Sea was traditionally believed to be completely barren of indigenous life until Volcani (1940, 1944) published his observations almost 40 years ago. He described the occurrence of the salt alga *Dunaliella*, a variety of different bacteria among which the halobacteria seemed to be prominent, and he described cyanobacteria, a ciliate and an amoeba. Since that time little was done to gain further understanding of the life in the Dead Sea until the work of Kaplan and Friedman (1970) who were the first attempting to quantify the biota of the Dead Sea (Table 3-4). The microbiology and biogeochemistry of the Dead Sea was ably reviewed and discussed by Nissenbaum (1975).

In the upper, aerobic water mass (<80 m) *D. viridis* is the dominating alga and possibly the only alga indigenous to the sea. It occurs in surface water of the Dead Sea in numbers of about 4×10^4 cells/ml. This corresponds to a biomass of the same order as that observed by Post (1977) for *D. salina* in the northern basin of Great Salt Lake. The number of *D. viridis* decreases rapidly with depth in the Dead Sea. At 50 m are found only 1/100 of that at the surface; at 100 m no algal cell was detected.

The dominating bacteria of the upper water mass are, as in Great Salt Lake, members of the genera *Halobacterium* and *Halococcus*. The halobacteria are the most common but appears to occur in much smaller numbers than in Great Salt Lake. The highest counts were found at the surface, about 7×10^6 cells/ml, corresponding to a biomass of 30 g/m³. This is only 1/10 of that observed for this bacterium in Great Salt Lake. At 100 m depth the bacterial count was decreased about tenfold; at 250 m the decrease was still another tenfold. The numbers did not seem to vary much through the year.

In the lower anoxic water mass of the Dead Sea (>80 m) the biological activity seems to be very modest. However, already Volcani (1944) at the early date demonstrated by the use of elective culture technique that in mud, sediments and deeper layers of water, there are the potential abilities to carry out glucose fermentation, peptone decomposition, denitrification, sulfur oxidation and cellulose decomposition. The responsible organisms, chemotrophic bacteria, were not isolated and characterized. In recent years sulfate reducing bacteria from deeper waters and sediments have been studied, and are believed to play a

TABLE 3-4.

Characteristic organisms of the Dead Sea (Kaplan and Friedmann, 1970). Less conspicuous or less known types are shown in brackets.

	nó./ml	g/m ³
<u>Surface water</u>		
Algae		
<i>Dunaliella viridis</i> (max.)	4 x 10 ⁴	ca. 50
Chemotrophic bacteria		
<i>Halobacterium</i> (av.)	7 x 10 ⁶	ca. 30
(<i>Halococcus</i>)		
(Others)		
<u>Deep water/sediments</u>		
(Chemotrophic bacteria)		
(Sulfate reducing bacteria)		
(Others)		
(Cyanobacteria)		
(Amoebae)		
(Protozoa)		

significant part in the S-cycle. The indications are that the bacterial sulfate reduction is extremely slow in the water column, but considerably more intense in the sediment (Nissenbaum, 1975).

On the basis of the information available it seems that the biomass of the Dead Sea is completely dominated by two types of organisms only: *D. viridis* and halobacteria. *D. viridis* is the primary producer; the halobacteria are the dominating mineralizing agents. In interesting contrast to Great Salt Lake zooplankton seems to be absent from the Dead Sea. This means that there are no animals grazing on the alga and controlling the size of its population. The bacteria are envisioned to feed on excretion products and lysates of the alga plus organic matter brought into the lake with water flowing in. The major source of in-flowing water is the river Jordan.

Dissolved organic material in the Dead Sea is about 10 mg/l, which is a fairly high figure for water bodies, but only 1/10 of that of Great Salt Lake. In addition NH₃ (2–6 mg/l) and organic N (0.3–3 mg/l) are quite high, but phosphate is very low and may possibly be a limiting factor (Nissenbaum, 1975).

It has been suggested that light might be an important limiting factor for *Dunaliella*, and may possibly be the dominating one since a high content of suspended material prevents the light from penetrating deep into the lake. Measurements have shown that the light intensity is reduced to 1% at 30 m depth (Kaplan and Friedman, 1970).

No clear conclusions have yet been reached as to which are the factors limiting and controlling the biota of the Dead Sea, its size and turn-over rates. In addition to the factors mentioned above, O₂, temperature, the high concentration of Mg⁺⁺ (and Ca⁺⁺),

and also the high concentration of certain trace elements, have been suggested to play a limiting role.

ALKALINE HYPERSALINE LAKES

Hypersaline lakes of high alkalinity are found in many places. Baas Becking (1928) reported the occurrence of red-colored bacteria and cyanobacteria from such localities in western United States. The red-colored bacteria may be present in such numbers that they color the brine, and Jannasch (1957), studying "die bakterielle Rotfärbung" in the alkaline salines of Wadi Natrun in Egypt, found that the dominating red-colored bacteria there were phototrophic sulfur bacteria (*Thiorhodaceae*). Some of the organisms contained sulfur globules inside the cells and seemed to belong to the *Chromatium/Thiocystis* group. There were also other types: vibrios and spirilla. Jannasch reported that these bacteria seemed to be extremely halophilic (no development at salt concentration below 20%) and some even alkaliophilic (best development in the pH-range 8.5–10.5).

More recently pure cultures of phototrophic sulfide-oxidizing spirilla that store sulfur out-side the cells, have been isolated from alkaline, hypersaline lakes. These organisms belong to the genus *Ectothiorhodospira*. Raymond and Sistrom (1967, 1969) described an organism isolated from Summer Lake, Oregon, which they named *E. halophila*; Imhoff and Trüper (1977) described an organism isolated from Wadi Natrun, Egypt, which they named *E. halochloris*. Both organisms are extremely halophilic; best growth at around 20% NaCl and still good growth at 30% NaCl.

At present Trüper and his collaborators are engaged in an extensive study of the ecology of the alkaline hypersaline lakes of the Wadi Natrun area in Egypt (Imhoff *et al.*, 1978). The Wadi Natrun is located northwest of Cairo. The bottom of this area is 23 m below sea level and a chain of shallow lakes extends along the deepest part. Water is slowly supplied from the ground, originating from the Nile. An extensive evaporation takes place and the water becomes highly enriched with mineral components.

Imhoff *et al.* (1978) report on the chemical composition of six of the lakes of the Wadi Natrun. The data listed in Table 3-2 are from two of the lakes of extremely high salinity and show some characteristic patterns: The dominating cation is Na^+ . The dominating anion is Cl^- , but also $\text{SO}_4^{=}$ is high and so may $\text{CO}_3^{=}$ be. All the six lakes investigated showed pH values of about 11. Some of the lakes contained sulfide in the water, others did not at the time of the sampling. It has, however, been shown that sulfate-reducing bacteria are quite active in the ground, supplying H_2S to the water and playing an important role in the development of alkalinity (Abd-el-Malek and Rizk, 1963a, b).

None of the six lakes investigated by Imhoff *et al.* (1978) contained animals ranking above unicellular protozoa. The biota of microbes differed somewhat from one lake to the next, but still there were characteristic patterns, and only few types seemed to be represented in significant numbers (Table 3-5).

Four of the six lakes had an extremely high salinity (>30% total salts) and these lakes were all red in color. The absorption spectra, supported by observations in the microscope, revealed that the color was mainly due to the presence in large numbers of halobacteria. This finding was in some contrast to that of Jannasch (1957) who reported that the red

TABLE 3-5.

Characteristic organisms of the hypersaline lakes of Wadi Natrun — Egypt (Imhoff et al., 1978). Less conspicuous or less known types are shown in brackets.

Algae
<u><i>Dunaliella salina</i></u>
Phototrophic sulfur bacteria
<u><i>Chromatium</i></u>
<u><i>Ectothiorhodospira halophila</i></u>
<u><i>Ectothiorhodospira halochloris</i></u>
Cyanobacteria
<u><i>Spirulina</i></u>
Others
Chemotrophic bacteria
<u><i>Halobacterium</i></u>
Sulfate reducing bacteria
(Others)
(Protozoa)

color of the Wadi Natrun brines examined by him was due to phototrophic sulfur bacteria. However, phototrophic sulfur bacteria (*Chromatium*, *Ectothiorhodospira*) were found in large numbers in the extremely saline Wadi Natrun brines also by Imhoff *et al.*, but the latter investigators pointed out that in the cases examined by them masses of phototrophic sulfur bacteria, and also cyanobacteria, tended to grow attached to the sediment often forming dense mats, while the halobacteria more tended to occur evenly suspended in the brine. The phototrophic bacteria were, however, also found in large numbers suspended in the water of the lakes, preferably near the bottom. *Dunaliella salina* was reported to give mass development. Flagellated protozoa were observed feeding upon the bacteria, but seemed to be relatively few in numbers.

The findings of Imhoff *et al.* strongly supported Jannasch' contention of a simple food chain in the Wadi Natrun lakes, based to a large extent upon a cycling of sulfur. Decaying halotolerant grass, of which there is an ample supply around the lakes, provides suitable organic matter for sulfate reducing bacteria. These bacteria produce CO₂ and H₂S which, in turn, serve as substrates for the phototrophic sulfur bacteria producing new organic matter and oxidizing the sulfide via elemental sulfur to sulfate. In addition to the grass of the surroundings and the phototrophic sulfur bacteria, also cyanobacteria and *Dunaliella* serve as primary producers; the cyanobacteria may play a role in the oxidation of sulfide. In addition to the sulfate reducing bacteria, the halobacteria seem to play an important role in the mineralization of the organic matter.

Imhoff *et al.* (1978) reported biologically important compounds of carbon, sulfur, nitrogen and phosphate to be present in abundance in all the Wadi Natrun lakes studied, and these elements can therefore not be considered growth limiting, although certain compounds of certain of the elements, *e.g.* CO₂ and H₂S, may be limiting at certain

times. The same holds true for O_2 . Furthermore, the lake waters are deficient in metal ions other than sodium and potassium, and this may influence the growth of the biota.

MARINE SALTERNS

Marine hypersaline ecosystems are found in coastal areas many places in the world. Natural hypersaline lagoons may form from almost enclosed shallow coastal waters exposed to a bright sun so that an extensive evaporation takes place. More frequently such hypersaline lagoons or ponds are man-made for the purpose of commercial production of NaCl from sea water. The manufacture of salt by solar evaporation of sea water is an ancient art (Baas-Becking, 1931a), and the way of making it is in principle the same today as in the ancient times: Sea water is led into a system of shallow ponds arranged in series. The water is retained in each pond for a certain period while evaporation by the sun takes place. In this way a brine results with an increasing concentration of salts from one pond to the next, and a fractional precipitation of the salts of the sea water occurs in the various ponds. The least soluble salts, $CaCO_3$ and $CaSO_4$, will first precipitate. Then NaCl, being the dominating component of sea water, is crystallized out in the following ponds, and is harvested, often washed, and dried in the sun. The remaining brine, which is strongly enriched in $MgCl_2$ (bitter salt) and KCl, may go to other ponds for precipitation upon further evaporation.

Few thorough studies have been carried out on the ecology of marine salterns. It seems, however, from the observations reported that a certain pattern is common to many, if not most, of them and largely independent of geographical locations. The pattern is the following.

The biological productivity in the dilute brine of the first evaporation pond(s) is quite high (Carpelan, 1957). A number of different primary producers, notably cyanobacteria but also green algae, develop. The cyanobacteria (*Spirulina*, *Oscillatoria*, *Coccochloris*, *Lyngbya* and others) tend to form a mat at the bottom of the pond, while the green alga, *Dunaliella viridis*, grows planktonic. The brine shrimp, *Artemia salina*, and the brine ciliate, *Fabrea salina*, are characteristic organisms of the zooplankton.

As the water evaporates not only do the concentrations of total salts increase, but the relative proportions of the salt components in solution also change. The number of species able to develop decreases rapidly as the brine becomes more concentrated, and so does the biological productivity of the ecosystem (Copeland and Jones, 1965). The cyanobacteria eventually die at the higher salt concentrations. The brine algae, *Dunaliella viridis* and *D. salina* become the dominating primary producers (Gibor, 1956a, b; Nixon, 1970). At the higher salt concentrations also a considerable population of bacteria develop, notably members of the genera *Halobacterium* and *Halococcus* which are red in color.

In the ponds where NaCl crystallizes out the concentration of total salt is about 10 times that of the sea water. The brine in these ponds is frequently red in color, mainly due to the presence of a very large number (appr. 10^8 /ml) of the above-mentioned red-colored, halophilic bacteria. Also *D. salina* may be present in the concentrated brines in such numbers that it contributes to the red color, but the alga appears to be considerably

TABLE 3-6.

Characteristic organisms of concentrated brines from marine salterns (Nixon, 1970). Less conspicuous or less known types are shown in brackets.

Algae
<u><i>Dunaliella salina</i></u>
<u><i>Dunaliella viridis</i></u>
Chemotrophic bacteria
<u><i>Halobacterium</i></u>
<u><i>Halococcus</i></u>
(Others)
(Brine shrimp)

hampered in its metabolism in concentrated salt solution (Gibor, 1956a). *D. viridis* is less dominating at the highest salinities (Table 3-6).

The red-colored bacteria of the genera *Halobacterium* and *Halococcus* have been considered the dominating types of concentrated brines in marine salterns (Larsen, 1962). When the salt precipitates myriads of the bacteria adhere to the crystals and may stay alive in the salt for a long time if no special precautions are taken to kill or remove them. Much of the salt is later used as a preservative against bacterial spoilage of proteinaceous material such as fish, hides, etc. This salt may contain as many as 10^5 – 10^6 viable cells/g of the halophilic, red-colored bacteria which, when given the proper conditions (high humidity and temperatures) may develop in the proteinaceous materials and spoil them. This is a well-known phenomenon in many parts of the world, and a reason why these bacteria have been so extensively studied.

Dundas (1977) reported recently that he had found the majority of bacteria in red-colored brines from the marine salterns to be colorless. A description of these organisms is needed to complete our knowledge of the biota of marine salterns.

It seems as if aerobic conditions normally prevail in the brine of marine salterns, although dissolved oxygen may be low. The bottom floor of the shallow ponds, beneath the mat of cyanobacteria if present, often contains black mud regardless of the concentration of the brine in the pond. This indicates anaerobiosis in the mud beneath the brine and activity of sulfate reducing bacteria, but they have not been studied.

The brine shrimp, *Artemia salina*, is found also in the concentrated brines of marine salterns. Nixon (1970) reports 3500 individuals/m² from a marine saltern of 35% salinity in Puerto Rico. This is a modest number.

Dissolved organic matter in the concentrated brines of marine salterns is high. Nixon (1970) reported figures of about 100 mg/l of organic C, which should roughly correspond to 200 mg/l of organic matter. The high content of dissolved organic matter obviously stems from the primary producers abounding in the brines at the lower salt concentrations. As the salt concentration increases most of the algae die, they autolyze and leave in the brine organic material which serves as food for the population of chemoorganotrophic organisms developing, notably the red-colored, halophilic bacteria, but also other bacteria.

Nixon (1970) found a ratio of organic C to organic N of 14:1 in the concentrated brine of the Puerto Rico marine saltern investigated by him. This indicates that proteinaceous material, including amino acids, makes up a modest part of the soluble organic matter in the brine. The organic components of the brines of marine salterns have not been further identified, but Nixon (1970) remarks that excretion products of the algae may make up a substantial part.

The gradual increase in salt concentration from sea water level to at least 10 times sea water, makes the brine of the marine salterns a considerably more complex ecosystem than for example the brines of Great Salt Lake and the Dead Sea which are of relatively constant composition. The increase in the concentration of salt results in a strict selection of organisms, as outlined above. In addition, the removal of water by evaporation causes shifts in the relative proportions of the components of the brine, which may also affect the biota. Upon evaporation Ca^{++} of the sea water is precipitated as CaCO_3 and CaSO_4 , so that the strong brines contain relatively little of this element. On the other hand Mg^{++} , K^+ and SO_4^- become relatively enriched in the strong brines.

Nixon (1970) reported that the levels of inorganic phosphate and nitrate were very low in brines from marine salterns. Enrichment experiments indicated that nitrogen was a major limiting factor in the brines studied by him, meaning that lack of nitrogen in suitable form limited growth of the primary producers.

GENERAL CONSIDERATIONS

From the specific cases discussed in the foregoing, it appears that life may not be sparse in hypersaline brines and lakes. On the contrary, such brines, even at the highest salt concentrations, may be as densely populated and contain a biomass as high as only rarely encountered in sea water or fresh water bodies. However, in contrast to the dilute and fresh water environments, the hypersaline environments are characterized by a low species diversity. In the most concentrated brines the number of types of dominating organisms is, indeed, limited to very few. This is clearly seen from Tables 3-3-6 which list the characteristic organisms of four different, extremely saline environments, namely Great Salt Lake, the Dead Sea, Wadi Natrun and marine salterns. In this connection should be particularly emphasized that the quantitatively dominating organisms of the different brines are, quite regularly and consistently, the same, or closely related, organisms, notably the red-colored bacteria of the genera *Halobacterium* and *Halococcus* and the algae *Dunaliella salina* and *D. viridis*. Admittedly, our knowledge of the biota of the brines are still far from complete, and future work may well reveal additional organisms indigenous to such environments. Still, it seems justified to conclude from what we know at present that the ability to live in strong brines is limited to few organisms, and that these specialized organisms are ubiquitously distributed in such environments all over the world.

The red-colored bacteria of strong brines have attracted considerable attention, and some insight in their physiological and biochemical peculiarities has been gained (Larsen, 1973; Dundas, 1977). These organisms are truly halophilic in the sense that generally they require at least 10–15% NaCl in their environment for growth, and best growth is

attained in almost saturated salt solutions, *i.e.* 20–30% NaCl. Their requirement for NaCl is specific. NaCl cannot be replaced by other salts. These organisms have overcome the osmotic problem created by the strong salt solution in the way that they take up salt in the cells to a concentration roughly the same as that in the environment. The internal salt is mainly KCl, but also NaCl. Surprisingly, the metabolic machinery is not adversely affected by the extremely high salt concentration inside the cells. On the contrary, the enzymes are stimulated by the salt; in its absence the enzymes become inactivated and irreversibly denatured. This exceptional behavior of the metabolic units appears to be due to some very special properties of the proteins of these organisms. Most, if not all, proteins are strongly acidic in nature, and the high concentration of salt seems to be required for neutralization of the proteins so that they are kept in a conformational state at which they are metabolically active. In the absence of salt a denaturation takes place; the proteins may even go into solution and the cells may lyse. In addition to ionic interactions hydrophobic phenomena of the proteins seem to play an important role in relation to the salt (Lanyi, 1974).

Most of the halobacteria and halococci seem to have the same extreme salt requirement, regardless of the extremely halophilic environment they live in. An exception – or possibly better, a variant – to this rule is described from the Dead Sea. *Halobacterium volcanii* (Mullakhanbhai and Larsen, 1975) isolated from Dead Sea mud has an optimum requirement for NaCl of only about 10%, and this is less than half of that required optimally by other halobacteria. As a matter of fact other halobacteria have a minimum NaCl requirement considerably higher than the optimum requirement for *H. volcanii*. On the other hand, *H. volcanii* is seriously hampered in its development at NaCl concentration being optimum for the other halobacteria. This behavior of *H. volcanii* is interesting in view of the fact that the NaCl content of the Dead Sea is relatively modest compared to other extremely saline water bodies (Table 3-2). The extreme salinity of the Dead Sea is to a large extent due to its content of $MgCl_2$, and it could be shown that *H. volcanii* is extremely tolerant to $MgCl_2$. In other words, *H. volcanii* is remarkably well adapted for life in the Dead Sea.

In all cases investigated the halobacteria and the halococci have been found to have a considerable requirement also for Mg^{++} (1–5% $MgCl_2$), and this has been reported as possibly a general property of these organisms (Larsen, 1967). Recently has been described mass occurrence of halobacteria in the lakes of Wadi Natrun which are extremely low in Mg^{++} and Ca^{++} (Table 3-2, Imhoff *et al.*, 1978). A further characterization of the salt requirements of the Wadi Natrun halobacteria might thus reveal variants, and may confirm the importance of the apt remark by Dundas (1977) that “it might be fruitful to pay more attention to the original habitat in future work on extracellular salt relationship of halobacteria”.

The frequent red coloration of the strong brines due to the mass occurrence of bacteria is one of the most striking features of these ecosystems, and has led to speculations as to the possible function of the pigment in these organisms. Colorless mutants display the same behavior towards salt as the red-colored parent strains. There is thus no direct relation between coloration and halophilism. However, the colorless mutants are quite sensitive towards light. At the light intensity of a bright sun, the colorless mutants are strongly hampered in their development. The red-colored parent strains

are not affected by the light (Dundas and Larsen, 1962). A physiological role of the red pigment is thus to protect the cells against the bright sun to which these organisms are so often exposed in their natural habitats. On the other hand, the absorption of light by the pigment may increase the temperature of the brine which, in turn, may stimulate the metabolism of these bacteria having an optimum temperature as high as 40–50°C.

It has been shown that halobacteria under oxygen limiting conditions form a protein-pigment with properties similar to that of rhodopsin, and that light energy absorbed by this chromoprotein, called bacteriorhodopsin, may be converted to chemical energy within the cells, and without the participation of chlorophyll of which these cells are devoid. It has been suggested that the halobacteria use this system as an auxiliary device for ATP production in the cell under oxygen limiting conditions where a limited amount of ATP is synthesized via the respiratory chain (Oesterhelt, 1976).

When freshly isolated from nature the halobacteria often produce gas-filled vacuoles which provide buoyancy to the cells (Walsby, 1975). Petter (1932) who first described gas vacuoles in the halobacteria, put forth the reasonable hypothesis that a function of these structures is to lift the obligate, aerobic bacteria, which live in an environment of low oxygen solubility, towards air.

It is as yet not understood how the red-colored, extremely halophilic bacteria have evolved in nature. The halobacteria and the halococci both have their non-halophilic counterparts to which they seem closely related except for the halophilic character. This character seems to be a stable one. The most convincing experiments to demonstrate an adaptation to higher or lower salt concentrations have failed. Biochemical considerations on the basis of our present knowledge imply an extremely large number of mutations in order to convert a non-halophile to an extreme halophile or *vice versa* (Larsen, 1962, 1967, 1973). Also the way these bacteria spread in nature is obscure. From numerous tests in the laboratory we know that, with few exceptions, they are killed when the salt concentration goes somewhat below 10%. Still they seem ubiquitous in nature where the salt concentration is high. These findings have nourished an idea that the extremely halophilic character might come about by some special gene-transfer mechanism (Dundas, 1977). This remains, however, to be shown.

Also the brine alga, *Dunaliella*, is regularly found in the strongest brines. Two species are referred to, *D. viridis* and *D. salina*. Authors are sometimes in doubt about species designation which indicates the need for a closer taxonomic study. Both species mentioned are strongly halophilic, but with a optimum salt requirement somewhat lower than the halobacteria and the halococci.

Dunaliella belong to the green algae and is thus an eukaryote. The halophilic representatives are equipped with a mechanism to overcome the osmotic strain of the hypersaline environment which is quite different from that of the extremely halophilic bacteria. *Dunaliella* quite effectively excludes the salt from the cells, and compensates for the osmotic strain by accumulating inside the cells a corresponding amount of glycerol. For example, *D. viridis* growing in 4.25 M NaCl was reported to accumulate glycerol to a concentration of 4.4 molal. At lower salt concentrations correspondingly lower contents of glycerol were found inside the algal cells (Ben-Amotz and Avron, 1973; Borowitzka and Brown, 1974). A similar mechanism is known to operate in other eukaryotes withstanding high salt concentrations, e.g. holotolerant yeasts accumulating

glycerol when exposed to a hypersaline environment (Gustafsson and Norkrans, 1976).

Mass occurrence of phototrophic sulfur bacteria has thus far only been reported from strong brines of high alkalinity, e.g. Wadi Natrun in Egypt. There is at present no particular reason to believe that a high pH is essential for the occurrence in strong brines of this type of organisms. The phototrophic sulfur bacteria require H_2S , CO_2 and the simultaneous presence of light; a requirement for a high pH is not a general characteristic of these organisms. As pointed out by Imhoff *et al.* (1978) a mass development of phototrophic sulfur bacteria cannot be expected in the Dead Sea because of the lack of H_2S in the photic zone. The same holds true for the shallow ponds of the marine salterns where aerobiosis prevails throughout the brine. It is more difficult to explain the absence of such bacteria in Great Salt Lake where H_2S is present near the bottom at less than 10 m depth. May be phototrophic sulfur bacteria will be found in Great Salt Lake when carefully looked for. Or could it be that the salt influences these particular organisms so that they come to prefer an alkaline environment? These problems and also the general problem of the biochemical basis for their salt requirement, remain to be elucidated.

A number of halophilic cyanobacteria are known, but their halophilism is not known to be so extreme as in the case of the halobacteria, the halococci, and the phototrophic sulfur bacteria. They are therefore not so frequently encountered, and appear unhealthy, in the strongest brines (Nixon, 1970). There is, however, one outstanding exception to this rule, namely the mass occurrence of apparently healthy cyanobacteria in the strongly saline lakes of Wadi Natrun (Imhoff *et al.*, 1978). A possible explanation may be sought in the old report by Baas Becking (1931b) that while cyanobacteria are tolerant of Ca^{++} and Mg^{++} at lower salinities, they become sensitive to these ions at high salt level. At 4 M NaCl the cyanobacteria tested would grow only when no Ca^{++} or Mg^{++} was present. The brines of Wadi Natrun are precisely characterized by their low contents of Ca^{++} and Mg^{++} , whereas other strong brines contain considerable amounts of one or both of these ions (Table 2).

The biochemical basis for the halophilic character of the cyanobacteria has only recently been looked into, and our knowledge is limited. When grown at 3 M NaCl *Aphanothece halophytica* accumulates K^+ to a concentration of somewhat less than 1 M. Little or no Na^+ is taken up (Miller *et al.*, 1976). It thus appears that K^+ plays the role of an osmoregulator as in the halobacteria and the halococci, but the intracellular concentration of K^+ is far from adequate to compensate for the external salt. An organic solute of a polyol nature, and possibly free amino acids in addition, seem to be the main osmoregulators, thus displaying a regulatory mechanism reminding of *Dunaliella* and other eukaryotes (Tindall *et al.*, 1977).

It appears from the foregoing that some insight has been gained in the ecology of hypersaline environments, but much remains to be elucidated. In some cases suggestions have been set forth as to which are the dominating or limiting ecological factors for the display of life in such environments. One feels the need for more data to support these suggestions. Possibly will more information about these ecosystems lead to different explanations.

A special point of interest is the fact that hypersaline environments often contain a relatively large amount of dissolved organic matter, and thus a potentiality to support a

population of organotrophs far larger than actually found. It would be of considerable interest to have some information about the chemical composition of the dissolved organic matter.

Sulfur metabolism represents another area which may prove interesting to study. Many of the hypersaline brines contain a considerable amount of $\text{SO}_4^{=}$ (Table 3-2) and the sediment of the bottom floor of the hypersaline lakes and ponds is often characteristically black and contains H_2S , indicating activity of sulfate reducing bacteria. Very little is known about these bacteria, but in view of the findings that about half of the organic matter in marine sediments may be mineralized by anaerobic sulfate respiration (Fenchel and Jørgensen, 1977), such a process may possibly be of considerable importance also in the element cycling of the hypersaline ecosystems.

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