4. The microflora

Adaptations to life in extremely saline lakes

Lesley J. Borowitzka
Roche Research Institute of Marine Pharmacology, Dee Why, Australia

Introduction

Extremely saline lakes, long thought to be lifeless, contain primary producers (phototrophs), heterotrophs and predators, like other lakes. The ecosystem is simplified, however, because the number of species in each trophic level is low. The more saline the lake, the lower the species diversity and simpler the structure of the system.

In lakes where the salt concentration is below 100‰, many primary producers are found, ranging from diatoms (eg. *Navicula*), through filamentous green algae (eg. *Rhizoclonium, Cladophora*) to the seagrass *Ruppia*. Photosynthetic and heterotrophic bacteria and blue-green algae (cyanobacteria) abound, as do the larger heterotrophs in the food chain, the protozoa, branchiopods and so on. I am pleased to leave the animals to those who know them better, and confine myself to reviewing the microflora: the bacteria, blue-green algae, and algae. I am further restricting my review to the organisms which are found in extremely saline lakes where NaCl is the dominant salt, in concentrations from 100‰ up to saturating (about 350‰). The microflora, plus several small animals, make up the entire normal biota of these lakes. The microflora is simple enough to attempt to list entirely, and each member has apparently unique physiological adaptations which suit it to its extreme environment.

Extremely high salt concentration is not the only environmental stress encountered. Because of their geographical locations (particularly in Australia) most salt lakes receive little rainfall, but have high light intensities and high day temperatures. Salt lakes in desert areas can also attain very low temperatures; for example, the Great Salt Lake, Utah, and Antarctic saline lakes can reach temperatures below 0°, but do not freeze because of their high solute content.

High salt concentrations, particularly when combined with high temperature, restrict the solubility of gases such as oxygen and carbon dioxide. CO_2 limitation may be a major factor governing photosynthetic carbon fixation rate, that is, primary production, in extremely saline lakes.

Table 4.1 lists the members of the microflora of extremely saline lakes which are discussed in detail in this review. It summarises the data on ranges and optima of temperature and salt concentration for their growth.

An important difference between unicellular and multicellular organisms is that each cell of the former is completely in contact with the brine solution that is the salt lake environment, and must individually cope with the stresses exerted by the environment. For example, each unicellular microorganism must regulate the composition of its intracellular fluid with respect to its environment to prevent net water flow and consequent cell shrinkage or swelling. This is called 'osmoregulation' in the unicellular organisms. There is no 'buffering' of environmental stress, by fluids such as haemolymph which bathe the individual cells.

Yet unicellular organisms are generally the sole colonisers of extreme or very changeable environments. Perhaps this is because a simple, immediate physiological response is the only alternative to death in an extreme environment. Such a response is more characteristic of unicellular, comparatively

Hydrobiologia 81, 33–46 (1981). 0018-8158/81/0811-0033/$ 02.80.

Table 4.1 Microflora of extremely saline lakes.

Organism	NaCl concentration for growth		Growth temperature	
	Range (‰)	Optimum (‰)	Range (°C)	Optimum (°C)
1. Algae				
Dunaliella	20–350[a]	120	0–48	32
2. Bacteria				
2.1. Heterotrophs				
Halobacterium	150–350	200–300	?–50	37–50
Halococcus	117–350	200–300	?–50	37–50
2.2. Phototrophs				
Ectothiorhodospira halophila	90–300	110–220	n.d.	47
Ectothiorhodospira halochloris	100–340	200	n.d.	47–50
3. Blue-green algae (Cyanobacteria)				
Aphanothece halophytica	30–350	60–110	n.d.	43
Oscillatoria limnetica, *Microcoleus* sp.	80–250[b]	n.d.	25–48[b]	n.d.

[a] See text for discussion and sources of data presented in this table.
[b] Approximate values. These represent the range in Solar Lake.
n.d. = Not determined.

simple organisms than more complex organisms.

Halobacterium spp. and *Dunaliella* spp. from extremely saline lakes have been intensively studied in culture. Other organisms, such as the photosynthetic anaerobic bacteria have barely been described, though they may contribute significantly to primary production and participate in nutrient cycles such as the sulphur cycle. Studies with laboratory cultures are needed to investigate physiology and environmental adaptations, but what has been neglected is the important step of relating these physiological studies back to the salt lake situation.

The time and effort expended on studying the microflora of Australian salt lakes has been much less than, for example, for the Great Salt Lake, where Dr. Thomas Brock, Dr. Fred Post and many others have been active. Work in Australia began with the energetic Prof. Baas-Becking, and I am pleased to note a recent increase in studies, inspired in part by commercial interests.

The algae: Dunaliella

Both prokaryotic and eukaryotic algae grow, and contribute to primary production in lakes which have salt concentrations above 200‰. The prokaryotic, blue-green algae (cyanobacteria) are dealt with later. The only eukaryotic alga which grows in extremely saline lakes is *Dunaliella.*

Dunaliella is a unicellular, motile green alga, belonging to the Order Volvocales and Division Chlorophycophyta. Two halophilic species were originally described by Téodoresco (1905, 1906). These are *D. salina,* whose cells accumulate carotenoids when grown in high salt concentrations and therefore appear red, and *D. viridis,* which remains green in all salt concentrations. Both species are present in salt lakes around the world, though red *D. salina* cells usually predominate, often giving extremely saline lakes a characteristic red colour. The green species *D. minuta* Lerche, *D. parva* Lerche and *D. euchlora* Lerche also live in salt

lakes, and have all been isolated from Lake Eyre in South Australia (Baas-Becking & Kaplan 1956).

Dunaliella has several growth forms and reproduces both sexually and asexually. Growth form, and type of reproduction are determined by salt concentration and probably other environmental parameters. Growth form and reproductive mode may therefore be regarded as a type of response to particular environmental conditions, but this aspect of the response to stress has been neglected in recent work.

Lerche (1937) and Hamburger (1905) were among early workers who described several growth forms, particularly for *D. salina:* vegetative cells, palmelloid cells and aplanospores. Encysted zygotes, the diploid cells resulting from gamete union in sexual reproduction, have also been described.

(i) Vegetative cells and asexual reproduction

Generally, when the salt concentration of the growth medium lies between 40‰ and saturating (about 350‰) and nutrients are not severely limiting, *D. salina* exists as motile vegetative cells, red or green coloured according to conditions. These cells usually reproduce asexually, by longitudinal division.

(ii) Palmelloid stage

Lerche (1937) reported that in concentrations below 10‰ NaCl, vegetative cells of *D. salina* settle, lose their flagella and form 6 μm round, green non-motile cells. This change was difficult to reverse in culture, but motile vegetative cells reappeared on raising the concentration to 50–100‰. In a brackish pond in Poland (below 10‰ NaCl), *Dunaliella* occurred only as a palmelloid form attached to sediments and rocks (Lerche 1937). In very dilute parts of the Great Salt Lake, palmelloid forms of *Dunaliella* are found in benthic algal mats (Brock 1976). Palmelloid forms have not been reported in Australian lakes, but have probably not been sought or recognised.

(iii) Aplanospores, asexual resting cysts

Hamburger (1905) reported asexual resting cysts in *D. salina* cultures. This was disputed by Lerche (1937) but Loeblich (1972) has recently described apparently the same forms as haploid aplanospores. She found that when the salt concentration of the medium was below 40‰, 37% of *D. salina* cells formed round, haploid, non-motile cysts, 12 μm in diameter and with a thick, bumpy outer layer. They were brown coloured, and canthaxanthin was the major carotenoid, not β-carotene as in the vegetative cells.

(iv) Sexual reproduction and encysted zygotes

D. salina and *D. viridis* are both capable of sexual reproduction, but some other species, for example *D. tertiolecta,* apparently are not (Butcher 1959). Sexual reproduction in *Dunaliella* is induced by a reduction in salt concentration, optimally from 100‰ to 30‰ (Lerche 1937).

In *D. salina,* motile gametes are either identical with the vegetative cells or form from them with no apparent morphological change. The gametes are isogametes, with each *D. salina* clone producing (+) or (–) gametes, but not both. Fusion of the gametes appears to occur in a manner similar to that of *Chlamydomonas* (Brown *et al.* 1968). First the flagella touch, and then the gametes form a cytoplasmic bridge and fuse.

The zygote has a thick outer layer and red pigmentation; it is large and heavy and sinks to the bottom of the lake. It can withstand freshwater or dry salt surroundings. Zygotes germinate about 3 weeks after being transferred from a very low NaCl concentration to about 100‰ NaCl. Two to thirty two motile haploid daughter cells are released through a tear in the cell envelope (Lerche 1937).

In general, it appears that the stress of low salt concentration possibly accompanied by nutrient limitation, triggers the morphological changes to produce a cell (or cyst) more able to survive in the dilute environment than is the vegetative cell. Nothing is known of the physiology of cell forms other than the vegetative cells.

In summary, the adaptation of *D. salina* to the stress of a salt concentration below 20‰ in the growth medium appears to require morphological changes. Physiological changes in the vegetative cells cope with salt concentrations within the range of 20‰ to saturating salts.

Salt concentration

Not only do *Dunaliella* spp. grow in salt concen-

trations higher than any other eukaryotic alga or plant, but they adapt very quickly to sudden changes in salt concentration. Their halotolerance has been the subject of a recent review (Brown & Borowitzka 1979).

D. salina has the widest reported growth range of all the species, 20‰ to saturated NaCl (Loeblich 1972). *D. viridis* is slightly less salt tolerant, as its growth range is 9‰ to 304‰ NaCl (0.15 M to 5.2 M) (Brock 1975), though it is still viable in saturated salt solution. Optimum NaCl concentrations for growth are 58–89‰ (1.0–1.5 M) for *D. viridis* (Borowitzka *et al.* 1977) and 120‰ (2.04 M) for *D. salina* (Loeblich 1972). Both optima are considerably below the actual concentrations in the lakes where *Dunaliella* spp. dominate the microflora. As Brock (1975) points out, the numerical dominance of *Dunaliella* in NaCl concentrations above 200‰ is not because it grows optimally there, but because it can tolerate that salt concentration better than its competitors, though growing suboptimally. The relatively small effect of widely different NaCl concentrations on growth rate is illustrated by a *Dunaliella* isolate from the Great Salt Lake (van Auken & McNulty 1973). It had a minimum doubling time in 192‰ NaCl of 30 h, whereas in 280‰ and 110‰ the doubling time was 42 h, and in 350‰ (saturating salt) the doubling time was 78 h. So the doubling time of the culture merely increased by a factor of two although the salt concentrations tripled.

With regard to *Dunaliella's* physiological adaptations to salt, it is noted that it can withstand transitions of hundreds of parts per thousand in salt concentration with no damage to the cells, except a temporary loss of shape and motility (Borowitzka *et al.* 1977; Ben-Amotz & Avron 1973a). *Dunaliella* has no cell wall, and changes shape when salt-stressed.

Up to ten years ago it was believed that the basis of *Dunaliella's* tolerance of high salt concentrations and tolerance of changes in those concentrations was a mechanism of NaCl uptake by the cells, followed by free water flux to equalise intracellular and extracellular osmotic pressures (Marrè & Servettaz 1959; Trezzi *et al.* 1966; Ginzburg 1969). *Dunaliella* employs the strategy of accumulating a high intracellular concentration of solute to balance the concentration of salts in the external medium, but the solute it accumulates is neither NaCl nor KCl. At least five cytoplasmic and membrane bound enzymes, and crude chloroplast preparations are as salt sensitive as their counterparts in other types of cells (Johnson *et al.* 1968, Ben-Amotz & Avron 1972; Borowitzka & Brown 1974).

D. tertiolecta, D. viridis (Borowitzka & Brown 1974), *D. parva* (Ben-Amotz & Avron 1973a) and *D. salina* (Norton & Borowitzka, unpublished results) all have very high intracellular concentrations of glycerol; the concentrations in each case are comparable to the concentrations of salts in the growth medium. Furthermore, glycerol is a compatible solute, which means that it is a low molecular weight, neutral solute which can be accumulated to high concentrations within cells without causing enzyme inhibition (Borowitzka & Brown 1974). When *D. viridis* is grown in 247‰ (4.25 M) NaCl, the cytoplasm contains 4.4 molal glycerol, plus 0.17 molal K^+, plus low levels of other solutes and macromolecules. The concentration of intracellular glycerol varies directly with the NaCl concentration in the growth medium (Borowitzka & Brown 1974).

The ability to produce and accumulate a compatible solute is therefore the physiological basis for the ability of *Dunaliella* to grow over a range of salt concentrations.

Apparently no alteration of enzyme structure is required for enzymes to function in extremely high glycerol concentrations, as *Dunaliella* enzymes appear to be indistinguishable from those from other sources (Heimer 1973). Since the enzymes function equally well in high and low glycerol concentrations, their function is almost independent of extracellular salt concentration. This last factor means that *Dunaliella* can grow over a much wider concentration range than *Halobacterium* (see below).

Osmoregulation in *Dunaliella* relies on controlled synthesis and degradation of glycerol; healthy cells do not release glycerol even under hypotonic stress (Ben-Amotz & Avron 1973a). Because the adaptation to changes in external solute concentration takes about 1 h, it is proposed that the first step in the sequence of changing the intracellular glycerol concentration is activation or inhibition of a pre-existing allosteric regulatory enzyme. This would be followed by synthesis or inhibition of synthesis of the same enzyme and further enzymes in the biosynthetic pathway (Borowitzka *et al.* 1977). An $NADP^+$-specific glycerol dehydrogenase has been

shown to have kinetic characteristics appropriate to an enzyme involved in the regulation of intracellular glycerol levels (Ben-Amotz & Avron 1973b; Borowitzka & Brown 1974; Borowitzka *et al.* 1977).

How the cell senses external salt concentration, and senses changes in that concentration, then transmits the information to regulatory enzymes and genes is unknown.

Temperature

Like most halophiles, halophilic species of *Dunaliella* have relatively high growth temperature optima. A *Dunaliella* sp. isolated from the Great Salt Lake grew best at 32° (van Auken & McNulty 1973). *D. salina* isolated from a Western Australian salt lake has its highest photosynthetic rate between 35° and 40°, with a fall to half that rate at 45° (Kessly & Borowitzka unpublished results).

D. viridis and *D. salina* are also very resistant to cold. The temperature of the Great Salt lake can drop below zero in winter, and both species remain viable and motile at -3°. At -18° their activity ceases (Post 1977).

Glycerol may serve a dual role in *Dunaliella* cells: as major osmoregulatory compatible solute, and also as a solute which reduces the effects of heat and cold stress on enzymes. The presence of a high concentration of the compatible solute, glycerol, limits change in water structure and formation of ice crystals at low temperature. Changes in water structure, and ice crystal formation alter enzyme function and damage enzyme and cell structure. Glycerol is commonly used as a cryopreservative because it reduces ice crystal damage of macromolecules. The molecular basis of compatible solute interactions with water, and with macromolecules are discussed in detail by Borowitzka (1981).

Light intensity

Halophilic *Dunaliella* spp. are often exposed to extremely high light intensities, because they live in shallow lakes. Furthermore, salt lakes are frequently found in geographical regions with few cloudy or rain-days *per annum.*

D. salina has been reported to grow best in 10 000 lux continuous white light (Yurina 1966) but this is probably an underestimate of its optimum light intensity. A Great Salt Lake isolate grew best in CO_2-enriched air at 25 000–35 000 lux (van Auken & McNulty 1973).

D. salina is the only *Dunaliella* species that accumulates large quantities of carotenoids, mainly β-carotene, plus smaller amounts of canthaxanthin, neoxanthin and astaxanthin (Aasen *et al.* 1969). High salt concentration, high light intensity and high temperature cause *D. salina* to accumulate carotenoids (Lerche 1937).

D. salina cells grown in medium containing 250‰ NaCl are red due to accumulation of carotenoids. *D. salina* grown in half that NaCl concentration (125‰) have no obvious accumulations of carotenoids and are green because the chlorophyll colour is not masked by carotenoid. Comparing cultures grown in otherwise identical conditions, the maximum photosynthetic rates are the same, about 600 μmole O_2 mg chlorophyll^{-1} h^{-1}, but occur at different light intensities: 4 000 μEinstein m^{-2} s^{-1} for a green (125‰ NaCl) culture and 7 000 μEinstein m^{-2} s^{-1} for a carotenoid-rich (250‰ NaCl) culture (Kessly & Borowitzka unpublished results). Since both intensities are higher than found even at salt lakes, *D. salina* must normally photosynthesise below maximum rate in highly saline lakes. The green *D. salina* cells have a lower compensation point than the red cells, which means that for a longer time during the day the photosynthetic rate of the green cells is greater than their respiration rate, and there is net incorporation of new carbon into the cells (Kessly & Borowitzka unpublished results).

Similar quantities of chlorophyll are present in *D. salina* cells grown in 250‰ and 125‰ NaCl, but the presence of more carotenoid in the former appears to lower the light harvesting efficiency of the chlorophyll (Kessly & Borowitzka unpublished results). Loeblich (1972) showed that the carotenoids of *D. salina* have no light harvesting function, so the expenditure of energy on synthesis of carotenoids appears, at first, to be a liability to the cells. Yet it is the red form of *D. salina,* not the green form or any other green species of *Dunaliella,* that dominates the salt lake environment.

A photoprotective role has been proposed for the carotenoids of *D. salina* (Loeblich 1972). Post (1977) points out that the red form of *D. salina* is found throughout the water column of the Great Salt Lake, but green *Dunaliella* cells are found only in shaded areas. Microscopic examination of

Australian salt lake waters shows mostly red *D. salina* cells, though green *D. viridis* is usually culturable from the same water sample. Under laboratory conditions, *D. viridis* and green forms of *D. salina* are favoured over red *D. salina* cells, even in high salt concentrations. Probably the low light intensities (relative to daylight) that are generally used for culturing give the green forms a photosynthetic and growth advantage over the carotenoid-rich cells.

Bacteria

Halophilic bacteria are those which require high salt concentrations to live. Those which tolerate high salt concentrations, but do not require them for growth are accurately termed halotolerant.

Microscopic examination of water from Australian salt lakes usually reveals numbers of rod shaped, motile, pleomorphic bacteria. Typical total counts of bacteria from extremely saline lakes in Western Australia and South Australia in summer range from 10^3 to 10^6 ml^{-1}. (L. Borowitzka unpublished data). This population compares with 40×10^6 to 240×10^6 cells ml^{-1} of *Halobacterium* and *Halococcus* in the Great Salt Lake throughout the year (Post 1977) and 2.3×10^6 to 8.9×10^6 bacteria ml^{-1} in the Dead Sea in winter (Kaplan & Friedmann 1970). The biomass of halophilic bacteria in the Great Salt Lake is greater than in any other known lake, and is greater than the biomass of the much larger *D. salina* cells (200–10 000 cells ml^{-1}) (Post 1977).

A wide range of heterotrophic activities has been attributed to bacteria from extremely saline lakes. Volcani (1944) set up enrichment cultures from the Dead Sea, using 250‰ NaCl and different carbon sources in the growth media. Anaerobic incubation produced sugar fermenters, methanogenic bacteria, denitrifiers, cellulose digesters and others. Aerobic incubation produced cellulose digesters and petroleum and kerosene decomposers. In addition there were the more prosaic proteolytic and saccharolytic aerobes (those that use protein and sugars as their energy source).

Enrichment cultures from Australian salt lakes, using complex media containing 250‰ NaCl and incubated aerobically at 30–40°, yield many different types of bacteria. Colonies range in pigmentation from colourless through yellow, orange, red and purple. All grow well at 40° and some red-purple isolates grow in the light on solid medium with no added carbon source. These are probably *Halobacterium halobium,* which will be discussed later.

In view of this apparent variety of physiological types of bacteria in extremely saline lakes, it must be emphasised that no-one has yet separated the autochtonous flora (those which live and grow there) from the allochthonous flora (those which have arrived and survived, but are not active or growing). This means that little is known of the heterotrophic processes of extremely saline lakes, a major aspect of the ecology.

Only two genera of heterotrophic bacteria have been studied and shown to live and grow in extremely saline lakes. They are *Halobacterium* and *Halococcus,* and both live in the water column. They are proteolytic or saccharolytic and probably derive most of their carbon from glycerol and cell protein produced by *Dunaliella.*

Some green and purple anaerobic photosynthetic sulphur bacteria may also be present in the water column, if conditions are sufficiently anaerobic.

If the sediment is sufficiently anaerobic, the

Table 4.2 Concentrations of ions within the cytoplasm of *Halobacterium* and *Halococcus* cells.[a]

Organism	Intracellular		
	Na^+	K^+	Cl^-
	(molal, moles kg^{-1} cell water)		
Halobacterium salinarium	1.37 ± .21	4.57 ± .12	3.61 ± .70
Halococcus (Sarcina) morrhuae	3.17 ± .28	2.03 ± .36	3.66 ± .25

[a] Data from Christian and Waltho (1962).

photosynthetic sulphur bacteria *Ectothiorhodospira halophila* and *Ectothiorhodospira halochloris* grow there. In both aerobic and anaerobic sediments filamentous and unicellular blue-green algae may be present. The blue-green algae will be discussed separately later. The heterotrophic and photosynthetic bacteria will be treated separately because of their different responses to salinity, temperature and light.

Halobacterium and Halococcus

More is known about the physiology of the halophilic heterotrophs than the phototrophs, and *Halobacterium* has been more closely studied than *Halococcus.*

Salt concentration

The three best known species of *Halobacterium* are *H. salinarium, H. halobium* and *H. cutirubrum* although only the first two are recognised in Bergey's Manual (Gibbons 1974). They all grow in the range 150‰ (2.6 M) to saturated NaCl and best growth occurs between 200‰ (3.4 M) and 300‰ (5.0 M) NaCl (Larsen 1963). The cells lyse in less than 2.6 M NaCl. *Halococcus* will grow in 117‰ (2.0 M) NaCl, and up to saturating NaCl (Brown, 1976). Recently a new species, *H. volcanii,* was isolated from the Dead Sea and shown to grow best between 100‰ (1.7 M) and 146‰ (2.5 M) NaCl, about half the optimum concentration for the other *Halobacterium* spp. It also tolerates high Mg^{2+} concentrations; the combination of preferred NaCl range and Mg^{2+} tolerance make it specially suited for life in the Dead Sea which has a relatively high Mg^{2+} content (Mullakhanbhai & Larsen 1975).

Related to their high NaCl requirement, *Halobacterium* spp. and *Halococcus* spp. differ from non-halophiles in further respects: (i) in the intracellular concentrations of Na^+, K^+ and Cl^-; (ii) in the cell envelope structure, both protein and lipid components; (iii) in the primary structure of cell proteins, including enzymes.

Table 4.2 shows the concentrations of ions in the cytoplasm of *Halococcus* and *Halobacterium* grown to stationary phase in medium containing 234‰ (4.0 M) NaCl and 0.032 M KCl. The amounts and proportions of intracellular ions vary with conditions and age of culture, but remain near saturating (Ginzburg *et al.* 1970, 1971).

Most enzymes cannot function in the presence of high salt concentrations, but the enzymes of the halophilic bacteria not only function optimally in high salt concentrations but require molar concentrations of KCl or NaCl to prevent denaturation. Of more than 50 halophile enzymes examined, only one has been found to be inhibited by high salt concentrations. It is a fatty acid synthetase, and because it is salt sensitive it probably does not function *in vivo* (Pugh *et al.* 1971).

Lack of fatty acid synthetase activity is reflected in the unusual lipid composition of the cell envelope. Instead of the usual esterified fatty acids, there are hydrocarbon side chains bound to glycerol by an ether bridge (Kates 1972).

The amino acid composition of the bulk cell protein, individual enzymes and ribosomal and membrane proteins reflects a higher than normal proportion of acidic and neutral amino acid residues (Brown, 1976). It was thought that the presence of high concentrations of cations in the cytoplasm was needed to stabilise protein and enzyme structures by electrostatic binding to the negatively charged side chains of the acidic amino acids. Charge shielding was proposed to reduce the mutual repulsion of the negative charges and tend to stabilise the protein structure.

However, relatively low concentrations of cations (0.5 M) are actually needed to shield the known number of negatively charged side chains. Lanyi (1974) has suggested an additional role for the cations which requires higher concentrations. Nearly saturating levels of salts alter water structure in such a way as to promote hydrophobic interactions within macromolecules. Such interactions, involving the neutral amino acid side chains would tend to stabilise the tertiary structure of proteins against unwinding and denaturation.

The cell envelope of *Halobacterium* lacks muramic acid, α-ϵ-diaminopimelic acid, teichoic acid and D-amino acids. These are major components of the peptidoglycan which makes up most bacterial cell walls (Brown 1964). *Halococcus* also lacks muramic acid and thus peptidoglycan (Brown & Cho 1970). Like the enzyme proteins, the cell envelope proteins of both bacteria have a high proportion of acidic amino acids. Brown (1964) proposes that charge shielding by Na^+ at the outside surface of the membrane and K^+ and Na^+ on the inside, stabilises the envelope proteins just as

cation binding contributes to halophile enzyme stability. Electrostatic interactions of cations with the proteins of the cell envelope apparently replace the need for the peptidoglycan usually found in bacterial cell envelopes.

Hydrophobic associations between non-polar amino acid side chains and lipids in membranes probably also contribute to the stability of the halophile cell envelope. The requirement for high cation concentrations is obligate; below about 120‰ (2 M) the cells lose their shape and the plasma membrane disintegrates.

It has been shown that adaptation of *Halobacterium* and *Halococcus* to high NaCl concentrations is extensive. Their genes code for proteins with primary structures (i.e. amino acid sequences and ratios) different from non-halophile proteins. But the adaptation is irreversible: the primary structure of enzymes, and ribosomal and membrane proteins confers an obligate requirement for 117–146‰ (2–2.5 M) salt (100‰ in *H. volcanii*) both extracellularly and intracellularly. *Dunaliella* can grow in both low and high salt concentrations largely because its enzymes function equally well in low and high glycerol concentrations. However, halophilic bacteria cannot grow below 100‰ NaCl, because in adapting to function in high intracellular salt concentrations their enzymes (and cell envelope proteins) have lost the ability to function in low salt concentrations.

Temperature

Like all known halophilic microorganisms, *Halobacterium* and *Halococcus* have relatively high temperature optima for growth. The range of optima for Great Salt Lake aerobic heterotrophs is 37–50°, and the most extensive growth of bacteria occurs in summer, near the shore where the temperature is 40° (Brock 1979).

At the molecular level, adaptation to high temperatures is probably connected with the adaptation to high salt concentrations and depends on the presence of a high concentration of intracellular solute. Most proteins have thermal stability maxima below 10°. *Halobacterium* menadione reductase has a maximum close to 25–30°, among the highest found in any protein. Lanyi (1974) suggests that this unusually high thermal stability supports his proposal that there is extensive hydrophobic stabilisation within halophile proteins. Studies on nitrate reductase from *H. salinarium* have shown that the unusually high temperatures of 85° and 73° give maximum enzyme activity, but only in the presence of high salt concentrations, 4.27 M (250‰) NaCl and 2 M KCl respectively. At lower salt concentrations these temperatures cause thermal denaturation (Marquez & Brodie 1973). It was suggested that high salt concentrations confer thermal stability on halophile proteins because they promote stabilising hydrophobic interactions within the proteins.

Light intensity

Because *Halobacterium* and *Halococcus* live in the water column of salt lakes, they are often exposed to higher light intensities than other bacteria. *Halobacterium* and *Halococcus* accumulate a C_{50} carotenoid, α-bacterioruberin (Marshall *et al.* 1969; Kelly *et al.* 1970) and natural isolates are heavily pigmented pink, orange or red. It has been suggested that this and other, minor carotenoids play an important photoprotective role. A colourless mutant of *H. salinarium* was shown to be more vulnerable to visible light damage than the pigmented parent strain (Larsen 1963). However, there is evidence that carotenoids have a more complex photoprotective function; they are involved in an energy transfer capacity that facilitates photoreactivation by visible light after exposure to damaging doses of ultraviolet radiation (Hescox & Carlberg 1972).

A positive, and so far unique, utilization of light as an energy source has been demonstrated in *H. halobium*. The membrane of *H. halobium* contains a purple pigmented complex of bacteriorhodopsin. When grown anaerobically in the light, the bacteria excrete protons, respiration is inhibited and intracellular ATP increases. The proton flux produces an electrochemical gradient, which, according to Danon & Stoeckenius (1974) can be used for ATP production within the framework of Mitchell's chemiosmotic mechanism. It is emphasised that this process of transduction of light energy to chemical energy (ATP) is not photosynthesis involving CO_2 fixation. It is known only in *H. halobium* growing under anaerobic conditions and the importance of the process, and indeed *H. halobium* itself, in the salt lake ecosystem is unknown.

This is an illustration of what I have mentioned

earlier, that much of the data on the unusual physiology of the salt lake organisms in culture has not been related back to the ecosystem. Conversion of light energy to ATP by the purple membrane of *H. halobium* has been the subject of intensive research in the past five years, but to my knowledge no-one has established that the conversion occurs in nature in the salt lake. The abundance of *H. halobium* in salt lakes, relative to other halophilic bacteria, is also unknown; so we have no way of assessing the role of this light energy transduction process in the salt lake.

An interesting additional factor in the ecology of salt lakes is the population of bacteriophages specific for halophilic bacteria and capable of lysing them. Post (1977) has reported a variety of bacteriophages specific for the halophilic bacteria in the most saline arm of the Great Salt Lake. A bacteriophage specific for *H. salinarium* (Torsvik & Dundas 1974) and one for both *H. cutirubrum* and *H. halobium* (Wais *et al.* 1975) are also known. These are true 'halophages', requiring high salinities to be able to infect and lyse the *Halobacterium* spp.

The role of bacteriophages in the salt lake ecosystem is not known, but they have a potential bearing on halophilic bacterial numbers and species composition.

Photosynthetic anaerobic bacteria

Two species of halophilic photosynthetic sulphur bacteria have been isolated from salt lake sediments which are rich in H_2S. *Ectothiorhodospira halophila,* from a salt lake in Oregon, U.S.A., and *Ectothiorhodospira halochloris,* from an alkaline salt lake in Egypt, are both anaerobes which deposit elemental sulphur outside their walls. Both can function as photoautotrophs or photoheterotrophs. As photoautotrophs they use light for energy, CO_2 as carbon source and sulphide, sulphur, succinate or other organic compounds as photosynthetic electron donors. As photoheterotrophs they use light as their energy source, but employ organic compounds such as acetate, pyruvate and succinate both as carbon sources and electron donors (Raymond & Sistrom 1969; Imhoff & Trüper 1977). Cultures of *E. halophila* are purple and *E. halochloris* pale green, tending to brown-red in denser cultures. The species differ in their major photosynthetic pigments: *E. halophila* has bacteriochlorophyll *a*, and *E. halochloris* has bacteriochlorophyll *b*. *E. halophila* is probably identical with isolates reported from other salt lakes (van Niel 1931; Baas-Becking 1928). Many Australian salt lakes have H_2S-rich sediments and probably have one or both of these bacteria as an integral part of the sulphur cycle.

In the stratified water column of Solar Lake, Sinai, anoxic zones rich in H_2S (up to 39 ppm) develop. The presence and stability of these zones allows growth of anaerobic photosynthetic bacteria in the water column. A layer of purple bacteria, identified as *Chromatium violescens* lies above a layer of the more H_2S-tolerant green *Prosthecochloris* spp. (Cohen *et al.* 1977).

Under stratified conditions in Solar Lake, photosynthetic bacteria live in 70–200‰ NaCl and temperatures of 36–48°, and contribute 91% of the total primary production of Solar Lake. When mixing occurs, oxygen is introduced and the layers disperse; photosynthesis in the water column decreases because the conditions are no longer suitable for the photosynthetic anaerobes.

In any lake with enough mixing to limit stratification, the contribution of photosynthetic bacteria to primary production would be less than in Solar Lake. Figures of 20–85% contribution to total lake carbon fixation have been given for 10 other lakes (Cohen *et al.* 1977).

Chromatium spp. has also been reported in Great Salt Lake (Tew 1966, quoted by Brock 1979), but as far as I can determine, no culturing or physiological studies of salt lake *Chromatium* or *Prosthecochloris* have been done. More is known about the sediment-dwelling *Ectothiorhodospira* spp., so I will confine my remarks on physiology and adaptation to those bacteria.

Salt concentration

The salt responses of the two *Ectothiorhodospira* species are similar. The growth range for *E. halophila* is 90–300‰, and for *E. halochloris* 100–340‰ NaCl. The optimum for growth of *E. halophila* lies in the range 110–220‰ and for *E. halochloris* is 200‰ NaCl. *E. halophila* lyses in 10–30‰ salt, and *E. halochloris* does not grow below 50‰, probably also because of cell damage, then lysis (Raymond & Sistrom 1969; Imhoff & Trüper 1977).

Both are therefore obligate extreme halophiles.

The physiological basis for their NaCl requirement is entirely unknown; in fact both species have been isolated and described only in the last 10 years. The adaptive mechanisms may be similar to those of the halophilic bacteria, but the concentrations of salts required and the range preferred for growth are different from those of the halophilic heterotrophs. The two groups are not closely related taxonomically.

Temperature

In common with the halophilic heterotrophs, the phototrophs have very high temperature optima for growth, 47° in the case of *E. halophila* and 47–50° for *E. halochloris* (Raymond & Sistrom 1969; Imhoff & Trüper 1977). These temperatures are by no means uncommon in the sediments of shallow saline lakes, including Australian lakes. Without knowledge of intracellular solutes and proteins, we cannot guess at the molecular basis for this thermal stability.

Light intensity

Both species live on the sediment surface rather than in the water column, and probably receive and utilise only a portion of the light intensity and wavelengths incident at the water surface. Their major photosynthetic pigments, bacteriochlorophylls *a* and *b*, absorb strongly in the blue region of the spectrum with a peak at 398 and smaller peaks at 602 and 1 020 nm for *E. halophila* bacteriochlorophyll *a*, and peaks at 389, 598 and 1 018 nm for *E. halochloris* bacteriochlorophyll *b* (Raymond & Sistrom 1969; Imhoff & Trüper 1977). Most photosynthetic bacteria tolerate only low light intensities compared with the photosynthetic eukaryotes.

Rhodopin and two of its derivatives are the major carotenoids of *E. halochloris,* and spirilloxanthin is the major carotenoid of *E. halophila.* It is not known whether the carotenoids play a role in light harvesting.

Blue-green algae – Cyanobacteria

Many genera of blue-green algae live in lakes with up to 100‰ NaCl and may be cultured in media with a similar NaCl content. The predominant filamentous blue-green algae in lakes with 50–100‰ NaCl are *Phormidium* spp., *Oscillatoria* spp., *Microcoleus* spp. and *Spirulina* spp. (Brock 1979; Krishna Pillai 1955).

Above 100‰ NaCl, the number of species diminishes; *Phormidium tenue* (Krishna Pillai 1955) and species of *Oscillatoria* and *Microcoleus* (Cohen *et al.* 1977) have been reported in some extremely saline lakes. One unicellular blue-green alga, *Aphanothece halophytica* is found in lakes with more than 100‰ NaCl.

Photosynthetic aerobes – Aphanothece halophytica

Little is known of the adaptations of the aerobic filamentous blue-green algae to life in very saline lakes, or even the extent of their salt tolerance. I will therefore restrict this discussion to *A. halophytica,* a unicellular blue-green alga of the Order Chroococcales.

In culture, *A. halophytica* cells have very variable size and shape: 2–10 μm long and ellipsoidal, ovoid or cylindrical. They produce mucus and are found embedded in this mucilage.

There is some confusion in the generic designation of this organism. Salt lake isolates fitting the extended description have been identified as *Aphanocapsa* Nägeli (Volcani 1944, following Hof & Fremy 1933) and *Aphanothece halophytica* Nägeli (Brock 1976, also following Hof & Fremy 1933), but because of cell pleomorphism Brock (1976) suggests that these are isolates of the same organism.

Drouet and Daily (1956) have merged both genera into the genus *Coccochloris* Drouet & Daily and the *Coccochloris elabens* Drouet & Daily of Kao *et al.* (1973) is probably identical with the previously mentioned isolates.

Aphanothece fits Group 1A of the Chroococcales (Stanier *et al.* 1971) but may be distinguished from *Synechococcus* Nägeli and probably *Synechococcus* Stanier *et al.* by its salt lake habitat and the amorphous mucilage, in which the cells are loosely arranged. However, a recent isolate from an Egyptian salt lake (Imhoff *et al.* 1978) was named *Synechococcus;* Brock (1979) has suggested that this isolate may be identical with *A. halophytica.*

I have discussed in some detail the taxonomic assignments of these unicellular isolates because I believe that the use of different names in the literature for the same or very similar organisms

(*Aphanocapsa halophytica, Aphanothece halophytica, Coccochloris* sp. and *Synechococcus* sp.) has complicated the synthesis of data on physiology and ecology of the isolates. Good taxonomic work is needed to show whether the isolates are identical or not, so that the considerable literature can be used to advantage. On the evidence to date it appears that the isolates are similar and referable to *Aphanothece halophytica.*

A. halophytica has been isolated from Australian natural and man-made salt lakes as far apart as Bajool, Queensland, and Port Hedland, Western Australia (L. Borowitzka, unpublished data). The cells are ovoid to cylindrical, 6–9 × 5 μm, and embedded in irregular lumps of mucus 10–50 cm across, attached to rocks and sediment. The lumps usually smell of H_2S, though this may not be a product of *A. halophytica.*

Mucus production by *A. halophytica* has caused problems to salt makers, because in large quantity it spoils salt quality. Smaller amounts, on the other hand, can have the useful property of sealing the porous surfaces forming the evaporation ponds.

Planktonic *Aphanothece halophytica* and a morphologically distinct *Aphanocapsa littoralis* are found in the top, aerobic, 1 m of stratified Solar Lake, Sinai (Cohen *et al.* 1977), about 2×10^3 cells ml^{-1} in the equivalent of 70–100‰ salinity. *A. halophytica* is the predominant blue-green alga in the lake and is found throughout the water column when mixing makes the whole column aerobic.

A halophytica is found in a mixed mat of algae and photosynthetic bacteria in the South Arm of the Great Salt Lake, but may be cultured from the water column as well (Brock 1976).

Salt concentration

Aphanothece halophytica grows in media containing 30‰ to saturated (350‰) NaCl. However, when Great Salt Lake water was added to enrichment media covering that concentration range, cultures of *A. halophytica* developed only in the range 160–230‰ NaCl. This defines the optimum for *A. halophytica* with respect to its competitors. Below 160‰ a mixture of other algae, including diatoms and filamentous blue-green algae, grew faster than *A. halophytica* and dominated the cultures. Above 230‰ *Dunaliella* outgrows *A. halophytica* (Brock 1975).

A minimum of 30‰ NaCl is required to preserve the cell integrity of *A. halophytica*, 60‰–120‰ is the range for optimum growth and slow growth occurs up to 300‰ (Brock 1976; Tindall *et al.* 1978).

Osmoregulation in blue-green has been recently shown to involve changes in both organic and inorganic components of the cytoplasmic solution. Salt tolerant *Phormidium tenue* has been reported to accumulate SO_4^{2-}, Ca^{2+} and K^+ from the medium to maintain osmotic balance (Krishna Pillai 1955). More recently, Miller *et al.* (1976) have shown that the level of K^+ in *A. halophytica* varies directly with external NaCl concentration up to an intracellular concentration of 1 M. But the intracellular enzymes are salt sensitive: activities of selected enzymes from glycolysis, the pentose phosphate pathway and the Krebs cycle are severely repressed at levels of KCl and NaCl 'far below those of the external medium' (Tindall *et al.* 1977). Actual enzyme activity in 1 M KCl was not stated. However, activity of glucose-6-phosphate dehydrogenase from a similar marine *Synechococcus,* (grown in 80‰ NaCl), was reduced by 80% when 1 M KCl was added to the assay mixture containing crude cell extract (Borowitzka *et al.* 1980).

Sensitivity of cytoplasmic enzymes to the stated concentrations of salts is no proof that such concentrations do not exist in the cells. However, a search was made for organic 'compatible'-type organic solutes which may reduce the need for inorganic ions as osmoregulatory particles within the cytoplasm, or alleviate the inhibitory effect of the inorganic ions on enzymes. The marine *Synechococcus* was shown to accumulate a polyhydric compound, α-glucosylglycerol, in response to the salt concentration in the growth medium (Borowitzka *et al.* 1980). *Aphanothece halophytica* isolated from an Australian salt lake accumulates quantities of betaine and glutamate in response to the salt concentration of the growth medium (Mackay, Norton & Borowitzka, unpublished results). Betaine is accumulated by some salt tolerant higher plants and reduces salt inhibition of the plant enzymes (Pollard & Wyn Jones 1979). Hence it is likely that the combination of salts with betaine and glutamate osmotically balances the salinity changes in the salt lake environment with minimum inhibitory effect on the cytoplasmic enzymes of *A. halophytica.*

Temperature and light intensity

Physicochemical factors interact in their effect on the growth of *A. halophytica*. At 40°, 230‰ NaCl in the growth medium inhibits growth less than at 30°; this same type of synergism was seen in studies on growth and enzyme activity in halophilic bacteria. *A. halophytica* grows optimally at 43°, 5 300 lux light intensity and when NaCl concentration is between 60‰ and 120‰ (Tindall *et al.* 1978). The optimum CO_2 concentration for growth varies from 0.033% to 3% depending on NaCl concentration, light intensity and temperature. Tolerance of high temperatures is typical of members of the Chroococcales (Castenholz 1969) and consistent with the generalisation that halophiles prefer high growth temperatures. The optimum light intensity of 5 300 lux is much lower than that of *Dunaliella* and should favour benthic rather than planktonic growth.

Photosynthetic aerobic and anaerobic blue-green algae

When Solar Lake, Sinai, forms stratified layers, a benthic mat of *Oscillatoria salina, O. limnetica* and *Microcoleus* sp. forms 50 cm deep on the bottom sediment (Cohen *et al.* 1977). The temperature there is around 40° and the salinity 160–200‰ when the lake is stratified; after mixing, the salinity is lower (about 100‰) and so is the temperature (below 30°). The filamentous blue-green algae in the mat are able to survive the physico-chemical changes caused by the annual cycle of stratification and mixing. The physiological basis of their salt and temperature tolerance is not known.

Another important adaptation to the physiochemical environment in the lake has been studied by Cohen *et al.* (1977) and Jørgensen & Cohen (1977). When the water column is well mixed, conditions in the bottom mat are aerobic, and photosynthesis proceeds as normal. CO_2 is fixed using H_2O as electron donor. However, when the water column is stratified, the mat lies below layers or purple and green sulphur bacteria. The H_2S they generate can reach concentrations of 39 ppm near the mat, and the water is anaerobic. Under these conditions, *O. limnetica* (and probably the other mat algae) switch to anoxygenic photosynthesis. Photosystem I functions alone, and H_2S is used as electron donor for photosynthesis. Elemental sulphur is deposited around the filaments and no oxygen is evolved.

Anoxygenic photosynthesis only occurs when conditions are anaerobic and H_2S level is high, that is, when the lake is stratified. The ability to switch between oxygenic and anoxygenic photosynthesis with the cyclic changes in the hydrology of the water column is a supreme adaptation to an extreme environment.

A. halophytica occurred only in aerobic layers of Solar Lake and has not been reported capable of anaerobic anoxygenic photosynthesis. The *Synechococcus* strain from the Wadi Natrun, however, is capable of this function (Imhoff *et al.* 1978) so further investigations of *A. halophytica* are needed to see whether it is capable of anoxygenic photosynthesis.

Concluding remarks

The purpose of this paper has been to review the aspects of the physiology of the salt lake algae and bacteria that suit them to their environment. The ecology of the microorganisms is discussed in more detail in Chapter VI by Dr. Post, but I will mention two aspects of the microflora that I feel have considerable bearing on the salt lake system.

None of the blue-green algae isolated from extremely saline lakes have been shown to fix nitrogen, and none have heterocysts, the usual structure associated with nitrogen fixation. With little runoff from the land, lack of combined nitrogen may be severely limiting to growth in the lakes. The main contribution of the blue-green algae must be as primary producers, together with the photosynthetic bacteria and *Dunaliella.*

The contribution by the photosynthetic bacteria to primary production in some salt lakes is much greater than in the marine and soil environments. Their contribution is dependent on anaerobic conditions, but these are frequently present in stratified extremely saline lakes.

Stratification occurs to some extent in many extremely saline lakes, and is a crucial factor in determining the types of phototrophs, heterotrophs and predators in the system, and their spatial and temporal distribution.

References

Aasen, A. J., Eimhjellen, K. E. & Liaaen-Jensen, S., 1969. An extreme source of β-carotene. Acta chem. scand. 23: 2544–2545.

Baas-Becking, L. G. M., 1928. On organisms living in concentrated brine. Tijdschr. ned. dierk. Vereen. 3, Ser. 1: 6–9.

Baas-Becking, L. G. M. & Kaplan, I. R., 1956. The microbiological origin of the sulphur nodules of Lake Eyre. Trans. R. Soc. S. Aust. 79: 52–65.

Ben-Amotz, A. & Avron, M., 1972. Photosynthetic activities of the halophilic alga, Dunaliella parva. Pl. Physiol. Lancaster, 49: 240–243.

Ben-Amotz, A. & Avron, M., 1973a. The role of glycerol in the osmotic regulation of the halophilic alga, Dunaliella. Pl. Physiol. Lancaster, 51: 875–878.

Ben-Amotz, A. & Avron, M., 1973b. NADP specific dihydroxyacetone reductase from Dunaliella parva. FEBS Letters, 29: 153–155.

Borowitzka, L. J., 1981. Solute accumulation and regulation of cell water activity. In: L. G. Paleg and D. Aspinall (eds.). Physiology and Biochemistry of Drought Resistance in Plants. Academic Press.

Borowitzka, L. J. & Brown, A. D., 1974. The salt relations of marine and halophilic species of the unicellular green alga, Dunaliella. The role of glycerol as a compatible solute. Arch. Microbiol. 96: 37–52.

Borowitzka, L. J., Demmerle, D., Mackay, M. A. & Norton, R. S., 1980. Carbon-13 nuclear magnetic resonance study of osmoregulation in a blue-green alga. Science 210: 650–651.

Borowitzka, L. J., Kessley, D. S. & Brown, A. D., 1977. The salt relations of Dunaliella. Further observations on glycerol production and its regulation. Arch. Microbiol. 113: 131–138.

Brock, T. D., 1975. Salinity and the ecology of Dunaliella from the Great Salt Lake. J. gen. Microbiol. 89: 285–292.

Brock, T. D., 1976. Halophilic blue-green algae. Arch. Microbiol. 107: 109–111.

Brock, T. D., 1979. Ecology of saline lakes. In: M. Shilo (ed.) Strategies of Microbial Life in Extreme Environments. Dahlem Konferenzen, Berlin.

Brown, A. D., 1964. Aspects of bacterial response to the ionic environment. Bact. Rev. 28: 296–329.

Brown, A. D., 1976. Microbial water stress. Bact. Rev. 40: 803–846.

Brown, A. D. & Borowitzka, L. J., 1979. Halotolerance of Dunaliella. In: M. Levandowsky & S. H. Hutner (eds.) Physiology and Biochemistry of Protozoa. Vol. 1. Academic Press, New York.

Brown, A. D. & Cho, K. Y., 1970. The walls of extremely halophilic cocci. Gram-positive bacteria lacking muramic acid. J. gen. Microbiol. 62: 267–270.

Brown, R. M., Johnson, C. & Bold, H. C., 1968. Electron and phase contrast microscopy of sexual reproduction in Chlamydomonas moewusii. J. Phycol. 4: 100–120.

Butcher, R. W., 1959. An introductory account of the smaller algae of British Coastal Waters. Part 1. Fisheries Investigations, Series IV. Her Majesty's Stationary Office, London.

Castenholz, R. W., 1969. Thermophilic blue-green algae and the thermal environment. Bact. Rev. 33: 476–504.

Christian, J. H. B. & Waltho, J. A., 1962. Solute concentrations within cells of halophilic and non-halophilic bacteria. Biochim. biophys. Acta 65: 506–508.

Cohen, Y., Krumbein, W. E. & Shilo, M., 1977. Solar Lake (Sinai). 2. Distribution of photosynthetic microorganisms and primary production. Limnol. Oceanogr. 22: 609–620.

Danon, A. & Stoeckenius, W., 1974. Photophosphorylation in Halobacterium halobium. Proc. natn. Acad. Sci. U.S.A. 71: 1234–1238.

Drouet, F. & Daily, W. A., 1956. Revision of the coccoid myxophyceae. Butler University Botanical Studies, 12: 1–222.

Gibbons, N. E., 1974. Halobacteriaceae. In: R. E. Buchanan and N. E. Gibbons (eds.) Bergey's Manual of Determinative Bacteriology. Eighth Edition. Williams and Wilkins, Baltimore.

Ginzburg, M., 1969. The unusual membrane permeability of two halophilic unicellular organisms. Biochim. biophys. Acta 173: 370–376.

Ginzburg, M., Sachs, L. & Ginzburg, B. Z., 1970. Ion metabolism in a Halobacterium. I. Influence of age of culture on intracellular concentrations. J. gen. Physiol. 55: 187–207.

Ginzburg, M., Sachs, L. & Ginzburg, B. Z., 1971. Ion metabolism in a Halobacterium. II. Ion concentrations in cells at different levels of metabolism. J. Membrane Biol. 5: 78–101.

Hamburger, C., 1905. Zur Kenntnis der Dunaliella salina und einer Amöbe aus Salinenwasser von Cagliari. Arch. Protistenk. 6: 111–130.

Heimer, Y. M., 1973. The effects of NaCl, KCl and glycerol on the activity of nitrate reductase of a salt-tolerant and two non-tolerant plants. Planta, 113: 279–281.

Hescox, M. A. & Carlberg, D. M., 1972. Photoreactivation in Halobacterium cutirubrum. Can. J. Microbiol. 18: 981–985.

Hof, T. & Fremy, P., 1933. On myxophyceae living in strong brines. Recl. Trav. Bot. Neerl. 30: 140–162.

Imhoff, J. F., Hashwa, F. & Trüper, H. G., 1978. Isolation of extremely halophilic phototrophic bacteria from the alkaline Wadi Natrun, Egypt. Arch. Hydrobiol. 84: 381–388.

Imhoff, J. F. & Trüper, H. G., 1977. Ectothiorhodospira halochloris sp. nov., a new extremely halophilic phototrophic bacterium containing bacteriochlorophyll b. Arch. Microbiol. 114: 115–121.

Johnson, M. K., Johnson, E. J., MacElroy, R. D., Speer, H. L. & Bruff, B. S., 1968. Effects of salts on the halophilic alga Dunaliella viridis. J. Bact. 95: 1461–1468.

Jørgensen, B. B. & Cohen, Y., 1977. Solar Lake (Sinai). 5. The sulphur cycle of the benthic cyanobacterial mats. Limnol. Oceanogr. 22: 657–660.

Kao, O. H. W., Berns, D. S. & Town, W. R., 1973. The characterisation of c-phycocyanin from an extremely halotolerant blue-green alga, Coccochloris elabens. Biochem. J. 131: 39–50.

Kaplan, I. R. & Friedmann, A., 1970. Biological productivity in the Dead Sea. 1. Microorganisms in the water column. Isr. J. Chem. 8: 513–528.

Kates, M., 1972. Ether-linked lipids. In: F. Snyder (ed.) Ether lipids. Chemistry and Biology. Academic Press, New York.

Kelly, M., Norgard, S. & Liaaen-Jensen, S., 1970. Bacterial carotenoids XXXI. C_{50}-carotenoids 5. Carotenoids of H. salinarium, especially bacterioruberin. Acta chem. scand. 24: 2169–2182.

Krishna Pillai, V., 1955. Observations on the ionic composition of blue-green algae growing in saline lagoons. Proc. natn. Inst. Sci. India, 21B: 90–102.

Lanyi, Y. K., 1974. Salt dependent properties of proteins from extremely halophilic bacteria. Bact. Rev. 38: 272–290.

Larsen, H., 1963. Halophilism. In: I. C. Gunsalus and R. Y. Stanier (eds.) The Bacteria. Vol. 4. Academic Press, New York.

Lerche, W., 1937. Untersuchungen über Entwicklung und Fortpflanzung in der Gattung Dunaliella. Arch. Protistenk. 88: 236–268.

Loeblich, L. A., 1972. Studies on the brine flagellate, Dunaliella salina. Dissertation, University of California at San Diego.

Marquez, E. D. & Brodie, A. F., 1973. The effect of cations on the heat stability of a halophilic nitrate reductase. Biochim. biophys. Acta, 321: 84–89.

Marrè, E. & Servettaz, O., 1959. Sul meccanismo di adattamento a condizioni osmotiche estreme in Dunaliella salina. II. Rapporto fra concentrazioni del mezzo esterno e composizione del succo cellulare. Atti. Accad. Naz. Lincei Cl. Sci. Fis. Mat. Natur. Rend. Ser. 8, 26: 272–278.

Marshall, C. L., Wicken, A. J. & Brown, A. D., 1969. The outer layer of the cell envelope of Halobacterium halobium. Can. J. Biochem. 47: 71–74.

Miller, D. M., Jones, J. H., Yopp,. J. H., Tindall, D. R. & Schmid, W. D., 1976. Ion metabolism in a halophilic blue-green alga, Aphanothece halophytica. Arch. Microbiol. 111: 145–149.

Mullakhanbhai, M. F. & Larsen, H., 1975. Halobacterium volcanii spec. nov., a Dead Sea Halobacterium with a moderate salt requirement. Arch. Mikrobiol. 104: 207–214.

Pollard, A. & Wyn Jones, R. G., 1979. Enzyme activities in concentrated solutions of glycinebetaine and other solutes. Planta 144: 291–298.

Post, F. J., 1977. The microbial ecology of the Great Salt Lake. Microbial Ecol. 3: 143–165.

Pugh, E. L., Wassef, M. K. & Kates, M. 1971. Inhibition of fatty acid synthetase in Halobacterium cutirubrum and Escherichia coli by high salt concentrations. Can. J. Biochem. 49: 953–958.

Raymond, J. C. & Sistrom, W. R., 1969. Ectothiorhodospira halophila: A new species of the genus Ectothiorhodospira. Arch. Mikrobiol. 69: 121–126.

Stanier, R. Y., Kunisawa, R., Mandel, M. & Cohen-Bazire, G., 1971. Purification and properties of unicellular blue-green algae (Order Chroococcales). Bact. Rev. 35: 171–205.

Téodoresco, E. C., 1905. Organisation et développement du Dunaliella, nouveau genre de Volvocacée-Polyblepharidée. Beihefte zum Botanischen Zentralblatt, 18: 215–232.

Téodoresco, E. C., 1906. Observations morphologiques et biologiques sur le genre Dunaliella. Revue gén. bot. 18: 353–371.

Tindall, D. R., Yopp, J. H., Miller, D. M. & Schmid, W. E., 1978. Physico-chemical parameters governing the growth of Aphanothece halophytica (Chroococcales) in hypersaline media. Phycologia, 17: 179–185.

Tindall, D. R., Yopp, J. H., Schmid, W. E. & Miller, D. M., 1977. Protein and amino acid composition of the obligate halophile Aphanothece halophytica (Cyanophyta). J. Phycol. 13: 127–133.

Trezzi, F., Galli, M. G. & Bellini, E., 1966. The resistance of D. salina to osmotic stresses: Ultrastructure researches. Giorn. Bot. Ital. 72: 255–263.

Torsvik, T. & Dundas, I. D., 1974. Bacteriophage of Halobacterium salinarium. Nature, Lond. 248: 680–681.

Van Auken, O. W. & McNulty, I. B., 1973. The effect of environmental factors on the growth of a halophilic species of algae. Biol. Bull. mar. biol. Lab., Woods Hole, 145: 210–222.

Van Neil, C. B., 1931. On the morphology and physiology of the purple and green sulphur bacteria. Arch. Mikrobiol. 3: 1–112.

Volcani, B. E., 1944. The Microorganisms of the Dead Sea. In: Papers collected to Commemorate the 70th Anniversary of Dr. Chaim Weizmann. Collective Volume. Daniel Sieff Research Institute, Rehovoth, Israel.

Wais, A. C., Kon, M., MacDonald, R. E. & Stollar, B. D., 1975. Salt-dependent bacteriophage infecting Halobacterium cutirubrum and H. halobium. Nature, Lond. 256: 314–315.

Yurina, E. V., 1966. Experiments in the cultivation of the halobiont algae Asteromonas gracilis and Dunaliella salina. Vestn. Mosk. Univ. 21: 76–83.