HALOPHILY AND HALOTOLERANCE IN CYANOPHYTES*

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Abstract. The survival, growth and distribution of organisms in hypersaline environments is discussed using cyanophytes (cyanobacteria) as examples. The distinction between halophilic (Na^+-requiring) and halotolerant organisms is not adequate to describe the entire spectrum of adaptations to salt. The classical division into stenohaline (narrow) and euryhaline (wide) adaptational types, with optima identified as oligo-, meso- and polyhaline, better reflects both organismal adaptations and the environmental conditions to which these are adjusted and is therefore recommended as a conceptual model.

Two independent properties of organisms are growth and survival. Organisms requiring narrow ranges of salt concentration are considered specialists and are restricted to environments with relatively constant salinities at any particular concentration. Organisms which tolerate wide ranges of fluctuation in salinity are considered generalists. The existence of separate and distinct microbial assemblages in these two types of environments is demonstrated in marine intertidal zones and seasonal salt works, representative of fluctuating salinity, and in the open ocean. The hypersaline ponds of Yallahs, Jamaica, and Solar Lake, Sinai represent different but relatively constant salinities. It is concluded that cyanophytes speciate along the salinity gradient, and that separate halophilic taxa occupy environments with relatively constant salinities.

1. Introduction

Many aquatic environments become periodically reduced in volume by evaporation, passing through stages of increasing ionic concentrations and sequential mineral precipitation. Such conditions are common in arid regions of the world, and, judging from the abundance of evaporitic sediments in the fossil record, have also been common in the past. Evaporation posed difficulties to the survival of aquatic organisms, which responded by evolving a variety of mechanisms to cope with high salinities; this distinguishes them from freshwater organisms. To express this distinction such terms as halotolerant and halophilic organisms, facultative and obligate halophiles, or extreme and moderate halophiles have been coined; however, these terms have not been clearly defined nor do they adequately reflect the full diversity of phenomena associated with life in the presence of salt.

The adaptations of various organisms to high salt concentrations differ qualitatively, although in all cases studied the intracellular ionic strength correlates closely to that of the saline environment. While eukaryotes, such as *Dunaliella* use photosynthetic production of glycerol to achieve internal osmotic balance with the external salinity, most prokaryotes (including halobacteria and cyanophytes) accumulate intracellular K^+ (Miller *et al.*, 1976). A specific requirement for high Na^+ that cannot be substituted by sucrose, K^+, Ca^{2+}, Mg^{2+} or other ions seems universal for all halophiles, (Mohr and Larsen, 1963; Waterbury, 1979). Enzymes, electron transport and ribosomes, once adjusted to an elevated salt concentration, lose their structural stability without it (Brock, 1969). An extreme case is that of *Halobacterium* which, unlike other prokaryotes, has a glycoprotein

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cell wall that is structurally dependent on Na^+ (Blaurock *et al.*, 1976). Halobacteria can grow in saturated brines, but cease growth and lose their shape below 15% NaCl; below 12% their cell walls disintegrate (McLeod and Matula, 1962). Prokaryotes show different physiological responses to sea water: some grow well in sea water without requiring Na^+, others require Na^+ only, while specialized marine forms have a suite of additional ion requirements (McLeod, 1971; Reichelt and Baumann, 1974; Waterbury, 1979).

In addition to qualitative differences, organisms show quantitative differences with respect to the minimum requirements, growth optima and tolerance levels of the NaCl concentrations to which they can adjust. However, the criteria for distinguishing between extreme and moderate halophily and halotolerance have not been established.

Furthermore, there is variability in salt-related phenomena in the variety of saline environments inhabited, ionic compositions, concentrations and changes over time. The selective pressures in the evolution of salt-adapted organisms differ in each of these cases.

In the present paper I will address the following questions using cyanophytes as examples: (1) how to define halophily and halotolerance in terms of the survival value of these properties for cyanophytes in their natural environments; (2) are environments with constant salinity inhabited by different taxa than those with fluctuating salinity; and (3) do cyanophytes speciate along a gradient of increased salinity and evolve taxa specialized to various salinity levels?

2. Halophily or Halotolerance

Organisms living under conditions of high salinity and requiring a higher minimum concentration of NaCl than marine organisms, are usually called halophiles. The first part of this definition implies tolerance to salt, while the second part indicates a dependence on salt.

It has been recognized for half a century that a distinction should be drawn between organisms able to *withstand* high salt concentrations and those able to *grow* and *multiply* under such conditions (Hoff and Frémy, 1933). Hoff and Frémy defined as true halophiles those organisms that grow in 3M (17.55%) NaCl solution, labeling others that survive this concentration but do not grow and develop as halotolerant. The same definition was accepted by Fogg *et al.* (1973, p. 299). On this basis Hoff and Frémy identified several species, including *Aphanothece halophytica* Frémy as true halophiles, and called *Microcoleus chthonoplastes* Thuret (a filamentous cyanophyte known to form mats in salt works that persist throughout the full evaporation sequence) a 'typical halotolerant species'. Their isolate of *M. chthonoplastes* did not grow above 2M (11.7%) NaCl concentration. Lower limits and the minimum requirement for NaCl in *Microcoleus* have not been established.

Flannery (1956) in his definition of bacterial halophily chose 2% NaCl as the lower boundary between obligate halophiles and facultative halophiles. The latter can grow below this limit but grow better above it.

Batterton and Van Baalen (1971) studied the growth preferences of several cyanophytes isolated from marine environments and found that these particular isolates grew better at lower salinities. They could not isolate cyanophytes from brines and concluded that cyanophytes found in brines are not halophilic but merely tolerate high salinities. This view has been challenged independently by Miller *et al.* (1976) and by Brock (1976). Both groups isolated truly halophilic cyanophytes from brines. In addition, Brock explained that a similar 'extremely tolerant' cyanophyte isolated earlier by Kao *et al.* (1973) is, in fact, a true halophile, differing from his own isolate in the level of its salinity adjustment. Significantly lower salt requirements have been found in many marine cyanophytes isolated by Waterbury (1971), and yet these requirements have proved to be just as obligatory as those of the halophiles living in brines.

We can see from the preceding discussion that halophily has been defined in at least two ways. One definition is based on the various minima Na^+ concentrations required for growth. The other refers to the maximum salt concentration at which the organism can grow. Similarly, tolerance to salt has been used in two ways. One describes the range of salinities within which the organism can grow. The second is concerned with the ability of an organism to survive saline conditions although the organism does not grow.

3. Growth and Salinity

The growth of several cyanophytes isolated from saline environments and grown under various salt concentrations is summarized in Figure 1. Each of the strains presented has a characteristic range within which growth is permitted and a specific range of optimum growth. Growth can be suppressed either by salt concentrations below the minimum required or by those above the maximum that limits growth. Eukaryotic algal taxa isolated from brines have a similar spread along the salinity gradient (Gibor, 1956). Information on growth suppression by salt in marine heterotrophic bacteria indicates a similar salinity gradient distribution (McLeod, 1965). Thus, halophily cannot be defined as growth above or below any particular salinity value. It is rather a property that allows an organism to adjust its growth to a specific salinity range, which can be defined in terms of three characteristic values: minimum, optimum and maximum. Species with an optimum adjustment to high salinities also have higher minimum salt requirements and higher maxima at which growth still occurs; many of those adjusted to lower salinities may grow in freshwater, although not optimally. Those truly freshwater species with a negligible minimum Na^+ requirement also show the lowest tolerance to salt. The ranges of growth tolerance vary from taxon to taxon.

The distribution of growth optima along the salinity gradient is best described by the classical ecological scheme that relates the organisms to the limiting ecological determinant of salinity (see Ruttner, 1962). Organisms having narrow ranges of growth may be referred to as stenohaline and those having wide ranges as euryhaline (Figure 2). Extreme halophiles in the upper ranges of salinity are polyhaline; moderate halophiles, mesohaline, and those at the lower salinity ranges, such as many marine and brackish water species, may qualify as oligohaline. Combined names, e.g. oligo-euryhaline, are used to express both

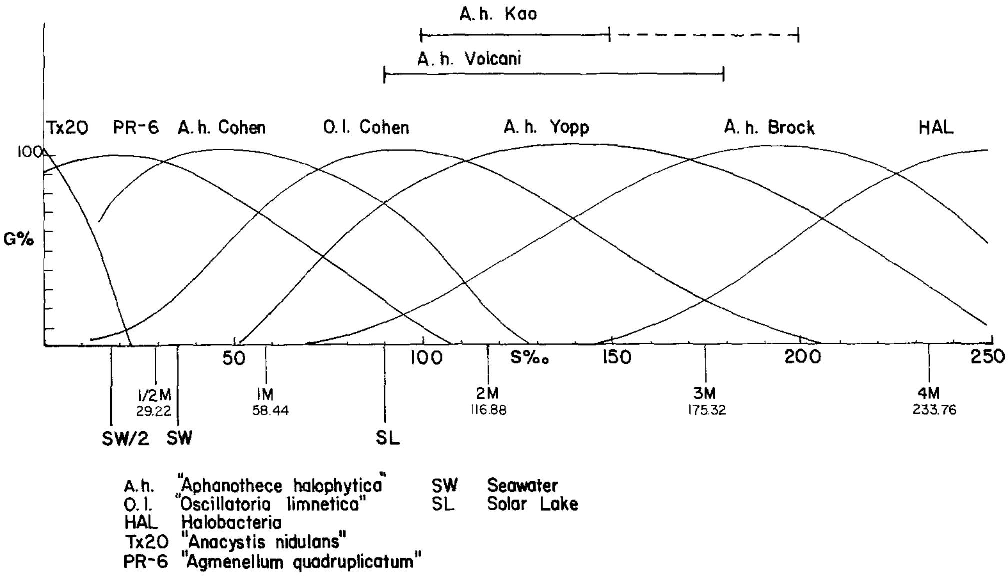


Fig. 1. Distribution of growth optima of cyanophytes isolated from environments of various salinities, expressed in percents of their optimal growth. Salinity is plotted in parts per thousand (°/oo), and in moles of NaCl (M).

range as well as optimum. This scheme restrains setting fixed boundaries to categories, stressing the gradient of increasing salinity, and thus can accommodate a large number of phenomena. It should be noted, however, that the data in Figure 1 refer to active organismal growth in 'unialgal' cultures, a condition which eliminates the effects of competition. When such organisms grow in mixed natural populations, a competitive pressure may be expected to exclude organisms from suboptimal salinity ranges, thus narrowing the width of the actual salinity niche around the optimum of each particular species.

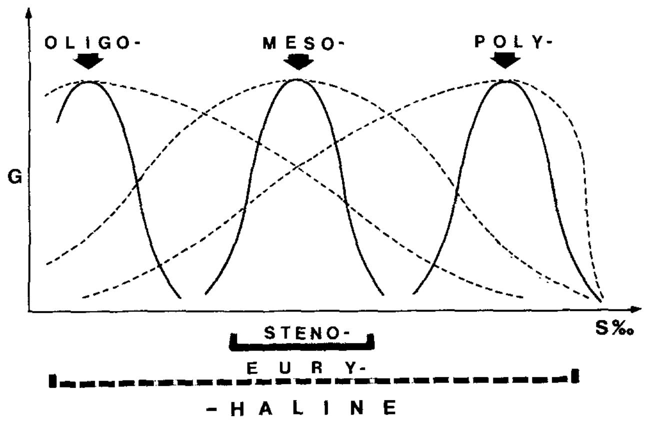


Fig. 2. Schematic presentation of the relationship of organismal adaptations to salinity. G = growth; S°/oo = salinity in parts per thousand NaCl.

4. Survival and Salinity

In the preceding section 'tolerance' to salt refers to the growth of organisms, yet the *halotolerance* of Hoff and Frémy (1933) refers to the property of withstanding, i.e. surviving, certain salinity conditions after growth has ceased. The distinction between halophilic and halotolerant organisms, arbitrarily set at 3M NaCl by these authors, is impractical in view of other criteria for halophily. It does not include moderate halophiles with lower, although obligate, salt requirements and limitations. For example, Hoff and Frémy found *Microcoleus chthonoplastes* to be halotolerant above 3M NaCl. With respect to the salt requirement and growth preference, however, the same strain might behave as a halophile at salinities below 2M NaCl concentration. Nevertheless, Hoff and Frémy's definition of halotolerance is important because it stresses *survival* under high salinity conditions.

Specific differences in the ability to survive high salinity have been shown by Stewart (1964) who compared at various salinities, the growth of two cyanophytes isolated from the marine environment. The taxa he compared, *Calothrix scopulorum* and *Nostoc endophytum*, cease growth at their respective salinity maxima, but only *Calothrix* resumes growth when lower salinities are re-established. Thus *Calothrix* shows a reversible growth inhibition.

While the growth of an organism may be confined to wider or narrower ranges of salinity (eury- vs steno), its ability to survive adverse salinity conditions may stretch far beyond the conditions that permit active growth. Thus, growth and survival represent two independent properties. The selective advantage of the ability to survive adverse conditions is obvious in an environment with fluctuating salinity. For example, an organism which can survive a wide range of salinities, but grows actively only within a narrow range, may dominate such habitats as the intertidal and wave spray zones of the sea coast by alternating short spurts of growth with long periods of dormancy, while its competitors are being eliminated by one or the other extreme of the fluctuating range. Therefore, the occurrence of an organism and its dominance in a natural habitat may reflect either its ability to survive or its ability to grow under the prevailing conditions of that particular habitat.

Survival of organisms or of their dormant stages under conditions not permitting their growth is a phenomenon which accounts for the common enrichment, isolation and culturing of organisms alien to the habitat under study, while the culturing of indigenous organisms fails. This may explain, for example, why Batterton and Van Baalen obtained no halophiles from brines, why some halophiles were isolated from terrestrial environments, and why many bacteria isolated from marine environment behaved as non-marine organisms (McLeod, 1965). However, the abundance or dominance of an organism in the habitat implies also an ability to grow and compete for this habitat within certain recurring periods when growth is permitted. Therefore, the presence or dominance of an organism in its natural habitat should be noted at the time of its isolation in culture.

5. Saline Environments and Their Inhabitants

Both halophily and halotolerance constitute different evolutionary strategies and thus offer different selective advantages. Accordingly, we can predict that stable environments with constant salinity at any concentration will select for halophily and stenohaline specialization. Conversely, environments with fluctuating salinity will select for halotolerance and euryhalinity. We have observed that stable and fluctuating environments are inhabited by different taxa as will be exemplified in the following discussion.

The open ocean is probably the most constant saline environment, while the rockpools and wave spray zone of its coasts are probably the least constant ones. Lagoons, tidal flats, salt marshes, and estuaries are intermediate. In arid regions where evaporation rates exceed atmospheric precipitation, water bodies of various sizes secluded from the ocean, embayments, lagoons, salt ponds gradually evaporate. In the process, the concentration of solutes increases, creating an array of environments with elevated salinities. Under humid climatic conditions, such secluded embayments are gradually diluted by freshwater resulting in a similar range of low salinity environments. The relative constancy of salinity depends on the size of a bounded saline environment. Salinity fluctuation in a semi-bounded water body is regulated by exchange with the ocean waters.

The tropical open ocean is populated by a few unique cyanophyte taxa, which form blooms of considerable population density. The genus *Trichodesmium* is the best known (Figure 5A). The nitrogen fixation in natural populations of *Trichodesmium* has been studied although pure cultures have not been achieved, indicating that the growth requirements of *Trichodesmium* are complex and the niche specialization, narrow. Fluctuating environments in the intertidal zone harbor epilithic and endolithic (carbonate boring) species on rocky shores, many of which can be distinguished from freshwater forms on morphological grounds. These have a wide distribution, although some regionality and latitudinal zonation in cyanophytes and microalgae has been observed (LeCampion, 1970; Schneider, 1976; Golubic *et al.*, 1975). A different set of microorganisms, primarily cyanophytes, is found in sand and mudflats; several of these taxa form laminated stromatolitic structures analogous to fossil stromatolites, particularly to those of Precambrian age (Golubic, 1976a, 1976b; Golubic and Hofmann, 1976). Microbial communities similar to those occurring in the intertidal flats of tropical lagoonal environments have been noted in salt works which are active during a short season in the summer. All salt pans used in the salt works of Piran, Yugoslavia, maintain a salinity close to that of the sea water throughout the winter. Only during 6 to 8 weeks in the summer do a series of these pans contain water of increasing salinity up to the precipitation of halite (Herrmann *et al.*, 1973).

Relatively constant salinity is maintained in large ponds that are adjacent to the ocean waters as well as in large secluded water bodies and inland lakes of arid regions, and all known halophilic cyanophytes have been isolated from such environments: the Great Salt Lake (Brock, 1976), a solar evaporation pond, Redwood City California (Yopp and Tindall, 1974), a salt pond in Puerto Rico (Kao *et al.*, 1973), the Dead Sea (Elazari-Volcani, 1944), Solar Lake, Sinai (Cohen, 1975), or isolated from salt that precipitated in these environments (Hoff and Frémy, 1933).

We have conducted field studies and investigated the occurrence and dominance of cyanophytes in a series of hypersaline ponds at Yallahs, south Jamaica and of the Solar Lake, Sinai, near Eilat, Israel.

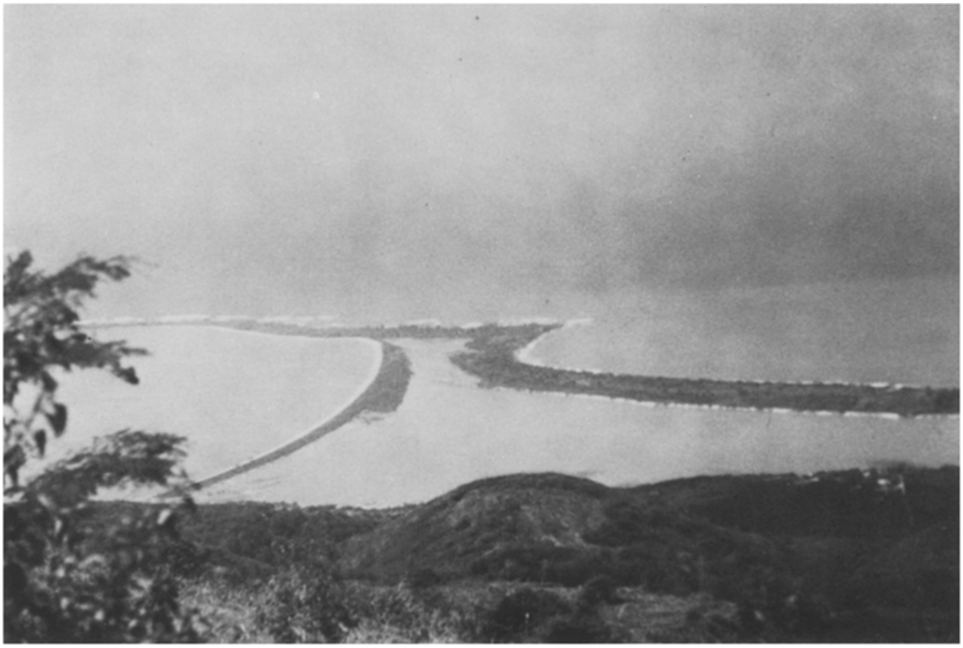


Fig. 3. View of the Yallahs salt ponds, south Jamaica. Great Salt Pond is to the left and Little Salt Pond to the right, Caribbean Sea in the background.

The Yallahs ponds, Jamaica (Figure 3) are three shallow hypersaline basins, Great Salt Pond, Little Salt Pond and West Pond separated from the ocean by a barrier beach and mutually connected by narrow channels. At the beginning of the dry season (February, 1978) the salinity in these ponds was 2.2M, 2.5M, and 4.5M NaCl. Both Great and Little Salt Ponds had localized seepage areas of lower salinity (0.54M) where sea water entered th ponds in the form of 'springs'. In addition to these seepage areas, small temporary puddles along the shores receive periodic windblown pond water, which either evaporates or becomes diluted by rain. These seepage locations and littoral puddles have the highest salinity fluctuation. In the course of the dry season the salinity gradually increases in all three ponds, but only the West Pond evaporates completely.

The Solar Lake, (Sinai peninsula) is smaller and deeper than the Yallahs Ponds. Seepage from the adjacent Red Sea occurs profusely through a beach barrier. The fluctuation of the water level in this pond is a function of the ratio of water seepage rate to the evaporation rate, as rain rarely occurs in this desert region. Seepage of lighter sea water during the winter results in overlayering of the heavier brine water of the Solar Lake forming one or more chemoclines. During the summer, evaporation causes a salinity increase at the surface ultimately resulting in mixing of the entire water column at the end of the summer (Cohen *et al.*, 1977).

Within these hypersaline ponds, three distinct habitats could be identified which are occupied by different microbial communities: (1) the seepage littoral community is

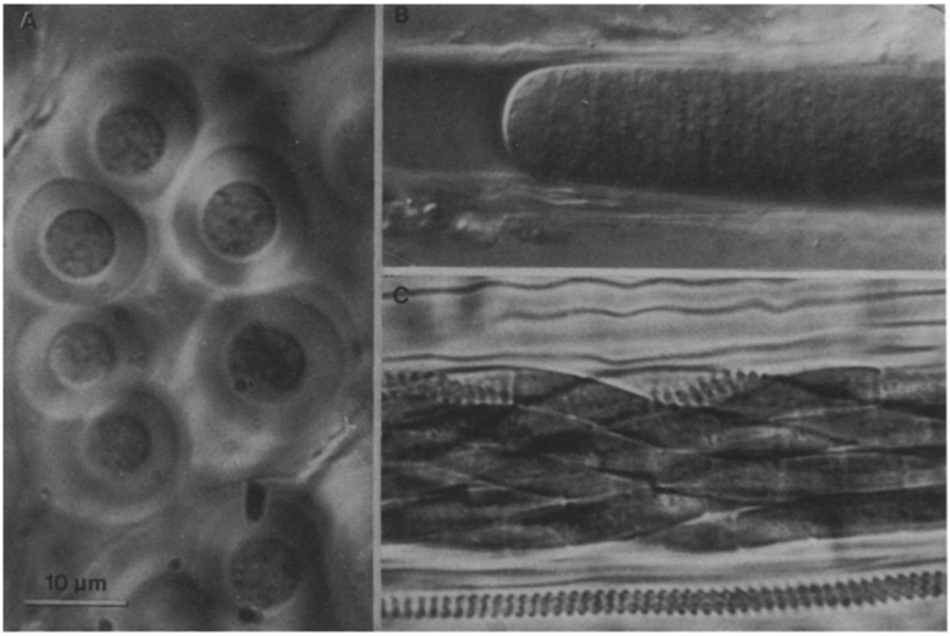


Fig. 4. Cyanophytes that dominate environments with fluctuating salinities (intertidal zone of tropical lagoons). (A) *Entophysalis major* Erceg. (B) *Lyngbya aestuarii* (Mertens) Liebman; (C) *Microcoleus chthonoplastes* Thuret with *Spirulina subtilissima* Kuetz. (Migrated into the *Microcoleus* sheath.) Scale bar = 10 μm.

composed of organisms that are in contact with varying salinity and form mats; (2) the pond plankton refers to organisms which are suspended in the water or loosely settled on the bottom; and (3) the pond benthos community refers to organisms firmly attached to the bottom or coating gypsum crusts and stones.

(1) The microbial community of the seepage areas forms typical algal mats of firm, gelatinous to leathery consistency. It is dominated at the primary producer level by the cyanophytes *Entophysalis major, Lyngbya aestuarii* and *Microcoleus chthonoplastes* (Figures 4A, B, and C). Other cyanophytes such as *Spirulina tenerrima, S. subsalsa* (Figures 4C, 5N), *Johannesbaptistia pellucida* and various species of *Schizothrix* form a minor component of these mats. The microorganisms inhabitating seepage areas are not significantly different from those that dominate intertidal flats and temporary saline ponds of tropical and subtropical coasts or those found in salt works with fluctuating salinity (e.g. Piran, Yugoslavia (Golubic, 1973, 1976)).

(2) The main water body of the shallow Yallahs ponds is mixed daily and contains dense populations of suspended microorganisms. During calm periods some of these organisms settle loosely on the bottom and are resuspended with the onset of wind. These suspended organisms give the ponds their characteristic color (Figure 3). The Great Salt Pond has a brownish-green color caused by a dominant coccoid cyanophyte (Figure 5B, C). This yet undescribed organism has unique properties which place it intermediate

to the genera *Aphanocapsa* and *Coelospherium*. Small spheroid cells (1.5–2 μm in diameter) divide in two planes but remain attached by the thin envelopes they shed, forming, hollow spherical colonies. Little Salt Pond and West Salt Pond were orange-pink during the time of study. The dominant planktonic organism was an equally unusual, new coccoid cyanophyte morphologically close to the genera *Dactylococcopsis* and *Synechococcus* (Figures 5G, H, J, K). The shape of its extremely long cells (up to 300 μm long) is a thin spindle (3.2 μm wide), which, when fully differentiated, has attenuated ends. Cell division takes place transversally and starts with a constriction midway along the cell (Figures 5G, H). The cytoplasm of these cells is arranged in distinct spirals around the periphery of the spindle; small highly refractive granules which could be gas vesicles are often seen within the spiral bands of cytoplasm (Figure 5J). Another small species of the same genus, not yet described, occurs in the Solar Lake.

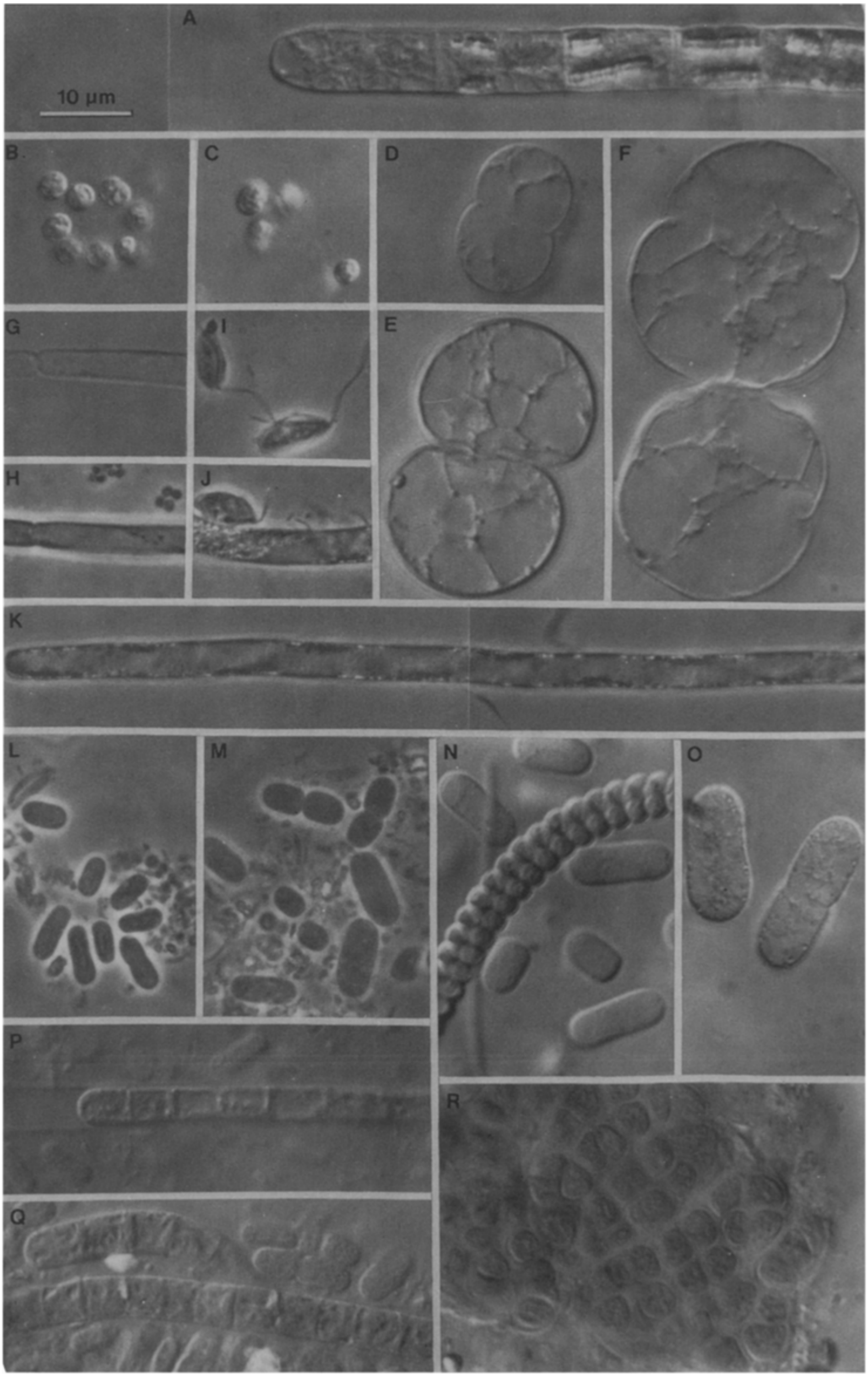
Minor components of the plankton community in all three Yallahs ponds are several taxa of coccoid cyanophytes with highly 'keratomized' cytoplasm (Figures 5D–F). Keratomization involves a reticulate separation of cytoplasm and a vacuole-like space. This phenomenon is not pathological (Geitler, 1930). These new coccoid cyanophytes are similar in morphology to the genera *Synechocystis* and *Synechococcus*. Their spheroid, isodiametric shape is consistent with the description of *Synechocystis*. However, the cells divide in one plane (see Figure 5F), which is a characteristic of *Synechococcus*. A eukaryote commonly encountered in Yallahs ponds in a colorless flagellate with four anterior flagella (*Tetramitus salinus*? Figures 5I, J).

(3) Benthic micorbial populations either form crusts that adhere firmly to hard substrates such as stones or gypsum crystals or coat the bottom with soft, flocculent mats. Crusty colonies adhering firmly to hard substrate in Yallahs ponds are formed by a pleurocapsacean cyanophyte similar to the genus *Xenococcus* (Figure 5R). Another common constituent of the Yallahs pond benthos is a soft mat forming *Phormidium*, characterized by keratomized cytoplasm, and quite similar to the *Phormidium* sp. isolated by Hoff and Frémy (1933, Figure 2) from salt crusts. At the time of this study the benthic cyanophyte cover in Yallahs ponds was very sparse, although *Phormidium* did form coherent mats during previous collection visits (A. Weiss, personal communication, 1978) (Figures 5P, Q).

An extremely dense population of brine flies, their larvae and puppae was present in the ponds in February, suggesting that brine fly larvae may have overgrazed algal mats as Brock *et al.* (1969) postulated for thermal springs.

The most conspicuous benthic cyanophyte population of the 4–5 m, deep Solar Lake, Sinai, is the flocculent, dark blue-green mat composed of several strains of a halophilic oscillatoriacean of genus *Phormidium* (Campbell and Golubic, unpublished results). One of these strains isolated in culture under the provisional name *Oscillatoria limnetica* was shown to be halophilic, and, in addition, to have the unusual property of facultative anaerobic photosynthesis using H_2S as a hydrogen donor (Cohen *et al.*, 1975).

Aphanothece is the most common genus that forms mucilagenous coatings in the benthos of many salt ponds. Examples of two forms of *Aphanothece* that coexist in Yallahs ponds are shown in Figures 5L, M. Two larger *Aphanothece* coexisting in Solar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Pond are shown in Figures, 5N, O. *Aphanothece* of various sizes occur in Yallahs ponds, on slopes and margins of the Solar Lake, in the Great Salt Lake, in ponds in the upper intertidal zone of the Arabian Gulf coast and in subtidal of the lagoons of tropical seas. Several strains isolated in culture have specifically different halophilic properties as shown in Figure 1. The existence of morphologically, physiologically and ecologically distinct populations of *Aphanothece* in saline environments raises the question of their species identity: Is it scientifically justified to call them all *Aphanothece halophytica*?

6. Cyanophyte Speciation in Saline Environments

The discovery of unusual organisms with unique properties that enable their growth and survival in saline environments raises the following, taxomically relevant questions: Do cyanophytes speciate along a salinity gradient and what is the best taxonomic reference for naming and comparing cyanophytes from saline environments?

So far we have established that the total number of discernible taxa decreases with the increase of salinity, that as the salinity increases, the species composition shifts, and that environments with constant and with fluctuating salinity are inhabited by different organisms. We have further established that the properties which characterize halophilic and halotolerant organisms are adjusted within a limited, genetically determined, range of tolerance to salt. In addition to a particular adjustment to NaCl, these organisms have other mutually correlated properties (growth requirements, cytological properties, morphology) that distinguish them from similar organisms in other environments.

From these considerations the first question can be answered affirmatively: cyanophytes did evolve specialized taxa which acquired adaptations that permit their survival, growth, and dominance in various saline environments. Thus halophilic, halotolerant and marine cyanophytes represent separate taxa that can be distinguished from each other and from similar forms in freshwater and terrestrial environments.

The second question concerns the nomenclatorial reference for these taxa and requires that the hierarchical level of taxonomic entities be established. This is more difficult as

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— Fig. 5. Cyanophytes that dominate environments with constant salinity: Tropical open ocean (A), Great Salt Pond (B–D) and Little Salt Pond (E–M, P–R), Jamaica and Solar Lake (N, O) Sinai. Scale bar for all organisms is 10 μm long (upper left). (A) *Trichodesmium thiebautii* Gom. from the Atlantic Ocean north of Puerto Rico; note the cytoplasmic reticulation and packets of gas vesicles; (B–C) *Coelosphaerium* sp., cells are held together by discarded envelopes forming hollow sphere colonies; (D–F) *Synechococcus* sp., characterized by strong reticulation (keratomization) of the cytoplasm, and a cell division in one plane (1D), the globular cell shape is similar to *Synechocystis* (2D); (G, H, J, K) *Dactylococcopsis* sp., with long spindle-shaped cells that divide transversally by constriction (G, H), cytoplasm spirally arranged with refractive inclusions (J, K); (I, J) *Tetramitus* sp. ?; (L, M) *Aphanothece* sp. from Yallahs pond benthos (phase contrast); (N, O) *Aphanothece* sp. from Solar Lake (Nomarski contrast) with *Spirulina subsalsa* Oerst. intermingled (N), and a larger taxon from the littoral of the Solar Lake (O); (P, Q) *Phormidium* sp., coating benthos of Yallahs ponds, intermixed with *Aphanothece* sp.; (R) *Xenococcus* sp. forms hard crust on gypsum crystals in Little Salt Pond of Yallahs.

it is dependent on the very concept of a species in prokaryotes, and to date consistent criteria have not been used.

Bacteriological classification is based on cultured strains, i.e. clonal lineages with uniform genetic properties. A strain is comparable to a tissue culture derived from an individual and is representative of a *very* small sample of a natural population. In bacteriology a species represents a group of strains that share a certain unspecified number of traits.

Taxonomic practice in ecology should not be based on individuals or clones, but rather on frequency distributions and combinations of traits that determine the establishment and continuity of a population in its natural environment. The boundaries of a species should be derived from a composite view of surviving populations of common descent that perform the same range of ecological functions, i.e. that occupy the same ecological niche.

Several criteria can be employed in determination and characterization of species. First, discernible properties should be recorded, evaluated and, if possible, quantified at the population level, using morphometry and statistics. Second, these properties should not be assessed separately but correlated as combinations, thus increasing the confidence in identifying individuals and populations with common properties. Third, a population so described should be compared with published species descriptions and oriented within the system of the basis of similarities and differences to several related taxa. Fourth, Natural populations are rarely identical with those described as types, and the degree of difference encountered can be defined as a 'distance' from a point of reference. If more than 3 properties are simultaneously evaluated, a conceptual framework of taxonomic hyperspace may be employed (Sneath and Sokal, 1973), in which populations separate as clusters. Fifth, a need for a description of a new species arises only if the newly identified population shows major differences from all described species, which constitutes a gap in the hyperspace reference matrix.

The problem of determination of species and genera is cyanophytes is exemplified by a group of rod-shaped unicells which are common in saline environments. A number of isolates of such organisms (Figure 1) have all been assigned to one species: *Aphanothece halophytica* Frémy. Occurrence of a single species across a wide range of salinites would imply either an extreme euryhalinity of a single, functionally adaptible taxon with capacity for reversible accommodation to salt, or that the species has been defined too broadly and contains physiologically and genetically distinct and stable taxonomic subunits. These should be characterized and named separately.

Attempts to change halophilic properties of at least one of these strains have failed (Tindall *et al.*, 1978), and some of the strains show differences other than growth optima with respect to salinity. Growth optima of cyanophytes from saline environments summarized in Figure 1 show a distribution along the salinity gradient analogous to that of tree growth on the slopes of Smoky Mountains (Whittaker, 1965), where dominance of tree species reflects their optimal adaptation to average temperature and availability of water. Thus, the halophilic cyanophytes behave in a way analogous to distinct species of plants. The *Aphanothece* illustrated in Figures, 5L–O show significant differences in shapes and sizes with little variation within each subpopulation. However, it was also reported that size and shape change with changing culture conditions (Brock, 1976; Yopp *et al.*, 1978). Whether the taxa under discussion should be grouped in one or more species remains inconclusive. A correlation of both morphological and physiological properties in a cluster analysis is needed in order to resolve the species identity of these forms.

The question of genus assignment of these forms has also been a subject of controversy. In their study of halophilic cyanophytes, Hoff and Frémy (1933) were confounded by the morphological variability of unicellular cyanophytes grown in high salt concentration media and, particularly, by the presence of the highly irregular 'giant cells' in their cultures. They could not agree on the taxonomic identity of the forms observed: Frémy distinguished between *Aphanocapsa litoralis* Hansgirg and a newly described species *Aphanothece halophytica* Frémy, while Hoff considered all coccoid forms observed to be variants of the former species. Brock (1976) used the name *Aphanothece halophytica* 'for simplicity' to describe his isolate from the Great Salt Lake and then used the name *Aphanocapsa halophytica* interchangeably (probably as a compromise between *Aphanocapsa litoralis* and *Aphanothece halophytica*).

Genera in coccoid cyanophytes are defined on the basis of cell division patterns, cell shape, production of extracellular envelopes and colony formation (Geitler, 1930; Golubic, 1976b). The genus *Aphanothece* (as well as *Synechococcus* and *Gloeothece*) is characterized by division of usually rod-shaped cells in a single plane, while the genus *Aphanocapsa* (as well as *Synechocystis* and *Gloeocapsa*) by division of usually spherical cells in two or three planes. These cell division patterns are constant within each taxon and persist until cell division is halted by excess salt (Schiewer and Jonas, 1977a, b), although they can be masked by pathological cell deformation under these conditions.

Thus, all rod-shaped unicellular cyanophytes that divide in one plane and are embedded in an amorphous mucilage belong to the genus *Aphanothece* (Figures 5L–O), while those suspended singly and without coherent mucilage are classified as the genus *Synechococcus*. Both genera are common in saline environments. Some round-celled forms which also divide in one plane (Figures 5D–F) should also be classified within the latter genus. Long, transversely dividing rods with pointed or curved ends should be classified within the genus *Dactylococcopsis*.

In conclusion, all forms under discussion, which have been classified as *Aphanothece halophytica* belong to the same genus, but probably represent separate species. The halophily has been demonstrated to date only in a few cyanophytes which were isolated in cultures. These represent a small fraction of the unique cyanophyte assemblages that coexist and dominate in environments of high salinity. It is predicted that these taxa once isolated in culture, will prove to have similar halophilic properties.

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