Chapter 18

Arthrospira (*Spirulina*): Systematics and Ecophysiology

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Summary

Notwithstanding the official recognition in 1989 of the cyanobacteria *Arthrospira* and *Spirulina* as distinct genera, the term "*Spirulina*" has been used to indicate the species of both genera indifferently, particularly the species "*S. platensis*" and "*S. maxima*" cultivated and sold as food, feed and a specialty product source. The successful commercial exploitation of *Arthrospira,* because of its high nutritional value, chemical composition and the safety of its biomass, has made it one of the most important industrially cultivated microalgae. This

chapter describes the ecology of *Arthrospira*, together with morphological and ultrastructural features relevant for supporting the systematic position of this organism. While the confused taxonomy of *Arthrospira* and its relationship with *Spirulina* have been resolved by 16S rRNA sequence analysis, the long debated problem of species definition is still ongoing. One study of ten strains suggested that the morphological criteria used to identify the species (*A. maxima, A. platensis*) corresponded to the molecular data obtained by total DNA restriction profile analyses. However, another study based on a wider range of classical species and forty different strains failed to show a clear correspondance with molecular data obtained for one part of the genome. This emphasises the need to address the problem of the definition of a species by using a polyphasic approach. Knowledge of the ecophysiology of *Arthrospira*, essential for understanding the growth requirements of this alkaliphilic organism in the natural environment, has been used in developing suitable technologies for mass cultivation. The relationships between environmental and cultural factors, which govern productivity in outdoor cultures, are discussed in connection with growth yield and efficiency. The response of *Arthrospira* and its modification under stress are described, together with the strategy of osmotic adjustment and the mechanism of internal pH regulation to alkalinity. The metabolic plasticity of the response of this cyanobacterium to disparate environmental stimuli is demonstrated in the natural environment, but is also well-expressed in the maintenance of highly productive monoculture in intensive outdoor cultivation systems.

I. Introduction

Ever since *Arthrospira* was first reported in 1852 by Stizenberger, many species of this genus of helically coiled cyanobacteria have been described and isolated. However, the classification of this genus has long been a source of confusion. Geitler (1932) invalidated the genus *Arthrospira* in his revision of the Cyanophyceae and included all regularly helically coiled oscillatorian organisms in the previously described genus *Spirulina* Turpin 1829. Since then, the term *Spirulina* has mostly been used to indicate species of either genus indifferently. As described later, it is now recognized that there are two very distinct genera (Castenholz, 1989). Therefore, many species that currently bear the generic epithet, *Spirulina*, should be re-included in the original genus *Arthrospira*. However, the term Spirulina is widely used to indicate the economically important species, "*S. platensis*" and "*S. maxima*", so we use it in parts of this chapter to avoid confusion with previous studies.

Arthrospira has been reported to exist in environments varying in their osmoticum, temperature and salt concentrations, most of them being of high alkalinity (Iltis 1969a, b; Busson, 1971). Filaments of the genus *Spirulina* in the strict sense also occur in many of these environments.

There is great interest in past use of the use of *Arthrospira* as a traditional food. Dangéard (1940), Brandily (1959), Léonard (1966) and Léonard and Compère (1967) all described how African tribes living along Lake Chad collect this microscopic "alga" (*A. platensis*). It is collected from water bodies near the lake and sun-dried on the shores to produce a hardened dark cake called "dihé", which is broken into small pieces and used in different forms by the local populations as part of their daily diet. At about the same time, and on the other side of the globe, *Arthrospira* was also recorded in the water of Lake Texcoco, Mexico. Here, it had been used as food by the natives living in the area (Clément, 1968). Travelers to Mexico during the 16th century described how the Aztecs used a soft a blue-green material, harvested with fine nets from the lake, for making a kind of bread called "tecuitlatl" (Ciferri, 1983). It is clear that these two different cultures, living far apart, discovered the nutritional value of *Arthrospira* independently.

Later attention was re-focused on "*Spirulina*" by the pioneering work done at the Institute Française du Pétrole, with studies on the cyanobacterial blooms occurring in the evaporation ponds of the industrial soda production facility at Lake Texcoco near Mexico City. This led to the first detailed study of the growth requirements and physiology of *Arthrospira*. The Ph.D. study by Zarrouk (1966) was the basis for establishing the first large-scale production plant of *Arthrospira*. The work was followed up by several groups in Italy, France and Israel and was summarized in a detailed review paper by Ciferri (1983). The extensive research on cell biology, biochemistry and biotechnology carried out since then is reviewed in the book edited by Vonshak (1997a).

This chapter provides a perspective on the systematics and the ecophysiology of "*Spirulina*", now correctly re-assigned to *Arthrospira* Stizenberger 1852.

II. Morphology

A. The Helical Shape of the Filament

The main morphological feature of *Arthrospira* is the patterned arrangement of its multicellular cylindrical trichomes in an open helix. The trichomes are composed of cylindrical cells that undergo binary fission in a single plane, perpendicular to the main axis. Trichome elongation occurs through multiple intercalary cell division along the entire filament. Multiplication occurs only by fragmentation. Trichome breakage is transcellular and involves destruction of an intercalary cell. The mechanism as it occurs in "*S. maxima*" and in "*S. platensis*" was described in detail by Tomaselli et al. (1981). In *Spirulina*, trichome breakage is intercellular (Rippka et al., 1979; Anagnostidis and Komárek, 1988).

The cell width ranges from about 3 to 12 µm, though occasionally reaches 16 µm. The helix pitch typically ranges from 10 to 70 µm and its diameter from 20 to 100 µm. These two parameters which define the shape of the helix architecture are highly dependent on growth and environmental conditions (Plate 27d).

The effect of temperature on filament structure was studied by Van Eykelenburg (1979), who subsequently described the rapid reversible change from the helix to the spiral shape on solid media (Van Eykelenburg and Fuchs, 1980). A detailed study of the effect of physical and chemical conditions on the helix geometry of *Arthrospira* was done by Jeeji Bai and Seshadri (1980) and Jeeji Bai (1985). They reported the occurrence of straight or nearly straight spontaneous culture variants also repeatedly observed by Lewin (1980) and by other authors. Though there are considerable variations in the degree of coiling between different strains of the same species and within the same strain, the helical shape of the trichome is regarded as a peculiar property of the genus. Once a strain has converted to the straight form, either naturally or due to physical or chemical treatments, it does not usually revert to the helical form. Jeeji Bai (1985) suggested that this may be due to a mutation affecting some trichomes under certain growth conditions. The common occurrence of straight trichomes in cultures of *Arthrospira* also suggests that the helical character may be carried on plasmids, but no one has yet demonstrated the existence of plasmids in *Arthrospira* or *Spirulina* strains. Fox (1996) successfully reverted several straight filaments to the typical coiled shape by exposing the filaments to high light irradiance. He believes the shape to be associated with helical protection against photolysis, which occurs frequently in natural water bodies. Perhaps because laboratory cultures grown at low light intensity, or well-mixed, dense outdoor cultures do not need to protect themselves against photolysis, straight filaments are frequently observed under both sets of conditions.

The ability to reverse filament configuration is further confirmed by the observation that it is possible to cause a straight filament to revert to the helical shape by growing it without intensive mixing at optimal light and temperature conditions, after inducing filament breakage with sonication (A. Vonshak, unpublished). We have also observed that under certain stress conditions (high growth temperature and very high alkaline medium) filaments have a more tightly coiled appearance.

In general, it seems that artificial laboratory conditions favor the development of straight forms. Trichomes of several *Spirulina* species also straighten after they have been streaked on plates during isolation procedures or after repeated transfers onto solid media (L. Tomaselli, unpublished).

B. Ultrastructure

The cell organization of *Arthrospira* is typical of prokaryotes, with a lack of membrane-bound organelles (Van Eykelenburg, 1979; Tomaselli et al., 1976; Tomaselli et al., 1993b; Fig. 1). The gram-negative multilayered cell wall is surrounded by a diffluent mucilaginous polysaccharide envelope. The thin cell wall has four layers, with an easily detectable electron-dense layer corresponding to the peptidoglycan layer (Van Eykelenburg, 1977). The regularly spaced cross-walls divide the trichome into cells connected by plasmodesmata. Cross-walls are formed by centripetal growth and extensions of both the peptidoglycan and the more internal layer of the cell wall toward the center of the cell. The cell has a number of inclusions mostly corresponding to the typical ones for cyanobacteria described in previous volumes (Fogg et al., 1973; Carr and Whitton, 1973; 1982). Thylakoid membranes with phycobilisomes are arranged in parallel bundles. The other main subcellular inclusions are (in addition to carboxysomes, ribosomes and DNA fibrils) gas vacuoles, polyglucan granules (especially near the cross-walls), polyphosphate granules and large

Fig. 1. Thin section showing the fine structure of *Arthrospira maxima*. Note: cell-wall (cw), septum (s) and thylakoids (t). Bar marker = 1 μm (photo courtesy of M.R. Palandri)

cyanophycin granules. Structures like the cylindrical bodies reported for some other members of the Oscillatoriaceae are often present. These inclusions,whose explanation is still unknown, are lacking in *Spirulina* (Tomaselli et al., 1996).

III. Systematics

The first report of a taxon which can be assigned to *Arthrospira* Stizenberger 1852 dates back to more than 150 years ago, when Wittrock and Nordstedt (1844) reported the occurrence near Montevideo of a blue-green alga with helical shaped filaments, which they described as "*Spirulina jenneri* f. *platensis*", even though it possessed septa. At that time the genus *Spirulina* was considered to be unicellular. Some years later, Stizenberger (1852), who had observed septa in some helically coiled oscillatoriacean forms, proposed that the forms, with a multicellular structure, be included in a new genus, *Arthrospira*. This distinction was confirmed by Gomont (1892) in his taxonomic study on the Oscillatoriaceae. He left the apparently aseptate forms in *Spirulina*, and placed the septate forms in *Arthrospira* Stizenberger 1852. However, Geitler's revision (1932) of the Cyanophyceae re-unified the members of these two genera in *Spirulina* Turpin 1829. He based classification on the close similarity in morphology and ignored the presence of septa, visible or otherwise, and thus made no distinction between the forms previously recognized as separate genera. Geitler actually split the genus into two subgeneric taxa (Section I. Arthrospira and Section II. Euspirulina) on the basis of the criterion originally used by Stizenberger (1852) to separate the genera *Spirulina* and *Arthrospira*, i.e. visible or non-visible cross-walls. The forms with septa that were easily observable under a light microscope were classified in the Section Arthrospira and those with unlikely or only artificially observable septa (after trypsin treatment or staining with neutral red) in the Section Euspirulina.

In Bergey's Manual of Systematic Bacteriology, Castenholz (1989) attempted to resolve the matter by restoring *Arthrospira* to its original rank of genus. He suggested using three features to separate *Spirulina* and *Arthrospira* in Subsection III of the Order Oscillatoriales, which shares the property of helically coiled trichomes. These are:

1) degree of inclination of the pitch of trichome helix (from transverse axis)
2) aspect and visibility (light microscope) of cross-walls between the cells in the filament
3) distribution (electron microscopy) of junctional pores in the cell wall.

Thus *Arthrospira* can be differentiated from *Spirulina* on the basis of the degree of inclination of the pitch of the trichome helix, which forms an angle $> 45^{o}$

Table 1. Main features separating the genera *Arthrospira* Stizenberger 1852 and *Spirulina* Turpin 1829.

Criteria	**Arthrospira**	**Spirulina**
Trichome diameter	2.5-16 μm	0.5-5 μm
Type of helicity	loosely coiled	tightly coiled
Cross-walls	visible under light microscope	invisible under light microscope
Cell wall pore pattern	single row around the trichome	several rows on concave side of the coil
Mode of trichome fragmentation	intracellular (necridium)	intercellular
Cylindrical bodies	present	absent
Anoxygenic photosynthesis	absent	present in some strains
C-phycoerythrin	not found	present in some strains
γ-linolenic acid	present	absent

from the transverse axis, the presence of easy visible septa and the distribution of junctional pores in one circular row around the cross-walls.

Tomaselli et al. (1996) suggest that there are other meaningful criteria to distinguish between the two, including 16S rRNA sequence data which clearly show that *Spirulina* and *Arthrospira* are phylogenetically distant (Giovannoni et al., 1988; Nelissen et al., 1994). Another useful criterion is that, unlike *Spirulina*, *Arthrospira* contains the unsaturated fatty acid γ-linolenic acid) (Cohen and Vonshak, 1991). *Spirulina* and *Arthrospira* are both aquatic, but, unlike *Spirulina*, *Arthrospira* has gas-vacuolated cells. The main criteria for distinguishing the two genera are listed in Table 1.

Although *Arthrospira* is helically coiled in natural populations, the occurrence of straight or slightly straight filamentous forms, similar to *Oscillatoria*, have been reported repeatedly in culture, making simple morphological observation invalid in the identification of *Arthrospira* (Lewin, 1980). The presence of straight forms of *Arthrospira* and their similarity to some members of the tribe "Oscillatoriae" (Castenholz, 1989) due to a similar junctional pore distribution (Guglielmi and Cohen-Bazire, 1982) emphasize the need to find additional phenotypic features to distinguish *Arthrospira*.

According to the Botanical Code, *A. jenneri* (Hass.) Stizenberger is the type species and *A. platensis* PCC 7345, which was isolated from a saline marsh (Del Mar Slough, California, USA), is the reference strain for the Bacteriological Code (Rippka and Herdman, 1992) (Table 2).

Hindák (1985) assigned all the planktonic forms of *Arthrospira* (including those previously described as "*S. platensis)* in alkaline saline lakes to "*S. fusiformis*" Voronichin 1934 (synonyms *A. maxima* Setchell et Gardner 1917 and *A. geitleri* De Toni 1935), since, in agreement with Fott and Karim (1973), he considered "*S. platensis*" to be a benthic species. On the basis of this assumption, most of the African populations were included under *A. fusiformis*. This opinion, also shared by Komárek and Lund (1990), has been debated by Tomaselli (1997). Komárek and Lund (1990), in their revision of the taxonomy and nomenclature of the three related species "*S. platensis*", "*S. maxima*" and "*S. fusiformis*", suggested that within the planktonic forms there are two taxa ("*maxima*" and "*fusiformis*"), and furthermore that these two taxa, which could represent two species (*A. fusiformis* Komárek and *A. maxima*), have different geographical distributions. Hence they consider *A. maxima* to be pantropical, *A. fusiformis* to be limited to Africa and tropical and central Asia, and consider the 'benthic' *A. platensis* to be essentially restricted to South America.

More recently Desikachary and Jeeji Bai (1992) proposed that all the calyptrate forms of *A. fusiformis* be included in the new species *A. indica* Desikachary

Table 2. Species of the genus *Arthrospira* Stizenberger 1852.

Species	First description	References
A. fusiformis Komárek 1990	Siberian steppe, Russia, Lake Tunatan	Voronichin, 1934
A. gomontiana Setchell 1895	North America, stagnant water	Geitler, 1932
A. indica Desikachary & Jeeji Bai 1992	Madurai, India, natural pond	Desikachary & Jeeji Bai, 1992
A. jenneri Stizenberger 1852	Europe, stagnant water	Gomont, 1892
A. khannae Drouet & Strickland 1942	Rangoon, Myanmar, natural pond	Desikachary, 1959
A. massartii Kufferath 1914	Luxemburg, spring water	Geitler, 1932
A. maxima Setchell & Gardner 1917	Oakland, Californiawarm, saline pond,	Gardner, 1917
A. platensis Gomont 1892	Montevideo, Uruguay, stagnant water	Wittrock & Nordstedt, 1844
A. spirulinoides Ghose 1923	Lahore, India, standing rain water	Geitler, 1932
A. tenuis Brühl & Biswas 1922	Bengal, India, artificial basin	Geitler, 1932

and Jeeji Bai 1992; this implies that the thickening of the apical cell wall (calyptra) is a significant taxonomic feature. The proposal is based on the fact that the calyptra was never reported in *A. fusiformis*. If the existence of the calyptra can be demonstrated in the original material, *A. indica* will became a later synonym of *A. fusiformis* (Desikachary and Jeeji Bai, 1996). Nevertheless, the proposal for a new species *A. indica* is questionable since other authors (Fott and Karim, 1973) consider "*S. geitleri*" which is a synonym of the calyptrate "*S. maxima"* to be identical to "*S. fusiformis* ".

The traditional separation of the common species into *A. maxima* and *A. platensis* should be maintained until more detailed studies have been completed (Tomaselli, 1997). Considering the great variations in size and shape of trichome geometry in natural and cultured *Arthrospira* populations, taxa based on morphological features should be confirmed through use of molecular biology techniques. Total DNA restriction profile analysis, performed on ten *Arthrospira* strains, led to the recognition of two well-separated genotypic groups (Viti et al., 1997) coinciding with the botanical species *A. maxima* and *A. platensis*, to which the strains had previously been assigned (Tomaselli et al., 1993a).

A further phylogenetic study, using ARDRA (Amplified Ribosomal DNA Restriction Analysis) of the ITS (Internal Transcribed Spacer) as a molecular taxonomic marker, was made on more than 40 strains of *Arthrospira*, which differed (with one exception) from the previous ones (Scheldeman et al., in press). The results of this study led the strains being grouped into only two main genotypic clusters with no clear correlation to their geographic origin or phenotypic features. This is in contrast to the previous study by Viti et al. (1997), which showed a large consensus between the genotypic clusters and the other features of the strains. The lack of a close concordance between the results of these studies emphasizes the need to address the long debated problem of the definition of species through a polyphasic approach using phenotypic and different types of genotypic information.

The question of the proper designation of *Arthrospira* extends to the commercial products and human health, since *Arthrospira* biomass is sold as a health food. The widespread use of the term Spirulina for such material clearly causes problems in accepting the correct terminology, but it is highly important to be aware of the type of organism being cultivated. Some *Spirulina* spp. (e.g. *S. subsalsa*) are in fact cultivated for commercial purposes in mistaken belief that they have the nutritional quality and toxicological safety of the traditional edible species of *Arthrospira* (Shimada et al., 1989; Brieba and Merinos, 1993).

IV. Occurrence and Distribution

A. General

Species of *Arthrospira* have been isolated mainly from alkaline, brackish and saline waters in tropical and semitropical regions (Castenholz, 1989). *A. jenneri* and *A. platensis* were described by Geitler

(1932) as cosmopolitan. Though most of the *Arthrospira* species have been found in alkaline water, where they form massive blooms, some other species have been reported to occur in fresh waters, but without such predominance. Other species with more restricted distributions occur in running fresh waters (*A. okensis*) and springs *(A. massartii*). *A. spirulinoides*, *A. gomontiana* and *A. tenuis* are reported for stagnant fresh waters. Desikachary (1959) considers *A. jenneri* and *A. platensis* as typical of tropical, saline environments.

Among the various species included in the genus *Arthrospira*, the most widely distributed, *A. platensis*, is mainly in Africa (Chad, Kenya, Egypt, Ethiopia, Sudan, Libya, Algeria, Congo, Zaire, Zambia), Asia (Pakistan, India, Sri Lanka, Myanmar, Thailand) and South America (Uruguay, Peru). *A. maxima* (syn. *A. geitleri*) appears to be confined to Central America (Mexico, California-USA). This species is the main component of the phytoplankton of the solar evaporation channels in Lake Texcoco (Mexico), while *A. platensis* dominates the alkaline saline lakes of the semi-desertic Sudan-Sahel area (Chad) and of the Rift Valleys (Kenya). The abundant occurrence of the species *A. platensis* and *A. maxima* in saline, alkaline, tropical and sub-tropical waters has led to recognition of these environments as typical for the entire genus.

The geographical distribution of the main populations of *Arthrospira* is given in Table 3. *Arthrospira* species have apparently not been reported from marine environments. However, when an adequate supply of bicarbonate, N and P, together with suitable pH and salinity values are provided, *Arthrospira* species can be highly productive in sea water (Tredici et al., 1986).

The most detailed studies on the ecology of *A. platensis* are those of Iltis (1968; 1969a, b; 1970a, b; 1971a, b; 1972) for tropical African lakes, and that of Busson (1971), who described past and recent use of "*S. geitleri*" (syn. *A. maxima*) as a food source in Mexico, in addition to its ecology. Other studies include those of Jeeji Bai and Seshadri (1980), Hindák (1985), Komárek and Lund (1990) and Desikachary and Jeeji Bai (1992). The last deals with the distribution of Indian isolates and revises the taxonomy of some culture collection strains, assigning them to the calyptrate species, *A. indica*.

B. Water Basins of the Sudan-Sahel Zone

The extensive survey of the phytoplankton of the permanent and temporary lakes near Lake Chad performed by Iltis for ORSTOM (Office de la Recherche Scientifique et Technique Outre-mers) has contributed much to our knowledge of *A. platensis*. Its main distribution is in the water basin of the Sudan-Sahel zone in Central Africa, north-east of Lake Chad towards the region of Ounianga Kebir, and the basins of the East African Rift Valleys. It also occurs in Ethiopia (Lake Chitu and Lake Aranguadi) and Zaire (Lake Kivu) (Table 3).

Most of the water bodies in which *Arthrospira* thrives are permanent or temporary saline alkaline lakes (natron lakes); these are scattered in the hollows of the fossil dune system of the Sahara and the Sudan-Sahel areas and fed by aquifers and the scanty rain (about 300 mm per year). The salinity of these lakes is highly variable; when evaporation exceeds inflow the salinity rises and leads to an accumulation of salt deposits on the shores (Iltis, 1971b). The mean water temperature is ~25^{o}C, with oscillations of about ± 10^{o}C. During the warm season, the water temperature in shallow lakes may reach 38^{o}C. Mean length of daylight is about 12 hours and solar radiation is over 2000 µmol photon m^{-2} s^{-1}. The disposition and slope of the impermeable clay and water-bearing sandy layers favor subterranean seepage of water away from Lake Chad (Maglione, 1969). The relationships between the superficial and the subterranean waters affect both the salinity and the ionic composition. The latter is characterized by prevalence of monovalent ions, by Na^{+} much higher than K^{+}, and by high concentrations of carbonate and bicarbonate, which lead to pH values from 9.5 to 11.0. Ca^{2+} and Mg^{2+} concentrations are low. SO_4^{2-} content is usually high, while that of Cl^{-} is very low. The water of the lakes is often blue-green in color due to the massive development of phytoplankton, which can attain very high densities.

A. platensis is present as nearly monospecific populations in the highly saline lakes (Iltis 1969a), only a few other phototrophs being found. In contrast, in the mesohaline lakes, *Arthrospira* coexists with a number of other phototrophs (especially diatoms and dinoflagellates) in addition to the dominant cyanobacteria. *Anabaenopsis arnoldii* and many *Oscillatoria* spp. are often important components of the cyanobacterial population. Several *Spirulina* species may be present, especially *S. laxissima*, *S. major* and *S. subsalsa*. A smaller form of *A. platensis,* var. *minor* Rich, was also present (Iltis, 1971b). In the studies performed on samples of two African natron lakes, Lake Mombolo and Lake Rombou, of the region of Kanem lying north-east of Lake Chad, Margheri et al. (1975) found

Table 3. Geographical distribution of the main populations of *Arthrospira* spp.

COUNTRY	ENVIRONMENT	SPECIES	REFERENCES
AFRICA			
Chad	natron lakes (Bodou, Mombolo, Rombou, Yoan) and pools (Latir, Iseirom, Latir, Liva), Kanem region	*A. platensis, A.platensis* f. *minor*	Léonard & Compère, 1967; Iltis, 1972, Rich, 1931
Kenya	natron lakes (Bogoria, Crater, Elmenteita, Nakuru), Rift Valley	*A. platensis*	Rich, 1931; Vareschi, 1982
	Lake Bogoria	*A. platensis, A.platensis* f. *minor, A. fusiformis*	Tuite, 1981; Hindák, 1985
	Lake Simbi	*A. platensis, A. platensis = A. fusiformis*	Melack, 1979 Kebede & Ahlgren, 1996
Ethiopia	Lake Aranguadi, Lake Chitu, Green Lake	*A. platensis, A. platensis = A. fusiformis*	Melack, 1979 Kebede & Ahlgren, 1996
Egypt	Lake Maryut	*A. platensis*	El-Bestawy et al., 1996
Sudan	Lake Dariba, Jebel Marra	*A. geitleri*	Fott & Karim, 1973
Algeria	pond Tamanrasset	*A. platensis*	Fox, 1966
Congo	Laka Mougounga	*Arthrospira* sp.	Fox, 1966
Zaire	Lake Kivu	*Arthrospira* sp.	Fox, 1996
Zambia	Lake Bangweolou	*Arthrospira* sp.	Fox, 1966
ASIA			
India	ponds	*A. maxima*	Desikachary & Jeeji Bai, 1996
	Lonar Lake; ponds; tank (Madurai, MCRC isolate)	*A. indica*	Desikachary & Jeeji Bai, 1992
Myanmar	Crater lakes	*Arthrospira* sp.	Min Thein, 1993
Pakistan	fish pond, Lahore	*Arthrospira* sp.	Fox, 1996
Sri Lanka	Lake Beria	*Arthrospira* sp.	Fox, 1996
China	fish ponds, Nanking	*A. platensis*	Tsen & Chang, 1990
Thailand	tapioca factory effluent lakes, Bangkok	*Arthrospira* sp.	Fox, 1996
Russia	Tunatan lake, Siberian steppe	*A. fusiformis*	Voronichin, 1934
Pakistan	pond, Lahore	*Arthrospira* sp.	Fox, 1996
AMERICA			
Mexico	Lake Texcoco solar evaporator	*A. maxima*	Busson, 1971
California	pond, Oakland; coastal lagoon, Del Mar	*A. maxima, A. platensis*°	Gardner, 1917; Lewin, 1980
Peru	Lake Huachachina	*A. platensis, A. maxima*	Busson, 1971; Desikachary & Jeeji Bai, 1992, 1996
Uruguay	Montevideo	*A. platensis*°°	Gomont, 1892
EUROPE			
Spain	Lake Santa Olalla	*Arthrospira* sp.	Rippka & Herdman, 1992
France	tiny lake, Camargue	*Arthrospira* sp.	Fox, 1996
Hungary	Adasztevel-Oroshaz	*Arthrospira* sp.	Busson, 1971
Azerbaidzan	water basin, Khumbasha	*Arthrospira* sp.	Fox, 1996

° reference strain PCC 7345.

°° holotype

the above-mentioned *Spirulina* species and several *Arthrospira* spp. The overwhelming majority of the isolated strains of *Arthrospira* were identified as *A. platensis*; one strain was identified as *A. platensis* var. *minor* and one other as *A. maxima* (Tomaselli et al., 1993a). Within the photosynthesizing population of the permanent and temporary lakes, Iltis and Riou-Duwat (1971c) observed a very high, albeit seasonally variable, density of rotifers (mainly *Brachionus* spp.), up to several hundred individuals per liter. In some lakes *Arthrospira* constitutes an excellent feed for the cichlid fish *Tilapia*. Another consumer of *Arthrospira* and other large filamentous cyanobacteria such as *Anabaenopsis* is the flamingo (*Phoenicopterus minor*). The pink color of these birds comes from the carotenoid pigments originating in cyanobacteria.

The detailed studies performed by Iltis over the course of several years have established a direct correlation between the biomass density of *Arthrospira* and the salinity of the water. The massive *Arthrospira* blooms occur at salinity levels from 22 to 60 g L^{-1}, high carbonate-bicarbonate concentrations (pH8.5 - 11.0), and temperatures from 25 - 40°C. Generally, the waters populated by *Arthrospira* have a mean salinity of 37 g L^{-1}. Nevertheless, *Arthrospira* has been found to occur at salinity levels ranging from 8.5 to 200 g L^{-1} (in some cases, up to 270 g L^{-1}: Iltis, 1968). Biomass density can exceed 1 g L^{-1}.

Tuite (1981) found the density of *Arthrospira* to be highly variable. The decline in the *Arthrospira* population was preceded by a period of increased water conductivity. In many of the natron lakes, *Arthrospira* was absent or present only at low densities. Marked fluctuations were recorded in other lakes. In some cases *Arthrospira* maintained high densities for periods up to several years, and appeared to be the only significant cyanobacterial species in the phytoplankton.

Arthrospira biomass density was lower in the temporary lakes than in the permanent lakes, but reached very high levels (up to 5 g L^{-1}) in the former immediately prior to their desiccation. The phytoplankton of the temporary water bodies is usually dominated by cyanobacteria and diatoms. *Arthrospira* blooms tend to develop after a bloom of green flagellates and other microalgae following the refilling of a lake by rainwater (Iltis, 1969b; 1970a). *Arthrospira* flourishes all year round, with a small decline during the short and scarce rainy season in the permanent lakes, but lasts no more than two months in the temporary water bodies. In these lakes the density of the populations depends not only on the mean salinity, but also on the time period during which the basin is flooded. *Arthrospira* blooms were not observed in water bodies flooded for less than four months.

C. Water Basins of the East African Rift Valley

In the volcanic regions of the East African Rift Valley there are many highly-alkaline saline lakes (HCO_3^- and CO_3^{-2} more than 1000 meq L^{-1}); these lakes are developed along the valley floor, the geology of which is dominated by alkaline, trachyte lavas rich in Na^+. The high alkalinity is the result of geological and climatic features:

i) presence of carbonates and bicarbonates derived from leaching of carbonatic volcanic rocks;
ii) absence of significant amounts of Ca^{+2} and Mg^{+2} in the surrounding land;
iii) concentration of ions due to evaporation.

Underground seepage through soluble volcanic rocks may also contribute to the increase in the salinity of the waters (Grant et al., 1990). Due to the generally hot and dry climate and the high solar irradiance, evaporation proceeds through brine saturation to crystallization. Therefore, the salinity and the ionic composition of the water are both subject to marked changes, due to the different solubility of the ions and the origins of the soluble salts. The saltiness of these extremely alkaline lakes in the East African Rift Valley ranges from 5 to 30%, according to seasonal weather conditions; the pH is almost consistently above 10 (Beadle, 1974). The waters are low in SO_4^{-2} and high in Na^+. In many of these lakes, *A. platensis* is dominant, as it is in the Chad area. In Lake Elmenteita and Lake Nakuru, Grant and Tindall (1986) found *A. platensis* to constitute the major bloom, although they also observed significant numbers of other cyanobacteria as well as diatoms, which became dominant as the cyanobacterial blooms decreased. In his review on "*Spirulina*", Ciferri (1983) summarized most of the studies on the distribution of this cyanobacterium in Africa, with particular emphasis on the alkaline lakes of the Rift Valley.

Arthrospira is also found in some lakes of the northern Algerian Sahara having saline waters (1 - 8%) and pH levels ranging from 7 to 9. These waters, which have lower alkalinity (about 20 meq L^{-1} HCO_{3-1} plus CO_3^{-2}), but Na^+ levels similar to those of the highly alkaline saline waters of the East African Rift Valley, may be considered to be sulfate-

chloride rich waters (with Cl^- the major anion). Fott and Karim (1973) found that the phytoplankton population of a saline alkaline lake (pH about 10) in Jebel Marra, Sudan, comprised only one cyanobacterium, which they named as *Spirulina geitleri*. Later, Fott (1977) identified this species as *S. fusiformis* (today *A. fusiformis*). Hindák (1985) assigned the *Arthrospira* populations occurring in Lake Bogoria (Kenya) to be *S. fusiformis.*

D. Water Basins of the Solar Evaporator at Lake Texcoco, Mexico

As previously mentioned, a massive population of *Arthrospira* was found in some of the external sectors of the gigantic spiral-shaped solar evaporator used to extract salts from the saline carbonate-bicarbonate rich waters of Lake Texcoco, Mexico. The evaporator lies 2200 m above sea level and is about 1 m in depth. The alkaline (pH 10) saline water is slowly moved from the outer part of the evaporator towards the center (Gallegos, 1993). The water is high in HCO_3^-, CO_3^{-2}, Cl^- and Na^+; the salt concentration ranges from 11 to 39 g L^{-1} (Busson 1971). In the past, the company that operated the plant, Sosa Texcoco (and later Spirulina Mexicana), had its Spirulina production facilities in the outside rim of the solar evaporator. In the alkaline saline waters, the species *A. maxima* is present in almost monospecific populations, being associated with only a few other cyanobacteria such as *Synechocystis aquatilis*.

V. Physiology of *Arthrospira*

Ecological studies performed during the last three decades have led to understanding of the conditions that can favor its abundant growth in phytoplankton populations. This information has been useful for developing the technology for mass cultivation of *Arthrospira* (Richmond, 1988).

One of the basic principles of this technology is the re-creation of determinate environmental and cultivation conditions such as to permit high productivity in a monoalgal culture. The main environmental factors which govern the productivity of a phototrophic microorganism are light intensity and temperature. Nutrient concentration, pH, salinity, cell density, mixing, etc., are strictly cultural factors (Vonshak, 1987a).

In nature, the organism uses its gas vacuoles to regulate its position along the underwater light gradient and follow the daily and seasonal light changes. Yet in the shallow culture ponds, light availability has to be optimized by modifying operational parameters such as optimal cell density, the degree of mixing and culture depth in order to obtain the highest productivity (Vonshak et al., 1982).

Photoinhibition and photooxidative damage due to prolonged exposure to high solar irradiance of the outdoor culture can be prevented to a certain degree by constant mixing of the culture and by maintenance of high cell densities. This also provides a more efficient light utilization (Richmond, 1996). The light regime imposed by mixing in the *Arthrospira* ponds at the very least prevents excessive exposure to high irradiance by repeatedly shifting the cells from the lighted into the dark layers and vice-versa. Intensive mixing also ensures an even distribution of nutrients and at the same time prevents filament aggregation and sinking (Grobbelaar, 1996).

The fact that *Arthrospira* requires high alkalinity for growth ensures selective conditions in the growth medium and for this reason *Arthrospira* culture growing outdoors in open ponds remains in quasi-monoculture (Vonshak et al., 1983). Moreover, when other essential growth elements are present in sufficient amounts, the elevated concentration of bicarbonate-carbonate favors the high productivity observed in the natural habitat.

A. Growth: Yield and Efficiency

The growth of *Arthrospira* follows the pattern common to many other micro-organisms which undergo simple cell division involving no differentiation or sexual steps. Thus, the specific growth rate (μ) is described by the following equation:

$$\mu = \frac{1}{x} . dx / dt$$

where x is the initial biomass concentration.

Ogawa and Terui (1970) made the first assessment of quantum yield (Y_{kcal}) (measured as the amount of dry algal biomass harvested per kcal light energy absorbed) for *Arthrospira.* Calculated values of Y_{kcal} range from 0.01 to 0.02 g cell $kcal^{-1}$. These values correspond to an efficiency of 6 - 12%. In a later study, Ogawa and Aiba (1978) estimated the quantum requirement of *Arthrospira* cultures grown at steady state conditions; the Q_{CO2} value was about 20 mol quanta mol^{-1} CO_2, which corresponds to an efficiency of 10%.

The relationship between the specific growth rate and the specific light energy absorption rate was used to establish a mathematical equation describing the growth of *Arthrospira* in a batch culture (Iehana, 1987). The equation indicates that in cultures with a high cell concentrations the specific growth rate increases linearly with the increase in the specific absorption rate of light energy. Two other groups, Lee et al. (1987) and Cornet et al. (1992a, b) have published detailed studies on attempts to establish a mathematical model for the growth of *Arthrospira* under a variety of growth conditions. It seems that all of the models fit the experimental growth data well under normal steady state conditions, where light is either limiting or at its saturation level. These models require modification if stress conditions, such as photoinhibition or environmental stress (i.e. temperature or salinity) are introduced.

B. Response to Environmental Factors

1. Effects of Light

a. Effects on Growth and Photosynthesis

Zarrouk (1966) was the first to study the response of "*Spirulina maxima*" to light; he reached the conclusion that growth of this organism saturates at levels of 25 - 30 Klux. Yet it is very difficult to compare these results to more recent studies because the method used to measure the light intensity and the light path in the vessel cultures is not described. From data obtained in our laboratory, growth of *Arthrospira platensis* is saturated at a range of 150 - 200 μmol photon m^{-2} s^{-1}. This is about 10 to 15% of the total solar irradiance in the 400 - 700 nm range. This value is highly dependent on growth conditions and correlates with the chlorophyll to biomass ratio. Using a turbidostatic culture, we have estimated that the μ_{max} of some *Arthrospira* strains corresponds to a doubling time of 8 - 10 h. For the strain *A. maxima*, 6Mx the value of μ_{max} was 0.0692 h^{-1}, corresponding to a doubling time of 10 hours (Tomaselli et al., 1997). This strain was able to alter its light harvesting capacity during acclimation to sudden irradiance changes. This is an important feature in selecting strains for outdoor cultivation.

The most common way to study the photosynthetic response of algal cultures to light is to measure the photosynthetic activity (P) versus irradiance (I) (i.e. the P-I curve). A typical P-I curve is shown in Fig. 2. In the dark, the rate of oxygen evolution or carbon fixation will be negative, due to respiration. As irradiance is increased, a point is reached at which the photosynthetic rate is just balanced by the rate of respiration. This is the compensation point (I_c). As irradiance is further increased, the rate of photosynthesis increases linearly. Following further increase in irradiance, the curve eventually levels off, as photosynthesis becomes saturated and reaches a maximum, P_{max}. The initial slope of the P-I curve is a useful indicator of quantum yield, i.e. photosynthetic efficiency.

Fig. 2. Typical curve of specific photosynthetic rate (P) as a function of light intensity (I), illustrating the maximum photosynthetic rate (Pmax), the saturation onset parameter (Ik) and the slope (α) of the linear part of the curve (P-I curve).

The irradiance at onset of light saturation is defined by the value of I_k, which represents the point at which the extrapolation of the initial slope of the P-I curve crosses P_{max}. Exposing *Arthrospira* cultures to high photon flux densities above the saturation point may result in a reduced rate of photosynthesis; this phenomenon is defined as photoinhibition. The classical view that photoinhibition is observed only at high irradiance values seems to be a very simplistic one. Photoinhibition may be observed even at relatively low irradiance levels when other environmental stresses are involved. This will be discussed later.

The P_{max} and I_k levels are highly dependent on growth conditions. *Arthrospira* cultures grown at high or low light intensities will have different P_{max} and I_k values. Changes in these values may represent the organism's ability to photoadapt, or acclimate, to different light environments, as demonstrated in another cyanobacterium *S. subsalsa* grown at two photon flux densities (Tomaselli et al., 1995). Table 4 summarizes experiments carried out by Vonshak et al. (1996a) indicating different values of measured photosynthetic parameters in three different *Arthrospira* strains. Although the strains were grown

under the same temperature and light conditions, they have different α and I_k values. Similar observations were reported by Tomaselli et al. (1993a).

b. Light Stress –Photoinhibition

Photoinhibition, as mentioned earlier, is defined as a loss of photosynthetic capacity due to damage caused by photon flux densities (PFDs) in excess of those required to saturate photosynthesis. The phenomenon of photoinhibition has been studied extensively and is well documented in algae and higher plants (Powles, 1984; Kyle and Ohad, 1986). Photoinhibition in laboratory *Arthrospira* cultures was first studied by Kaplan (1981), who observed a reduction in the photosynthetic activity when the cells were exposed to strong light under CO_2-depleted conditions. It was suggested that the reduction in photosynthetic activity was due to the accumulation of H_2O_2.

Different strains of *Arthrospira* may differ in their sensitivity to light stress (Vonshak et al., 1988b). In at least one strain such a difference was probably due to a difference in the turnover rate of a specific protein, D1, which is part of PSII. The different responses of different *Arthrospira* strains to photoinhibitory stress may be genotypic characteristics as well being dependent on growth conditions. Cultures grown at high light intensities exhibit a higher resistance to photoinhibition (Vonshak et al., 1996a). It should be emphasized that photoinhibition affects not only the P_{max} level, but actually has a stronger effect on light-limited photosynthetic activity. Thus a significant reduction in α is observed in photoinhibited cultures.

Table 4. Specific growth rate (μ) and photosynthetic parameters of three *Arthrospira* strains. μ = h^{-1}; I_k = irradiance at onset of light saturation (μmol photon m^{-2} s^{-1}); Pmax = maximal light-saturated rate of O_2 evolution (μmol O_2 h^{-1} mg Chl a^{-1}); α = light-limited slope of the P-I curve (μmol O_2 mg Chl a^{-1}/μmol photon m^{-2}). Values are mean ± SE (*n*=3).

PARAMETERS	STRAINS		
	BP	P4P	Z19/2
μ	0.047	0.044	0.045
I_k	258 ± 20	185 ± 15	325 ± 40
Pmax	536 ± 4	585 ± 10	585 ± 12
α	2.3 ± 0.3	3.85 ± 0.9	2.1 ± 0.5

2. Effects of Temperature

a. Effect of Temperature on Growth

Fig. 3. Temperature response of 3 *Spirulina* isolates measured as the increase in Chlorophyll concentration after incubating the cells for 4 days on a temperature block under constant light (100 μmol m^{-2} s^{-1})

In nature, *Arthrospira* is found in permanent or temporary water bodies at relatively high temperatures. The optimal temperature for laboratory cultivation of this organism ranges from 30 - 38°C. However, many *Arthrospira* strains differ in their optimal growth temperature as well as in their sensitivity to extreme values. For instance, in the comparison of strains shown in Fig. 3, strain SA2 has a relatively low optimal growth temperature (24 - 28°C), while the EY strain grows well up to 40 - 42°C. The isolate marked K has a relatively wide range of optimal growth temperatures, 28 - 36°C.

Growth temperature also affects the biochemical composition of the cells (Tomaselli et al., 1988). When "*S. platensis*" M2 is grown under light-limited turbidostatic conditions at its maximum growth temperature (42°C), a marked decrease in the photosynthetic pigments and proteins level is observed. Under these conditions, an increase in carbohydrate cell content associated with changes in the degree of fatty acid saturation (i.e. reduction in linolenic acid synthesis in favor of linoleic acid accumulation) is also observed.

b. Effect of Temperature on Photosynthesis and Respiration

The net productivity of an algal culture is directly correlated with the gross rate of CO_2 fixation, or O_2 evolution (photosynthesis), and with the rate of

respiration. These two metabolic activities are temperature dependent, while only CO_2 fixation, or O_2 evolution, is both light- and temperature-dependent. A detailed study of the response of an *Arthrospira* strain marked M2, performed by Torzillo and Vonshak (1994), determined the optimal temperature for photosynthesis (35°C) and also measured the effect of temperature on the dark respiration rate of *Arthrospira* by following the O_2 uptake rate in the dark. A temperature-dependent exponential relationship was obtained, with the respiration rate increasing as temperature increased. The temperature-dependent dark respiration rate is given by:

$$R = 0.771.e^{(0.0636.T)}$$

where R is the respiration rate (μmol O_2 mg^{-1} chl h^{-1}) and T is the temperature (°C). At 50°C and 15°C, dark respiration rates dropped almost to zero. An Arrhenius plot for respiration showed an activation energy of 48.8 kJ mol^{-1} for *Arthrospira*. The temperature coefficient (Q_{10}) calculated for the 20 - 40°C range was 1.85. The respiration to photosynthesis ratio in *Arthrospira* was 1% at 20°C and 4.6% at 45°C (Torzillo and Vonshak 1994). These low values confirm the general assumption that cyanobacteria have low respiration rates (van Liere and Mur, 1979). The respiration-to-photosynthesis rates measured in these experiments were much lower than those reported for outdoor cultures of *Arthrospira*, where up to 34% of the biomass produced during daylight may be lost through respiration at night (Guterman et al., 1989; Torzillo et al., 1991). In the *Arthrospira* strain M2, the optimum temperature for respiratory activity was much higher than for photosynthetic activity. Nevertheless, the photosynthetic activity of the cells was more resistant to temperature extremes than was dark respiration, at the minimum and maximum temperatures tested.

c. Interaction with Light

Deviations from optimal growth temperatures have an inhibitory effect on photosynthetic capacity. This reduction in activity represents a limitation that is immediately overcome after a shift back to the optimal temperature, if no other damage was occurred. The kinetics of recovery from low temperature incubation indicate that some repair process must take place before the original level of photosynthetic activity is reached. This observation concerns solely cultures incubated at a low temperature in the light. It is thus suggested that *Arthrospira* cultures grown at less than the optimal temperature are more sensitive to photoinhibition than those grown at the optimal temperature. The latter will be able to better handle excessive light energy, since they have a higher rate of electron transport, an active repair mechanism and more efficient means of energy dissipation (Vonshak, 1997b).

3. Response to Salinity

Cyanobacteria inhabit environments characterized by drastically differing saline levels. In the past 15 years many studies have been published on the response of cyanobacteria to different saline environments. The studies mainly focus on the specific role of organic compounds as osmoregulants (Borowitzka, 1986), the modifications occurring in photosynthetic and respiratory activities (Vonshak and Richmond, 1981), and the variations in the protein synthesis pattern (Hagemann et al., 1991). Different *Arthrospira* species have been isolated from a variety of alkaline environments. We will describe the work done using strains isolated from alkaline and brackish waters.

a. Effect of Salinity on Growth

The occurrence of *Arthrospira* spp. in marine habitats has not been documented, at least not as dominant populations like those found in the natron lakes. This is most likely due more to the very low content of bicarbonate than to the high content of NaCl. *Arthrospira*, unlike marine organisms, does not require high NaCl concentration for growth, rather it only tolerates the salt. The exposure of *Arthrospira* cultures to high NaCl concentrations at first results in an immediate cessation of growth; after a lag period, a new steady state of growth is established (Vonshak et al., 1988b). The length of the time lag is exponentially correlated to the degree of salinity stress imposed on the cells. In many cases, this lag is associated with a decline in chlorophyll and biomass concentrations in the culture (Vonshak et al., 1988b). The response of *Arthrospira* to salinity in terms of the degree to which its growth is inhibited, its adaptation to salt levels and the rate of such adaptation varies widely, depending on the strain used in the study. A decrease in the growth rate due to salt stress has also been demonstrated in other cyanobacteria such as *Anacystis* (Vonshak and Richmond, 1981) and *Nostoc* (Blumwald and Tel-Or, 1982).

b. Effect of Salinity on Photosynthesis and Respiration

It has been suggested that exposure to high salinity is accompanied by a higher demand for energy by the stressed cells (Blumwald and Tel-Or, 1982). Changes in the photosynthetic and respiratory activities of an *Arthrospira* strain were measured over a period of 48h beginning 30 min. after exposure to 0.5 and 1.0 M NaCl. A marked decrease in the photosynthetic oxygen evolution rate was observed 30 min. after exposure to the salt. This decline was followed by a recovery period, characterized by a lower steady state rate of photosynthesis (Vonshak, 1988b).

The immediate inhibition of the photosynthetic and respiratory systems after exposure to salt stress have been explained by Ehrenfeld and Cousin (1984) and Reed et al. (1985), respectively in *Dunaliella* and in *Synechocystis*. They showed that a short-term increase in cellular sodium concentration was due to a transient increase in the permeability of the plasma membrane during the first seconds of exposure to high salt concentration. It has been suggested that the inhibition of photosynthesis due to the rapid entry of sodium, might be the result of the detachment of phycobilisomes from the thylakoid membranes (Blumwald et al., 1984). High rates of dark respiration in cyanobacteria due to salinity stress had been reported previously (Vonshak and Richmond, 1981; Fry et al., 1986; Molitor et al., 1986). This high activity may be associated with the increased level of maintenance energy required for pumping out the excess of sodium ions.

c. Osmoregulation and Strain-Specific Response of Arthrospira *to Salinity*

During the course of adaptation to salinity, an osmotic adjustment is required. In *Arthrospira*, low molecular weight carbohydrates accumulate. These have been identified as a nine carbon heteroside called glucosyl-glycerol, plus a trehalose (Martel et al., 1992). When the biomass compositions of two *Arthrospira* strains grown under different salt stress conditions were compared, significant changes, mainly an increase in carbohydrates and a decrease in the protein level, were observed. These changes correlated with the degree of stress imposed (i.e., a higher level of carbohydrates at a higher salt concentration). The difference in the level of carbohydrates accumulated by the two strains may also reflect a difference in their ability to adapt to salt stress. Changes in lipid synthesis have also been demonstrated; in salt-stressed cells, an increase in lipid content and in the degree of fatty acid saturation is observed with specific modifications in the long-chain fatty acids (C18). In *Arthrospira* strain M2 the oleic acid content doubled when the organism was grown in presence of 0.5 M NaCl (Tomaselli et al., 1993a).

C. The Alkaliphilic Nature of Arthrospira

As previously indicated, most *Arthrospira* species were isolated from alkaline and saline or brackish waters characterized by high levels of carbonate-bicarbonate and high pH levels. The physiological characteristics which enable a micro-organism to thrive in such an extreme environment are still not fully understood. It should be pointed out that *Arthrospira* not only survives at high pH values, but actually thrives in such conditions. Belkin and Boussiba (1991), who compared the growth pH optima for the two cyanobacteria *Anabaena* and *Arthrospira*, demonstrated that while the optimal pH for *Anabaena* was in the range of 6.8 - 7.2, the maximal growth rate for *Arthrospira* was obtained in the 9.5 - 9.8 range. When incubated at pH7.0, the growth rate of *Arthrospira* was severely inhibited and was only 20% of that obtained under the optimal conditions. This high pH (> 8) requirement clearly defines *Arthrospira* as an obligatory alkaliphile (Grant et al., 1990).

One of the major problems faced by cells in an alkaliphilic environment is that of regulating their internal pH. Belkin and Boussiba (1991) were the first to measure the ability of *Arthrospira* to maintain a pH gradient across its cytoplasmatic membrane. It was demonstrated that at external pH values of 10.0 and 11.5 the intracellular pH values were only 8.0 and 8.5 respectively.

Many alkaliphiles require sodium in order to survive in extreme alkaline environments (Horikoshi and Akiba, 1982). Padan et al. (1981) demonstrated that an active sodium-proton antiporter is required in order to maintain a low internal pH. In a more recent study, Schlesinger et al. (1996) demonstrated that "*Spirulina platensis*" requires sodium in order to maintain optimal growth at pH10. The authors demonstrate that under sodium deprivation the pH gradient collapses and the cells undergo fast lysis. Depriving the cells of sodium causes inactivation of the sodium-proton antiporter that is responsible for the inward pumping of protons and the outward pumping of sodium. This mechanism is one way to

maintain lower internal pH levels in cells under alkaliphilic conditions. Further studies on the energy requirement and involvement of light in the regulatory process are yet to be carried out.

D. How Does Arthrospira *Compete in Culture ?*

Studies performed on different *Arthrospira* isolates from various sources demonstrate highly variable responses to different ecological factors (Tomaselli et al., 1987; Vonshak 1987b, Vonshak et al., 1996a, b). It is on the basis of such variability that strains suitable for biotechnological applications are selected, such as photo-, thermo- or osmotolerant ones (Tomaselli et al., 1993a, Vonshak and Guy, 1992; Vonshak et al 1988b). Detailed studies aimed at measuring the ecological specificity of the different species, particularly those used for commercial purposes, are scarce (Kebede and Ahlgren., 1996). Nevertheless, full knowledge of the ecological demands of the chosen species and the use of more productive selected strains is important if high productivity is to be achieved in intensive outdoor cultivation systems. The use by commercial producers of multistrain inocula which respond positively to changes in abiotic factors (due to their heterogeneity) represents another way of compensating for the present lack of well-characterized strains.

Like other components of the phytoplankton, *Arthrospira* plays a role in cycling nutritional elements and in their passage into different food chains; moreover, it provides an important direct food source for zooplankton, fishes, birds and humans. The same factors that in the original habitat regulate the occurrence of *Arthrospira* in monospecific populations are those required for both successfully maintaining a monoculture in intensive production ponds outdoors and for achieving high productivity. Factors such as high alkalinity and salinity are the key features that selectively limit the growth of other organisms and predators.

Even if high pH and salt concentration reduce contamination from both air and water, *Arthrospira* cultures may, when effective pond management is lacking, be greatly contaminated by other organisms such as heterotrophic bacteria, fungi, protozoa, rotifers, other cyanobacteria and microalgae. Contamination of outdoor *Arthrospira* cultures by other microalgae is of frequent occurrence; these contaminants are usually green algae (*Chlorella*) and diatoms. As the diatoms live tightly attached to surfaces (such as paddle wheels), they are difficult to wash out of the culture system. More problematic is the presence of other filamentous *Oscillatoria*-like cyanobacteria, which have similar ecological demands and may invade the culture. An increase in the organic load subsequent to deterioration processes (cell lysis) also stimulates the development of fungi and bacteria. Nevertheless, once the optimal conditions for the growth of *Arthrospira* are maintained in ponds, this organism successfully out-competes other occasionally occurring phototrophs for the primary resources and may then reach high population densities. This is attributable to its fast rates of growth and photoacclimation, and its high tolerance to environmental stress (irradiance, alkalinity, salinity, temperature).

VI. Concluding Remarks

Arthrospira is a cosmopolitan cyanobacterium, yet occupies very selective habitats. The main ecological demands are stable alkaline, saline waters, warm growth temperatures, high irradiance, and eutrophic conditions, (i.e. abundance of bicarbonate and other macronutrients). Consequently, primary productivity of *Arthrospira* in some tropical and subtropical lakes can be very high.

The use of *Arthrospira* as food by indigenous populations in different parts of the world is well documented. In recent years, this cyanobacterium has gained a considerable reputation as a health food additive and is marketed commercially. In many cases the term "Spirulina" has become a practical synonym for cultivated microalgae. At present, *Arthrospira* represents the second most important commercial microalga for the production of biomass as a health food and an animal feed (after *Chlorella*). With the current rapid increase in production and a demand reaching 1500t in 1996 (predicted to reach 2000t before the turn of this millennium), there is no doubt that *Arthrospira* will be the most important mass-produced microalga for commercial purposes in the near future.

The accumulated knowledge of the ecology, physiology and biochemistry of *Arthrospira* has made such results feasible, yet much more work is required, in terms of strain selection and better understanding of the factors which govern growth and light utilization in dense cultures, to support this growing agro-industry.

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