Evaluation of growth and biomass production of *Arthrospira* (*Spirulina*) *fusiformis* in laboratory cultures using waters from the Ethiopian soda lakes Chitu and Shala

Tadesse Ogato · Demeke Kifle · Tadesse Fetahi · Baye Sitotaw

Abstract The cost of nutrient media is the major challenge for biomass production of *Spirulina*. Although much effort has been made to use enriched seawater for the cultivation of this microalga, little attention has been given to the potential of water of soda lakes. In this study, growth (μ, day^{-1}) and biomass production (B) of *Arthrospira fusiformis* cultivated using waters of the soda lakes Chitu and Shala with or without supplementation were evaluated. Comparable μ and B values were achieved in both Lake Chitu water-based media (CBM) and Lake Shala water-based media (SBM), with slightly higher values in the latter. Both CBM and SBM supplemented with the standard *Spirulina* medium (SM) by 25 % and 50 % supported considerably higher μ and B. The pH and salinity of the cultures showed significant variations ($P<0.05$) among the media and had considerable effect on μ and B. The observed higher μ and B were probably associated with the reduction in pH and salinity of the supplemented media due to addition of bicarbonate–carbonates and dilution, and provision of the limiting nutrient nitrogen. The higher μ and B in SBM may have resulted from some of their aggregate chemical parameters, which were closer to those in the SM, and abundant PO$_4$-P. This seems to suggest that Lake Shala water is more conducive to *Arthrospira*. We contend that 25 % and 50 % supplemented Lake Shala water can be preferably used to produce *Arthrospira* biomass, thereby reducing the cost of nutrients by 75 % and 50 %, respectively.

Keywords *Arthrospira* production · Culture medium · Ethiopian soda lakes · Lake Shala · *Spirulina*

T. Ogato (✉)
Department of Biology, Debre Berhan University, PO Box 445,
Debre Berhan, Ethiopia
e-mail: tadesseogato@gmail.com

D. Kifle · T. Fetahi
Fisheries and Aquatic Sciences Stream, Department of Zoological
Sciences, Addis Ababa University, PO Box 1176, Addis Ababa,
Ethiopia

B. Sitotaw
Department of Applied Biology, Hawassa University, PO Box 05,
Hawassa, Ethiopia

Introduction

Mass production of *Arthrospira* (commercially known as *Spirulina*), an edible photosynthetic cyanobacterium, has gained worldwide attention due to its invaluable nutritional and health benefits. It has received worldwide acceptance as a natural food and source of several pharmaceuticals (Belay and Ota 1993; Belay et al. 1996; Chen et al. 1996). *Arthrospira* is used as a source of protein (50–70 % protein by dry weight, surpassing all known standard plant proteins), rare fatty acids (gamma-linolenic acid), vitamins (e.g., β-carotene and B$_{12}$), minerals (e.g., iron) and essential amino acids. It has also therapeutic effects on various health problems including hyperlipidemia, nephrotoxicity, diabetes, obesity, hypertension, cancer and even HIV (Belay and Ota 1993; Belay et al. 1996; Belay 2002). *Arthrospira* is one of the microalgae that reproduce fast and has high productivity (Kilic et al. 2006). It can be produced in small or large scale using different types of production systems and growth media. Large commercial scale production of *Arthrospira* is currently used by several big companies around the world including Earthrise Nutritionals and Cyanotech in the USA and Hainan DIC Microalgae in China (Ayala et al. 1988; Jourdan 1993; Belay 1997). *Arthrospira* is also produced in small scale pond cultures for local consumption to fight malnutrition in various countries including India.

Growth and biomass production of *Arthrospira* depends on many environmental factors, especially carbonate–bicarbonate alkalinity, pH, nutrient availability, temperature and light

(Vonshak 1997; Habib et al. 2008). High biomass of the alga can be obtained when these environmental factors are optimized. It preferably dominates in tropical and sub-tropical water bodies characterized by high carbonate–bicarbonate alkalinity and pH (Ciferri 1983; Cogne et al. 2001). However, optimization of growth conditions is the major challenge to biomass production of *Arthrospira* in cultivation systems and incurs high production cost. Out of the total production cost of *Arthrospira* biomass, 15–25 % is accounted for by nutrients, which form the second major cost item (Belay 1997; Vonshak 1997; Habib et al. 2008). As a result, production of *Arthrospira* biomass using standard carbonate medium is unaffordable.

Much effort has been made to investigate alternative methods of cultivation through biotechnological research and innovation in order to cut down the cost of production without compromising biomass productivity (Vonshak 1997). Promising results have been found in experiments using seawater enriched with phosphate and urea. In such experiments, Materassi et al. (1984) and Tredici et al. (1986), in laboratory and outdoor mass cultivations, respectively, obtained biomass yields which are slightly less than those obtained with the standard medium. Studies in Brazil by Costa et al. (2003) have also shown that comparable *Arthrospira* biomass could be obtained using lagoon water supplemented with bicarbonate and urea. Use of different organic sources of carbon, nitrogen and phosphorus has also resulted in increased biomass yield of *Arthrospira* (Neilson and Larsson 1980; Baldia et al. 1991; Habib et al. 2008). However, the potential of water of soda lakes for biomass production of *Arthrospira* under laboratory or outdoor conditions has received little attention to date though these lakes are conducive for *Arthrospira*, with some of them supporting its abundant populations.

Ethiopia has alkaline soda lakes such as lakes Chitu and Shala, which have several interesting features. Lake Chitu is a small shallow lake best known for its natural monoculture of *Arthrospira* (Talling et al. 1973; Kebede 1996). Harvesting *Arthrospira* biomass from the natural ecosystem of the small lakes like Chitu will not be sustainable ecologically and economically. Lake Shala is a large and deep soda lake whose gross water chemistry is very similar to that of the adjacent Lake Chitu and which is suitable for *Arthrospira* production (Wood and Talling 1988; Kebede 1996). The lakes are situated in areas with high temperature and irradiance and nearly constant photoperiod, which seem to be ideal tropical climatic conditions favoring the high productivity of *Arthrospira* (Richmond and Grobbelaar 1986; Vonshak 1997; Talling and Lemoalle 1998). These conditions presumably indicate potential of the large Lake Shala water for *Arthrospira* production. However, natural populations of *Arthrospira* have not been reported for Lake Shala for reasons not known to date. This calls for an in-depth experimental testing of the suitability of the lake water for *Arthrospira* production under laboratory and outdoor conditions. The purpose of this work was, therefore, to evaluate growth and biomass production of *Arthrospira* in waters of the soda lakes Chitu and Shala with or without supplementation with standard Spirulina medium (SM) at a constant light and temperature condition in the laboratory. The results that emanate from such investigations are crucial to efforts being made to develop suitable low-cost media for *Arthrospira* cultivation.

Materials and methods

Description of the soda lakes

Lakes Chitu and Shalla are tropical crater lakes located in the Ethiopian rift some 285 km south of Addis Ababa. These lakes are typical examples of African soda lakes characterized by highly saline–alkaline waters in which the concentrations of the major cations are in the order Na>K>Ca>Mg in contrast to that of most temperate lakes in which ionic dominance is in the order Ca>Mg>Na>K (Tudorancea et al. 1999). The lakes are closed and evaporative concentration is the prime factor for their saline–alkaline nature (Wood and Talling 1988; Zinabu 2002). The abundance of alkali elements in the lakes is attributed to their large concentration in the trachytic and rhyolitic rocks of the Ethiopian rift (Klemper and Cash 2007). The lakes' region has semi-arid to sub-humid type of climate with mean annual precipitation and temperature of 600 mm and 25 °C, respectively (Legesse et al. 2002). The region has higher rate of evaporation than precipitation, which causes rainfall deficit.

Lake Chitu is a small closed and highly productive soda lake with ideal environmental conditions for the formation of monoalgal population of *A. fusiformis* (Kebede et al. 1994; Kebede 1996). The lake is characterized by high salinity, pH and $HCO_3^- + CO_3^{2-}$ alkalinity. The water surface temperature of the lake ranges from 21 to 24 °C. Lake Shala, the deepest (maximum depth 266 m) soda lake of the Ethiopian rift lakes, is also characterized by high salinity and alkalinity, which are less than those of Lake Chitu. The water surface temperature of this lake ranges from 22 to 26 °C. The lake is unproductive and devoid of *Arthrospira* although some features of its gross water chemistry are supposed to be conducive for this alga. These highly saline–alkaline conditions of the lakes could be suitable for the production of *Arthrospira*.

Measurement of some physicochemical parameters of the soda lakes

Before using for experimental culture, some chemical parameters of the lake waters were determined in situ or in the

laboratory to see their variation under the two conditions. pH and conductivity were measured in situ and in laboratory using digital pH meter (model HI 9024, Hanna Instruments) and conductivity meter (model CC-505, Elmetrron). Salinity (g L^{-1}) was calculated from conductivity measurements according to UNESCO (1983). Surface water samples, collected with 10-L plastic containers, were used for the analysis of chemical features in laboratory. Alkalinity of unaltered water sample was determined by titration with 1 N HCl to pH 4.5 using a mixed indicator (Bromocresol green-methyl red). Carbonate–bicarbonate alkalinities as $CaCO_3$ and their ions were calculated from total alkalinity and pH according to APHA et al. (1999). NO_3^-, NH_3, PO_4-P and SO_4^{2-} were determined, using samples filtered through Whatman GF/F, by sodium salicylate (APHA 1995), phenate, ascorbic acid and turbidimetric methods, respectively (APHA et al. 1999). Samples acidified to a pH of 2 with HNO_3, were used for the analysis of major ions and some micronutrients according to the standard analytical methods outlined in APHA et al. (1999): Na^+ and K^+ by flame photometric method, Ca^{2+} by direct nitrous oxide-acetylene flame method, Cl^- by argentometric method, Mg^{2+}, Fe, Zn, Mn, Cu, and Co by direct air-acetylene flame method. Boron (B) was determined by azomethine H-colorimetric method (FAO 2008).

Isolation of *Arthospira* and scaling-up of its seed cultures

Trichomes of *A. fusiformis* (Fig. 1) were isolated from Lake Chitu by the serial dilution technique (Andersen and Kawachi 2005). An aliquot from a sample of the lake water was diluted with liquid SM, Zarrouk medium as modified by Aiba and Ogawa (1977), in a small test tube from which drops were transferred to multi-well plate using Pasteur micropipette and concentration of trichomes was checked under an inverted microscope. After a series of similar dilutions and observations, some trichomes were picked up with the micropipette and introduced into two small test tubes of 15 mL capacity containing about 4 mL of SM. The trichomes in the test tubes were allowed to grow at a photon flux density of about 20 μmol photons m^{-2} s^{-1} (produced by two fluorescent lamps, 36 W each) and temperatures of 22–24 °C. Dense cultures of the microalga were diluted by the addition of the SM and scaled up to a large volume (125 mL). All these cultures, which served as sources of inocula, were mixed manually four to five times a day.

Preparation of growth media for experimental cultures

Soda lake water was first filtered through a plankton net (64 μm pore size) to remove large microorganisms and particulate materials, within a few hours of its collection. The filtered water was then sterilized by a chemical sterilization technique (bleaching) and neutralized with sodium thiosulfate

solution following the procedure outlined in Kawachi and Noel (2005). To evaluate growth responses of *Arthrospira*, with the aim to reduce the cost of growth media by substituting standard medium with soda lake waters (by 50 %, 75 % or 100 %), nine different types of media were prepared as indicated in Table 1. These media include SM, Lake Chitu water-based media (CBM) and Lake Shala water-based media (SBM). CBM and SBM consist of unsupplemented lake waters and lake waters supplemented in different proportions with SM and SM^- (SM lacking carbonate–bicarbonate components — Na_2CO_3, $NaHCO_3$ and NaCl). The idea of using SM^- was to determine if these chemicals (Na_2CO_3, $NaHCO_3$ and NaCl), which are required in large quantities in the standard SM, are replaceable by the carbonate- and bicarbonate-rich lake waters.

Experimental design and growth conditions

The experiment was designed to test growth and biomass production of *Arthrospira* in a laboratory using media produced from soda lake waters. The independent variable to be optimized was the growth medium, keeping such growth conditions as temperature, light and mixing constant. The response variables analyzed were specific growth rate (μ), biomass production (B) and doubling time (dt).

The experiment was carried out in 3000 mL Erlenmeyer flasks holding 150 mL culture medium without replication. The culture flasks were inoculated with 15-mL aliquots, constituting 10 % of the final culture volume, removed from exponentially growing cultures. The cultures were exposed to an artificial light source from fluorescent lamps (three, 36 W each), providing a photon flux density of about 50 μmol photons m^{-2} s^{-1} on the surface of the culture. The experimental cultures were grown in a temperature-regulated water bath (model DKZ series) at 35 °C in a light–dark cycle of 10:14 for 18 days. These light and temperature conditions were reported in several research articles as the optimal levels for *Arthrospira* growth in the laboratory (Vonshak 1997; Oliveira et al. 1999; Andersen and Kawachi 2005). Mixing of the cultures was achieved manually by gentle shaking of the culture flasks four times a day.

Analytical methods

Measurement of pH and conductivity and estimation of chlorophyll-a and growth parameters

Before inoculation, initial value of the biomass index — chlorophyll-a (chl-*a*) of the inocula and the initial pH and conductivity of all culture media were measured. During the experimental period, similar measurements were made every 2 days except for the first set of measurements, which were taken after 24 h to check the occurrence of a lag phase. The

Fig. 1 Isolates of *Arthrospira fusiformis* from Lake Chitu used for experimental culture. Scale bar, 20 μm

measurement and analyses were not replicated for the variables.

Chl-*a* was determined spectrophotometrically from 5-mL samples filtered onto GF/F and extracted in 90 % acetone. The absorbance of pigment extracts was measured at 665 and 750 nm with a UV–VIS Spectrophotometer (model 6405, Jenway) and chl-*a* concentration was estimated using the equation in Vonshak ([1997]). Biomass production (*B*, mg L^{-1}) was calculated as the change in biomass per volume of sample filtered (Colla et al. [2007]). Specific growth rate (*μ*) and doubling time (d*t*) were calculated using the following equations used for batch culture of microalgae in the exponential growth phase (Guillard [1973]; Vonshak [1997]).

$$\mu = \frac{\ln N_2 - \ln N_1}{t_2 - t_1}, \quad dt = \frac{\ln 2}{\mu} = \frac{0.6931}{\mu},$$

where N_2 and N_1 are concentrations of the indicator of biomass (chl-*a*, mg L^{-1}) at the end and beginning of the time intervals, t_2 and t_1, respectively.

Table 1 Types and composition of growth media used for the experimental laboratory cultures of *Arthrospira fusiformis*

Types of media	Designation	% (v/v) media composition
Standard: Spirulina medium	SM	100 SM
CBM		
Chitu medium	CM	100 CM
25 % supplemented Chitu medium	25CM	25 SM:75 CM
50 % supplemented Chitu medium	50CM	50 SM:50 CM
50 % supplemented Chitu medium (supplemented with SM$^-$)	50CM$^-$	50 SM$^-$:50 CM
SBM		
Shala medium	SHM	100 SHM
25 % supplemented Shala medium	25SHM	25 SM:75 SHM
50 % supplemented Shala medium	50SHM	50 SM:50 SHM
50 % supplemented Shala medium (supplemented with SM$^-$)	50SHM$^-$	50 SM$^-$:50 SHM

SM$^-$ SM lacking Na$_2$CO$_3$, NaHCO$_3$ and NaCl; *CBM* Chitu water-based media; *SBM* Shala water-based media

Statistical analysis

The differences in growth parameters and some growth conditions of the various media were analyzed by one-way ANOVA, and Tukey's LSD post-hoc test was used for multiple comparisons. The variables, which contributed to the observed variations in growth and biomass production of *Arthrospira* in various media, were determined by multiple regression analysis. All statistical analyses were done using SPSS statistical program (Version 20).

Results and discussion

Some chemical features of the study lakes

Certain physicochemical features of Lakes Chitu and Shala including those chemical features, which seem to be of overriding importance to the growth of *Arthrospira* are presented in Table 2. In laboratory and in situ measurement of some variables such as pH, conductivity and salinity did not show perceptible differences, and those physicochemical data measured in the laboratory were used in this study. The observed high levels of carbonate–bicarbonate alkalinity (as mg L^{-1} CaCO$_3$), pH, salinity, conductivity, Na$^+$, Cl$^-$ and PO$_4$-P in both lakes are characteristic of soda lakes. Most of the recorded chemical parameters were considerably higher in Lake Chitu than in Lake Shala and SM, with the levels in Lake Shala approaching those measured in the SM (Table 2). In both lakes, these parameters did not show considerable seasonal variations although the variation was slightly higher in Lake Chitu due to its productivity and small size. Na$^+$ and HCO$_3^-$ + CO$_3^{2-}$ and Cl$^-$ ions accounted for large proportions of the total cations and anions, respectively, in both lakes. The main mechanisms for the formation of these ions are leaching of rock materials rich in Na from volcanic rocks of the catchment areas and evaporative concentration enhanced by the dry and warm climate (Klemper and Cash [2007]). In most saline lakes of arid regions, these dominant ions are responsible for the high pH, alkalinity, conductivity and salinity of their waters (Wood and Talling [1988]; Kebede et al. [1994]). The concentrations of the divalent cations, Ca^{2+} and Mg^{2+}, are low

Table 2 Some chemical features of lakes Chitu and Shala waters and SM, which were used for experimental cultures

Parameters	Lake Chitu	Lake Shala	SM
pH	10.1	9.4	8.85
Salinity (g L^{-1})	37.50	15.74	13.53
Conductivity (µS cm^{-1})	56,330	25,800	22,460
HCO$_3^-$ + CO$_3^{2-}$ alkalinity	36,848.5	10,990.8	–
HCO$_3^-$	12,428.7	7,606.2	9,880
CO$_3^{2-}$	15,996.6 (1.3)	2,853.7 (0.4)	2,280 (0.23)
NO$_3^-$ (µg L^{-1})	20	ND	1,820,000
NH$_3$ (µg L^{-1})	50	ND	–
PO$_4$-P (µg L^{-1})	460	830	273,000
SO$_4^{2-}$	187.5	33.4	634
Na$^+$	20,500	8,000	6,550
K$^+$	1,200	260	672
Ca^{2+}	0.39	ND	11
Mg^{2+}	0.06	0.03	20
Cl$^-$	12,336.6	3,701	626
Fe	0.57	0.20	–
Zn	0.043	0.027	–
Mn	ND	ND	–
Cu	0.118	0.122	–
Co	ND	ND	–
B	16.23	11.5	–

Units are mg L^{-1} unless otherwise indicated; data in *bracket* are CO$_3^{2-}$ to HCO$_3^-$ ratio

ND not detectable

compared to those of the dominant ions. The low level of these divalent cations is a salient feature of tropical saline–alkaline lakes (Kebede et al. 1994; Zinabu 2002; Klemper and Cash 2007) as these cations are precipitated from solution as their respective carbonates at high alkaline pH.

PO$_4$-P, whose concentration in Lake Shala was about two times that in Lake Chitu, was quite high in both lakes considering its levels commonly recorded for freshwater lakes. In contrast, the concentration of nitrogen compounds NO$_3^-$ and NH$_3$, which were often low or undetectable, were rather higher in Lake Chitu than in Lake Shala. In both lakes, the concentration of these macronutrients was considerably low compared to that of SM.

High pH and salinity and a large reserve of inorganic carbon sources and PO$_4$-P are characteristic of some of the East African soda lakes, which favor dense populations of microalgae (Talling et al. 1973; Melack et al. 1982; Wood and Talling 1988; Kebede et al. 1994; Jones and Grant 1999). The pH and salinity of a medium affect different physiological processes such as growth, photosynthesis and chemical production of *Arthrospira* (Guterman et al. 1989; Vonshak et al. 1996; Kebede 1997). The high level of carbonate–bicarbonate is important as a buffer system to maintain optimum alkaline

pH and provides carbon source for aquatic autotrophs such as alkaliphilic cyanobacteria (Richmond 1990; Vonshak 1997). It has been suggested that high level of CO$_3^{2-}$ to HCO$_3^-$ ratio at pH >10 may indicate a low level of HCO$_3^-$, which is the principal and preferred carbon source for *Arthrospira* (Binaghi et al. 2003) and buffer against a pH rise. The low CO$_3^{2-}$ to HCO$_3^-$ ratio (or high HCO$_3^-$) in Lake Shala compared to Lake Chitu may indicate that its water is well buffered against a pH rise and presence of surplus carbon source for growth of *Arthrospira*.

Growth and biomass production of algae are enhanced when nitrogen and phosphate sources are sufficiently available (Melack et al. 1982; Larned 1998; Miller et al. 1999). In some African freshwaters, inorganic sources of nitrogen and phosphorus were often suggested to be limiting to algal production (Talling and Talling 1965; Melack et al. 1982). Very high concentration of PO$_4$-P was, however, recorded in the present study lakes. This is consistent with the results of earlier studies, which also recorded similarly high concentration of PO$_4$-P in Lakes Chitu, Shala and Arenguade, with concentrations sometimes exceeding 2 mg L^{-1} in Lake Chitu despite the large algal standing crop it supported (224 µg L^{-1} chl-*a*) (Wood and Talling 1988; Kebede et al. 1994). Thus, PO$_4$-P may not be limiting algal growth in these soda lakes but its concentration may not be adequate to support optimal growth of *Arthrospira* from the production point of view.

Nitrogen sources were low or undetectable in both lakes in the present study, corroborating the contention that nitrogen limitation of algal production is more likely in these soda lakes (Wood and Talling 1988; Kebede et al. 1994; Talling and Lemoalle 1998). In productive soda lakes like Chitu, over 90 % of the total inorganic nitrogen is in the algal biomass (Wood and Talling 1988) making nitrogen sources less available in the water body but which can be regenerated by recycling from the anoxic layer during destratification (Kebede et al. 1994). It is also argued that the high denitrification activities by abundant populations of some bacteria (e.g., *Halomonas* spp.) associated with tropical temperature in productive soda lakes may contribute to low nitrogen levels (Jones and Grant 1999).The unusually low level of nitrogen sources in Lake Shala may be attributed to the low concentration of decomposable organic matter and reduced rate of recycling owing to the great depth of the lake.

Salinity and pH of the experimental culture

In all media, pH increased while salinity decreased progressively with time during the cultivation period (Fig. 2). The decrease in salinity and increase in pH was gradual during early phase of the growth period, but became abrupt towards the end starting from days 11 to 12 in all media. These changes in pH can result from the chemical reactions among the carbonate systems and photosynthetic activities by dense algal

Fig. 2 Changes in pH (**a** and **b**) and salinity (**c** and **d**) with time during the cultivation of *Arthrospira fusiformis*. **a** and **c** for Lake Chitu water-based media, **b** and **d** for Lake Shala water-based media
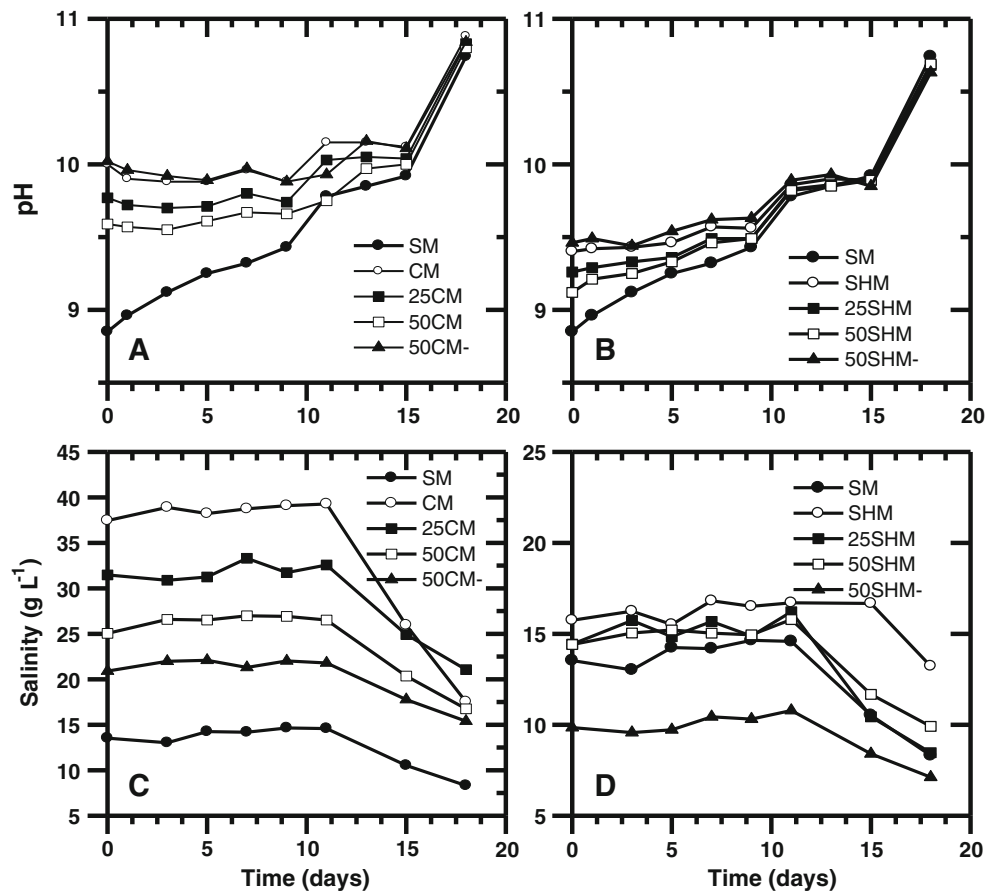
biomass. According to Talling et al. (1973), dense algal biomass and vigorous photosynthesis in Lake Arenguade caused a rise in pH more than one pH unit despite the high buffering capacity associated with the lake's large carbonate–bicarbonate alkalinity. During growth, *Arthrospira* preferably consumes bicarbonate and releases carbonate into the medium and this carbonate/bicarbonate imbalance causes a progressive pH rise. The association of carbonate with proton (H^+) to produce bicarbonate, which is rapidly consumed by the alga, and dissolution of bicarbonate to CO_2 causes a rapid increase in pH (Richmond and Grobbelaar 1986; Richmond 2002). pH increased more slowly in lake waters-based media than in the SM, which may be attributable to the high buffering capacity of the lake waters owing to their high carbonate–bicarbonates contents. The rapid decline of salinity observed after exponential phase may have resulted from the chemical transformation of carbonate–bicarbonate species, the major salinity components, and dilution of the culture through growth.

The mean pH values of all CBM and SBM were higher than that of SM (9.52). Compared to CBM, SBM had relatively low pH and supplementation with SM reduced it further to values approaching the SM. Statistically significant differences in pH ($F_{(8, 81)}=2.45$, $P<0.05$) were observed among all media, with significantly ($P<0.05$) higher values in all

CBM except 50CM. In contrast, the pH of all SBM (SHM, 25SHM, 50SHM and 50SHM⁻) were not significantly different from that of SM despite the decreased pH levels in 25SHM and 50SHM. Mean salinity also showed significant differences ($F_{(8, 63)}=40.61$, $P<0.01$) among the media, with significantly ($P<0.01$) higher salinity being in all CBM than those of other media. The salinities of 25CM, 50CM and 50CM⁻ were significantly lower ($P<0.05$) than that of CM although they were still higher than that of SM. Among SBM, those supplemented with SM showed some insignificant reduction in salinity (SHM > 25SHM > 50SHM), particularly that of 25SHM and 50SHM was closely approaching the salinity of SM (12.88 g L⁻¹), but that supplemented with SM⁻ (50SHM⁻) was significantly reduced ($P<0.05$).

The pH of Lake Chitu water was close to the upper limit of the optimal range for the growth of *Arthrospira* (8–10) (Richmond 1990; De Oliveira et al. 1999). Supplementation of the lake water with SM, however, led to significant pH reduction, which was pronounced in 50CM. The pH of Lake Shala water was lower than that of Lake Chitu water and supplementation caused its further reduction, though not significant, close to that of SM. The reduction in pH of the lake waters shortly after supplementation with SM (pH: 8.9) may be due to addition of carbonate–bicarbonates from SM, as the

dissociation of bicarbonate to carbonate releases protons (H^+) in the system causing a short term pH decline. The change in pH due to supplementation was not large in Lake Shala because it had low ratio of CO_3^{2-} to HCO_3^-, which probably made it more resistant to pH change. On the other hand, supplementation with SM^- (pH: 9.87) did not cause changes in pH (almost similar to the lake water), which may be due to the absence of the carbonate–bicarbonates in the supplement.

Lake Chitu water had relatively high salinity but supplementation with SM (salinity: 13.53 g L^{-1}) and SM^- (salinity: 5.23 g L^{-1}) significantly reduced its salinity closer to that of SM. The salinity of Lake Shala water was relatively low and supplementation reduced it further very close to that in the SM. The reduction in salinity following supplementation could result from the dilution effect of the SM and SM^-, which had low salinity compared to both CBM and SBM. The large reduction in salinity of CBM and SBM when supplemented with SM^- seems to have resulted from the greater dilution effect of SM^- as it was more dilute than SM due to the lack of the major ions (Na^+, Cl^- and CO_3^{2-} + HCO_3^-). In general, supplementation of the lake waters with SM lowered pH and salinity to values approaching that of the standard SM while supplementation with SM^- resulted in a large decline in salinity but not pH. Supplementation of the soda lake waters, by 25 % and 50 % with SM is, therefore, necessary to adjust pH and salinity to the optimal levels and also to provide deficient nutrients including nitrogen and phosphorus.

Growth and biomass production

Figure 3 shows growth curves of *Arthrospira* cultured in different complex media in comparison with that in SM. The growth curves show the typical pattern of exponential growth of microlagae without distinct lag phase. High growth of *Arthrospira* was observed in the SM, 25CM and 50CM of CBM and 25SHM and 50SHM of SBM although better

growth was exhibited by the cultures in the SBM. Low growth was observed in the CM, SHM, $50CM^-$ and $50SHM^-$.

Responses of *Arthrospira* reflected in such growth parameters as specific growth rate (μ), biomass production (B) and doubling time (dt) determined from chl-*a* measurements are given in Table 3. Higher μ and B and the shortest dt were achieved in SM, 25CM and 50CM of CBM and 25SHM and 50SHM of SBM, with higher values comparable to that of SM in the SBM. The least values of μ and B and the largest dt were observed in CM and $50CM^-$. The μ and B in both $50CM^-$ and $50SHM^-$ showed a slight increase compared to SHM and CM. Analysis of variance (ANOVA) of the growth parameters μ and B did not, however, show significant differences among the various media, implying that comparable growth rate and biomass production can be achieved using the soda lake waters with or without supplementation.

The results of multiple regression analysis of the causal relationship between the response variable (B) and the chemical factors — pH and salinity indicated the statistically significant ($F(2,69)=21.52$, $P<0.01$) effect the two factors had on the biomass production of *Arthrospira*. pH was the main factor accounting for more than 52 % of the variations in biomass. The relationships of μ and B with mean pH and salinity in different media of the cultures (Fig. 4) show that the increased μ and B in SM, 25CM and 50CM of CBM and 25SHM and 50SHM of SBM coincided with decreased mean pH and salinity while the lowest values of μ and B in CM and $50CM^-$ corresponded to the highest pH.

Saline–alkaline conditions and availability of nutrients in the soda lakes are ideal conditions for the growth and production of *Arthrospira*. Better growth and biomass production, comparable to those observed in the SM, occurred in both CBM and SBM supplemented with SM by 25 % and 50 %, which had relatively low pH and salinity. This seems to have resulted from the reduction in pH and salinity by supplementation to levels, which were closer to those in the standard SM,

Fig. 3 Growth curves of *Arthrospira fusiformis* cultivated in various media at 35 °C and photon flux density of 50 μmol m^{-2} s^{-1} during the cultivation period. **a** for Lake Chitu water-based media, **b** for Lake Shala water-based media, the control (SM) in both **a** and **b**
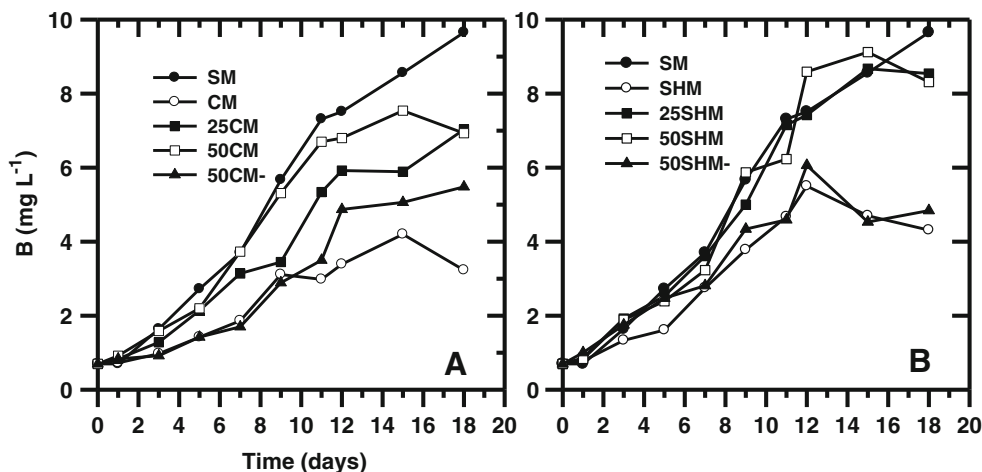
Table 3 The growth parameters of *Arthrospira fusiformis* cultured in various media for 18 days

Types of culture media	Growth parameters			
	B_{max}	Mean B	μ	dt
SM	9.65	4.82±2.3	0.20	3.47
CM	4.20	2.26±0.8	0.13	5.22
25CM	7.03	3.57±1.3	0.18	3.85
50CM	8.54	4.15±1.9	0.19	3.62
50CM$^-$	5.48	2.74±1.1	0.16	4.24
SHM	5.50	3.01±1.0	0.17	3.99
25SHM	8.67	4.64±2.1	0.20	3.48
50SHM	9.12	4.72±2.3	0.21	3.28
50SHM$^-$	6.06	3.31±1.1	0.18	3.81

Maximum (B_{max}) and mean of index of biomass (mg chl-a L^{-1}, $n=10$), μ (/day) and dt (days).The parameters μ and dt are calculated from the exponential growth phase (12 days)

due to dilution and addition of bicarbonate–carbonates. In contrast, the relatively low growth rates and biomass production observed in the CM and 50CM$^-$ may be attributable to the high pH of the media. The pH determines the formation of carbonate–bicarbonate species and the solubility of minerals in the lake waters thereby influencing the metabolism of algae (Guterman et al. 1989). Supplementation with SM adds more carbonate–bicarbonates that maintain alkaline pH and prevents carbon depletion, which is an important condition for the optimal growth of *Arthrospira* (Richmond 1990; Vonshak 1997). In an experiment carried out using lagoon water supplemented with sodium bicarbonate and urea, Costa et al. (2003) also obtained the highest biomass production with the addition of 2.88 g L^{-1} sodium bicarbonate. Salinity of a medium affects different physiological processes such as growth, photosynthesis and chemical production of *Arthrospira* (Vonshak et al. 1996; Kebede 1997). Several studies made on the growth and production of *A. fusiformis* in media with different salinity levels have demonstrated the association of higher growth rate and production of the alga with media of relatively low salinity (Kebede 1997; Vonshak 1997; Mussagy 2006). Exposure of *Arthrospira* culture to high salinity was found to result in the reduction and eventual cessation of growth (Vonshak 1997; Mussagy 2006), which was associated with substantial decrease in photosystem II activities due to osmotic shock (Zeng and Vonshak 1998). In addition, availability of limiting nutrients such as nitrogen and phosphorus enhances growth and biomass production of algae (Larned 1998; Miller et al. 1999). Supplementation may have provided these nutrients, particularly nitrogen, which is limiting in these soda lakes. The results of experiments carried out using seawater enriched with phosphate and urea, in both laboratory and outdoor mass cultivation of *Arthrospira*, showed high biomass yield, which was slightly less than that
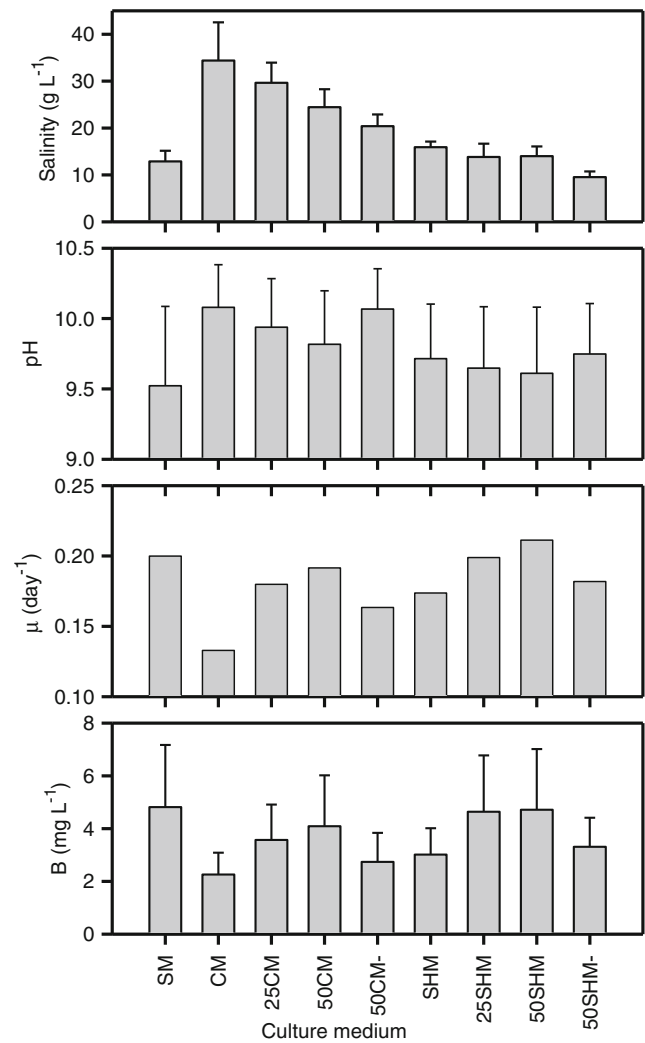
Fig. 4 Growth rate (μ) and index of biomass (B) of *Arthrospira fusiformis* in relation to pH and salinity of the various media used for laboratory cultures

in the standard medium (Tredici et al. 1986; Materassi et al. 1984).

The higher growth rate and biomass production with shorter doubling time observed in SBM seems to be associated with some chemical features of Lake Shala. Primarily, pH, salinity, conductivity, CO_3^{2-} + HCO_3^- and Na$^+$ of this lake were closer to those of the SM, indicating suitable conditions and huge carbon source (HCO_3^-) for the alga. Secondly, the abundant PO_4-P level in Lake Shala, which was about twice higher than that in Lake Chitu, could favor better growth and higher biomass production of the species under consideration. Furthermore, Lake Shala was deficient in nitrogen sources compared to that of Lake Chitu, and hence supplementation would provide this nutrient thereby enhancing the production of the alga.

The large reserve of inorganic carbon sources, which prevails even at high pH values, is a salient feature of soda lakes that favor dense populations of *A. fusiformis* (Talling et al. 1973; Wood and Talling 1988). In the natural ecosystem, Lake

Chitu supports superabundant populations of *Arthrospira* while the exceptionally deep adjacent Lake Shala, which has broadly similar gross chemical features, is devoid of *Arthrospira* and much less productive. On the basis of the high light extinction coefficient and optical depth, and undetectable concentration of nitrogen compounds in Lake Shala, light and nitrogen were previously identified as the major factors associated with its low phytoplankton productivity (Wood and Talling 1988; Kebede et al. 1994; Zinabu 2002). As the results of the present study show, Lake Shala water supported better growth of *Arthrospira* with provision of light and nutrients, and its aggregate chemical features except nitrogen were in the range required for *Arthrospira* growth. Light and nitrogen nutrients together with other hydrographic and morphometric variables, therefore, probably have contributed to the low productivity and absence of *Arthrospira* in the natural ecosystem of Lake Shala. Pilot-scale experimental testing of *Arthrospira* production in shallow ponds using Lake Shala water may enable one to provide further plausible explanations for the low productivity of the lake.

Conclusion

Comparable growth and biomass production of *Arthrospira* was achieved using water of the soda lakes Chitu and Shala. Supplementation of the waters of the soda lakes with SM by 25 and 50 % further enhanced the growth and biomass production of *Arthrospira*. This appears to be attributed to the adjustment of the pH and salinity close to the optimal range by dilution and addition of carbonate–bicarbonates and provision of some deficient nutrients such as nitrogen by supplementation. pH and salinity differed significantly among the media and caused considerable effect on the growth and biomass production of *Arthrospira* in this study. Supplementation with SM reduced pH and supported better growth and more production than SM⁻, indicating the importance of addition of carbonate–bicarbonates even to the lake waters rich in these compounds. Lake Shala-based media supported better growth and higher biomass of *Arthrospira* than Lake Chitu-based media, indicating that some of the chemical features of Lake Shala are more conducive for *Arthrospira* production. This was interesting and desired result as the main goal was to show the use of the large volume of Lake Shala water for mass production. In general, *Arthrospira* can be produced using 25 and 50 % supplemented soda lake waters, preferably the large lake Shala water, thereby reducing the cost of nutrient media by 75 % and 50 %, respectively, which is a rough estimation based on the proportion of lakes water that could be combined with SM to produce a culture medium. In order to make use of soda lake water as a low cost medium for *Arthrospira* production, further optimization studies and nutrients (nitrogen) enrichment of the lake waters are deemed essential.

References

Aiba S, Ogawa T (1977) Assessment of growth yield of a blue-green alga *Spirulina platensis* in axenic and continuous culture. J Gen Microbiol 102:179–182

American Public Health Association (APHA) (1995) Standard methods for the examination of water and wastewater, 19th edn. Washington, DC

American Public Health Association (APHA), American Water Works Association (AWWA), Water Environment Federation (WEF) (1999) Standard methods for the examination of water and waste water, 20th edn. Washington, DC

Andersen RA, Kawachi M (2005) Traditional microalgae isolation techniques. In: Andersen RA (ed) Algal culturing techniques. Elsevier, London, pp 83–100

Ayala F, Vargas T, Cardenas A (1988) Chilean experiences on microalgae culture. In: Stadler T, Mollion J, Verdus MC, Karamanos Y, Morvan H, Christiaisen D (eds) Algal biotechnology, proceedings of the 4th international meeting of the SAA. Elsevier, London, pp 229–236

Baldia SF, Nishijima T, Hata Y (1991) Effects of physicochemical factors and nutrients on the growth of *Spirulina platensis* isolated from Lake Kojima Japan. Nippon Suisan Gakkaishi 57:481–490

Belay A (1997) Mass culture of *Spirulina* outdoors—the Earthrise Farms experience. In: Vonshak A (ed) *Spirulina platensis* (*Arthrospira*): physiology cell-biology and biotechnology. Taylor & Francis, London, pp 131–158

Belay A (2002) The potential application of *Spirulina* (*Arthrospira*) as a nutritional and therapeutic supplement. JANA 5:27–48

Belay A, Ota Y (1993) Current knowledge on potential health benefits of *Spirulina*. J Appl Phycol 5:235–241

Belay A, Kato T, Ota Y (1996) *Spirulina* (*Arthrospira*): potential application as an animal feed supplement. J Appl Phycol 8:303–311

Binaghi L, Borghi AD, Lodi A, Converti A, Borghi MD (2003) Batch and fed-batch uptake of carbon dioxide by *Spirulina platensis*. Process Biochem 38:1341–1346

Chen F, Zhang Y, Guo S (1996) Growth and phycocyanin formation of *Spirulina platensis* in photoheterotrophic culture. Biotechnol Lett 18:603–608

Ciferri O (1983) *Spirulina*, the edible micro-organism. Microbiol Rev 47: 551–578

Cogne G, Lasseur C, Cornet JF, Dussap CG, Gros JB (2001) Growth physiology of microorganism (*S. platensis*) by pressure measurement. Biotechnol Lett 23:1309–1314

Colla LM, Reinehr CO, Reichert CJ, Costa AV (2007) Production of biomass and nutraceutical compounds by *Spirulina platensis* under different temperature and nitrogen regimes. Bioresour Technol 98: 1489–1493

Costa JAV, Colla LM, Filho FD (2003) Spirulina platensis growth in open raceway ponds using freshwater supplemented with carbon nitrogen and metal ions. Z Naturforsch 58c:76–80

Food and Agriculture Organization of the United Nations (FAO) (2008) Guide to laboratory establishment for plant nutrient analysis. FAO Fertil Plant Nutr Bull 19, Rome

Guillard RRL (1973) Culture methods and growth measurements division rates. In: Stein JR (ed) Handbook of phycological methods. Cambridge University Press, Cambridge, pp 289–311

Guterman H, Vonshak A, Ben-Yaakov S (1989) Automatic online growth estimation method for outdoor algal biomass production. Biotechnol Bioeng 34:143–152

Habib MAB, Parvin M, Huntington TC, Hasan MR (2008) A review on culture production and use of Spirulina as food for humans and feeds for domestic animals and fish. FAO Fisheries and Aquaculture Circular No 1034, Rome, pp 33

Jones BE, Grant WD (1999) Microbial diversity and ecology of the soda lakes of East Africa. In: Bell CR, Brylinsky M, Johnson-Green JP (eds) Microbial biosystems: new frontiers, proceedings of the 8th international symposium for microbial ecology. Antic Canada Society for Microbial Ecology, Halifax, pp 681–687

Jourdan JP (1993) Solarium Spirulina farm in the Atacama desert (North Chile) In: Doumenge F, Durand-Chastel H, Toulemont A (eds) Spiruline algue de vie. Bull Inst Oceanogr Monaco Mus Oceanogr 12:191–194

Kawachi M, Noel MH (2005) Sterilization and sterile techniques. In: Andersen RA (ed) Algal culturing techniques. Elsevier, London, pp 65–81

Kebede E (1996) Phytoplankton in a salinity–alkalinity series of lakes in the Ethiopian rift valley. Dissertation, Uppsala University

Kebede E (1997) Response of Spirulina platensis (Arthrospira fusiformis) from Lake Chitu Ethiopia to salinity stress from sodium salts. J Appl Phycol 9:551–558

Kebede E, Zinabu GM, Ahlgren I (1994) The Ethiopian rift valley lakes: chemical characteristics of a salinity–alkalinity series. Hydrobiologia 288:1–12

Kilic C, Goksan T, Ak I, Gokpinar S (2006) Iki farkli Spirulina paltensis supunun buyume ozelliklerinin karsilastirilmasi. EU J Fish Aquat Sci 23:189–192

Klemper SL, Cash MD (2007) Temporal geochemical variation in Ethiopian lakes Shala, Arenguade, Awasa and Beseka: possible environmental impacts from underwater and borehole detonations. J Afr Earth Sci 48:174–198

Larned ST (1998) Nitrogen- versus phosphorus-limited growth and sources of nutrients for coral reef algae. Mar Biol 132:409–421

Legesse D, Gasse F, Radakovitch O, Vallet-Coulomb C, Bonnefille R, Verschuren D, Gibert E, Barker P (2002) Environmental changes in a tropical lake (Lake Abiyata; Ethiopia) during recent centuries. Palaeogeogr Palaeoclimatol Palaeoecol 187:233–258

Materassi R, Tredici M, Balloni W (1984) Spirulina culture in seawater. Appl Microbiol Biotechnol 19:384–386

Melack JM, Kilham MP, Fisher TR (1982) Responses of phytoplankton to experimental fertilization with ammonium and phosphate in an African soda lake. Oecologia 55:1–6

Miller MW, Hay ME, Miller SL, Sotka E, Szmant AM (1999) Effects of nutrients versus herbivores on reef algae: a new method for manipulating nutrients on coral reefs. Limnol Oceanogr 44:1847–1861

Mussagy A (2006) The cyanophyte Arthrospira fusiformis in African waters-ecophysiology and potential use in tropical aquaculture. Dissertation, Lund University, Lund, pp 118

Neilson AH, Larsson T (1980) The utilization of organic nitrogen for growth of algae: physiological aspects. Physiol Plant 48:542–553

Oliveira MACL, Monteiro MPC, Robbs PG, Leite SGF (1999) Growth and chemical composition of Spirulina maxima and S. platensis biomass at different temperatures. Aquacult Int 7:261–275

Richmond A (1990) Large scale microalgal culture and applications. Prog Phycol Res 7:269–330

Richmond A (2002) Microalgal biotechnology at the turn of the millennium: a personal view. J Appl Phycol 12:441–451

Richmond A, Grobbelaar JU (1986) Factors affecting the output rate of Spirulina platensis with reference to mass cultivation. Biomass 10:253–264

Talling JF, Lemoalle J (1998) Ecological dynamics of tropical inland waters. Cambridge Univ. Press, Cambridge

Talling JF, Talling IB (1965) The chemical composition of African lake waters. Int Rev Gesamten Hydrobiol 50:421–463

Talling JF, Wood RB, Prosser MV, Baxter RM (1973) The upper limit of photosynthetic productivity by phytoplankton: evidence from Ethiopian soda lakes. Freshw Biol 3:53–76

Tredici M, Papuzzo T, Tomaselli L (1986) Outdoor mass culture of Spirulina maxima in seawater. Appl Microbiol Biotechnol 24:47–50

Tudorancea C, Zinabu GM, Elias D (1999) Limnology in Ethiopia. In: Wetzel RG, Gopal B (eds) Limnology in developing countries, vol. 2. International Association for Limnology (SIL) International Scientific Publications, New Delhi, pp 63–118

UNESCO (1983) Algorithms for computation of fundamental properties of seawater. UNESCO technical papers in marine science 44, 58 pp

Vonshak A (1997) Outdoor mass production of Spirulina: the basic concept. In: Vonshak A (ed) Spirulina platensis (Arthrospira): physiology, cell-biology and biotechnology. Taylor and Francis, London, pp 79–99

Vonshak A, Torzillo G, Accolla P, Tomaselli L (1996) Light and oxygen stress in Spirulina platensis (cyanobacteria) grown outdoors in tubular reactors. Physiol Plant 97:175–179

Wood RB, Talling JF (1988) Chemical and algal relationships in a salinity series of Ethiopian inland waters. Hydrobiologia 158:29–67

Zeng MT, Vonshak A (1998) Adaptation of Spirulina platensis to salinity-stress. Comp Biochem Physiol A Mol Integr Physiol 120/121:113–118

Zinabu GM (2002) The effect of wet and dry seasons on the concentrations of solutes and phytoplankton biomass in seven Ethiopian rift valley lakes. Limnologica 32:169–179