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Ecophysiological Studies on *Arthrospira fusiformis*

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Mary Nakabungo Kaggwa

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Dr. Steve O. Oduor

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Nakuru, 2010 – *'Food for thought'*

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Zusammenfassung

Arthrospira fusiformis (Voronich.) Komárek und Lund, (Cyanobakterien; früherer Name: *Spirulina plantensis*) ist für ihre hohe Anpassungsfähigkeit bekannt, die es der Alge ermöglicht, eine Vielzahl von Habitaten zu besiedeln, von alkalischen Binnengewässern bis zu Sodaseen. Das Taxon stellt den Hauptanteil – oft auch die einzige Komponente - des pelagischen Phytoplanktons der Seen Nakuru und Bogoria dar. Die beiden Seen befinden sich in der semi-ariden Zone Zentralkenias und bilden einen Teil der Kette an alkalischen Sodaseen im Ostafrikanischen Grabenbruch. Sie beherbergen Millionen von Flamingos (*Phoeniconaias minor*), die sich hauptsächlich von *Arthrospira* ernähren. Lange stand *Arthrospira* im Mittelpunkt der ökologischen Forschung, vor allem wegen ihrer fundamentalen Rolle für die Funktion von Sodaseen, aber auch wegen ihrer wohlbekannten Rolle als nährstoffreiche Nahrungsquelle. In der vorliegenden Arbeit werden wichtige, bis jetzt nicht oder nicht ausreichend erforschte Aspekte der Physiologie und Ökologie dieser faszinierenden Organismen beleuchtet.

Die praktischen Arbeiten für diese Studien wurden in den Seen Nakuru und Bogoria vom Juli 2008 bis Oktober 2009 durchgeführt. Die Datenerhebung erfolgte in jedem See wöchentlich. Die erste Studie beschäftigte sich mit dem Einfluss des Nährstoffgehalts (Kohlehydrate, Proteine und Fette) und der Qualität der pelagischen Phytoplanktongemeinschaft auf das Auftreten der Flamingopopulationen. Es zeigte sich, dass im See Nakuru das Flamingovorkommen signifikant positiv mit dem Fettgehalt und der Biomasse von *Arthrospira* korreliert war, während ein negativer Zusammenhang mit dem Auftreten kleiner Blaualgen herrschte (*Synechococcus minutus*, *Synechocystis* sp., *Raphidiopsis* sp.). Dagegen konnten beim See Bogoria keine derartigen signifikanten Zusammenhänge festgestellt werden. Wir ziehen den Schluss, dass die räumlich-zeitlichen Abundanzschwankungen des Flamingos von Nährstoffgehalt und Qualität des Phytoplanktons beeinflusst wird, wenn auch fallweise andere Faktoren – etwa die vorherrschenden Umweltbedingungen – an Wichtigkeit gewinnen können.

In der zweiten Studie wurden die zeitlichen Veränderungen in der Morphologie von *Arthrospira* in Bezug auf Zelldurchmesser, Zellhöhe, Höhe der Windung, Windungsdurchmesser und Anzahl der Windungen in den zwei Seen untersucht und die zugrunde liegenden Schlüsselfaktoren identifiziert. Zellen mittlerer Größe und große, weit gewundene Filamente dominierten in beiden Seen. Der Prozentsatz an großen, weit

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gewundenen Filamenten nahm bei hohem Orthophosphatgehalt, sowie erhöhter Windgeschwindigkeit, Temperatur und Leitfähigkeit zu. Gegenteilige Bedingungen förderten das Auftreten von kleinen Filamenten. Große, eng gewundene Filamente traten bei einer Zunahme des *Arthrospira*-fressenden Zooplanktons und der Infektionen durch Cyanophage (Viren) auf, während weit gewundene Spiralen hauptsächlich bei starker Turbulenz zu beobachten waren. Bei den weit gewundenen Filamenten ergaben Fluoreszenzmessungen eine hohe Vitalität. Diese Studie zeigte erstmals die morphologischen Muster von *Arthrospira* in der freien Natur. Die Morphologie von *Arthrospira* kann als Indikator für die biologische Stabilität in Sodaseen herangezogen werden.

Die Schlüsselfaktoren, welche die Phytoplanktongemeinschaft strukturieren und die Umweltvariablen, die die Änderungen in der Biomasse vom *Arthrospira* beeinflussen, untersuchten wir in der dritten Studie. In beiden Seen dominierte *Arthrospira fusiformis* hinsichtlich der Biomasse. Im Lake Nakuru konnten plötzliche Zusammenbrüche bei *Arthrospira* beobachtet werden. Bei den drei Faktoren Salinität, pH-Wert und Aschegewicht war ein signifikanter Zusammenhang mit dem Muster des Auftretens der Phytoplanktontaxa festzustellen. *Arthrospira* fehlte bei sehr hohen Aschegewichten ($> 0.677 \text{ g L}^{-1}$) und den höchsten Salinitätswerten ($> 57.8 \text{ ‰}$). Unter diesen Bedingungen war *Picocystis salinarium* konkurrenzstärker. Picoplankton war auch bei hohen pH-Werten und während der Zusammenbrüche der *Arthrospira*-Populationen im See Nakuru zu beobachten. Generell war festzustellen, dass sich *Arthrospira* nur bedingt an rasche Umweltänderungen anpassen kann. Unter solchen Bedingungen wird es von anderen Phytoplanktern auskonkurrenziert, was zu einer höheren Diversität in der Phytoplanktongemeinschaft führt. Lichteindringtiefe, Konsumenten von *Arthrospira* und Virenattacken (Cyanophagen), die dieses Taxon angreifen steuern die Abundanz von *Arthrospira* im See Nakuru. In dieser Studie konnte erstmals der Zusammenhang zwischen der Infektion durch Cyanophagen in *Arthrospira*-Filamenten und dem plötzlichen Zusammenbruch dieser Alge dokumentiert werden.

Unsere Studien sind Pionierarbeiten in den Sodaseen und die wöchentliche Probennahme ermöglichte eine detaillierte Analyse der kurzfristigen Veränderungen in diesen dynamischen Systemen. Die ökophysiologischen Studien an *Arthrospira* wiesen dieses Taxon als Basis des funktionierenden Ökosystems aus und die periodischen Zusammenbrüche schlugen sich nicht nur in signifikanten Veränderungen der Phytoplanktongemeinschaft nieder, sondern auch in der Abundanz der Primärkonsumenten – in diesem Fall der Flamingos. Die Ergebnisse der Studien vertieften nicht nur unser Verständnis der Funktionsweise dieser Ökosysteme,

sondern eröffneten auch neue Forschungshorizonte, vor allem in Bezug auf die Rolle von Viren bei der Populationsdynamik von *Arthrospira*.

Abstract

Arthrospira fusiformis (Voronich.) Komárek and Lund (Cyanobacteria; formerly called *Spirulina plantensis*) is known to have a high degree of adaptation which enables it to grow in a wide range of habitats, from fresh-alkaline to saline waters. This taxon is the largest contributor to the pelagic phytoplankton communities of lakes Nakuru and Bogoria often forming unialgal biomass. The two lakes are located in the semi-arid region of Central Kenya and form part of the chain of alkaline-saline lakes along the floor of the eastern Africa Rift Valley. They are primary hosts to millions of Lesser Flamingos (*Phoeniconaias minor*) which mainly feed on *Arthrospira*. For a long while now, *Arthrospira* has been a taxon of interest for ecologists because of the fundamental role it plays in the functioning of the soda lakes as well as commercial producers due to its well-known nutritional benefits. For the present work the aim was to delve further in the fascinating nature of the ecology and physiology of this organism particularly targeting those areas that until now knowledge about them is unavailable or if so still needed further research to derive definitive conclusions.

All the studies for this work were carried out in lakes Nakuru and Bogoria from July, 2008 to October, 2009 and data collected on a weekly basis for each lake. The first study was about the influence of the nutritional (carbohydrates, crude protein and lipids) composition and quality of the pelagic phytoplankton community on the occurrence of Lesser Flamingo populations. The findings showed that in Lake Nakuru, Lesser Flamingos had a significant positive relationship with lipids and *Arthrospira* biomass but a negative relationship with small cyanoprokaryotes (*Synechococcus minutus*, *Synechocystis* sp., *Raphidiopsis* sp.). For Lake Bogoria, no significant relationships were observed. We concluded that the nutritional composition and quality of the phytoplankton community influenced the temporal and spatial abundance of Lesser Flamingos although other factors such as the prevailing environmental conditions might take precedence.

The second study involved temporal morphological (cell diameter, cell height, height of coil, coil diameter and number) changes of *Arthrospira* in the two lakes and underlying key factors were identified. In both lakes, medium-sized cells and large, widely-coiled filaments

prevailed most. Percentage of large, widely-coiled filaments was promoted by elevated levels of soluble reactive phosphorous, wind speed, temperature and conductivity and the opposite for small filaments. Large, narrow-coiled filaments were associated with an increase in mainly *Arthrospira*-grazing zooplankton and cyanophage infections, widely-coiled spirals were promoted by increased turbulences. Based on fluorescence measurements, we found widely-coiled filaments representing high vitality. From this study we were able to demonstrate for the first time morphological patterns of *Arthrospira* in nature. Also *Arthrospira* morphology is suitable for indicating the biological stability in soda lakes as its shape shifts are highly subjective to the prevailing conditions.

The third study involved investigations on the key drivers for structuring the phytoplankton community and determining variables that significantly influenced the observed changes in *Arthrospira* biomass. In both lakes, *Arthrospira fusiformis* dominated algal biomass, but for Nakuru, sudden *Arthrospira* breakdowns were recognized. Three variables – salinity, pH and ash mass – significantly contributed to the phytoplankton taxa pattern. *Arthrospira* was absent at the highest ash mass ($> 0.677 \text{ g L}^{-1}$) and salinity ($> 57.8 \text{ ‰}$) levels being outcompeted by *Picocystis salinarium*. Picoplankton were also present at a higher pH and during *Arthrospira* crash in L. Nakuru. Overall it was observed that *Arthrospira*'s response to fast environmental changes is limited in nature hindering its growth and therefore being outcompeted by other taxa thus contributing to a more diverse phytoplankton community. Light penetration, consumers of *Arthrospira* and cyanophages attacking this taxon significantly influenced *Arthrospira* abundance in L. Nakuru. This was the first study to prove cyanophage infection in *Arthrospira* filaments and relate the virus attacks to its sudden breakdowns.

The above studies were all maiden research works in these soda lakes and the weekly sampling intervals offered an exceptional opportunity to capture the short term occurrences in these dynamic systems. The ecophysiological studies on *Arthrospira* depicted this taxon as the basis of the ecosystem functioning and its periodic disappearance revealed significant alterations not only in the phytoplankton community composition and abundances but also in other ecosystem components such as abundances of the primary consumers – Lesser Flamingos. The findings from these studies contributed to a further understanding of the functioning of these systems and at the same time opened new avenues of research for instance, the role of viruses in *Arthrospira* population dynamics.

Dissertation Outline and Contributors

This dissertation is a compilation of my research studies which I have presented in three papers;

1). Kaggwa N. M., Gruber M., Oduor S. O. & Schagerl M. 2012. A detailed time series assessment of the diet of Lesser Flamingos: further explanation for their itinerant behaviour. *Hydrobiologia* DOI 10.1007/s10750-012-1105-1

2). Kaggwa N. M., Burian A., Oduor S. O. & Schagerl M. (submitted). Ecomorphological variability of *Arthrospira fusiformis* (Cyanoprokaryota) in African soda lakes. *FEMS Microbiology Ecology*. Submission ID. FEMSEC-13-02-0077

3). Kaggwa N. M., Oduor S. O. & Schagerl M. Factors controlling the abundance of *Arthrospira fusiformis* (prepared for submission to The ISME Journal, Multidisciplinary Journal of Microbial Ecology).

In addition I have also contributed to other publications;

1). Straubinger N., Lawton L., Gruber M., Kaggwa N. M., Oduor S. O., Schagerl M. (submitted). Cyanobacterial toxins in Lakes Nakuru and Bogoria – do they really cause flamingo die-offs? *PLOS ONE*. Submission ID. PONE-S-13-04946

2). Burian A., Schagerl M., Kaggwa N. M., Singer G. and Yasindi A. (in prep.). A story of rapid rise and steep fall: Triggers and Ecological functioning of tropical rotifer blooms in saline lakes.

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Introduction

Arthrospira fusiformis (Voronich.) Komárek and Lund (formerly called *Spirulina plantensis*) is a filamentous cyanobacterium (Fig. 1) that grows extensively in some of the alkaline, saline waters having a high pH of up to 11. *A. fusiformis* often forms thick algal mats (Kebede, 1997). Other than the high pH, these lakes are also characterised by high temperatures, nutrients especially phosphorus and salinity. These growth conditions are unfavourable to most algal species other than a few Cyanobacteria taxa which in most cases tend to grow extensively and reach an almost unialgal population such as *A. fusiformis*.

A. fusiformis is the main food source for huge flocks of the Lesser Flamingos, *Phoenicanaias minor* Geoffroy, that inhabit most of these lakes. It has been estimated that an adult Lesser Flamingo consumes up to about 72 g dry weight (DW) of this cyanobacteria per day, mostly in shallow lake areas (Vareschi, 1978; Owino et al., 2001). Lesser Flamingos constitute the biggest part of the lakes bird population that are a world-famous tourist attraction contributing greatly to the much needed revenue to the countries that are home to the soda lakes. Be that as it may, their presence and abundances have been hampered by the frequent unpredictable episodes of mortalities at these lakes which have been well reported (e. g. Ndeti and Muhandiki, 2005; Krienitz and Kotut, 2010). The causes of these die-offs are yet to be fully understood but have been related to the degradation of their habitats (Krienitz and Kotut, 2010). Other explanations that have been brought forward include: changes in food quantity, composition and/or quality, cyanotoxins and bird diseases (Vareschi, 1978; Sileo et al., 1979; Codd et al., 2003; Krienitz et al., 2003; Ballot et al., 2004; Krienitz and Kotut, 2010).

Due to the uncertainties that still shroud the causes of these mortalities considerable research work over the years has been elicited aimed at unravelling this mystery. Since feeding is fundamental to an organism's survival, the ecology and physiology of *Arthrospira* offers a prime base in the quest for explanations. The main morphological feature of *Arthrospira* is the patterned arrangement of its multicellular cylindrical trichome in an open helix with a cell width ranging from about 3-12 µm together with the helix pitch (10-70 µm) and its diameter from 20-100 µm.

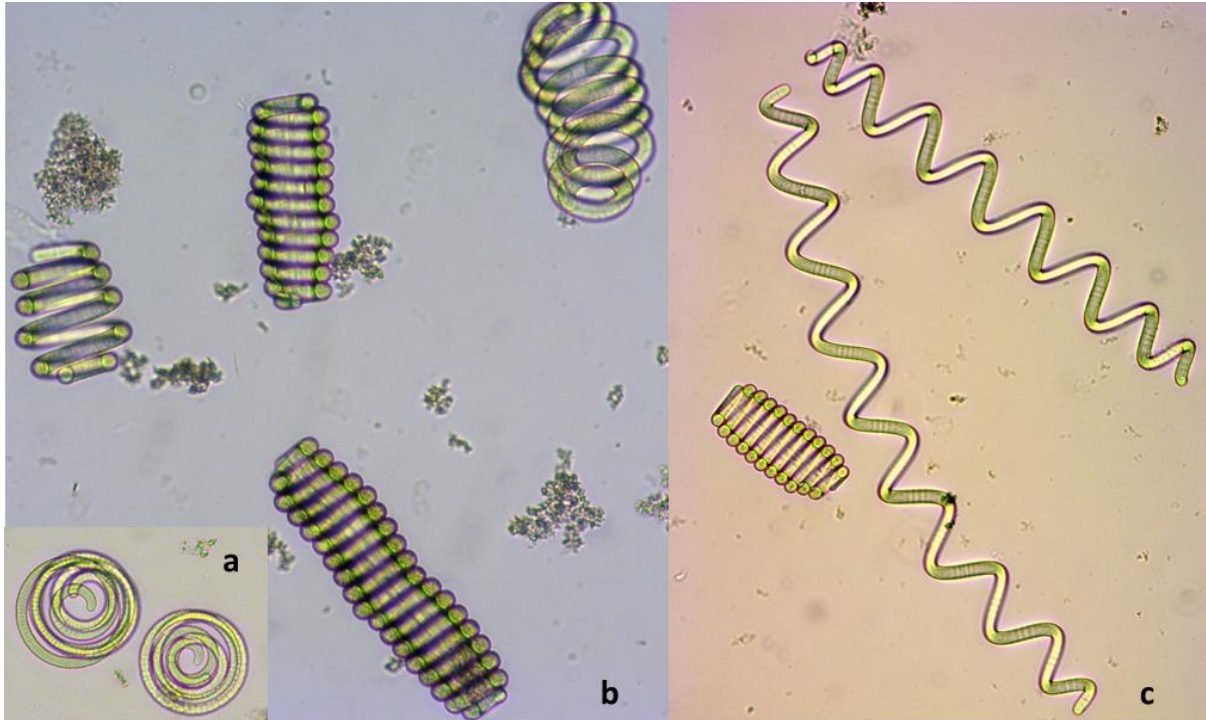


Fig. 1. *Arthrospira fusiformis* morphotypes from Lake Nakuru (a & b) and Lake Bogoria (c)

These two parameters which define the shape of the helix architecture are highly dependent on growth and environmental conditions (Vonshak and Tomaselli 2000) often resulting into different morphotypes (see Fig 1). This advantageous shape and size has been reported to be one of the reasons for its suitability as a food resource for the Lesser Flamingos and therefore a lower preference for the other taxa. A case in point is the observation by Krienitz and Kotut (2010) and Krienitz et al. (2013) that the large and slimy lumps of *Anabaenopsis* (300-2000 μm) and *Cyanospira* (several millimetres) were seen clogging the excluders and lamellae of the Lesser Flamingos hence hindering food uptake. The excluders are at $\sim 800 \mu\text{m}$ distance each, while the lamellae of the bills have $\sim 50 \mu\text{m}$ gaps. This may have led to starvation contributing to the massive die-offs of the birds.

Over the last few decades *Arthrospira* has been observed to collapse irregularly and unpredictably (Vareschi, 1982; Schagerl and Oduor, 2008; Krienitz and Kotut, 2010). These changes have been attributed to *Arthrospira*'s response to the changing environmental stress expressed as fluctuations in physical and chemical variables (Schagerl and Oduor, 2008). During such periods *Arthrospira* is out-competed by members of other genera such as *Anabaenopsis* and *Cyanospira* (Krienitz et al., 2013) which is a clear change from the common persistent occurrence of the *A. fusiformis*. Since Lesser Flamingos are specialised

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consumers of *Arthrospira*, such biomass changes in its food base are a sure threat to the abundance of the birds at these lakes.

To have a deeper understanding of the ecological and physiological milieu of *Arthrospira* it is, imperative for future research work to take on a much more holistic approach so as to obtain answers to the yet unanswered questions. Veritably, the pivotal role played by *Arthrospira* in the functioning of the soda lakes ecosystems resonates in most of the research work done so far. More so, there has been tremendous progress in laboratory and mass culture driven by the increasing knowledge of *Arthrospira* occurrence in nature, but still more has to be done.

Paradigm of research

The goal of this research work was to investigate the ecology and physiology of *Arthrospira fusiformis* so as to contribute to a better understanding of its role in interacting with Lesser Flamingos and other pelagic communities in soda lakes. Since *Arthrospira* plays a pivotal role in the functioning of these dynamic systems, a holistic approach was engaged which involved synthesizing data from climatic, physical, chemical and biological factors and related them to its abundance and behaviour in these systems. To be able to establish this synergy the investigation was carried out in two soda lakes, Nakuru and Bogoria, guided by the following aims:

- *To carry out a detailed time series study of shifts in the pelagic phytoplankton community*
- *To determine the nutritional quality of the pelagic phytoplankton community in terms of crude protein, lipids and carbohydrates over weekly intervals*
- *To investigate the temporal morphological changes of *Arthrospira* for a better understanding of its physiology.*
- *To identify key environmental and biological variables which are responsible for controlling the morphological changes of *Arthrospira**
- *To investigate the factors influencing the occurrence of pelagic algal taxa and the key drivers of *Arthrospira* occurrences.*

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Guiding questions

- *How does the phytoplankton biomass and composition fluctuate on short term basis?*
- *What is the nutritional composition of the pelagic phytoplankton community and how does it vary in the two lakes?*
- *Can changes in the nutritional composition of the pelagic phytoplankton community be related to the Lesser Flamingo deaths that were observed?*
- *How do the different *Arthrospira* morphotypes vary over time?*
- *What are the key factors controlling these morphological changes?*
- *What are the key factors governing the pelagic phytoplankton communities?*
- *Which are the main factors underlying *Arthrospira* biomass changes in soda lakes?*

Validity

Lakes Nakuru and Bogoria are important Ramsar sites in Kenya, famous for their spectacular bird sanctuaries and are a chief source of revenue for the country. In addition to other bird species, they host huge flocks of Lesser Flamingos with numbers sometimes going above 1 million birds between the lakes (Vareschi, 1978). They are recognized as being among the world's most productive ecosystems (Talling et al., 1973; Melack and Kilham, 1974). Talling et al. (1973) attributed the high photosynthetic rates typical of lakes with *Arthrospira* to a combination of high algal standing crops in the euphotic zone and high photosynthetic capacity.

There have been reports of frequent unpredictable episodes of Lesser Flamingo mortalities at both lakes Nakuru and Bogoria (e.g. Vareschi, 1978; Codd et al., 2003; Ballot et al., 2005; Krienitz and Kotut, 2010) which have been attributed to a number of possible reasons, including changes in food quantity and composition related to ingestion mechanisms during feeding (Krienitz and Kotut, 2010). However, no studies so far have been done to investigate the aspect of phytoplankton community nutritional composition and quality as a key factor in influencing the Lesser Flamingo abundance at these lakes and also as a possible explanation for the observed flamingo deaths. Addressing this aspect would contribute to a further understanding to the influence of this food base on the abundance and survival of the flamingos.

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Secondly, hitherto the research work on the phytoplankton community has mostly been on biomass dynamics and primary productivity with data collected over long sampling intervals and/or brief sampling periods. However, though such biomass dynamics are associated with changes in its morphological dimensions, no comprehensive study has so far been done on the morphological variability of *Arthrospira* and the factors that control these changes. Such information is currently unavailable yet it is important for providing knowledge on the physiology of *Arthrospira* in nature.

Thirdly, the recent changes from the largely *Arthrospira* dominated algal community to other algal taxa in the East African soda lakes, there has been a number of inferences put across to try and explain these unusual trends. Nonetheless, data driven explanations that are firmly conclusive and statistically supported are not available until now. There is therefore an urgent need to have a holistic study including climatic, physico-chemical and biological factors to determine the key factors responsible for *Arthrospira* disappearance. Finally, the overall purpose of this research effort would contribute tremendously to knowledge base greatly needed for effective management of these lakes as conservation centres and bird sanctuaries not to mention, a more sustainable source of revenue.

References

- Ballot A., Krienitz L., Kotut K., Wiegand C., Metcalf S.J., Codd A.G. & Pflugmacher S. 2004. Cyanobacteria and cyanobacterial toxins in three alkaline rift valley lakes of Kenya—Lakes Bogoria, Nakuru and Elmenteita. *Journal of Plankton Research* 26:925–935.
- Codd A. C., Metcalf S. J., Morrison F. L., Krienitz L., Ballot A., S. Pflugmacher, Wiegand C. & K. Kotut K. 2003. Susceptibility of flamingos to cyanobacterial toxins via feeding. *The Veterinary Record* 152:722–723.
- Kebede E., 1997. Response of *Spirulina platensis* (= *Arthrospira fusiformis*) from Lake Chitu, Ethiopia, to salinity stress from sodium salts. *Journal of Applied Phycology* 9:551–558.

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Krienitz L. & Kotut K. 2010. Fluctuating algal food populations and the occurrence of Lesser Flamingos (*Phoeniconaias minor*) in three Kenyan Rift Valley lakes. *Journal of Applied Phycology* 46:1088–1096.

Krienitz L., Ballot A., Kotut K., Wiegand C., Pütz S., Metcalf S. J., Codd A. G. & Pflugmacher S. 2003. Contribution of hot spring cyanobacteria to the mysterious deaths of Lesser Flamingos at Lake Bogoria, Kenya. *FEMS Microbiology Ecology* 43:141–148.

Krienitz L., Dadheech K. P. & Kotut K. 2013. Mass developments of the cyanobacteria *Anabaenopsis* and *Cyanospira* (Nostocales) in the soda lakes of Kenya: ecological and systematic implications. *Hydrobiologia* 703:79–93.

Melack M. J. & Kilham P. 1974. Photosynthetic rates of phytoplankton in East African alkaline, saline lakes. *Limnology and Oceanography* 5:743–755.

Ndeti R. & V. S. Muhandiki 2005. Mortalities of lesser flamingos in Kenyan Rift Valley saline lakes and the implications for sustainable management of the lakes. *Lakes & Reservoirs Research and Management* 10:51–58.

Owino A. O., Oyugi O. J., Nasirwa O. O. & Bennun A. L. 2001. Patterns of variation in waterbird numbers on four Rift Valley lakes in Kenya, 1991-1999. *Hydrobiologia* 458:45–53.

Schagerl M. & Oduor O. S. 2008. Phytoplankton community relationship to environmental variables in three Kenyan Rift Valley saline-alkaline lakes. *Marine and Freshwater Research* 59:129–136.

Sileo L., Grootenhuys G. J., Tuite H. C. & Hopcraft D. B. J. 1979. Mycobacteriosis in the Lesser flamingos of Lake Nakuru, Kenya. *Journal of Wildlife Diseases* 15:387–389.

Talling J.F., Wood B. R., Prosser V. M. & Baxter M. R. 1973. The upper limit of photosynthetic productivity by phytoplankton: evidence from Ethiopian soda lakes. *Freshwater Biology* 3:53–76.

Vareschi E. 1978. Ecology of Lake Nakuru (Kenya). I. Abundance and feeding of Lesser Flamingo. *Oecologia* 32:11–35.

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Vareschi E. 1982. Ecology of Lake Nakuru (Kenya). III. Abiotic factors and primary production. *Oecologia* 55:81–101.

Vonshak A. & Tomaselli L. 2000. *Arthrospira (Spirulina)*: Sytematics and Ecophysiology. *The Ecology of Cyanobacteria* (Whitton A. & Potts M., eds), pp. 505–522. Kluwer Academic Publishers, The Netherlands.

Chapter 1

A Detailed Time Series Assessment of the Diet of Lesser Flamingos - Further Explanation for their Itinerant Behaviour



Bogoria, Kenya, 2009. – *‘Oh yes, it can be this spectacular’*

A Detailed Time Series Assessment of the Diet of Lesser Flamingos - Further Explanation for their Itinerant Behaviour

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Mary Nakabungo Kaggwa¹, Martin Gruber¹, Steve Omondi Oduor² and Michael Schagerl¹

1 Department of Limnology, Faculty of Life Sciences, University of Vienna, Althanstrasse 14. 1090, Vienna, Austria.

2 Department of Biological Sciences, Faculty of Science, Egerton University, P.O.Box 536 Egerton, Kenya.

Key words: *Arthrospira*, Lesser Flamingos, migration, nutritional quality, phytoplankton biomass, saline lakes

Abstract

We hypothesised that changes in nutritional composition and quality of the pelagic phytoplankton community influence the occurrence of Lesser Flamingo populations in two Kenyan saline-alkaline lakes, Nakuru and Bogoria. This was achieved by carrying out a detailed time series assessment of the phytoplankton community composition and nutritional components (carbohydrates, crude protein and lipids) from July, 2008 to October, 2009 on a weekly basis for each lake. Lesser Flamingos were estimated visually from the lake shore of the sampling sites. In Lake Nakuru, Lesser Flamingos had a significant positive relationship with lipids and *Arthrospira* biomass but a negative relationship with small cyanoprokaryotes. For Lake Bogoria, no significant differences were observed as the *Arthrospira* was available

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throughout the sampling period though the flamingo numbers still fluctuated. We concluded that the nutritional composition and quality of the phytoplankton community influence the temporal and spatial abundance of Lesser Flamingos although other factors such as the prevailing environmental conditions may take precedence.

Introduction

In recent years, much work has been done to explain fluctuations and shifts in the phytoplankton community composition in the soda lakes of the East African Rift Valley and how they influence the abundances of Lesser Flamingos (*Phoeniconaias minor* Geoffrey) which inhabit these water bodies (Krienitz & Kotut, 2010; Harper et al., 2003, Vareschi, 1978). These saline-alkaline lakes are renowned for their abundant phytoplankton biomass dominated by the cyanobacterium *Arthrospira fusiformis* (Voronichin) Komárek & Lund (formerly called *Spirulina plantensis* Voronichin), which is the main food source for these birds (Owino et al., 2001; Vareschi, 1978). Besides having high pH, these lakes are also characterised by high temperatures, limited light penetration and high conductivity (Oduor & Schagerl, 2007a). These factors create an unfavourable environment for most phytoplankton species other than the few well adapted cyanobacteria taxa. *A. fusiformis*, for example, dominates the phytoplankton biomass in these extreme biotopes over long periods where they almost form a unialgal population.

Lesser Flamingos exhibit unpredictable, spontaneous and sporadic nomadic movements between African alkaline-saline lakes that harbour their preferred food supply. They are able to migrate several hundreds of kilometres each day to access sites with suitable living conditions. It has been estimated that an adult Lesser Flamingo consumes up to about 72 g dry weight of cyanobacteria per day, mostly in shallow lake areas (Owino et al., 2001; Vareschi, 1978). This highly sought after food resource has been observed to undergo significant changes in its biomass over time. Ballot et al. (2004) observed changing dominance between *A. fusiformis* (hereinafter referred to as, *Arthrospira*) and *Anabaenopsis abijatae* Kebede & Willén in some saline lakes, which have been attributed to their response to the changing environmental stress expressed as fluctuations in physical and chemical variables (Schagerl & Oduor, 2008). Since Lesser Flamingos are specialised consumers of *Arthrospira*, such changes in biomass and quality are bound to affect the population of these

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birds in these soda lakes and in some cases, may also contribute to the sporadic mass mortalities observed at times.

Indeed, there have been reports of frequent unpredictable episodes of Lesser Flamingo mortalities in Kenyan Rift Valley lakes. In L. Nakuru, reports indicated sudden mortalities from 1993 to 2003, with deaths going beyond 500 in 1998 (Ndeti & Muhandiki, 2005). Krienitz & Kotut (2010) reported the highest mortality of flamingos (approx. 30,000) in L. Nakuru in 2006. In 2008, the same authors also recorded about 30,000 carcasses of Lesser Flamingos that had accumulated along the shoreline of L. Bogoria. Among the possible causes for the sudden mortalities cited were the changes in food quantity (Sileo et al., 1979; Mlingwa & Baker, 2006) and composition (Ndeti & Muhandiki, 2005; Krienitz & Kotut, 2010). Problems with food composition are mainly associated with by mucilage clogging the filters in the beaks and the production of cyanobacterial toxins by some taxa of cyanobacteria, which are ingested during feeding (Vareschi, 1978; Codd et al., 2003; Krienitz et al., 2003; Krienitz & Kotut, 2010).

Arthrospira is commonly regarded as non-toxic (Jassby, 1988) but some investigations have indicated possible toxicity of some of its strains (Gilroy et al., 2000; Iwasa et al., 2002). A toxic strain producing microcystin-YR and anatoxin-*a* was isolated from L. Sonachi, Kenya (Ballot et al., 2005). Microcystins (cyanobacterial hepatotoxins) and anatoxin-*a* (cyanobacterial neurotoxin) have been found to be present in cyanobacterial mats of the hot springs along the shores of L. Bogoria (Krienitz et al., 2003). These toxins also have been detected in the livers of dead Lesser Flamingos collected from L. Bogoria and L. Nakuru (Codd et al., 2003). The presence of hot spring cyanobacterial cells and cell fragments in stomach contents and faecal pellets show that flamingos ingest toxic cyanobacteria whilst drinking and washing at the hot springs (Krienitz et al., 2003). From these observations, cyanotoxins could be one factor contributing to the sudden deaths of the birds.

On the other hand, variations in nutritional quality, in terms of lipids, carbohydrates and crude protein contents of phytoplankton communities may also be another key factor contributing to the migration of these birds from one lake to the other, in search of phytoplankton with high nutritional value. *Arthrospira* (*Spirulina* spp.) nutrient components have been widely studied (Tokuşogulu & Ünal, 2003; Mühling et al., 2005; Zielińska & Chojnacka, 2009). However, no studies so far have addressed variations in nutritional quality of phytoplankton community in terms of lipids, carbohydrates and crude protein content over

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short-term intervals in the natural environment. Krienitz & Kotut (2010) related flamingo movements to changes in algal food quantity and quality in terms of toxicity on an irregular basis. To our knowledge, a detailed time series assessment of the nutritional quality of the diet for the Lesser Flamingos on a regular basis has not previously been done in African saline lakes.

To address this gap, we carried out a detailed time series study of shifts in the pelagic phytoplankton community and the nutritional quality in terms of the crude protein, lipids and carbohydrates over short time intervals of seven days and observed their effect on Lesser Flamingos in L. Nakuru and L. Bogoria. We hypothesised that change in nutritional composition and quality of the pelagic phytoplankton community influence the occurrence of Lesser Flamingo populations.

Materials and Methods

Study area and sites description

This study was carried out in two Kenyan Rift Valley lakes, L. Nakuru and L. Bogoria (Fig. 1), which are known to host huge flocks of Lesser Flamingos (Vareschi, 1978) with numbers sometimes going above 1 million birds between the lakes. L. Nakuru is a shallow pan situated in Lake Nakuru National Park, next to Nakuru Town. It is mainly recharged by rainfall. It also occasionally receives inflows from three seasonal surface streams, namely Njoro, Makalia and Nderit, which flow from the Mau Forest, and a small spring, the Baharini Spring at the northern end. Municipality sewage is also discharged into the lake. The lake hosts only one fish species (*Oreochromis grahami alcalicus* Linnaeus). The sampling point in L. Nakuru was in the central location at 00° 21.387'S, 036° 05.519'E. This point was chosen based on our preliminary observations which showed that it commonly hosts more Lesser Flamingos compared to the northern and southern parts of this lake. Also, due to the complete daily mixing of the lake, the data for physical and chemical variables collected from this point are representative of the whole lake (Oduor & Schagerl, 2007a).

L. Bogoria lies in a semi-arid region in north-western Kenya. It is fed by few springs from the escarpment on the western side and some small impermanent tributaries that include the Emsoss on the eastern side and Wasagess River discharging from the northern side. The area

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around the lake is still volcanically active. Some boiling springs and fumaroles occur along the lake shore and discharge fresh to moderately alkaline-saline water into the lake. The lake receives erratic and stormy rainfall that erodes the scarcely vegetated areas around it, washing sediments into the lake. No macrophytes grow along its shoreline other than nearby the springs where some salt-tolerant grasses can be found (Harper et al., 2003). The lake does not support any fish life. The sampling point from this lake was at 00° 16.166'N, 036° 05.766'E. Similar to L. Nakuru, this sampling point was chosen as it is in the central area, which was observed to host higher flamingo numbers compared to the northern and southern parts of the lake.

Field sampling

Weekly sampling was carried out in each lake from July 2008 to October 2009. To capture seasonality, rainfall data was collected using HOBO RG3-M data loggers (Onset Computer

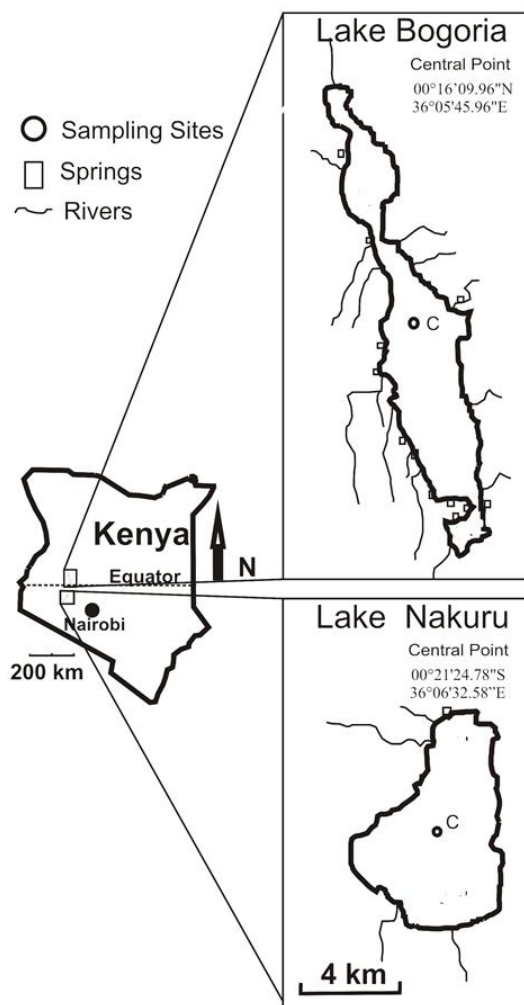


Fig 1. Map showing Kenya and the two studied saline-alkaline lakes, Nakuru and Bogoria

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Corporation, Massachusetts, USA), installed at weather stations located at the shores of both lakes. Salinity was measured at the sampling sites using a multiprobe (WTW Multi 340i Wissenschaftlich Technische Werkstätten Weilheim, Germany).

Flamingo numbers were estimated visually from the lake shore at least one sampling date per month at the sampling sites for both lakes from January to October 2009. The estimates were grouped into four size classes as follows: 1 = $< 10,000$, 2 = $10,000 < 100,000$, 3 = $100,000 \leq 500,000$ and 4 = $> 500,000$.

Phytoplankton community composition and biomass determination

Phytoplankton community composition was determined using lake water samples fixed with formalin. The taxa were identified with the aid of established identification keys (Komárek, 2003; Komárek et al., 2003; Shubert, 2003 and Kociolek & Spaulding, 2003). To determine the biovolume of the various community taxa, the identified cells for each taxa were then enumerated and measured using an inverted microscope (Nikon Diaphot, Nikon, Tokyo) at the 100 $\times$ and 200 $\times$ magnification (for *Arthrospira* and *Anabaenopsis*) and 400 $\times$ for other taxa according to Utermöhl (1958). Their biovolumes were estimated using geometric formulae of the shapes similar to the respective phytoplankton cells (Hillebrand et al., 1999). At least thirty cells for each taxon identified were measured to give the average size and biovolume. For conversion of cell volume into biomass, a conversion factor of 1 was used (Wetzel & Likens, 1991).

Dry and ash mass, carbohydrate, lipid and crude protein determination

Dry mass and ash mass was analysed by filtering a known volume of the raw sample using pre-weighed filters (45 μm pore size; Ederol BM/C, Battenburg, Germany). For dry mass, filters together with residue material were dried at 95 ± 5 °C until a constant weight was achieved. For estimation of the ash mass, dry mass filters were combusted in a muffle furnace for 2 hours at 550 °C. The weight loss on ignition gave the ash-free dry mass (organic content).

A plankton net of 30 μm was used to collect samples so as to harvest algal particles that can go through the excluders of the Lesser Flamingo bill and are retained and secondly to obtain adequate material for nutritional components analysis. These samples were analysed for carbohydrates, crude protein and lipids which were extracted according to the methods described by Schwörbel (1994) with appropriate modifications. Net samples were then filtered through filter papers with mesh size 0.45 μm (Ederol BM/C, Battenburg, Germany)

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and the residue together with the filter ground using a tissue grinder. Where immediate analysis was not possible, the samples were kept frozen at -20°C.

Carbohydrates were determined using the phenol-sulphuric acid complex reaction; controls were prepared in a similar way, using distilled water instead of the net sample, and β -D-glucose was used for preparation of the standards. The vanillin reaction method was used to determine the sample lipid concentration. Controls were prepared in a similar way using distilled water instead of raw material from the samples. A calibration factor of 1805 for algae was used (Schwörbel, 1994). Crude protein was measured following the Biuret method as described by Schwörbel (1994) with controls prepared with distilled water; bovine serum albumin was used for calibration.

Statistics

For group comparisons, flamingo numbers were classified within each lake into four categories (ref. field sampling) and tested with Kruskal-Wallis statistics for significant differences of potential explanatory variables like food quality in terms of lipids, carbohydrates, proteins and occurrence of *Arthrospira* and other algal groups. Calculations were done with the software package SPSS 20.0.

Results

Temporal changes in phytoplankton biomass were observed in both lakes as illustrated in Fig. 2. Biomass fluctuation was greater in L. Nakuru compared to L. Bogoria. The lowest phytoplankton biomass in L. Nakuru was recorded in December 2008 as 6.4 mg L^{-1} while the highest recorded value of 248.4 mg L^{-1} was in June 2009. Over time, the contribution of *Arthrospira* to the total phytoplankton biomass in L. Nakuru was quite variable (Fig. 3). Most of the time, *Arthrospira* contributed over 80% of the total biomass during the wet seasons that occurred between August to November 2008 and April to August 2009, with correspondingly low salinity levels (16–20 ppt) in the lake.

A notable phenomenon in L. Nakuru was the crash in the biomass of *Arthrospira* that occurred from January to April, 2009 and September to October, 2009 (Fig. 3), coinciding

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with high salinity levels (62 ppt) when rainfall was low (Fig. 2). During these dry periods, other phytoplankton taxa, in particular, *A. abijatae*, cryptomonads and other cyanobacteria (*Synechoccus minutus* West, *Synechocystis* sp., *Raphidiopsis* sp.) dominated the biomass of the phytoplankton community in the lake (Fig. 3). Additionally, in October 2009, a bloom of an unidentified filamentous cyanobacterium species (cf. *Pseudanabaena acicularis* (Nygaard) Anagnostidis et Komárek) emerged, forming a thick mat on the lake surface. Green algae (*Ankistrodesmus* sp., *Crucigenia* sp. and *Monoraphidium minutum* (Nägeli) Kormárková-Legnerová), diatoms (*Nitzschia* and *Navicula*) and euglenoids (*Euglena* sp.) contributed 6 % to the phytoplankton biomass.

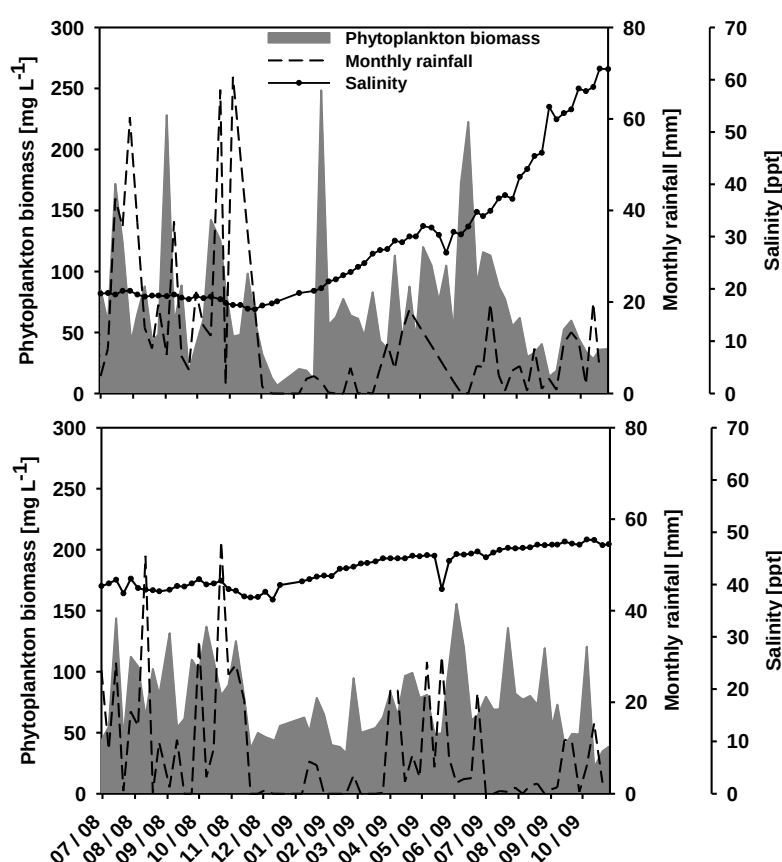


Fig. 2. Temporal trends of phytoplankton biomass, monthly rainfall and salinity (ppt = parts per thousand) in lakes Nakuru (top) and Bogoria (bottom) during the study period.

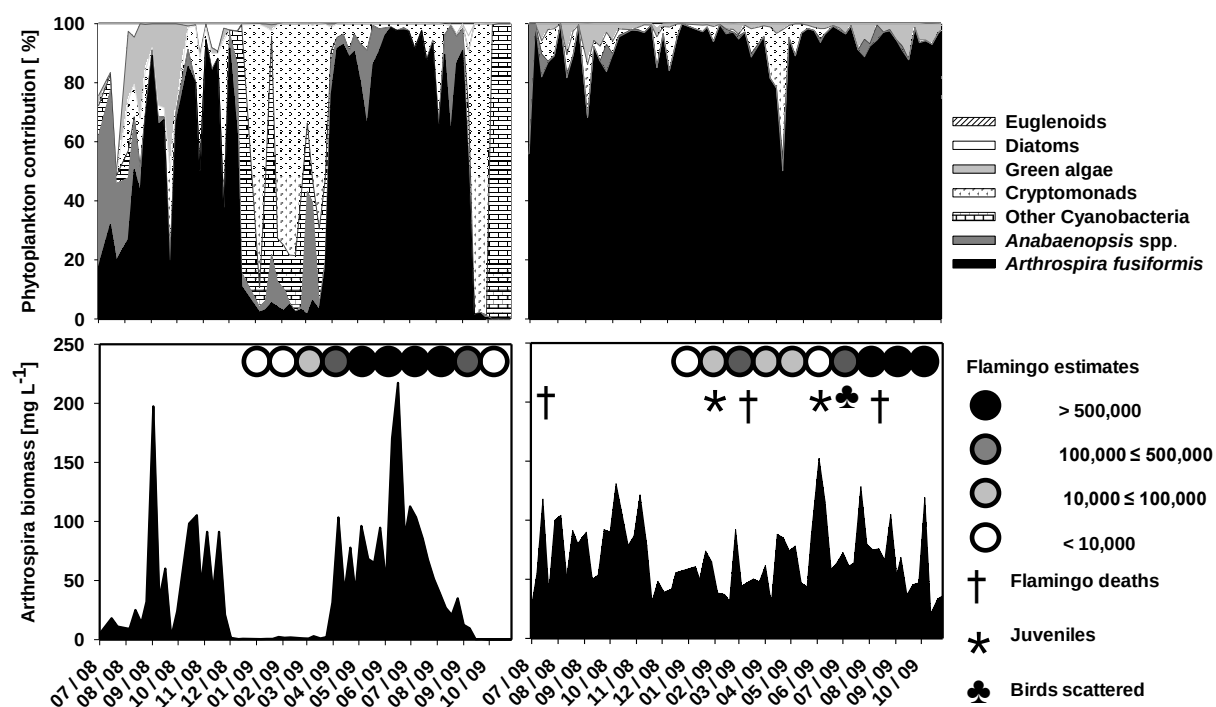


Fig. 3. Temporal changes in the biomass contribution of the main phytoplankton divisions (top), *Arthrospira* biomass and Lesser Flamingo (bottom) estimates in Lakes Nakuru (left side) and Bogoria (right side) during the study period. (Please note that counts are not available from July to December, 2008)

Carbohydrate and lipid composition of the organic matter in L. Nakuru ranged from 2.6–76.3 and 0.6–42.4 %, respectively, whereas crude protein ranged from 18.8–94.5 % (Fig. 4). The lowest carbohydrate and lipid content (2.6 and 0.6 %, respectively) occurred in December 2008 and February 2009, coinciding with the first *Arthrospira* biomass crash ($0.4\text{--}2.9\text{ mg L}^{-1}$). Crude protein composition (Fig. 4) was generally high between November 2008 and March 2009 (70–95%) when the salinity was low (16–28 ppt) but concentrations decreased, reaching 20% from March to October 2009 as salinity levels increased steadily to 62 ppt, which coincided with the period of low rainfall (see Fig. 2). On the other hand, it was observed that there was an increase in carbohydrate and lipid composition, with carbohydrate exceeding crude protein composition in October 2009 (Fig. 4). Dry mass concentrations in Lake Nakuru fluctuated between 0.04 and 1.43 g L^{-1} (Fig 4). The minimum value occurred in January 2009 during the period of the first *Arthrospira* crash while the maximum occurred in October 2009. For the greater part of the sampling period, the ratio of ash mass to ash free dry mass was 1:1 but the pattern changed in September 2009 to 2:1, respectively.

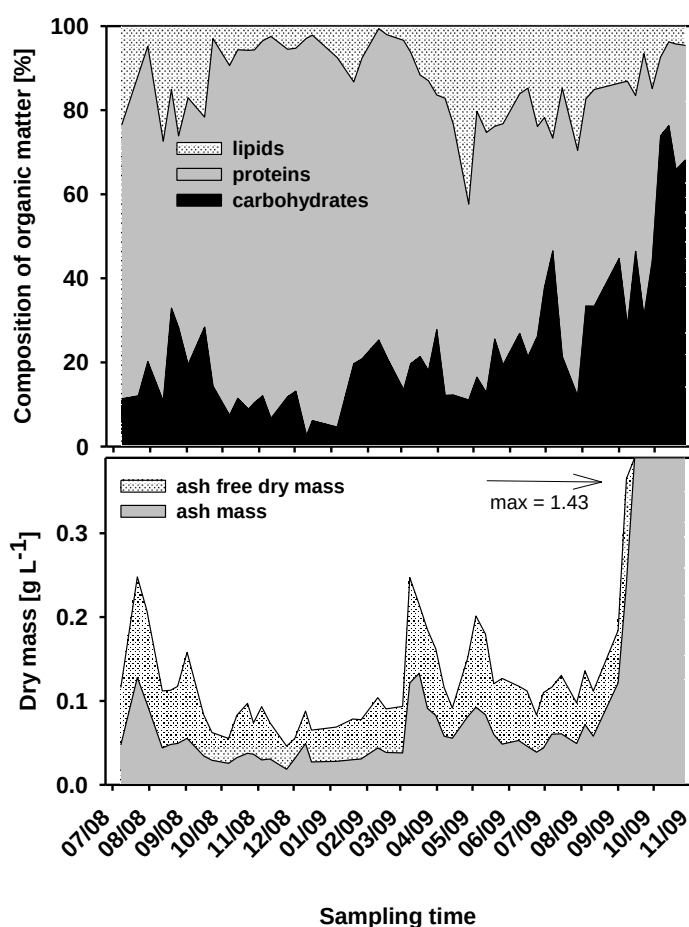


Fig. 4. Temporal trends of food quality composition of organic matter (top) and dry mass (bottom) in Lake Nakuru during the sampling period.

Generally, there was an inverse relationship between Lesser Flamingo populations in L. Nakuru and L. Bogoria for the greater part of sampling period (Fig. 5). Lesser Flamingo numbers in L. Nakuru showed temporal fluctuation that mirrored the changes in *Arthrospira* biomass (see Fig. 3). There were no more than 100,000 flamingos during the period of January to March 2009, coinciding with the first *Arthrospira* biomass crash observed during this study. In April 2009, flamingo numbers started to rise, increasing further from June to August to over 500,000, and matching with the increase in *Arthrospira* biomass during the same period. The numbers dropped again in September and October 2009 to less than 10,000, corresponding to the second *Arthrospira* biomass crash and a very low organic matter content (Fig. 4). During this time, *Arthrospira* was replaced by a mono-specific dominance of a filamentous cyanobacterium (cf. *P. acicularis*). It is also worth noting that no flamingo deaths were observed in L. Nakuru during this study. Kruskal-Wallis test indicated significant

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differences ($p < 0.01$) among the four categories of Lesser Flamingo estimates in relation to lipids, *Arthrospira* biomass and biomass of “other cyanoprokaryotes”, which subsume all cyanopokaryotes except *Arthrospira* and *Anabaenopsis* (Fig. 6).

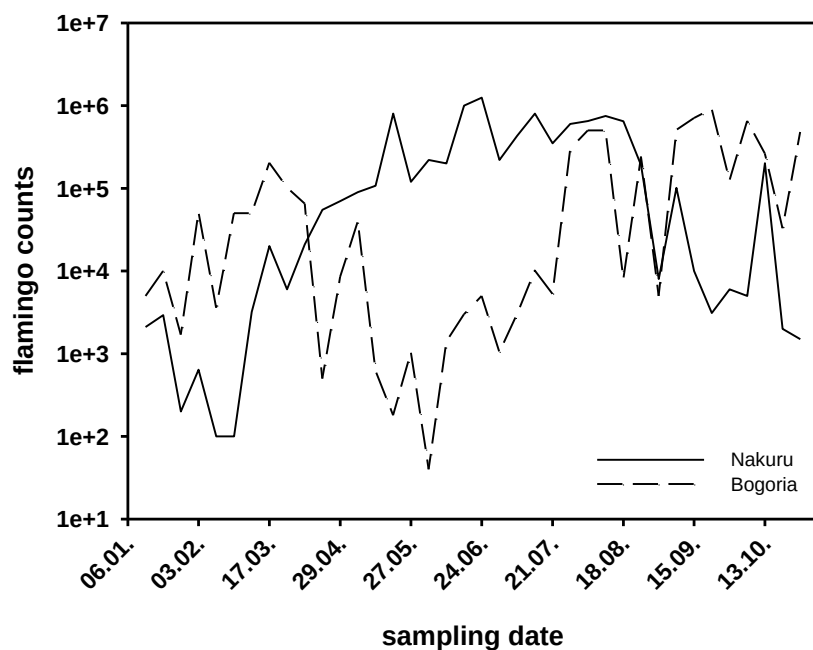


Fig 5. Temporal trends of Lesser Flamingo estimates from January to October, 2009 at Lakes Nakuru and Bogoria

L. Bogoria received a total of 353 mm of rainfall from July to December 2008 with a brief dry period from September to October, 2008. The marked temporal fluctuations in salinity levels and total phytoplankton biomass that were observed in L. Nakuru were not very evident in L. Bogoria even though rainfall was quite variable throughout the study. As reported for L. Nakuru, a distinct dry period was also evident from January to March 2009, followed by increase in rainfall from April to July 2009 and a brief dry period again from August to September 2009 in L. Bogoria. The salinity range in L. Bogoria was narrow, 37–41 ppt from July to December 2008, slightly increasing with the onset of the dry period to 49 ppt towards October 2009 (see Fig. 2).

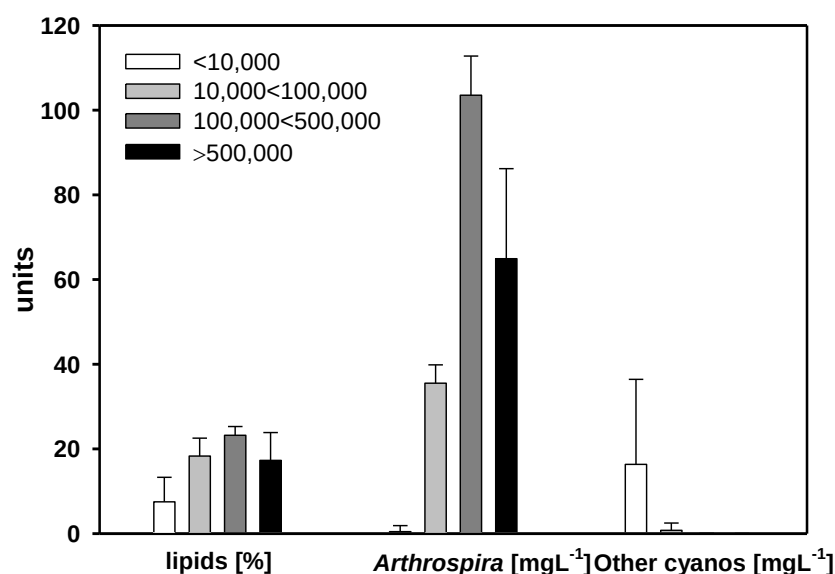


Fig. 6. Significant differences between Lesser Flamingo categories in relation to lipids, *Arthrospira* biomass and other cyanobacteria (*A. abijatae*, *S. minutus*, *Synechocystis* sp., *Raphidiopsis* sp. and *P. acicularis*) for L. Nakuru. (bars = median; deviation = 75% quartiles)

Phytoplankton biomass in L. Bogoria ranged from a minimum of 21.4 mg L⁻¹ in October 2009 to a maximum of 153.5 mg L⁻¹ recorded in June 2009 (see Fig. 2). As in L. Nakuru, *Arthrospira* contributed the largest proportion of biomass to the total phytoplankton biomass in L. Bogoria, averaging 92 ± 1.1 (s.e.) % (see Fig 3). Unlike L. Nakuru, no big variation was observed in the phytoplankton composition in L. Bogoria. *Arthrospira* exhibited a more or less mono-specific dominance in this lake, contributing more than 80% of the total phytoplankton biomass throughout the study. Other phytoplankton taxa that occurred in decreasing order of importance included cryptomonads, green coccal algae like *Picocystis salinarum* Lewin, *M. minutum*) and diatoms (*Nitzschia* sp.), which in total contributed about 8% to the total phytoplankton biomass (see Fig 3).

Carbohydrate content of organic matter in L. Bogoria varied between 8 and 70% whereas lipids ranged from 3 to 37% (Fig. 7). The variations in carbohydrate and lipid content in L. Bogoria did not follow any distinct seasonal pattern nor did they reflect the temporal *Arthrospira* biomass. Generally, crude protein content was higher than carbohydrate or lipid contents with values up to 88%. There was no marked variation in crude protein content even with the changes in the seasonal rainfall. Dry mass concentrations ranged from 0.1 (March

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2009) and 0.4 g L^{-1} (July 2008) throughout the sampling period (Fig. 7). Ash mass and ash-free dry mass concentrations occurred in a ratio of approximately 1:1 for the whole sampling period.

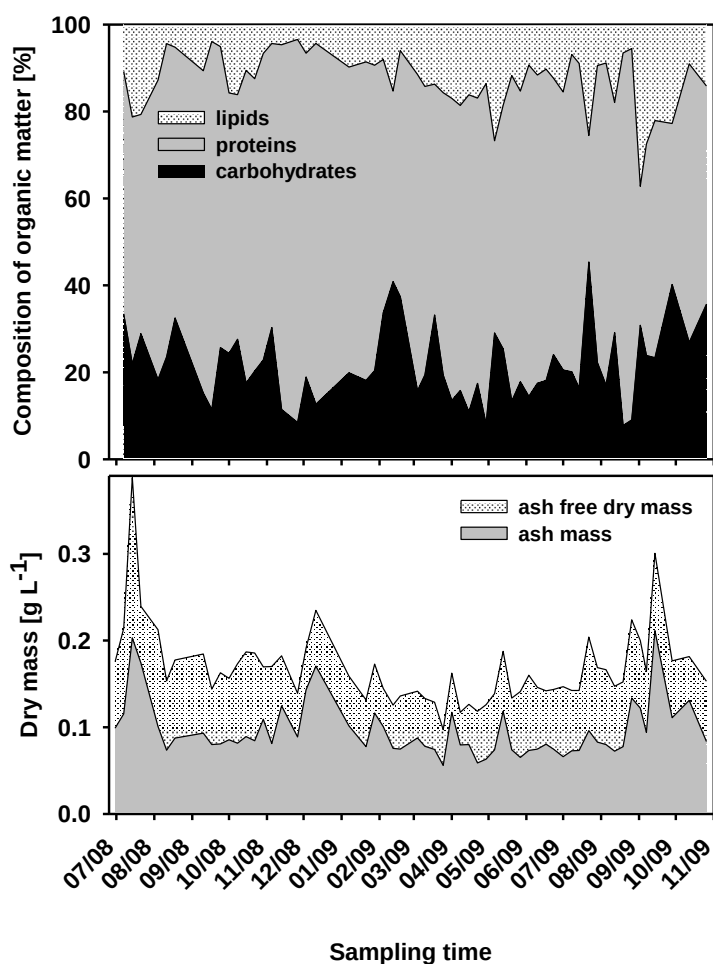


Fig. 7. Temporal trends of food quality composition of organic matter (top) and dry mass (bottom) in Lake Bogoria during the sampling period.

Though no Lesser Flamingo counts were done for the period of July to December 2008, it is worthwhile to mention that in July 2008, 50,000 flamingo carcasses were observed along the shores of L. Bogoria. Compared to L. Nakuru, changes in Lesser Flamingo numbers in L. Bogoria seemed unresponsive to *Arthrospira* biomass in the lake (see Fig. 3). For L. Bogoria there were no observed significant differences among the flamingo categories and the nutritional quality components, *Arthrospira* biomass and other algae.

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There were about 100,000 Lesser Flamingos in L. Bogoria in January and February 2009, with many juveniles observed in February as *Arthrospira* biomass levels remained below 100 mg L⁻¹. The number of Lesser Flamingos increased in March to about 500,000. At the same time, 150 flamingo carcasses were found scattered along the lake shore. From April to July 2009, Lesser Flamingo numbers ranged between 10,000 and 500,000 even though *Arthrospira* biomass increased beyond 100 mg L⁻¹. High numbers of juvenile Lesser Flamingos were observed again in June while in July, it was observed that the birds were scattered all over the lake, with *Arthrospira* biomass remaining relatively high. From August to October 2009, the lake hosted the highest number of Lesser Flamingos, with numbers above 500,000. Once more, about 100 flamingo carcasses were seen in August 2009 along the lake shore.

Discussion

In spite of the high temporal variations observed for phytoplankton biomass in L. Nakuru, with peaks sometimes surpassing those observed for L. Bogoria, a higher and more stable phytoplankton biomass occurred in L. Bogoria compared to L. Nakuru. This was as a result of the physical and chemical stability of L. Bogoria as similarly observed by other researchers (Harper et al., 2003). Alternatively, the high fluctuations of environmental conditions in L. Nakuru hamper stable growth of phytoplankton.

Being the main diet of Lesser Flamingos (Owino et al., 2001), these birds tend to migrate in response to changes in *Arthrospira* biomass in these lakes (Nasirwa, 2000). *Arthrospira* is known to have an exceptionally high protein content of about 65% of its dry mass while total lipids and carbohydrates constitute between 7% and 15%, respectively (Tokuşogulu & Ünal, 2003), which overall results into a relatively low caloric equivalent of 4,360 cal g⁻¹ DM (Vareschi, 1978). This nutritional composition was also reflected in our food analyses for both L. Nakuru and L. Bogoria.

In L. Nakuru, the Lesser Flamingos thrived best during the period of the highest *Arthrospira* biomass, which prevailed during suitable environmental conditions in the rainy season offering more fresh water and a cooler environment. However, with the increase in flamingo numbers the grazing pressure on the *Arthrospira* increased leading to a reduction in biomass

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and in turn a decline in Lesser Flamingos numbers. When *Arthrospira* biomass falls below a certain threshold, Lesser Flamingos are not able to obtain enough food to meet their energy requirements. As a result, they move to other lakes with a more favourable food base or at the worst, they are forced to change diets to the less desirable phytoplankton taxa (Sileo et al., 1979; Tuite, 2000). We opine that this may have been the reason for the flamingo fluctuations between the two lakes as the birds tend to favour the lake with better living conditions at a given time.

If the food supply becomes a limiting factor in L. Nakuru, the flamingos have to find other sources even moving towards the harsh environment of L. Bogoria leading to interlake movements (Chidress et al., 2004; Tuite, 1979). Lakes Nakuru and Bogoria are a part of the key site network consisting of eight alkaline lakes – Logipi, Elmenteita, Natron, Empakayi Crater Lake, Manyara and Eyasi, which are the main home to the Lesser Flamingos (Chidress et al., 2007) in the Eastern Rift Valley.

During the period of *Arthrospira* crash, *Anabaenopsis*, other cyanobacteria and cryptomonads dominated. These phytoplankton taxa are less preferred by the Lesser Flamingos, consequently the presence of very low flamingo numbers that occurred at this time. For instance, the large slimy lumps of *Anabaenopsis* may clog the excluders and lamellae of flamingos hence preventing food uptake, which could lead to starvation and consequently, malnutrition (Krienitz & Kotut, 2010). Moreover, single-celled cyanoprokaryotes, cryptomonads and diatoms are much too small to be held back effectively.

Increased grazing pressure on *Arthrospira*, affected the nutritional quality as well, as was shown by a similar reduction in the amount of lipids. Our results for carbohydrates and crude protein did not show any significant relationship with the Lesser Flamingo categories as expected, possibly due to interference from other food sources since the method of sampling used was non-selective thereby including other organic material. For instance, Vareschi (1978) observed that dense rotifer populations can substantially contribute to the Lesser Flamingo diet in times of very low *Arthrospira* densities.

In L. Bogoria, it is more likely that environmental conditions may have taken precedence over the nutritional composition and quality in regulating the abundance of Lesser Flamingos at this lake. For instance, the extremely hot weather conditions could have been stressful for the flamingos hence the fluctuation in numbers even when there was adequate *Arthrospira* biomass.

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The temporal pattern observed of higher crude protein composition during the wet period in L. Nakuru compared to the dry period was most probably as a result of the changes in salinity levels that occurred at that time. During the wet period, the lowered salinity levels may have been favourable for higher protein production by the phytoplankton and therefore offering a more nutritious diet to the Lesser Flamingos. This was supported by the exceptionally high numbers that prevailed during this period. Vonshak & Tomaselli (2000) have shown that at high salinities, protein levels in *Arthrospira* decrease while carbohydrates increase as an adaptation for efficient osmoregulation. Additionally, from the experiments conducted by Apte & Bhagwat (1989) on two *Anabaena* strains, they observed that exposure to high salinity resulted in inhibition of protein synthesis.

The high flamingo mortalities that were observed in July 2008 at L. Bogoria were comparable to those observed by Krienitz and Kotut (2010) who recorded about 30,000 flamingo carcasses. Although comparably fewer flamingo deaths occurred in March and August 2009, we noted that both mortalities occurred when the highest flamingo numbers were recorded at this lake. *Arthrospira* contributed above 80% of the total phytoplankton biomass in the lake which indicated that the food quantity was sufficient. Given that *Arthrospira* dominance remained unchanged in L. Bogoria and the nutritional quality, as reflected in carbohydrates, lipids and crude protein was also relatively stable, the observed flamingo fluctuations and mortalities at the lake could not be directly associated with low food availability nor could they be due to poor nutritional quality.

One possible food related cause for the observed mortalities could be that the filtration system of the juvenile Lesser Flamingos may have been clogged due to the high phytoplankton biomass resulting into death. Vareschi (1978) observed that Lesser Flamingos avoid areas of aggregated algae, which is a common occurrence in L. Bogoria, possibly because their filter system would become clogged. It is also thought that mortality in juvenile birds is higher than for adults (Vareschi, 1978). On several occasions in L. Bogoria, we observed especially in the afternoons, strong winds blow the phytoplankton towards the shores leading to formation of thick algal mats.

Ingestion of cyanobacterial toxins by the Lesser Flamingos during feeding has also been cited by Codd et al., (2003) and Krienitz et al., (2003) as another possible cause of Lesser Flamingo mortalities in these lakes. For this study, we did not find cyanotoxins cyanobacteria

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(unpublished results) so we can exclude toxins as a key factor for flamingo deaths during our study period.

We agreed with Krienitz & Kotut, 2010, that the problems of phytoplankton food quantity and quality facing the Lesser Flamingos are indeed still quite complex and there is still much more to be discovered. Therefore, we concluded that the nutritional composition and quality of the phytoplankton community influence the temporal and spatial abundance of Lesser Flamingos in these soda lakes although other factors such as the prevailing environmental conditions may take precedence over nutritional composition and quality.

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References

- Apte, K. S. & Bhagwat A., 1989. Salinity-Stress-Induced Proteins in Two Nitrogen-Fixing *Anabaena* Strains Differentially Tolerant to Salt. *Journal of Bacteriology* 171: 909–915.
- Ballot, A., L. Krienitz, K. Kotut, C. Wiegand, J. S. Metcalf, G. A. Codd & S. Pflugmacher, 2004. Cyanobacteria and cyanobacterial toxins in three alkaline rift valley lakes of Kenya - Lakes Bogoria, Nakuru and Elmenteita. *Journal of Plankton Research* 26: 925–935.
- Ballot, A., L. Krienitz, K. Kotut, C. Wiegand & S. Pflugmacher, 2005. Cyanobacteria and cyanobacterial toxins in alkaline crater lakes Sonachi and Simbi, Kenya. *Harmful Algae* 4: 139–150.

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Childress, B., D. Harper, B. Hughes, W. van den Bossche, P. Berthold & U. Querner, 2004. Satellite tracking Lesser Flamingo movements in the Rift Valley, East Africa: pilot study. *Ostrich*, 75: 57–65.

Childress, B., B. Hughes, D. Harper & W. van den Bossche, 2007. East African flyway and key site network of the Lesser Flamingo (*Phoenicopterus minor*) documented through satellite tracking. *Ostrich*, 78: 463–468.

Codd, A. C., J. S. Metcalf, L. F. Morrison, L. Krienitz, A. Ballot, S. Pflugmacher, C. Wiegand & K. Kotut, 2003. Susceptibility of flamingos to cyanobacterial toxins via feeding. *The Veterinary Record*, 152: 722–723.

Gilroy, D. J., K. W. Kauffman, R. A. Hall, X. Huang & F. S. Chu, 2000. Assessing potential health risks from microcystin toxins in blue-green algae dietary supplements. *Environmental Health Perspectives* 108: 435–439.

Harper, D. M., R. B. Childress, M. M. Harper, R. R. Boar, P. Hickley, S. C. Mills, N. Otieno, T. Drane, E. Vareschi, O. Nasirwa, W. E. Mwatha, J. P. E. C. Darlington & X. Escute-Gasulla, 2003. Aquatic biodiversity and saline lakes: Lake Bogoria National Reserve, Kenya. *Hydrobiologia* 500: 259–276.

Hillebrand, H., C. D. Durselen, D. Kirschtel, U. Pollinger & T. Zohary, 1999. Biovolume calculation for pelagic and benthic microalgae. *Journal of Phycology* 35: 403–424.

Iwasa, M., M. Yamamoto, Y. Tanaka, M. Kaito & Y. Adachi, 2002. *Spirulina*-associated hepatotoxicity. *American Journal of Gastroenterology* 97: 3212–3213.

Jassby, A. 1988. *Spirulina*: a model for microalgae as human food. In Lembi, C. A. & J. R. Waaland (eds), *Algae and Human Affairs*. Cambridge University Press, Cambridge, 149–179.

Krienitz, L. & K. Kotut, 2010. Fluctuating algal food populations and the occurrence of Lesser flamingos (*Phoeniconaias minor*) in three Kenyan Rift Valley lakes. *Journal of Phycology* 46: 1088–1096.

Chapter 1

Krienitz, L., A. Ballot, K. Kotut, C. Wiegand, S. Putz, J. S. Metcalf, G. A. Codd & S. Pflugmacher, 2003. Contribution of hot spring cyanobacteria to the mysterious deaths of Lesser Flamingos at Lake Bogoria, Kenya. *Fems Microbiology Ecology* 43: 141–148.

Kociolek P. J. & S. A. Spaulding, 2003. Symmetrical Naviculoid Diatoms. In Wehr, D. J. & R. G. Sheath (eds), *Freshwater Algae of North America, Ecology and Classification*. Academic Press, Massachusetts: 637–639.

Komárek, J., 2003. Coccoid and Colonial Cyanobacteria. In Wehr, D. J. & R. G. Sheath (eds), *Freshwater Algae of North America, Ecology and Classification*. Academic Press, Massachusetts: 59–110.

Komárek, J., H. Kling & J. Komárková, 2003. Filamentous Cyanobacteria. In Wehr, D. J. & R. G. Sheath (eds), *Freshwater Algae of North America, Ecology and Classification*. Academic Press, Massachusetts: 117–191.

Mlingwa, C. & N. Baker, 2006. Lesser Flamingo *Phoenicopterus minor* counts in Tanzanian soda lakes: implications for conservation. In Boere, G.C., C.A. Galbraith & D.A. Stroud (eds.), *Waterbirds around the world*. The Stationery Office, Edinburgh, UK. 230–233.

Mühling, M., A. Belay & B. A. Whitton, 2005. Variation in fatty acid composition of *Arthrospira* (Spirulina) strains. *Journal of Applied Phycology* 17: 137–146.

Nasirwa, O., 2000. Conservation status of Flamingos in Kenya. *Waterbirds* 23: 47–51.

Ndeti, R. & V. S. Muhandiki, 2005. Mortalities of lesser flamingos in Kenyan Rift Valley saline lakes and the implications for sustainable management of the lakes. *Lakes & Reservoirs Research and Management* 10: 51–58.

Oduor, S. O. & M. Schagerl, 2007a. Phytoplankton primary productivity characteristics in response to photosynthetically active radiation in three Kenyan Rift Valley alkaline-saline lakes. *Journal of Plankton Research* 29: 1041–1050.

Owino, A. O., J. O. Oyugi, O. O. Nasirwa & L. A. Bennun, 2001. Patterns of variation in waterbird numbers on four Rift Valley lakes in Kenya, 1991-1999. *Hydrobiologia* 458: 45–53.

Chapter 1

Schagerl, M. & S. O. Oduor, 2008. Phytoplankton community relationship to environmental variables in three Kenyan Rift Valley saline-alkaline lakes. *Marine and Freshwater Research* 59: 125–136.

Shubert, E., 2003. Nonmotile coccoid and Colonial Green Algae. In Wehr, D. J. & R. G. Sheath (eds), *Freshwater Algae of North America, Ecology and Classification*. Academic Press, Massachusetts: 253–308.

Schwörbel, J. 1994. *Methoden der Hydrobiologie*. 4 Edn. – Gustav Fischer Verlag, Stuttgart, Jena: 81, 238.

Sileo, L., J. G. Grootenhuis, C. H. Tuite & J. B. D. Hopcraft, 1979. Mycobacteriosis in the Lesser flamingos of Lake Nakuru, Kenya. *Journal of Wildlife Diseases* 15: 387–389.

Tokuşoglu, Ö & M. K. Ünal, 2003. Biomass nutrient profiles of Microalgae: *Spirulina platensis*, *Chlorella vulgaris* and *Isochrysis galbana*. *Food Chemistry and Toxicology* 68:4.

Tuite, C. H., 1979. Population size, distribution and biomass density of the Lesser Flamingo in the Eastern Rift Valley, 1974–76. *Journal of Applied Ecology* 16: 765–775.

Tuite, C. H., 2000. The distribution and density of Lesser Flamingos in East Africa in relation to food availability and productivity. *Waterbirds* 23: 52–63.

Utermöhl, H., 1958. Zur Vervollkommnung der quantitativen Phytoplankton-Methodik. *Mitteilungen der Internationalen Vereinigung für Theoretische und Angewandte Limnologie* 9: 1-38.

Vareschi, E., 1978. Ecology of Lake Nakuru (Kenya). I. Abundance and Feeding of Lesser flamingo. *Oecologia* 32: 11–35.

Vareschi, E., 1982. Ecology of Lake Nakuru (Kenya). III. Abiotic Factors and Primary Production. *Oecologia* 55: 81–101.

Vonshak A. & L. Tomaselli, 2000. *Arthrospira (Spirulina)*: Systematics and Ecophysiology. In Whitton A. B. & M. Potts (eds), *The Ecology of Cyanobacteria. Their Diversity in Time and Space*. Kluwer Academic Publishers, The Netherlands: 505–522.

Chapter 1

Wetzel, R. G. & G. E. Likens, 1991. *Limnological Analyses*. 2 edn. Springer Verlag, New York.

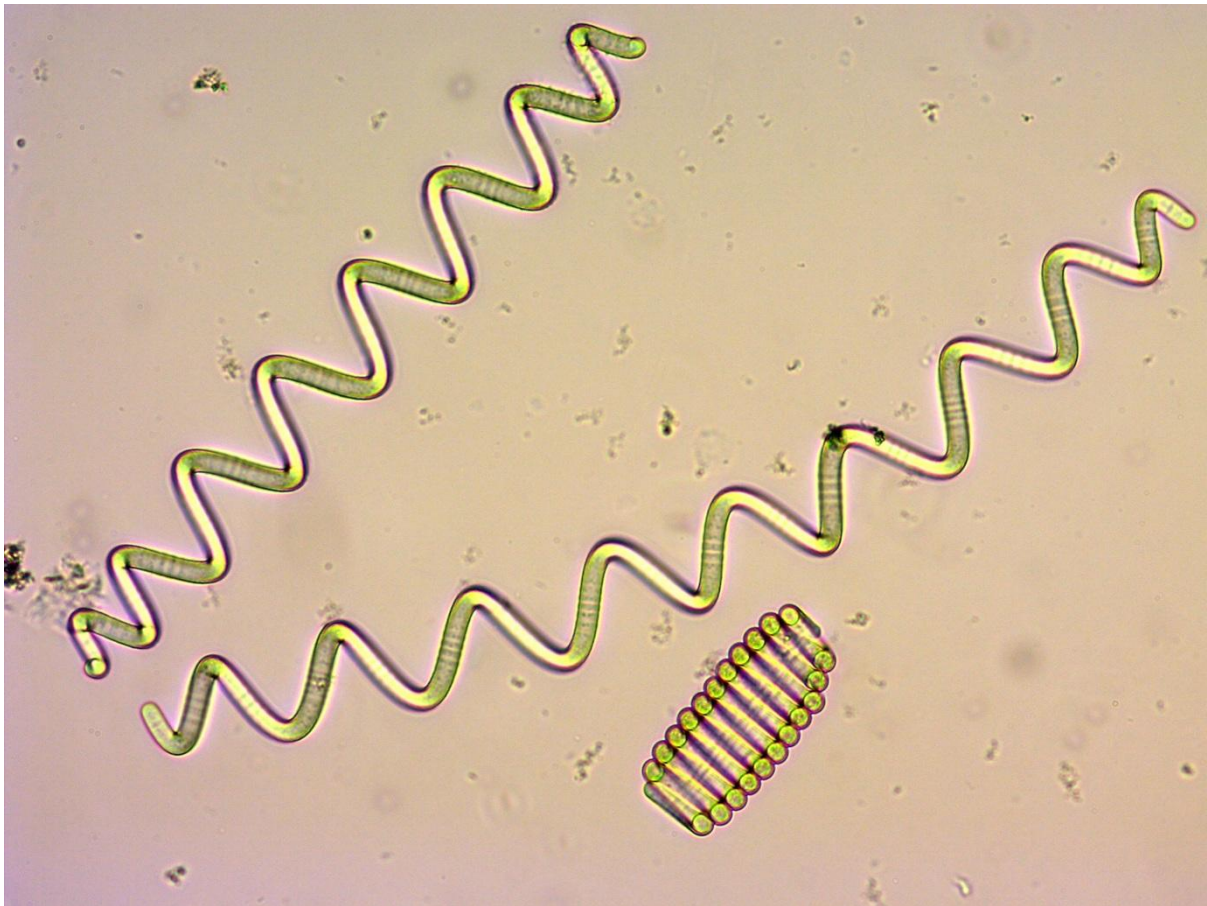
Zielińska, A. & K. Chojnacka, 2009. The comparison of biosorption of nutritionally significant minerals in single- and multi-mineral systems by the edible microalga *Spirulina* sp. *Journal of the Science of Food and Agriculture* 89: 2292–2301.



Bogoria, 2009. - '*Flamingos are normally shy birds*'

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Ecomorphological variability of *Arthrospira fusiformis* (Cyanoprokaryota) in African soda lakes



Arthrospira fusiformis morphotypes, Lake Bogoria (mag = 10×). - ‘*Arthrospira* fashions?!’

Ecomorphological variability of *Arthrospira fusiformis* (Cyanoprokaryota) in African soda lakes

Submitted: FEMS Microbiology Ecology

Mary Nakabungo Kaggwa¹, Alfred Burian², Steve Omondi Oduor³ and Michael Schagerl¹

1 Department of Limnology, Faculty of Life Sciences, University of Vienna, Althanstrasse 14. 1090, Vienna, Austria.

2 Department of System Ecology, Stockholm University, SE-106 91 Stockholm, Sweden.

3 Department of Biological Sciences, Faculty of Science, Egerton University, P.O.Box 536 Egerton, Kenya.

Key words: cell, environment, filament, phytoplankton morphology, saline lakes, *Spirulina*.

Abstract

The filamentous spirally-coiled cyanoprocaryote *Arthrospira fusiformis* is found in extremely high densities in tropical soda lakes acting as driving force of the food-web. We studied pronounced temporal morphological changes of *Arthrospira* in Kenyan soda lakes and identified underlying key factors. Cell (diameter and height) and filament (height of coil, coil diameter and number) dimensions were measured from weekly samples collected over a period of sixteen months. In both lakes, medium-sized cells and large, widely-coiled

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filaments prevailed most. Percentage of large, widely-coiled filaments was promoted by elevated levels of SRP, wind speed, temperature and conductivity and the opposite for small filaments. Large, narrow-coiled filaments were associated with an increase in mainly *Arthrospira*-grazing zooplankton and cyanophage infections, widely-coiled spirals were promoted by increased turbulences. Based on fluorescence measurements, we found widely-coiled filaments representing high vitality. From this study we were able to demonstrate for the first time morphological patterns of *Arthrospira* in nature. Moreover, from our findings we opine that *Arthrospira* morphology is suitable for indicating the biological stability in soda lakes as its shape shifts are highly conspicuous – this outcome might be also of interest for commercial ‘*Spirulina*’ farms.

Introduction

Effective resource exploitation under variable environmental conditions is one of the most important causes of intra- and interspecific morphological diversity in phytoplankton (Naselli-Flores & Barone, 2000; Naselli-Flores et al., 2007). Available literature on morphological variability of phytoplankton indicates that environmental and biological constraints such as nutrients, light availability and grazing pressure from herbivores influence phytoplankton morphology (Kagami & Urabe, 2001; O’Farrell et al., 2007). It follows therefore that morphological traits adapted by phytoplankton are a reflection of changes and regularities of seasonal and/or environmental patterns. They not only show regular periodicities in weather patterns, but also reflect effects of perturbations or other disturbances in ecosystem (Naselli-Flores et al., 2007).

Arthrospira fusiformis (Voronichin) Komárek and Lund is a filamentous cyanobacterium that forms almost unialgal blooms in soda lakes of the East African Rift Valley. These lakes are characterised by high levels of carbonate and bicarbonate contents and a pH of up to 11 (Vonshak, 1997; Oduor & Schagerl, 2007a). *A. fusiformis* is the main food source of the Lesser Flamingos, *Pheoniconaias minor* Geoffrey (Vareschi & Vareschi, 1984) linking *Arthrospira* abundance directly to the high number of these birds in African saline-alkaline lakes (Krienitz & Kotut, 2010; Kaggwa et al., 2012). Lesser Flamingos are a big tourist attraction in Lakes Nakuru and Bogoria in Kenya, which has economic importance for local

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people (Harper et al., 2003; Schagerl & Oduor, 2008; Krienitz & Kotut, 2010). At times, the dominance of *A. fusiformis* suddenly crashes and the lake shifts towards an unstable pelagic community of different organisms, which cause high degrees of food insecurity for top-level consumers like fish and flamingos. *A. fusiformis* is also sold as “*Spirulina platensis*” dietary supplement because of its high content of essential fatty acids, vitamins, proteins and minerals (Jassby, 1988; Tokuşogulu & Ünal, 2003; Mühling et al., 2003; Zielińska & Chojnacka, 2009). Pharmacists and nutritionists have additionally discovered antioxidant functions (Capelli & Cysewski, 2010) and anti-inflammatory effects (Rasool et al., 2006), further enhancing its economic importance.

A. fusiformis strains have been observed to occur in a varied range of saline habitats which shows its ability to adapt to freshwater-alkaline conditions as well as saline-alkaline and even hypersaline environments (Dadheech et al., 2010). In both natural and culture conditions, it shows high morphological variability (Mühling et al., 2003; Ballot et al., 2004; Wang & Zhao, 2005). The main morphological feature of *A. fusiformis* is the patterned arrangement of its multicellular cylindrical trichome in an open helix. Trichomes are composed of cylindrical cells that undergo binary fission in a single plane, perpendicular to the main axis. Cell width ranges from about 3-12 µm, though occasionally it may reach up to 16 µm. The helix pitch typically ranges from 10-70 µm and its diameter from 20-100 µm. These two parameters which define the shape of the helix architecture are highly dependent on growth and environmental conditions (Vonshak & Tomaselli, 2000).

Under laboratory conditions, Kebede (1997) detected differences in the length of its trichomes and degree of helicity when cultured at varying salinity levels expressing the physiological stress to which the cells were subjected to. The author observed that long trichomes occurred at the lowest salinity level (13 g L⁻¹) while very short but closely coiled trichomes were dominating in Cl⁻ rich and highly saline media (55–68 g L⁻¹). Additionally, very loose helices were distinctive for cultures grown in SO₄²⁻ rich media. The helix feature in *A. fusiformis* shows high variability (Mühling et al., 2003; Wang & Zhao, 2005) which probably is determined at the genetic level and induced by various environmental factors, hence the concept of ‘plasticity genes’ (Schlichting & Pigliucci, 1993). This refers to the regulatory loci that directly respond to a specific environmental stimulus by triggering a specific series of morphogenic changes (Pigliucci, 1996).

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In the shallow African saline-alkaline lakes, it has already been observed that there are large temporal fluctuations in *A. fusiformis* biomass (Oduor & Schagerl, 2007a; Schagerl & Oduor, 2008; Krienitz & Kotut, 2010). Even though such shifts in *A. fusiformis* biomass may go along with morphological changes, no comprehensive field study has been done on the morphological variability of *A. fusiformis* so far. In this study, we sought to address this gap by assessing the temporal morphological changes of *A. fusiformis* and identifying key environmental and biological variables that were responsible for these changes. Such shifts in morphology of the dominant primary producer probably have significant impacts on the food-web structure, as grazing might be promoted or hindered by certain morphological features. Additionally, the study allowed evaluating the potential of *A. fusiformis* morphology as a reliable indicator of the biological stability in soda lakes.

Materials and Methods

Study site

This study was carried out in the two Kenyan Rift Valley lakes Nakuru and Bogoria (Fig. 1), which are known to host huge flocks of Lesser Flamingos with numbers sometimes rising over 2 million birds (Vareschi, 1978), which is equivalent to 75% of its world population.

The sampling point in L. Nakuru is located in the central part at 00° 21.387'S, 036° 05.519'E which is representative of the whole ecosystem due to its polymictic character and the small size of the lake (Oduor & Schagerl, 2007b). In L. Bogoria, the sampling point is located at 00° 16.166'N, 036° 05.766'E in the central region of the lake and is one of the deeper parts of the whole lake; it gives a good representation of the physical and chemical conditions when compared to the shallower northern and southern parts of the lake.

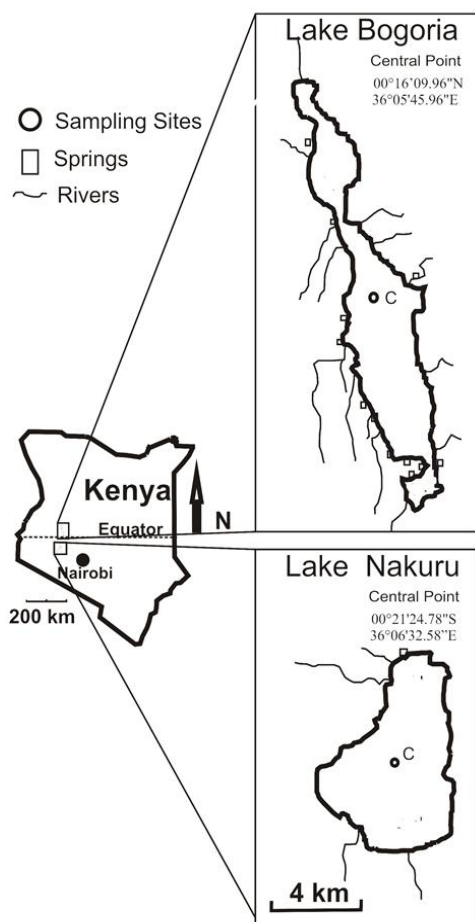


Fig. 1. Map showing Kenya and the two studied saline-alkaline lakes, Nakuru and Bogoria

Sample collection

Weather stations with complete data loggers of the HOBO RG3-M were installed at the shores of both lakes (Onset Computer Corporation, USA). Data were logged for wind speed, solar radiation, air temperature and precipitation. All limnological parameters were sampled weekly from July 2008 to October 2009 ($n = 130$ sampling occasions). Measurements of environmental *in-situ* parameters included light attenuation (PAR, Skye instruments, United Kingdom), pH, electrical conductivity and water temperature (multi-probe WTW Multi 340i, Wissenschaftlich Technische Werkstätten Weilheim, Germany).

Nutrient analysis

Filtered lake water was analysed for $\text{NO}_3\text{-N}$ and SRP which were both determined according to modified standard procedures (American Public Health Association, 1995) to cater for the high buffering capacity of the alkaline water.

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Cyanophages.

Samples collected with a plankton net (30 µm mesh size) were fixed with glutaraldehyde to a final concentration of 2%. *A. fusiformis* filaments were prepared for transmission electron microscopy (Peduzzi et al., unpublished) to identify infected cells.

Zooplankton

Surface water samples were taken with a 10 L Schindler sampler. Rotifers were concentrated with a 50 µm sieve, fixed with formalin (5% final concentration) and counted following the Utermöhl (1958) protocol. Presence of crustacean zooplankton was checked regularly with a plankton net (200µm), but densities were constantly below 0.1 ind. L⁻¹ and quantitatively not important. For ciliates, 250 mL of lake water was fixed with Bouin's solution (5%), stained using the Quantitative Protargol Staining Technique (QPS) by Montagnes & Lynn (1993) and counted with a compound microscope (1000x). For this study we used only the biomass of *A. fusiformis* ingesting taxa based on feeding experiments with dominant rotifers and ciliates of African soda lakes (Burian et al., 2012).

Phytoplankton

Quantitative lake water samples for microscopic examination, identification and measurement of phytoplankton cells were taken 5 cm below the water surface at the sampling locations and the samples fixed with 5 % formaldehyde. The taxa were identified with the aid of established identification keys (Komárek, 2003; Komárek et al., 2003; Kociolek & Spaulding, 2003; Shubert, 2003). To estimate the biovolume of the various community taxa, cells of each taxon were enumerated and dimensions measured using an inverted microscope (Nikon Diaphot, Nikon, Tokyo) at the 100× and 200× magnification for *Anabaenopsis* and 400× for other taxa according to Utermöhl (1958; details for *A. fusiformis* see below). Biovolumes were estimated using geometric formulae of the shapes similar to the respective phytoplankton cells (Hillebrand et al., 1999). At least thirty cells for each identified taxon were measured to give the average size and biovolume. For conversion of cell volume (mm³) into biomass (mg), a conversion factor of 1 was used (Wetzel & Likens, 1991).

Morphological measurements of *A. fusiformis*

Cell diameter and cell height were measured from the same cell at a high resolution (1000×) using a Zeiss AXIO Imager M1 (Göttingen, Germany) microscope. Filament dimensions which included height of coil, coil diameter and number of coils were measured from the same filament using an inverted microscope (Nikon Diaphot, Nikon, Tokyo) at 400× magnification (Fig. 2). We defined a surrogate parameter, the pitch of a filament, which is an indication of how tightly a filament is coiled. The pitch was calculated by dividing the height

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of a coil (μm) by the number of full turns. Each morphological variable was measured on 50 filaments per sample, which resulted in over 6000 measurements for each variable.

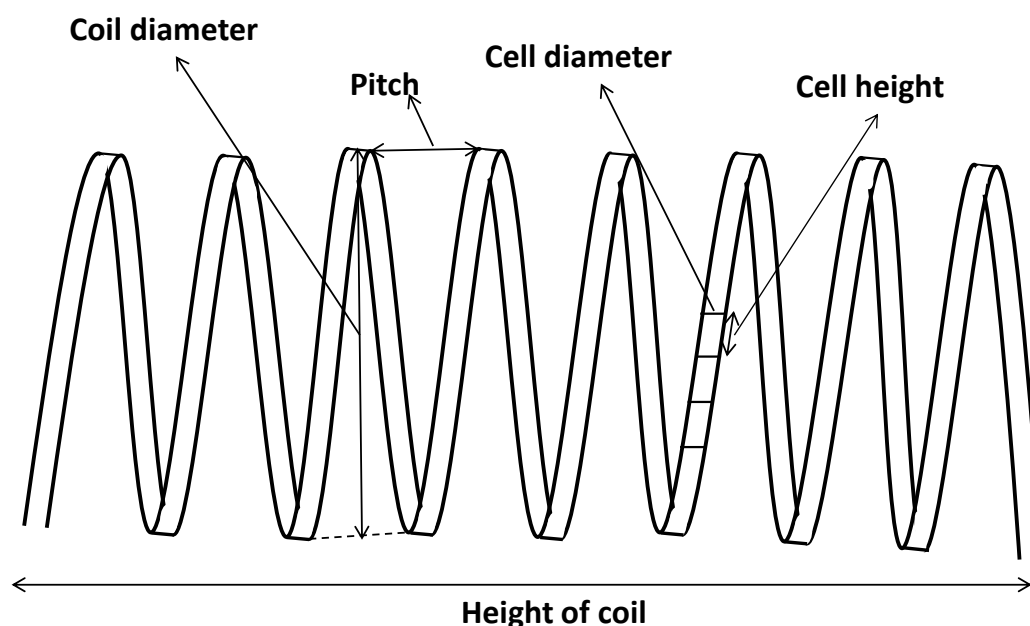


Fig. 2. Illustration of cell and filament morphological dimensions of *Arthrospira*. Pitch = height of coil divided by number of full turns

Pulse amplified modulation (PAM) measurements

PAM fluorescence was used to estimate the overall photosynthetic performance of the phytoplankton community between June 2008 and May 2009. A raw sample was filtered (Whatman GF/C) at the lake shore under dim light and the filter clamped in a leaf clip. The clip together with the filter was then placed on a wet sponge in a black petri dish for 10 min in order to achieve dark-acclimation (full relaxation of the photosystems). With a PAM fluorometer (FMS2, Hansatech, Great Britain), the initial (minimum) fluorescence (F_0) was measured and after application of a saturating light impulse, the maximal fluorescence (F_m) was estimated (intensity of saturation light pulse 85 relative units; duration of light pulse 0.7s). The so-called dark fluorescence yield F_v/F_m or maximal operation efficiency of PSII was calculated as follows: variable fluorescence (F_v) was obtained by subtracting F_0 from F_m . F_v/F_m is a parameter that provides information about the physiological state of the photosynthetic organism (Baker, 2008).

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Statistical analysis

Data of both lakes were pooled for groupings and ordinations. 3 groups of cell sizes (small: <4; medium: 4 – 6; large: >6 μm in diameter) were defined after examination of the size distribution of the cell diameter (Fig. 3). For pruning filament groups, a cluster analysis was run with SPSS 16.0 software: Standardized data for coil diameter (μm), number of coils per filament and pitch (μm) were used as variables defining dissimilarity by applying Ward's method. Three filament groups were obtained (small, large + wide pitch = largeW, large + narrow pitch = largeN filaments). To test for homogeneity within the morphology groups, a multi-response permutation procedure (mrpp) was performed with PC-ORD 5.33 software; applied distance measure was Sorensen (Bray-Curtis). Spearman's rank-order correlation was done to assess the relationship between the cell / filament morphology groups / F_v/F_m and *A. fusiformis* biomass. Unconstrained ordination was used to explore the placements of cell and filament group distributions along synthetic axes (software package CANOCO version 4.5; Microcomputer Power, New York). Standard deviation of the gradients was < 2.5, therefore the linear method of principal component analysis (PCA) was applied (Lepš & Šmilauer, 2005). Samples were centred; supplementary environmental and biological variables were standardized and projected post hoc into the plots to assist interpretation of the group orientation. Variance inflation factors (VIF) for each variable were checked and variables with a VIF above 5 were excluded to minimize the problem of multi-collinearity.

Results

Phytoplankton biomass

The main phytoplankton groups are presented in Fig. 3. Most abundant were Cyanobacteria including *A. fusiformis*, *Anabaenopsis* spp. and of lower biomass were *Synechococcus minutus* West, *Synechocystis* sp., *Raphidiopsis* sp. and *Haloleptolyngbya alcalis* Dadheech, Mahmoud, Kotut et Krienitz. Other groups comprised of cryptomonads, green algae (*Ankistrodesmus* sp., *Crucigenia* sp. and *Monoraphidium minutum* (Nägeli) Kormárková-Legnerová) and diatoms (*Nitzschia* sp. and *Navicula* sp.).

A. fusiformis contributed mostly to the overall biomass, though its abundance was highly variable in L. Nakuru compared to L. Bogoria (Fig. 3). In L. Nakuru, *Arthrospira* peaks were

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recorded from September to November, 2008 and from April to July 2009. *A. fusiformis* biomass crashed twice during the study period, between December 2008 to March 2009 and also from September to October 2009. For L. Bogoria, *A. fusiformis* dominated throughout the whole sampling period. There were no breakdowns observed though there was a drop in biomass coinciding with the *A. fusiformis* biomass crash in L. Nakuru (Fig. 3).

Cell dimensions

Cell size categories were based on diameter changes as illustrated in Fig. 4. This pattern was replicated with the cell biovolume; also for cell height a trend was noted although considerable scattering was observed. Mrpp revealed that the groups were homogenous and well separated from each other (chance-corrected within-group agreement $A = 0.46$; $p < 0.001$). In both lakes Nakuru and Bogoria, cell diameter ranged from 2 to 9 μm with much more temporal variation in L. Nakuru compared to L. Bogoria (Fig. 3). In L. Nakuru, increased number of small cells was recognized from July to October 2008 and February to May 2009. Most of the cells in this lake were medium sized category ($68.1 \pm 1.7 \%$, s.e.), followed by small cells ($30 \pm 2 \%$, s.e.) and a few large cells ($4 \pm 0.7 \%$, s.e.). Spearman's rank-order correlation indicated that there was no significant correlation between the small ($p = 0.65$), medium ($p = 0.58$) as well as large ($p = 0.09$) cells and *A. fusiformis* biomass.

In L. Bogoria, a different scenario was depicted with no distinct changes in cell size groups observed over time. The dominant group was however the medium cells ($74.6 \pm 0.9 \%$, s.e.) followed by the large cells ($15.6 \pm 1.2 \%$, s.e.) and fewer small cells ($9.8 \pm 0.8 \%$, s.e.). There was also no significant correlation observed between the small ($p = 0.96$), medium ($p = 0.93$) as well as large ($p = 0.634$) cells and *A. fusiformis* biomass.

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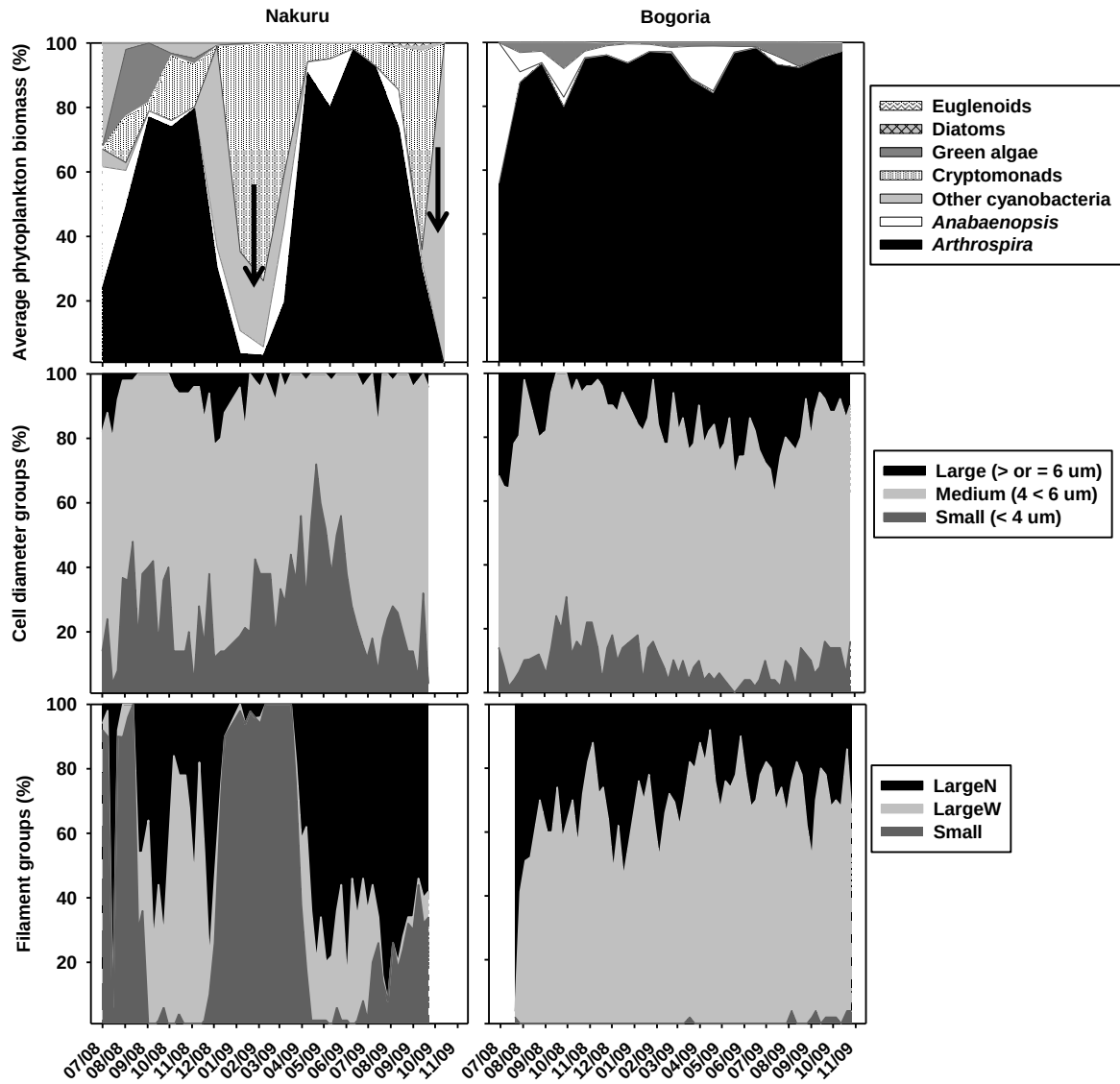


Fig. 3. Temporal trends of average biomass of phytoplankton (top), cell diameter (middle) and filament (bottom) groups in L. Nakuru and L. Bogoria (arrow = period of *Arthrospira* biomass crash)

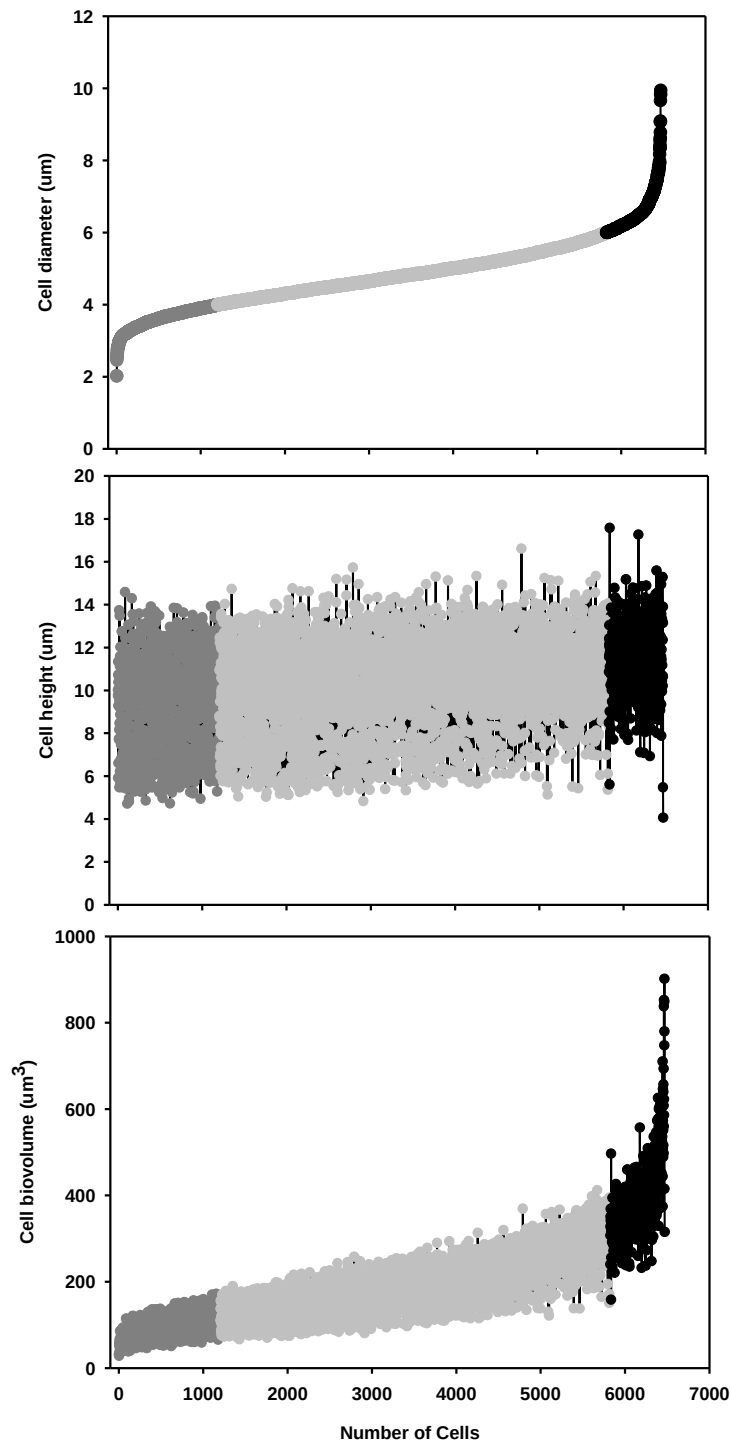


Fig. 4. An overview of the different cell diameter size classes and it's relation with cell height and cell biovolume derived from combined data of both lakes (total $n = 6469$)

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The PCA-model revealed that about 42% of the differences in the cell groups could be explained by the post-hoc projected environmental variables (Table 1 and 2). The PCA bi-plot (Fig. 5) did not provide a clear distinction between the large and medium cells but a discrete separation was observed of large/medium cells from small cells along PC1. The occurrence of large cells was supported by elevated levels in SRP (soluble reactive phosphorous) concentration, wind speed, temperature and conductivity whereas the opposite encouraged the prevalence of small cells. pH, light attenuation and NO₃ played a minimum role in the cell group pattern.

Table 1. Summary of statistics of principal component analysis.

Cell morphology axes	1	2	Total variance
Morphology-environment correlations	0.679	0.550	
Cumulative percentage variance			
of morphology data:	74.8	100.0	
of morphology-environment relations:	81.9	100.0	
Sum of all eigenvalues			1.000
Sum of all canonical eigenvalues			0.421
Filament morphology axes	1	2	Total variance
Morphology-environment correlations	0.773	0.703	
Cumulative percentage variance			
of morphology data:	70.5	100.0	
of morphology-environment relations:	74.4	100.0	
Sum of all eigenvalues			1.000
Sum of all canonical eigenvalues			0.568

Filament dimensions

Three filament morphology groups were obtained from the cluster analysis (Table 3). Mrpp revealed a high homogeneity within groups and a significant separation (chance-corrected within-group agreement $A = 0.43$; $p < 0.001$). Similar to the cell group scenario, we observed high temporal dynamics in filament group shifts in L. Nakuru compared to L. Bogoria (see Fig. 3). In L. Nakuru, the abundance of small filaments peaked to 100% in July and August 2008 and for a more extended period from December 2008 to March 2009. Between

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Table 2. Environmental and biological variables (mean $\pm$ s.d.) of L. Nakuru and L. Bogoria during the study period.

Variable	L. Nakuru	L. Bogoria
Soluble reactive phosphorus (mg L ⁻¹)	0.9 $\pm$ 1.1	3.1 $\pm$ 0.7
Water temperature (°C)	25.1 $\pm$ 1.9	28.6 $\pm$ 1.5
pH	10.1 $\pm$ 0.2	10 $\pm$ 0.2
Coefficient of attenuation	9.5 $\pm$ 2.9	8.2 $\pm$ 2.5
Specific conductivity (mS cm ⁻¹)	44.5 $\pm$ 18.2	67.2 $\pm$ 5.8
<i>Arthrospira</i> -biomass (mg L ⁻¹)	39.1 $\pm$ 47.1	68.3 $\pm$ 29
<i>Arthrospira</i> -grazing zooplankton (mg C L ⁻¹)	2.4 $\pm$ 2.4	0.8 $\pm$ 1.4
Nitrate-N (mg L ⁻¹)	16.1 $\pm$ 9.5	11.3 $\pm$ 3.4
Wind Speed (km h ⁻¹)	4.2 $\pm$ 1.8	6.6 $\pm$ 1.7
Cyanophages (% infected cells)	1.0 $\pm$ 3.6	1.1 $\pm$ 3.6

September and November 2008, alternating dominance between largeW and largeN filaments was observed while from May till end of the sampling period, largeN filaments prevailed. The peaks of the small filaments appeared to coincide with those of small cells and *A. fusiformis* biovolume but there was no overall significant relationship between the small cells and small filaments ($p = 0.76$). On the other hand, the small filaments had a strong negative correlation with *A. fusiformis* biomass ($p < 0.001$, $r = -0.75$) while the largeW and largeN filaments were positively correlated to *A. fusiformis* biomass ($p < 0.001$, $r = 0.69$ and 0.45 respectively). In L. Bogoria, the largeW filaments predominated throughout the study period at approximately 80% while the largeN filaments fluctuated around 20%. There was no significant relationship observed between small, largeW and largeN filaments ($p = 0.13$, 0.60 and 0.81 respectively) and *A. fusiformis* biomass.

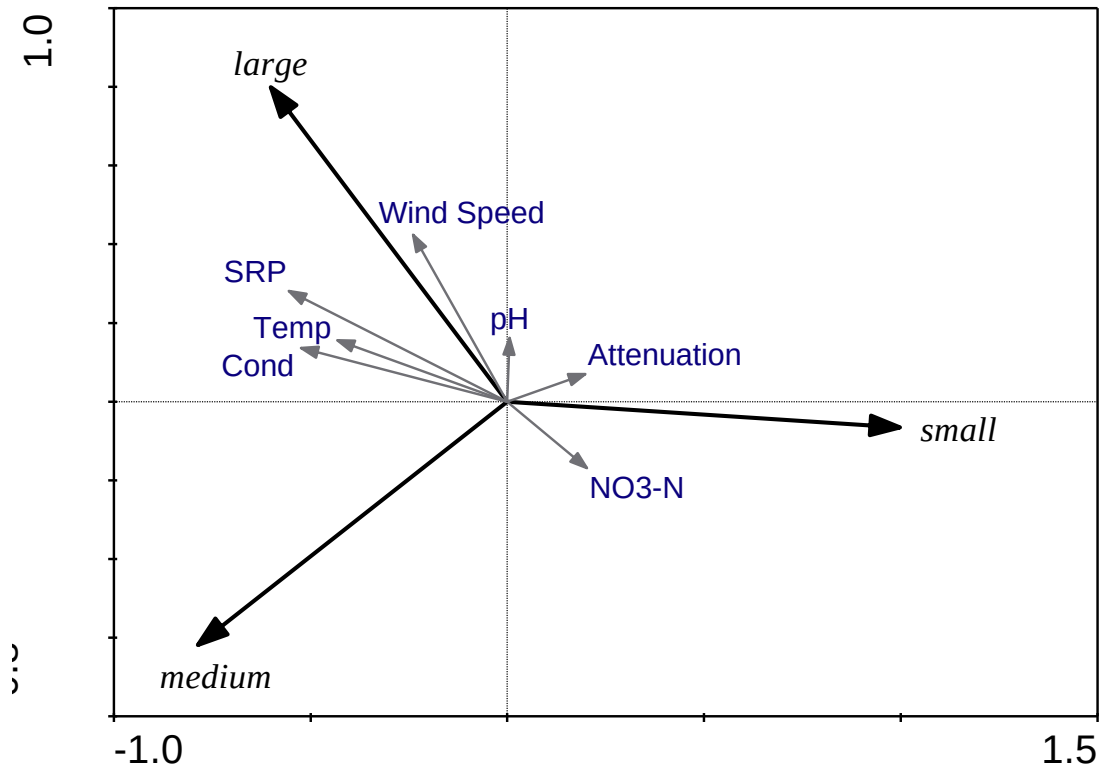


Fig. 5. PCA-biplot based on cell groups (small, medium, large) and supplementary environmental variables

Table 3. Characteristics of filament morphology groups of *Arthrospira* obtained from cluster analysis for both lakes Nakuru and Bogoria (dimensions, mean $\pm$ s.e.; n total = 6469; Large-W = Large wide pitch filaments; Large-N = Large narrow pitch filaments).

Group	Sketch	n	Coil diameter (μm)	Coils/filament	Pitch (μm)
Small		1336	36.6 ± 0.3	2.6 ± 0.1	11.1 ± 0.3
LargeW		2848	50.1 ± 0.2	7.3 ± 0.1	63.2 ± 0.3
LargeN		2285	60.5 ± 0.4	8.6 ± 0.1	12.6 ± 0.1

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PCA for filament morphology indicated that about 57% of the differences in the filament groups (see Table 1 and 2) could be explained by post-hoc projected variables. As was observed with the cell groups, there was a discrete separation of the small filaments from the largeN and largeW filaments along PC1 (Fig. 6). LargeW filaments dominated with increasing levels of conductivity, SRP, temperature and wind speed. The occurrence of largeN filaments was associated with increase in the *A. fusiformis*-grazing zooplankton (rotifers - *Brachionus plicatilis* Mueller and *Hexathra jenkiniae* De Beauchamps and all ciliates larger than 60 μm), NO_3 and cyanophage-visibly infected cells. pH and light attenuation appeared to have played a negligible role in the group separations. As expected, *A. fusiformis* biovolume increased when the largeW and largeN filaments predominated. Small filaments were negatively coinciding with conductivity, SRP, temperature and wind speed.

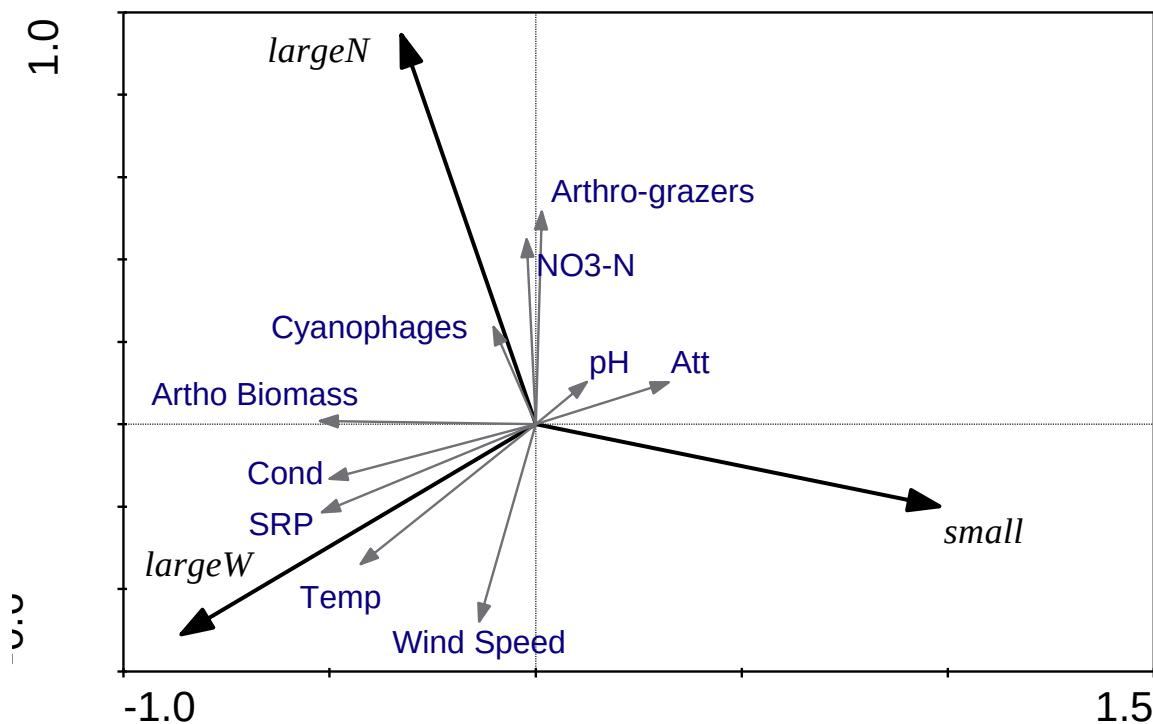


Fig. 6. PCA-biplot based on filament groups and supplementary environmental variables
largeN = largeN filaments; *largeW* = largeW filaments; *small* = small filaments

PAM fluorescence measurements

F_v/F_m ranged between 0.20 and 0.68 in both lakes. Between largeW occurrence and F_v/F_m , a significant positive correlation was calculated ($r = 0.703$, $p < 0.001$, $n = 69$; Fig. 7). Also for largeN, a significant, although weaker positive relationship could be shown ($r = 0.252$, $p = 0.037$, $n = 69$). For small filaments, a negative correlation was obtained ($r = -0.681$, $p < 0.001$, $n = 69$).

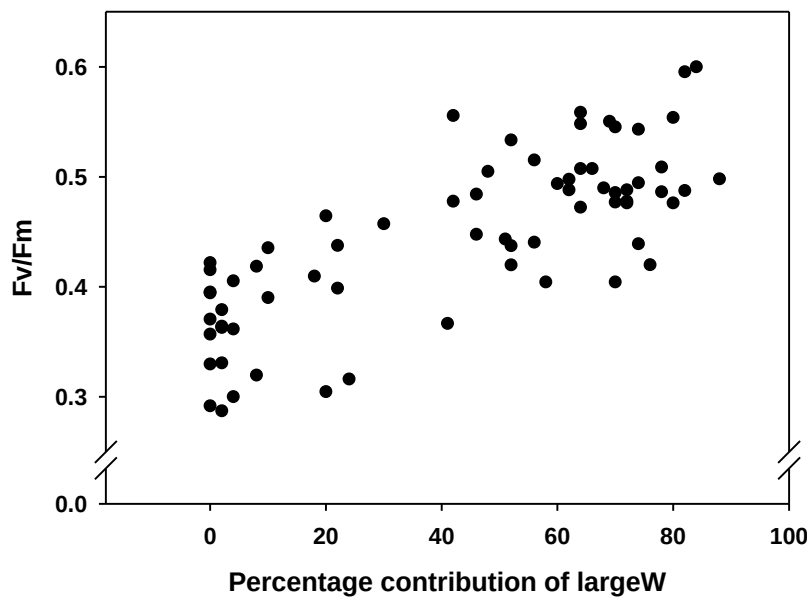


Fig 7. LargeW filament group related to F_v/F_m values

Discussion

The diverse ecological strategies adopted by phytoplankton can be related to their morphological variability (Reynolds, 1997); this acclimation value is directed towards adapting the best fitting trait to the prevailing environmental template (Naselli-Flores et al., 2007). Morphological modification of *A. fusiformis* has been well studied under laboratory conditions such as variable irradiance (Wu et al., 2005; Helbing et al., 2006), temperature (Gao et al., 2008; Vonshak & Novoplansky, 2008) and salinity (Kebede, 1997). Additionally due to problems that arose concerning the nomenclature of *Arthrospira* spp. (formerly classified under *Spirulina*) there was extensive research done concerning morphology and

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ultrastructure which resulted into the earlier research outputs of Eykelenburg (1979), Bai & Seshadri (1980), Hindak (1985) among others. So far, no extensive field studies exist that indicate whether these changes that have been observed under extreme laboratory situations also occur in nature.

Cell dimensions

The cell diameter measurements were in the range given by Vonshak and Tomaselli (2000). Even though cell height is not suitable for cell group classification as it is highly subjective to elongation during cell division as shown by the scatter observed in our measurements (Fig. 4), there was however, a noticeable trend of larger diameter cells depicting larger cell height. From our findings we were unable to conclude which group may indicate cells with high vitality for *A. fusiformis*, which supports the inconsistencies between cell and filament categories. The distinct changes in the prevalence of different cell sizes in L. Nakuru can be seen as a reaction to the changing environmental conditions, which however does not mean that cells are exposed to adverse conditions. L. Bogoria, on the other hand is both physically and chemically more stable (Harper et al., 2003). The lake's large volume enhances buffering capacity to the chemical changes expected to accompany rainfall dilution (Schagerl & Oduor, 2008) and therefore *A. fusiformis* cells are not confronted by pronounced changes in the physical and chemical variables of the water column. This is well illustrated by the observed lack of big variations in the proportions of each of the cell categories.

From the PCA bi-plot, it was observed that only non-biological factors played a significant role in the cell category pattern along the gradients. Biological factors such as *A. fusiformis*–eating zooplankton and cyanophage infections were considered in the post-hoc analysis but were not found to contribute significantly to the cell category pattern. Elevated conductivity, temperature and SRP concentration promoted the percentage of large and medium cells while wind speed coincided with elevated percentage of large cells. *A. fusiformis* develops aerotops to regulate its position along the underwater light gradient and follow the daily and seasonal light changes (Vonshak & Tomaselli, 2000). Although we were not able to obtain data concerning gas vesicle development, we assume that increased cell size results in a higher potential to form gas vesicles and therefore a better buoyancy regulation to counteract turbulent conditions. The importance of advection and turbulence has also been reported for marine ecosystems (Li, 2002). The author observed that phytoplankton size structure across

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various marine ecosystems varies with the ocean physics, which set the nutrient supply and irradiance levels to which phytoplankton are exposed.

Filament dimensions

Once again the temporal trend of filament morphology groups in L. Nakuru seemed to have been influenced by the frequent changes in physico-chemistry and biological parameters. During dry spells, *A. fusiformis* filaments were exposed to unfavourable conditions making filaments vulnerable for e.g., cyanophage attacks (Peduzzi et al., unpublished data). As a result, filaments were breaking off at weak spots where the cells were dying, giving rise to accumulation of short filaments. It was during such phases that *A. fusiformis* was out-competed by other phytoplankton groups (*Anabaenopsis*, cryptomonads and other cyanobacteria) and thus the occurrence of *Arthrospira* crashes.

On the other hand, largeW and largeN filaments thrived during *A. fusiformis* peaks depicting favourable conditions for *A. fusiformis* occurrence. A number of studies have demonstrated the adaptive morphological changes from loosened to tightened helix that *A. fusiformis* manifests under various environmental conditions such as, light, temperature and salinity. For instance, loose or straight *A. fusiformis* forms can change to tight helical forms under high light intensities (Bai & Seshadri, 1980) from about 8 h (Gao et al., 2008) to 4-5 d (Helbling et al., 2006). Wu et al. (2005) suggested that a reduced pitch could be an effective protective mechanism against self-shading to counteract ultra-violet or high photo-active radiation levels. During our field study, irradiance supply per se did not separate largeW and largeN filaments, but wind speed did. Turbulences induced by elevated wind-speed seem to be a key variable; the filaments are exposed more in dark areas of the water body and as a result, the pitch increases. During calm periods, cells tend to accumulate near the surface scums due to buoyancy forming densely coiled spirals as a protection against excess radiation. It is not only light penetration, but also the turbulence regime, which controls the shape.

Additionally, zooplankton might influence the pitch: an increase in *A. fusiformis*-eating zooplankton was coinciding with largeN filaments. Grazing is one of the most widely explored environmental constraints on size and shape spectrum of phytoplankton as it plays a key role in the size-scaling of phytoplankton (e.g., Salmaso & Padisák, 2007; Stoyneva et al., 2007). Burian et al. (2012) observed that zooplankton ingests *A. fusiformis* filaments in a spaghetti-like way. As densely coiled filaments are harder to grasp, the observed coincidence could be a reaction of *A. fusiformis* to reduce the grazing pressure. Such defence strategies of

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phytoplankton against grazing by the rotifer *Brachionus* have already be proved for green algae such as, *Scenedesmus* (Verschoor et al., 2004) and *Micractinium* (Luo et al., 2005); it is assumed that kairomones released by the rotifers promote defence structures like bristles.

The explanatory value of cyanophages is weak, but still significant (Fig. 6). It is an indicator that cyanophages attacking *Athrospira* are increased especially during periods of high *A. fusiformis* biomass, which is in accordance to other findings, as virus replication rates usually increase in conjunction with increases in host growth rates (Suttle, 2007). In *A. fusiformis*-dominated natural systems, this study therefore is the first to provide evidence of massive cyanophages attacks even though this aspect needs further in-depth studies on the phage-host relationship.

F_v/F_m values were within the typical range of cyanoprocaryotes (Campbell et al., 1998), which were with a few exceptions the dominant phytoplankton group throughout the investigation period (Fig. 3). For this group, lower F_v/F_m of 0.40 to 0.60 is common, because F_o increases especially at higher phycocyanin contents (Campbell et al., 1998) thus lowering F_v . A comparison between filament type groups and F_v/F_m revealed a clear pattern: large filaments, especially largeW, were highly related to increases in F_v/F_m . As this group is generally dominating the community in Bogoria and sometimes in Nakuru, we conclude that largeW indicates filaments of high vitality; this is also the case for largeN. Contrarily, small filaments are related to lower F_v/F_m , which can be seen as indicator for adverse conditions.

Summarizing up, the study was able to demonstrate detailed morphological changes of *A. fusiformis* in nature for the first time. Key variables responsible for the morphological changes were identified. More so, from morphology changes, a pronounced shift in *A. fusiformis* biomass can be deduced: largeW and largeN filaments indicating filaments of high vitality prevailed during the periods of *A. fusiformis* peaks. It was clearly demonstrated that morphological changes reflected the abiotic and biotic developments that took place in the two lakes during this study. Therefore, *A. fusiformis* morphotypes may reliably be used as one of the easily accessible monitoring tools for the prevailing environmental and biological variables in soda lakes as its shape and structure is highly subjective and therefore reflective of what is happening in its habitat. As an additional benefit, our findings may also be of interest for mass cultivation, as morphological features provide insight into the vitality of the cultures and induced changes of the filament shape might be used for optimizing harvesting.

Acknowledgements

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References

- American PHS (1995) Standard methods for the examination of water and wastewater. American Public Health Association, Washington DC.
- Bai JN & Seshadri VC (1980) On coiling and Uncoiling of trichomes in the genus *Spirulina*. *Arch Hydrobiol* 60:32-47.
- Baker NR (2008) "Chlorophyll fluorescence: A probe of photosynthesis in vivo." *Annu Rev Plant Biol* 59: 89-113.
- Ballot A, Krienitz L, Kotut K, Wiegand C, Metcalf SJ, Codd AG & Pflugmacher S (2004) Cyanobacteria and cyanobacterial toxins in three alkaline rift valley lakes of Kenya—Lakes Bogoria, Nakuru and Elmenteita. *J Plankton Res* 26: 925-935.
- Bjorkman O & Demmig B (1987) Photon yield for O₂ evolution and chlorophyll fluorescence characteristics at 77K among vascular plants of diverse origins. *Planta* 170: 489-504.
- Burian A, Schagerl M & Yasindi A (2012) Microzooplankton feeding behaviour: grazing on the microbial and the classical food web of African soda lakes. *Hydrobiologia* DOI 10.1007/s1050-012-1023-2.

Chapter 2

- Campbell D, Hurry V, Clarke AK, Gustafsson P & Öquist G (1998) Chlorophyll fluorescence analysis of cyanobacterial photosynthesis and acclimation. *Microbiol Mol Biol R* 62: 667-683.
- Capelli B & Cysewski G (2010) Potential health benefits of *Spirulina* microalgae. *Nutrafoods* 9:19-26.
- Dadheech KP, Ballot A, Casper P, Kotut K, Novelo E, Lemma B, Pröschold T & Krienitz L (2010) Phylogenetic relationship and divergence among planktonic strains of *Arthrospira* (Oscillatoriales, Cyanobacteria) of African, Asian and American origin deduced by 16S-23S ITS and phycocyanin operon sequences. *Phycologia* 49: 361-372.
- Eykelenburg vC (1979) The Ultrastructure of *Spirulina platensis* in relation to temperature and light intensity. *A van Leeu* 45:369-390.
- Gao K, Li P, Watanabe T & Hebling WE (2008) Combined effects of ultraviolet radiation and temperature on morphology, photosynthesis and DNA of *Arthrospira* (*Spirulina*) *platensis* (Cyanophyta). *J Phycol* 44: 777-786.
- Harper DM, Childress BR, Harper MM et al. (2003) Aquatic biodiversity and saline lakes: Lake Bogoria National Reserve, Kenya. *Hydrobiologia* 500: 259-276.
- Helbing WE, Gao K, Ai H, Zengling M & Villafañe EV (2006) Differential responses of *Nostoc sphaeroides* and *Arthrospira platensis* to solar ultraviolet radiation exposure. *J Appl Phycol* 18: 57-66.
- Hillebrand H, Durselen DC, Kirschtel D, Pollinger U & Zohary T (1999) Biovolume calculation for pelagic and benthic microalgae *J Appl Phycol* 35: 403-424.
- Hindák F (1985) Morphology of trichomes in *Spirulina fusiformis* Voronichin from Lake Bogoria, Kenya. *Arch Hydrobiol* 71: 201-218.
- Jassby A (1988) *Spirulina*: a model for microalgae as human food. *Algae and Human Affairs* (Lembi AC & Waaland JR, eds), pp. 149-179. Cambridge University Press, Cambridge.
- Kagami M & Urabe J (2001) Phytoplankton growth rate as a function of cell size: an experimental test in Lake Biwa. *Limnology* 2:111-117.

Chapter 2

Kaggwa NM, Gruber M, Oduor OS & Schagerl M (2012) A detailed time series assessment of the diet of Lesser Flamingos: further explanation for their itinerant behaviour. *Hydrobiologia* DOI 10.1007/s10750-012-1105-1.

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.

Kociolek PJ & Spaulding SA (2003) Symmetrical naviculoid diatoms. *Freshwater Algae of North America: Ecology and Classification* (Wehr DJ & Sheath GR, eds), pp. 637-639. Academic Press, San Diego.

Komárek J (2003) Coccoid and colonial cyanobacteria. *Freshwater Algae of North America: Ecology and Classification* (Wehr DJ & Sheath GR, eds), pp. 39-110. Academic Press, San Diego.

Komárek J, Kling H & Komárková J (2003) Filamentous cyanobacteria. *Freshwater Algae of North America: Ecology and Classification* (Wehr DJ & Sheath GR, eds), pp. 117-191. Academic Press, San Diego.

Krienitz L & Kotut K (2010) Fluctuating algal food populations and the occurrence of Lesser Flamingos (*Phoeniconaias minor*) in three Kenyan Rift Valley lakes. *J Appl Phycol* 46:1088-1096.

Lepš J & Šmilauer P (2005) *Multivariate analysis of ecological data using CANOCO*. Cambridge University Press, Cambridge.

Li WKW (2002) Macroecological patterns of phytoplankton in the northwestern North Atlantic Ocean. *Nature* 419: 154-157.

Luo W, Krienitz L, Pflugmacher S & Walz N (2005) Genus and species concept in *Chlorella* and *Micractinium* (Chlorophyta, Chlorellaceae): genotype versus phenotypical variability under ecosystem conditions. *Ver Int V Limn* 29: 170-173.

Mopntagnes DJS & Lynn HD (1993) A quantitative protargol stain (QPS) for ciliates and other protists. *Handbook of Methods in Aquatic Microbial Ecology* (Kemp et al., eds), pp. 229-240. Lewis Publishers, Boca Raton.

Mühling M, Harris N, Belay A & Whitton AB (2003) Reversal of helix orientation in the cyanobacterium *Arthrospira*. *J Phycol* 39: 360-367.

Chapter 2

Naselli-Flores L & Barone R (2000) Phytoplankton dynamics and structure: a comparative analysis in natural and man-made water bodies of different trophic state. *Hydrobiologia* 438: 65-74.

Naselli-Flores L, Padisák J & Albay M (2007) Shape and size in phytoplankton ecology: do they matter? *Hydrobiologia* 578: 157-161.

O'Farrell I, Pinto PdeT & Izaguirre I (2007) Phytoplankton morphological response to the underwater light conditions in a vegetated wetland. *Hydrobiologia* 578: 65-77.

Oduor OS & Schagerl M (2007a) Temporal trends of ion contents and nutrients in three Kenyan Rift Valley saline-alkaline lakes and their influence on phytoplankton biomass. *Hydrobiologia* 584: 59-68.

Oduor OS & Schagerl M (2007b) Phytoplankton primary productivity characteristics in response to photosynthetically active radiation in three Kenyan Rift Valley saline-alkaline lakes. *J Plankton Res* 29: 1041-1050.

Pigliucci M (1996) How organisms respond to environmental changes: from phenotypes to molecules (and vice versa). *Tree* 11: 168-173.

Rasool M, Sabina EP & Lavanya B (2006) Anti-inflammatory effect of *Spirulina fusiformis* on adjuvant-induced arthritis in mice. *Biol Pharm Bull* 29: 2483-2487.

Reynolds CS (1997) Vegetation Processes in the Pelagic: A Model for Ecosystem Theory. *Excellence in Ecology* (Kinne O, ed), pp. 371. Ecology Institute, Oldendorf Luke, Germany.

Salmaso N & Padisák J (2007) Morpho-functional groups and phytoplankton development in two deep lakes (Lake Garda, Italy and Lake Stechlin, Germany). *Hydrobiologia* 578: 97-112.

Schagerl M & Oduor OS (2008) Phytoplankton community relationship to environmental variables in three Kenyan Rift Valley saline-alkaline lakes. *Mar Freshwater Res* 59: 129-136.

Schlichting CD & Pigliucci M (1993) Evolution of phenotypic plasticity via regulatory genes. *Am Nat* 142: 366-370.

Shubert E (2003) Nonmotile coccoid and colonial green algae. *Freshwater Algae of North America: Ecology and Classification* (Wehr DJ & Sheath GR, eds), pp. 253-308. Academic Press, San Diego.

Chapter 2

Stoyneva PM, Descy J-P & Vyverman W (2007) Green algae in Lake Tanganyika: is morphological variation a response to seasonal changes? *Hydrobiologia* 578: 7-16.

Suttle CA (2007) Marine viruses – major players in the global ecosystem. *Nat Rev Microbiol* 5: 801-812.

Tokuşogulu Ö & Ünal KM (2003) Biomass nutrient profiles of microalgae: *Spirulina platensis*, *Chlorella vulgaris* and *Isochrysis galbana*. *Food Chem Toxicology* 68: 4.

Utermöhl H (1958) Zur Vervollkommnung der quantitativen Phytoplankton-Methodik. *Mitt Int Ver Theor Ang Limn* 9: 1-38.

Vareschi E (1978) Ecology of Lake Nakuru (Kenya). I. Abundance and feeding of Lesser Flamingo. *Oecologia* 32: 11-35.

Vareschi E (1982) Ecology of Lake Nakuru (Kenya). III. Abiotic factors and primary production. *Oecologia* 55: 81-101.

Vareschi E & Vareschi A (1984) Ecology of Lake Nakuru (Kenya). IV. Biomass and Distribution of Consumer Organisms. *Oecologia* 61: 70-82.

Verschoor AM, Van Der Stap I, Helmsing NR, Lüring M & Van Donk E (2004) Inducible colony formation within the Scenedesmaceae: adaptative response to infochemicals from two different herbivore taxa. *J Phycol* 40: 808-814.

Vonshak A (1997) *Spirulina: Growth, Physiology and Biochemistry*. *Spirulina platensis* (*Arthrospira*): *Physiology, Cell-Biology and Biotechnology*, pp. 43-65. Taylor and Francis Ltd., London.

Vonshak A & Novoplansky N (2008) Acclimation to low temperature of two *Arthrospira platensis* (Cyanobacteria) strains involves down-regulation of PSII and improved resistance to photoinhibition. *J Phycol* 44: 1071-1079.

Vonshak A & Tomaselli L (2000) *Arthrospira* (*Spirulina*): Sytematics and Ecophysiology. *The Ecology of Cyanobacteria* (Whitton A & Potts M, eds), pp. 505-522. Kluwer Academic Publishers, The Netherlands.

Wang PZ & Zhao Y (2005) Morphological reversion of *Spirulina* (*Arthrospira*) *platensis* (Cyanophyta): from Linear to Helical. *J Phycol* 41: 622-628.

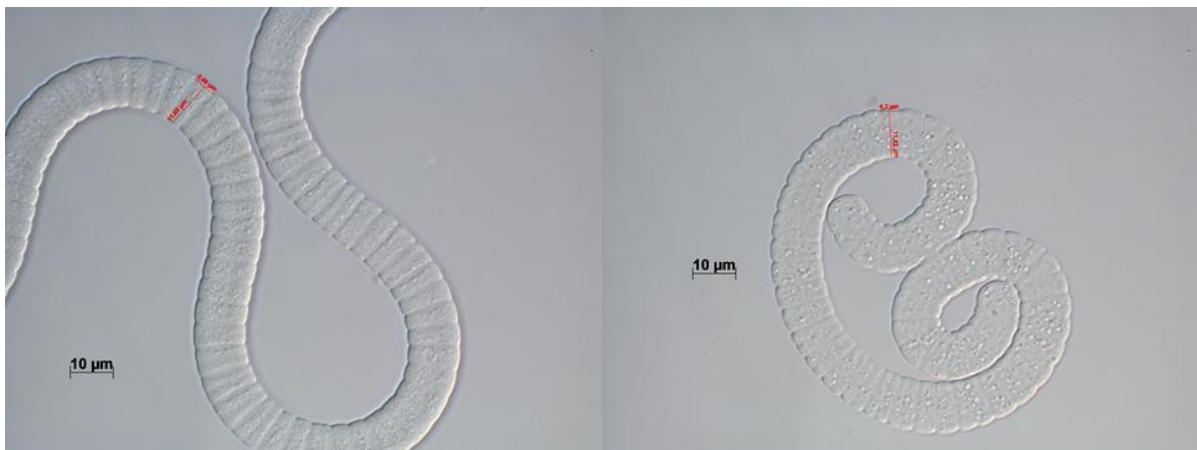
Chapter 2

Wetzel GR & Likens EG (1991) *Limnological Analyses*. Springer Verlag, New York.

Wu H, Gao K, Villafañe EV, Watanabe T & Helbling WE (2005) Effects of Solar UV Radiation on Morphology and Photosynthesis of Filamentous Cyanobacterium *Arthrospira platensis*. *Appl Environ Microb* 71: 5004-5013.

Young EB, Dring MJ, Savidge G, Birkett DA & Berges JA (2007) Seasonal variations in nitrate reductase activity and internal N-pools in intertidal brown algae are correlated with ambient nitrate concentrations. *Plant Cell Environ* 30: 764–774.

Zielińska A & Chojnacka K (2009) The comparison of biosorption of nutritionally significant minerals in single- and multi-mineral systems by the edible microalga *Spirulina* sp. *J Sci Food Agr* 89: 2292-2301.



A. fusiformis.- ‘Behold the beauty...’

Chapter 3

Factors controlling the abundance of *Arthrospira fusiformis*



Simbi, Kenya, 2010. 'Let's go green!'

Algal communities of African soda lakes with a special focus on *Arthrospira fusiformis*

Prepared for submission to The ISME Journal - Multidisciplinary Journal of Microbial Ecology

Mary N. Kaggwa¹, Steve Omondi Oduor², Michael Schagerl¹

¹ University of Vienna, Department of Limnology, Althanstrasse 14, A-1090 Vienna, Austria.

² Egerton University, Department of Biological Sciences, P.O. Box 536, Njoro, Kenya.

Abstract

Soda lakes tend to be very simple in their biodiversity, but due to their highly stochastic temporal environmental dynamics unexpected shifts in species composition do occur. The purpose of this research study was to investigate the key drivers for structuring the phytoplankton community and determine variables that significantly influence the observed changes in *Arthrospira* biomass in the Kenyan soda lakes, Nakuru and Bogoria. Environmental and biological data were collected weekly for sixteen months and related to phytoplankton abundances. In both lakes, *Arthrospira fusiformis* dominated algal biomass, but for Nakuru, sudden *Arthrospira* breakdowns were recognized. Three variables – salinity, pH and ash mass – significantly contributed to the phytoplankton taxa pattern. *Arthrospira* was absent at the highest ash mass ($> 0.677 \text{ g L}^{-1}$) and salinity ($> 57.8 \text{ ‰}$) levels being outcompeted by *Picocystis salinarium*. Picoplankton were also present at a higher pH and during *Arthrospira* crash in L. Nakuru. Overall it was observed that *Arthrospira*'s response to

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fast environmental changes is limited in nature hindering its growth and it therefore was outcompeted by other taxa thus contributing to a more diverse phytoplankton community. Light penetration, consumers of *Arthrospira* and cyanophages attacking this taxon significantly influenced *Arthrospira* abundance in L. Nakuru. This was the first study to prove cyanophage infection in *Arthrospira* filaments and relate the virus attacks to its sudden breakdowns. Since *Arthrospira* is a fundamental taxon for ecosystem production in these lakes, its linkage with other components is vital for the survival of these unique systems.

Keywords: Biological, cyanophage, environmental, phytoplankton, saline-alkaline, salinity, *Spirulina*, virus

Introduction

Arthrospira fusiformis (Voronichin) Komárek & Lund (formerly called *Spirulina plantensis* Voronichin) is a filamentous cyanobacterium which is quite ubiquitous (Table 1) and is capable of adaptation to a varied range of habitats, from freshwater-alkaline conditions to saline-alkaline and even hypersaline environments (Ciferri, 1983; Dadheech et al., 2010). In the soda lakes of East Africa, *Arthrospira fusiformis* (hereinafter referred to as *Arthrospira*) forms an exceptionally high algal crop due to its high photosynthetic capacity designating these lakes as part of the world's most productive ecosystems (Talling et al., 1973; Melack & Kilham, 1974; Oduor & Schagerl 2007a). It is the main food for the Lesser Flamingos, *Phoeniconaias minor* Geoffroy, that flock these lakes in millions (Vareschi, 1978; Krienitz & Kotut 2010).

The endorheic soda lakes are prone to hydrological influences since they are often located in semi-arid regions, and their levels can fluctuate considerably in response to seasonal changes and annual differences in rainfall (Melack, 1981). For these extreme habitats, the dependence on phytoplankton productivity as driving force is even greater than for freshwater systems since they tend to have minimal macrophyte growth and low allochthonous organic carbon input due to limited rainfall and inflows. The high phytoplankton biomass further makes the contribution of micro-phytobenthic community to overall productivity to be very minimal due to the resulting poor light climate in most of the water column other than the immediate surface zone (Oduor & Schagerl, 2007a).

Several studies have been done on the algal communities prevailing in African saline lakes (e.g. Krienitz et al., 2003; Ballot et al., 2005; Schagerl & Oduor, 2008; Krienitz & Kotut, 2010, Kaggwa et al., 2012), where temporal trends have often indicated periodic dominance and paucity among the different phytoplankton groups especially at extreme salinity. Apart from the predominant *Arthrospira*, other cyanobacteria also occur in these lakes for instance, *Anabaenopsis arnoldii* Aptekarj, *Anabaenopsis abijatae* Kebede et Willen, *Synechococcus minutus* Voronichin, *Haloleptolyngbya alcalis* Dadheech, Mahmoud, Kotut et Krienitz, *Chroococcus* sp., *Synechocystis* sp. and *Cyanospira* sp.. Additional phytoplankton groups include Chlorophyceae, Cryptophyceae and Bacillariophyceae (Schagerl & Oduor, 2008; Dadheech et al., 2012; Krienitz et al., 2013). Very recently, Luo et al., (2013) also detected a hidden diversity of eukaryotic plankton in L. Nakuru during a phase of low salinity.

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Table 1. Geographical occurrence of *Arthrospira* species in saline alkaline lakes

Continent	Country	Lake	Reference
Africa	Kenya	Bogoria, Nakuru, Sonachi, Simbi, Oloidien, Elmenteita Magadi	Melack & Kilham, 1974; Melack, 1979, 1981, 1988, 1996, 2009; Vareschi, 1982; Ballot et al., 2005; Schagerl & Oduor, 2008; Krienitz & Kotut, 2010.
	Ethiopia	Abijatae, Arenguade, Chitu, Kilotes	Melack & Kilham, 1974; Talling et al., 1973; Kebede, 1997
	Tanzania	Natron, Eyasi, Big Momella, Magad, Manyara, Reshitani, Tulusia	Melack & Kilham, 1974; Tuite, 1981;
	Uganda	Katwe, Masehe	Mungoma, 1990
	Chad	Chad, Rombou, Djikare, Mombolo, Macu-Leyla	Iltis, 1969a & b, 1971; Ciferri, 1981, 1983;
	Sudan	Dariba	Fott & Karim, 1973
Asia	Turkey	Van	Hammer, 1986
	India	Shambhar, Mansagar	Dadheech et al., 2010.
Central America	Mexico	Texoco	Ciferri, 1981; Dadheech et al., 2010.

To date, only a few comprehensive studies have been done on the primary consumers in African saline lakes. In their study on the ecology of L. Nakuru, Vareschi & Jacobs (1985) observed that most primary consumer organisms fed on the dominant primary producer, *Arthrospira*; the authors also deduced that Lesser Flamingos are the main primary consumers of *Arthrospira*. Other primary consumers included: calanoid copepod *Lovenula africana*

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Daday, three species of rotifers, *Brachionus dimidiatus* Bryce and *Brachionus plicatilis* Müller and *Hexarthra jenkiniae* De Beauchamps, a few insect taxa and two chironomid larvae species for benthic fauna. For Lake Bogoria, only a single chironomid species *Paratendipes* sp. was found (Harper et al., 2003). The tilapine fish *Oreochromis alcalicus grahami* Boulenger, also feeds on *Arthrospira* in lake Nakuru. This supports the suggestion that *Arthrospira* represents the basis of life for these lakes (Vareschi, 1982; Ballot et al., 2004).

Melack (1979) related the observed temporal variations in phytoplankton composition to changes in environmental conditions. However, community shifts related to the environment have so far not been explained very well (Schagerl & Oduor, 2008), which might be due to either long sampling intervals or brief sampling periods which are bound to miss out on short-term events in these highly dynamic systems. Over time, there have been stochastic biomass crashes of *Arthrospira* (Table 2) being replaced by members from other genera such as, *Anabaenopsis*, *Cryptomonas* and *Cyanospira*. A number of causal factors have been suggested as being responsible for this phenomenon including, changes in salinity, nutrient limitation, variation in the hydrological pattern, heavy grazing pressure and even viral attacks (Vareschi, 1982; Schagerl & Oduor, 2008; Krienitz & Kotut, 2010; Krienitz et al., 2013); these factors however remain speculative to date.

The purpose of this study was to address this knowledge gap by using a holistic approach which included a simultaneous collection of data on over 20 environmental variables and various biological parameters covering almost all ecosystem components. In order to acquire an explicit outcome, data were collected over short-term sampling intervals on weekly basis for sixteen months from two soda lakes, Nakuru and Bogoria. Data on environmental and biological variables and related to the community composition and its shifts as well as *Arthrospira* biomass changes which enabled us to make evidence derived ecological inferences on the interactions of primary producers and other ecosystem components in these lakes.

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Table 2. Historical dates of observed *Arthrospira* biomass crashes in alkaline-saline lakes

Date of crash	Lake	Reference
1971	Elmenteita	Melack & Kilham, 1974
1973	Nakuru	Vareschi et al., 1981
1973 (Mar- Sep)	Bogoria	Melack, 1976
1973,1974	Elmenteita	Melack, 1988
1974	Nakuru	Vareschi, 1982
1974, 1976	Elmenteita	Tuite, 1981
1974 (Jan-Mar)	Nakuru	Tuite, 2000
2001 (Jun)	Nakuru	Ballot et al., 2004
2001 (Oct)	Bogoria	Harper et al., 2003
2002 (Sep)	Elmenteita	Ballot et al., 2004
2004	Elmenteita	Schagerl & Oduor, 2008
2004 (Jul to Oct)	Bogoria	Schagerl & Oduor, 2008
2006	Bogoria	Krienitz & Kotut, 2010
2008 (Dec)-2009 (Apr) & 2009 (Sep-Oct)	Nakuru	Kaggwa et al., 2012

Materials and methods

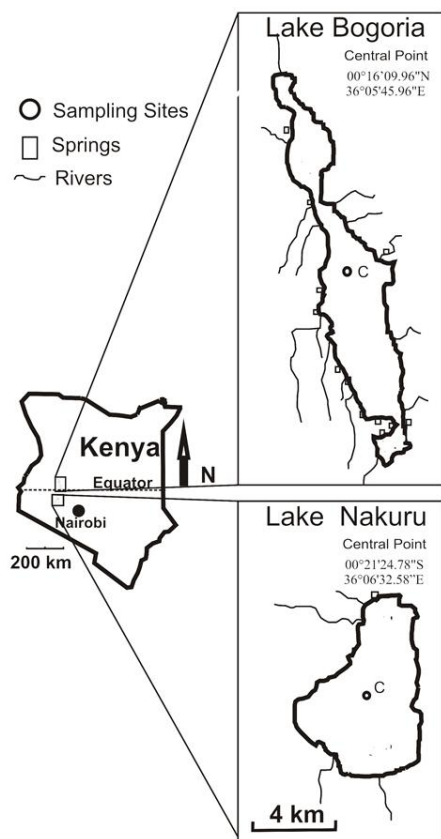


Fig 1. Map showing Kenya and sampling sites of the two studied saline-alkaline lakes, Nakuru and Bogoria

The data used for this study were collected from lakes, Nakuru and Bogoria located in Central Kenya (Fig. 1) over a period of sixteen months (July 2008 to October 2009). Salinity and pH were measured in-situ using a multiprobe (WTW Multi 340i Wissenschaftlich Technische Werkstätten Weilheim, Germany). Light intensity was measured as PAR using a light meter (Skye Instruments, Quantum sensor, Wales, Great Britain).

Dry and ash mass

Dry mass was analysed by filtering a known volume of the raw sample using pre-weighed filters (45 μm pore size; Ederol BM/C, Battenburg, Germany). It was obtained by drying the filters together with residue material at 95 ± 5 °C until a constant weight was achieved. For estimation of the ash mass, the dry mass filters were combusted in a muffle furnace for 2 hours at 550 °C.

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Nutrients analysis

Unfiltered lake water was analysed for total nitrogen and total phosphorus while filtered water samples were used for analysis of soluble reactive phosphorus, ammonium-N, nitrate-N and dissolved nitrogen. For some nutrients such as, soluble reactive phosphorus, the standard methods of analyses (American Public Health Association, 1995) were modified to cater for the high buffering capacity of the alkaline water. For the other nutrients the normal standard procedures were followed.

Cyanophages

Samples collected with a plankton net (30 µm mesh size) were fixed with glutaraldehyde to a final concentration of 2%. *Arthrospira* filaments were prepared for transmission electron microscopy (Peduzzi et al., unpublished) to identify infected cells.

Zooplankton

Surface water samples were taken with a 10 L Schindler sampler. Rotifers were concentrated with a 50 µm sieve, fixed with formalin (5% final concentration) and counted following the Utermöhl (1958) protocol. Presence of crustacean zooplankton was checked regularly with a plankton net (200µm), but densities were constantly below 0.1 ind. L⁻¹ and quantitatively not important. For ciliates, 250 mL of lake water was fixed with Bouin's solution (5%), stained using the Quantitative Protargol Staining Technique (QPS) by Montagnes & Lynn (1993) and counted with a compound microscope (1000x). For this study we used only the biomass of *Arthrospira* ingesting taxa based on feeding experiments with dominant rotifers and ciliates of African soda lakes (Burian et al., 2012).

Phytoplankton community composition and biomass determination

Phytoplankton community composition was determined using lake water samples fixed with formalin. The taxa were identified with the aid of established identification keys (Komárek, 2003; Komárek et al., 2003; Shubert, 2003 and Kociolek & Spaulding, 2003). To determine the biovolume of the various community taxa, the identified cells for each taxa were enumerated and measured using an inverted microscope (Nikon Diaphot, Nikon, Tokyo) at 100× and 200× magnification (for *Arthrospira* and *Anabaenopsis*) and 400× for other taxa according to Utermöhl (1958). Biovolumes were estimated using geometric formulae of the shapes similar to the respective phytoplankton cells (Hillebrand et al., 1999). At least thirty cells for each taxon identified were measured to give the average size and biovolume. For conversion of cell volume into biomass, a conversion factor of 1 was used (Wetzel & Likens, 1991).

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Statistical analysis

For searching species-environment relationships, an indirect gradient analysis was applied. Prior to analysis, species abundance was 4th root transformed to down-weight dominant taxa. Non-metric multidimensional-scaling (NMDS) was based on Bray-Curtis similarities and resulted in a 2-dimensional solution of 0.16 minimum stress and a 3-dimensional solution of 0.11 minimum stress (Kruskal stress value; 100 iterations). Taxa responsible for pruning groups according to their site similarities were obtained with LINKTREE (minimum group size = 5, minimum split size = 10; minimum split $r = 0.65$), SIMPROF was used to test significance of the clusters (1000 permutations $p < 0.05$).

The community pattern along artificial axes obtained by NMDS was post-hoc related to environmental variables by the non-parametric routine BIOENV, which seeks for significant contributions of specific variables to explain the species pattern (1000 permutations, $p = 0.001$; $Rho = 0.497$; resemblance matrix based on Euclidian distances). Environmental variables were normalized prior to calculations and where necessary log transformed to achieve normal distribution. LINKTREE was used to seek for variables and their values, which are responsible for dichotomous group separation (minimum group size = 5, minimum split size = 5; minimum split $r = 0.3$). All statistics mentioned above were performed with the statistical package Primer V6.1.15 (Clarke & Gorley 2006).

To get insight into variables significantly influencing *A. fusiformis* abundance, a linear regression with forward selection was performed based on Lake Nakuru data. Prior to analyses, variables were standardized to zero-means-unit variance. The problem of multicollinearity was minimized by considering only significant variables with variance inflation factors ≤ 1.2 ; Durbin-Watson-statistics ($= 1.57$) indicated only very low autocorrelation; heteroscedasticity was checked visually by a scatter plot of predicted values against predicted residuals (residuals were normally distributed). Statistics was performed with IBM SPSS Statistics 20 (IBM Corp.); graphs were plotted with Sigmaplot 12.2 (Systat Software Inc.).

Results

High amounts of nutrient concentrations were measured in both lakes with the exception of nitrate-N (Table 3). pH values ranged from 9–10 for both lakes while salinity concentrations ranged widely in L. Nakuru (16–62‰) and more conservatively in L. Bogoria (37–48‰). Sodium concentrations were exceptionally high coupled with elevated alkalinity levels typical of the saline-alkaline nature of these lakes. High chlorophyll-*a* values (46–1351 mg L⁻¹, L. Nakuru; 92–1059 mg L⁻¹, L. Bogoria) and coefficient of attenuation (4–18, L. Nakuru; 2–13, L. Bogoria) were also recorded. Average recordings from L. Nakuru (443.2 ± 308.1 (SD) mg L⁻¹) showed that total and dissolved organic carbon were 8 times more than in L. Bogoria (59 ± 13.2 (SD) mg L⁻¹). The abundance of rotifers (*Brachionus plicatilis* Mueller and *Hexathra jenkiniae* De Beauchamps) and ciliates (> 60 µm) in L. Nakuru were 2-3 times as much as in L. Bogoria.

A detailed report of the temporal variability of phytoplankton community that prevailed in both lakes has been elaborated in Kaggwa et al. (2012). Four main phytoplankton groups were present including, cyanobacteria, cryptomonads, green algae and diatoms. In L. Nakuru a total of 12 taxa were observed during the study period which included *A. fusiformis*, *Anabaenopsis* spp., *Synechococcus* sp., *Synechocystis minutus* West, *Cryptomonas* spp., *Chlorococcus* sp., *Nitzschia* sp., *Navicula* sp., *Monoraphidium minutum* Nägeli, *Euglena* sp., and *H. alcalis*. For L. Bogoria 9 taxa were observed namely, *A. fusiformis*, *Arthrospira platensis* (Nordstedt) Gomont, *Anabaenopsis abijatae* Kebede & Willen, *Picocystis salinarum* Lewin, *Chlorococcus* sp., *Cryptomonas* spp., *M. minutum*, *Nitzschia* sp. and *Synechocystis minuscula* Voronichin. In both lakes, *Arthrospira* dominated the overall biomass. Contribution of *Arthrospira* to the phytoplankton community in L. Nakuru ranged from 0 to 98 % of the phytoplankton biomass with an average of 39.1 ± 47.1 (SD) mg L⁻¹. There were pronounced shifts in community composition in L. Nakuru with two *Arthrospira* crashes occurring between December 2008 and March 2009 and September to October 2009. Comparably, in L. Bogoria the phytoplankton community composition was more homogenous, largely (80 %) made up of *Arthrospira* at an average of 68.3 ± 29 mg L⁻¹.

NMDS analysis of phytoplankton species revealed that the significant taxa in the communities responsible for the pattern prevailing in both lakes were *A. fusiformis*, *A. platensis*, *Anabaenopsis* spp., *P. salinarum*, *H. alcalis* and picoplankton not identifiable to a lower level (Fig. 2). The high variability of phytoplankton composition in L. Nakuru and

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predominance of *Arthrospira* in L. Bogoria throughout the sampling period was well depicted. The clustering observed in Fig. 2 was further supported by the groups pruned by LINKTREE routine (Figs 3 and 4). From the dendrogram, the first split A was explained by *A. fusiformis*, separating samples with no *A. fusiformis* (A, 0.0) from the rest of the samples with *A. fusiformis* (> 0.7). This was also illustrated in the MDS ordination (Fig. 2). Picoplankton determined the second split (C) and divided samples that contained other taxa including *A. fusiformis* (< 0.55) and those samples that had mostly picoplankton (> 0.74) resulting into group D. The right partition of nanoflagellates included samples with *P. salinarum* which split into two groups, F1 – associated with samples with no *P. salinarum* (F1, 0) but more of *Arthrospira* and F2 – subsuming samples with more abundance of *P. salinarum* (> 0.23) in addition to other species. The remaining splits were associated with *A. platensis*, with group H including samples with a high abundance (> 0.83) of the taxa and samples in group G containing none (G, 0).

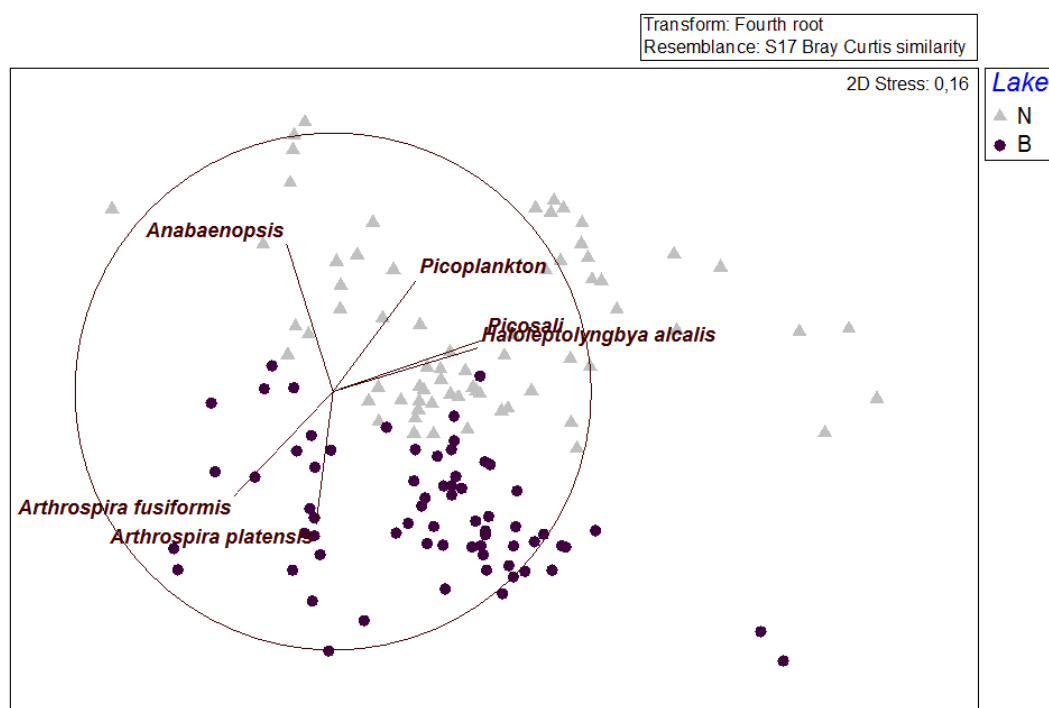


Fig. 2. MSD-plot based on species data. Species with correlations > 0.5 (Pearson) are presented.

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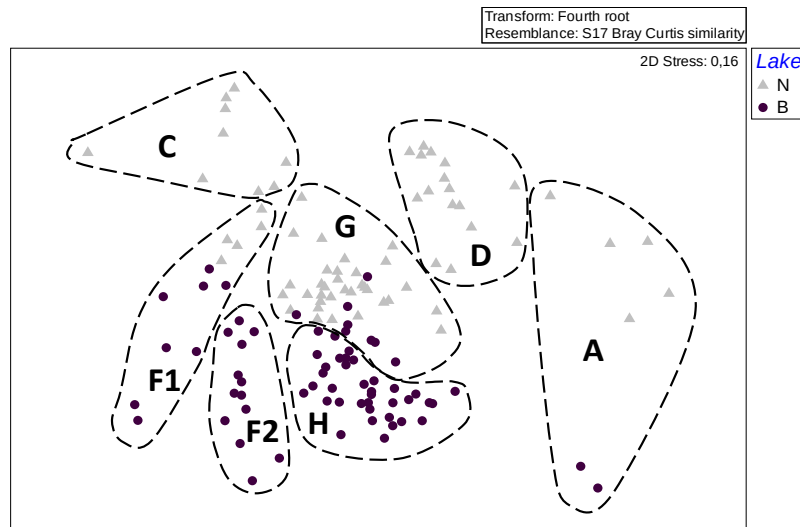


Fig. 3. NMDS-plot of algae taxa. Inclusions indicate groups pruned by the LINKTREE routine based on species data.

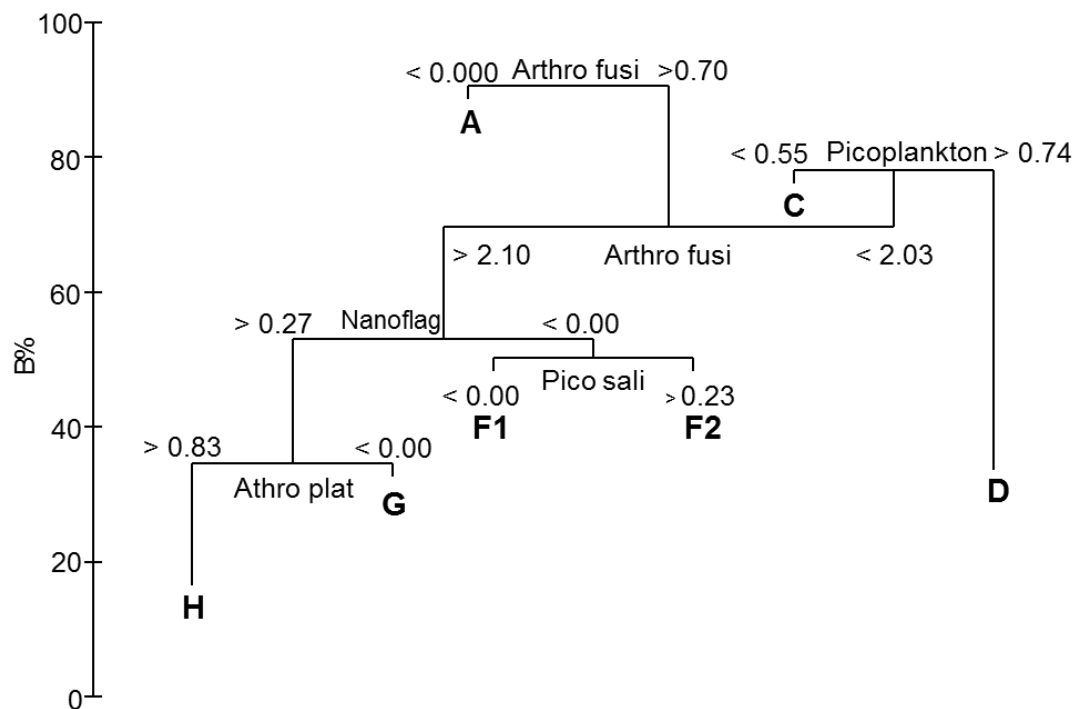


Fig. 4. LINKTREE dendrogram showing significant taxa for pruning groups; labeling corresponds to the NMDS plot (numbers = 4th root transformed taxa biomass).

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Table 3 shows the environmental and biological variables that were used in analyzing the species-environment relationship. Out of the 25 variables, only three significantly ($p < 0.001$) contributed to the species pattern and these included, salinity, pH and ash mass. Relating the species and environmental variables, non-parametric routine BIOENV revealed that *Arthrospira* was absent at the highest ash mass ($> 0.677 \text{ g L}^{-1}$) and salinity ($> 57.8 \text{ ‰}$) levels (Fig. 5) which conditions occurred from end of September to October 2009. Other taxa (see Fig. 4) including *A. fusiformis* (left, C) prevailed at a lower pH (< 10.3) whereas picoplankton (right, C) was present at a higher pH (> 10.4). Picoplankton (D) occurred during *Arthrospira* crash in L. Nakuru (Jan – Mar 09). *Arthrospira* (F1) occurred mainly during periods of *Arthrospira* peaks (Nakuru, Mar – Aug 09; Bogoria, Aug 08 – Oct 09) with salinity less than 48.5 ‰. *P. salinarium* (F2) was more common at high salinity levels ($> 48.6 \text{ ‰}$).

To get an insight into key variables significantly influencing *Arthrospira* abundance, a multiple linear regression was performed with data of L. Nakuru, where the complete dataset including cyanophages was available. Out of 11 variables potentially influencing species abundance like nutrients, irradiance supply or wind speed, grazers or virus infections, three contributed significantly to the model (Fig. 6). Nutrient compositions were of minor importance (high amounts are available), but light penetration (here given as the inverse coefficient of attenuation) seemed to be a key variable. Beside this environmental factor, two more biological variables contributed significantly to the model, i.e. consumers of *Arthrospira* (rotifers and all ciliates larger than $60 \text{ }\mu\text{m}$) and cyanophages.

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Table 3. Environmental and biological variables (mean $\pm$ s.d.) of L. Nakuru and L. Bogoria related post hoc to the phytoplankton community pattern using the non-parametric routine BIOENV method of analysis (variables in **bold** significantly explained the species-environment relationship).

Variable	Nakuru	Bogoria
pH	10.1 $\pm$ 0.2	10 $\pm$ 0.2
Salinity (‰)	29.3 $\pm$ 13.4	43.1 $\pm$ 3.5
Ash mass (g L⁻¹)	0.1 $\pm$ 0.2	0.1 $\pm$ 0.03
Total phosphorus (μ g L ⁻¹)	3371.1 $\pm$ 1876.1	6011.1 $\pm$ 1522
Soluble reactive phosphorus (μ g L ⁻¹)	905.2 $\pm$ 1085.3	3052.6 $\pm$ 679.8
Ammonium-N (mg L ⁻¹)	123.8 $\pm$ 129.1	12 $\pm$ 32.1
Total Nitrogen (mg L ⁻¹)	24 $\pm$ 11.8	4.7 $\pm$ 1.1
Dissolved Nitrogen (mg L ⁻¹)	24.8 $\pm$ 13.3	3.5 $\pm$ 0.4
Nitrate-N (g L ⁻¹)	16.1 $\pm$ 9.5	11.3 $\pm$ 3.4
Temperature (°C)	25.1 $\pm$ 1.9	28.6 $\pm$ 1.5
Coefficient of attenuation	9.5 $\pm$ 3	7.9 $\pm$ 2.1
Chlorophyll- <i>a</i> (mg L ⁻¹)	371.8 $\pm$ 252.4	284.4 $\pm$ 139.8
Dry mass (g L ⁻¹)	0.2 $\pm$ 0.3	0.2 $\pm$ 0.04
Ash free dry mass (g L ⁻¹)	0.1 $\pm$ 0.1	0.2 $\pm$ 0.02
Total alkalinity (meq L ⁻¹)	588.1 $\pm$ 322.8	992 $\pm$ 106.8
Total Organic Carbon (mg L ⁻¹)	443.2 $\pm$ 308.1	59 $\pm$ 13.2
Dissolved Organic Carbon (mg L ⁻¹)	364.3 $\pm$ 212.4	45.4 $\pm$ 2.6
Na (mg L ⁻¹)	17383.8 $\pm$ 9823.1	25446.1 $\pm$ 1657.1
K (mg L ⁻¹)	504.5 $\pm$ 239.7	411.6 $\pm$ 25.6
Si (mg L ⁻¹)	99.6 $\pm$ 26.3	50 $\pm$ 18.6
Wind Speed (km h ⁻¹)	4.2 $\pm$ 1.8	6.6 $\pm$ 1.7
Ciliates (mg C L ⁻¹)	1.8 $\pm$ 2.3	0.7 $\pm$ 1.4
Rotifers (mg C L ⁻¹)	2.1 $\pm$ 3.4	0.6 $\pm$ 1.6
Heterotrophs (mg C L ⁻¹)	4 $\pm$ 1.7	1.8 $\pm$ 0.7
Cyanophages (% infected cells)	1.0 $\pm$ 3.6	1.1 $\pm$ 3.6

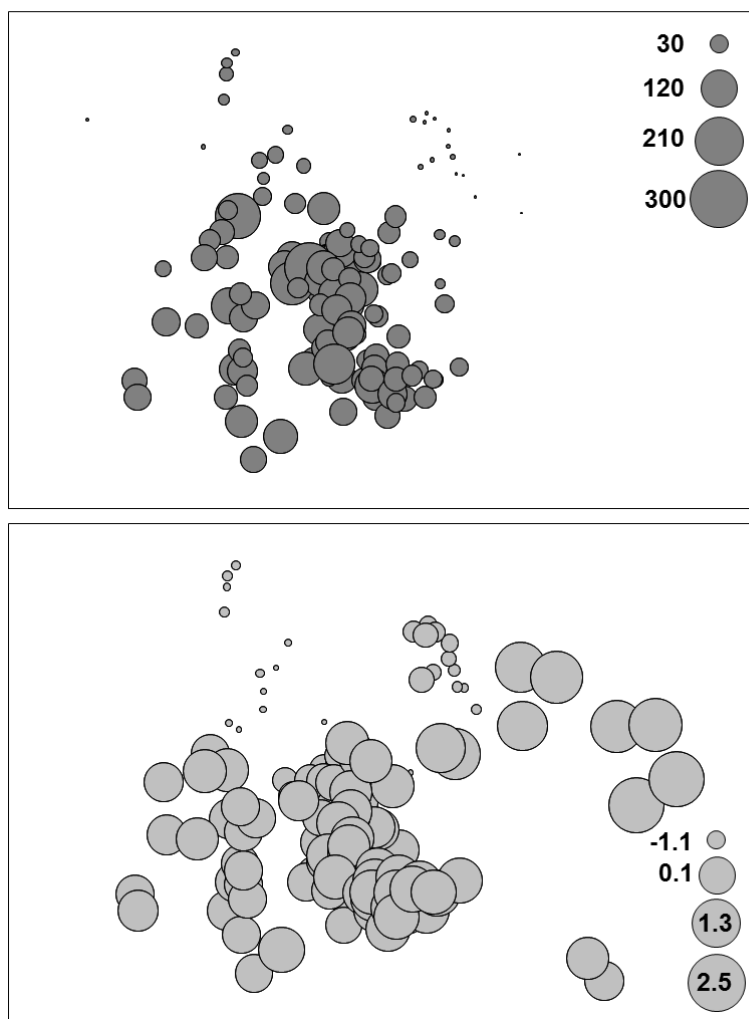


Fig. 5. Bubble plots of *A. fusiformis* biomass (top) and log-transformed salinity data (bottom).

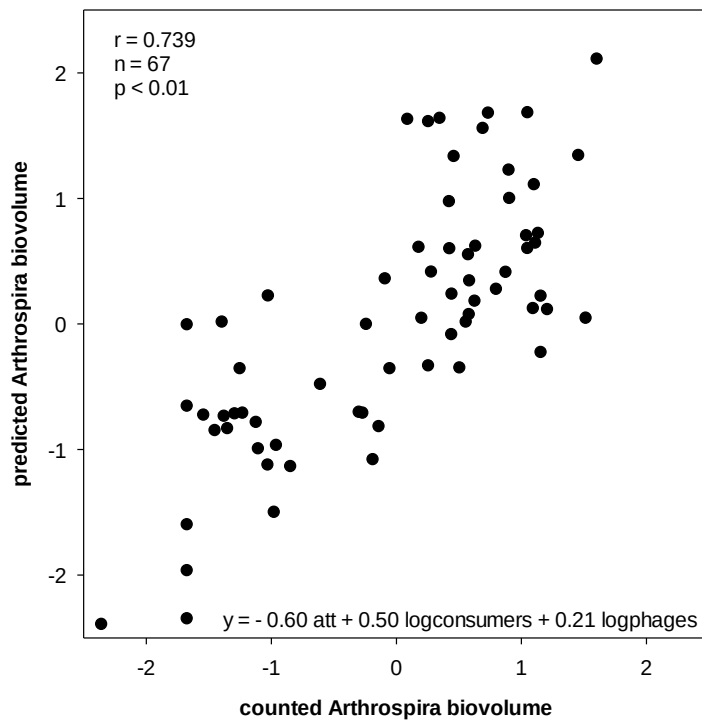


Fig. 6. Results of the linear regression model with forward selection. *Arthrospira* abundance could be explained significantly by irradiance supply, consumers of *Arthrospira* and cyanophages infection.

Table 4. Summary statistics of the linear regression model

explaining variable	non-standardised coefficient	Std-error	Beta	T	p	VIF
Attenuation	-.604	.090	-.603	-6.726	.000	1,117
logConsumr	.504	.090	.506	5.617	.000	1,125
logPhage	.207	.086	.208	2.395	.020	1,047
ANOVA-model	sum of squares	df	mean square	p	F	
regression	36.059	3	12.020	.000	25.282	
non-standardised residuals	29.951	63	.475			
total	66.010	66				
standardised residuals	min	Max	mean	Std-dev	n	
predicted value	-2.464	1.947	.000	1.000	67	
residuals	-2.394	2.173	.000	.977	67	

Discussion

Soda lakes are commonly located in arid (25-200 mm annual precipitation) and semiarid (200-500 mm) regions (Schagerl & Oduor, 2008). Similar to saline lakes elsewhere in the world, they respond quickly and significantly to even small climatic changes because of their discrete nature (Williams, 1981). Garcia et al. (1997) observed that as saline-alkaline lakes become shallower and the surface-to-volume ratio increases, they become more physically controlled. Changes in the seasonal hydrological budget greatly affect such endhoreic lakes which at times may be extreme, resulting in drastic algal biomass crashes (see Table 2) and big changes in community composition (Schagerl & Oduor, 2008, this study).

A discrete separation of the two lakes (Fig. 3) based on taxa abundances pronounced their unique nature. This can be seen as differences in basin morphometry which in turn governs the magnitude of response to the prevailing environmental conditions. L. Bogoria for

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example has been reported to be hydrologically more stable than other endhoreic lakes in Kenya, because of its higher depth (max. about 10 m) in an area of 3000 ha and therefore does not dry out (Harper et al., 2003) as has been observed for L. Nakuru. This may account for the high phytoplankton community shifts accompanied by *Arthrospira* crashes observed in L. Nakuru which is a shallow pan (max. depth depending on the volume about 1 m). The influence of basin morphometry on the stability of the water column has also been observed in other saline lakes, such as, L. Sonachi (MacIntyre & Melack, 1982). In their study MacIntyre & Melack (1982) cited basin morphometry to be one of the main factors contributing to the maintenance of meromixis in this lake as the short fetch reduced the impact of wind mixing by limiting the size of the waves. L. Sonachi is a small volcanic crater lake that is sheltered from wind by crater walls 30–115 m above its surface.

Phytoplankton composition in natural lakes in the temperate climates is influenced mainly by the annual cycles of temperature and thermal stratification, photoperiodicity, nutrient supply and grazing pressure on phytoplankton (Reynolds, 1984; Wetzel, 2001). Conversely in the tropics, the key driving force appears to be the hydrologic cycle, which influences the chemical dynamics of the water column and ultimately their biota (Sondergaard et al., 1990; Schagerl & Oduor, 2008). Schagerl and Oduor (2008) found out that the variation in phytoplankton groups was associated with hydrological stability rather than water chemistry, which only explained 44% of the variance in taxa composition. Due to the high salinity levels (mesosaline, 16–62 ‰) that prevailed during this study, the phytoplankton community was composed largely of cyanobacteria. In a recent genetic study on the phytoplankton community in L. Nakuru, Luo et al. (2013) observed a hidden diversity of eukaryotic plankton which was exhibited during a phase of low salinity (9.7 ‰). *Arthrospira* crashes in L. Nakuru (December 2008 and March 2009 and September to October 2009) created a niche-vacuum that was easily colonized by other phytoplankton taxa which were otherwise suppressed at high *Arthrospira* biomass levels. This led to a manifestation of a more diverse phytoplankton community composition though different governed by the high salinity levels compared to the hidden diversity of eukaryotic plankton observed by Luo et al. (2013) at low salinity levels. L. Nakuru therefore, depending on the prevailing salinity levels has a potential of hosting a diverse range of algal species.

The role of nutrients in community structuring was muted suggesting an absence of nutrient limitation in these lakes as also previously observed by Oduor and Schagerl (2007a).

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Furthermore, our findings were in contrast to the commonly observed control of zooplankton grazers on structuring of phytoplankton communities (e.g., Salmaso & Padisák, 2007; Stoyneva et al., 2007). Microzooplankton in tropical soda lakes have the potential to shape phytoplankton communities (Vareschi & Vareschi, 1984) though when *Arthrospira* dominates the influence of zooplankton is very limited because of low consumer densities and a very high phytoplankton biomass (Vareschi, 1982). We opine therefore that the low biomass levels and feeding rates may have been the reason as to why zooplankton had an insignificant influence in the structuring of the phytoplankton communities in these lakes.

Salinity per se has often been regarded as the major player in structuring biological communities especially in saline lakes (Williams, 1998; Tweed, 2011). From the laboratory experiments carried out by Vareschi (1982) on salinity tolerance of *Arthrospira* the author observed this taxon has a wide salinity tolerance beyond levels that are recorded in the saline lakes. However, Vareschi et al. (1981) reported that changes of algal composition and density in lakes Elmenteita and Bogoria resulted from severe salinity shifts which exceeded the physiological tolerance of *Spirulina platensis* (now *Arthrospira fusiformis*). In the current study *Arthrospira* disappeared at very high salinities (beyond 50‰) which exceeded its physiological tolerance (for optimum growth of *Arthrospira*, 20–70g L⁻¹, FAO, 2008), thereby being outperformed by the chlorophyte, *P. salinarium*. The latter taxon has been observed by Krienitz et al. 2012 to occasionally replace *Arthrospira* in East African saline lakes. The occurrence of this small sized chlorophyte species is of profound significance to the food chain in African soda lakes as such taxa are undesirable to the primary *Arthrospira* consumers - Lesser Flamingos (Krienitz & Kotut, 2010) and may trigger movement of the birds between these lakes.

Alkaliphiles have the ability to adapt to high (9 – 12) pH conditions and extensive studies have been done on species that exist in soda lakes (López-Archilla et al., 2004). The high pH levels in African soda lakes originate from the geochemistry arising from the calcareous rock-beds. It is enhanced further by exceptionally high primary production levels (Talling et al., 1973; Melack 1981) resulting into the hyper-eutrophic nature of these systems which was reflected by the high chlorophyll-*a* values recorded in this study. During this study lakes Nakuru and Bogoria were highly alkaline with a dominance of Na > K > Si > Ca in cations and HCO₃ > CO₃ > Cl > F > SO₄ in anions (Jirsa et al., 2012). This ionic composition was the reason for the observed salinity and pH that significantly influenced the community structure

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with high pH levels coinciding with high salinity levels. Even though *Arthrospira* is known to be well adapted to alkaliphilic conditions (Vonshak & Tomaselli, 2000), its growth was observed to be restricted at pH levels > 10.3 and was outcompeted by picoplankton such as *Synechococcus* and *Synechocystis*. Picoplankton have the advantage of a faster molecular diffusion - very crucial for preventing cellular salt accumulation - and higher generation times of hours (Reynolds, 2006).

Ash mass acted as a surrogate for light attenuation and water turbulence caused by strong winds and consequently leading to turbidity resulting from re-suspension of bottom sediments. Inorganic turbidity tends to be higher in lake basins with larger fetch distances and shallower depths (Wetzel, 2001) which description fits L. Nakuru for example. The significant bird population hosted by L. Nakuru could have contributed further to the sediment re-suspension as they disturb the sediments during feeding. Commonly, in soda lakes inorganic suspended particles are associated with precipitated CaCO_3 contributing to scattering of solar radiation reflected by the high light attenuations recorded in these lakes and are visually conspicuous as 'whitings' (Wetzel, 2001). Some of the observed phytoplankton taxa that occurred in this study are known to have features that enable them to maintain their position in turbid waters so as to maximise light absorption. *Arthrospira* for example, contains gas vesicles which facilitate vertical movement to the light-supplied surface layer (Oduor & Schagerl, 2007b). However, in our case *Arthrospira* appeared to tolerate these conditions only to a certain extent beyond which its growth was hindered as was indicated by its disappearance at the highest ash mass concentrations ($>0.677 \text{ g L}^{-1}$) measured.

Arthrospira is morphologically adapted to withstand the harsh and often unstable conditions as has been proven so far in laboratory settings for some environmental factors such as irradiance (Wu et al., 2005; Helbing et al., 2006), temperature (Gao et al., 2008; Vonshak & Novoplansky, 2008) and salinity (Kebede, 1997), where it modifies the shape of the filament. In L. Nakuru high light attenuation levels arise not only from re-suspended solids but also from self-shading of phytoplankton caused by increased biomass levels. The observed inverse relationship between light attenuation and *Arthrospira* biomass in the current study was indicative of light limitations that prevail especially during *Arthrospira* biomass peaks. Schagerl and Oduor (2008) also observed that variation of *Arthrospira* biomass was related to light attenuation. Algal scums at the surface occurring especially during periods of

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Arthrospira biomass peaks create self-shading problems consequently limiting its growth due to the reduced light penetration.

It has already been shown by Burian et al. (2012) that usually *Arthrospira* zooplankton consumer number is too low to affect *Arthrospira* biomass. The positive relationship rather indicated that the consumer density was controlled by *Arthrospira* abundance (bottom-up control) emphasizing the fundamental role played by *Arthrospira* in ecosystem functioning of these lakes also noted by other authors (Vareschi 1982; Ballot et al., 2004). This observation differed from the common top-down control of phytoplankton by zooplankton grazers (e.g. in L. Tanganyika, Stoyneva et al., 2007) and confirms that the feeding activity of the zooplankters in L. Nakuru is minimal in order to have a top-down control on *Arthrospira* biomass. Reynolds (2006) explains that limitations in filtering activities of grazers depend upon the relation of nutrition, growth and recruitment of grazers and the quantity and quality of food available. Filamentous cyanobacteria that commonly dominate these soda lakes are mostly considered as low-quality food leading to low zooplankton population growth rates (Arnold, 1971; Brett et al., 2006).

The significance of cyanophage (viruses) infections in controlling *Arthrospira* has not been illustrated before in African soda lakes. In a recent study carried out on mass cultures of the related taxon *A. platensis* it was proven that cyanophages are able to infect this genus (Jacquet et al., 2013). In this study we were able to demonstrate for the first time that *Arthrospira* is susceptible to cyanophages in nature Peduzzi et al., 2013. A positive relationship between *Arthrospira* and cyanophages indicated however that in nature *Arthrospira* biomass is not controlled by the phages. They rather occur in a more mutualistic relationship, which already has been observed by other authors (Suttle & Chan, 1994; Wang & Chen, 2004; Mühling et al., 2005). Despite cyanophages having not had a negative effect on *Arthrospira* in this study, their ability to cause breakdowns of phytoplankton communities in these lakes should not be overlooked. In natural waters viruses are known to cause phage-induced mortality (Reynolds, 2006) though their effect varies considerably (Weisse, 2003). Therefore more detailed studies on viruses and their ecological importance in these systems is greatly required as currently almost no information exists.

Shifts in phytoplankton community composition have been associated to the response of the different taxa to environmental stress expressed as changes in physical and chemical variables by various authors (Vareschi, 1982; Oduor & Schagerl, 2007a; Schagerl & Oduor,

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2008; Krienitz & Kotut, 2010). The short-sampling intervals engaged in the current study allowed a comprehensive assessment of the phytoplankton community composition. Phytoplankters have mostly short generation times (Reynolds, 2006) and therefore it was beneficial to conduct data collection over short intervals so as not to miss out on any prevailing taxon and their interactions especially in such dynamic systems. It is most likely that such interactions can easily be muted and therefore a significant part of the plankton ecology may be lost when longer sampling intervals are engaged (Schagerl & Oduor, 2008; Krienitz & Kotut, 2010).

Withal, our findings seem to indicate that phytoplankton community stability maybe strongly governed by the stability of the physical habitat as was depicted by the varied response of the phytoplankton community to environmental oscillations that prevailed in the lakes. However, the magnitude of the effect of the physical habitat needs to be calculated to be proven so, an aspect that was not included in this study. This work also contributed to a further understanding of the important role played by other phytoplankton taxa which though may be less preferred by the largest consumers –Lesser Flamingos– but still may contribute significantly to the energy flow in the overall production in these lakes. Even though *Arthrospira* suffers break downs in L. Nakuru it appears that the system always has an ‘ecological memory’ from which the taxon can be recruited at the onset of favourable conditions. It has a high survivorship because of its superior adaptations and therefore is resilient to the unpredictable changes.

Taking into account the findings from this study together with other research so far conducted on African soda lakes, their subjective response to environmental changes has frequently been highlighted. We hypothesise that over extended periods (for instance, a decade) the factors controlling the phytoplankton community composition and more especially *Arthrospira* will be altered as driven by the changing climatic conditions. This will result into entirely new populations of phytoplankton species emerging and contributing further to phytoplankton diversity in these lakes but also may alter the *Arthrospira*-driven ecosystem functioning. Therefore for effective management purposes of these systems it is worthwhile to have an on-going ecological monitoring to assess these changes and also obtain knowledge on whether they are stochastic or they follow a predictable pattern.

Acknowledgements

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References

- Arnold DE. (1971). Ingestion, assimilation, survival and reproduction by *Daphnia-Pulex* fed / species of blue-green algae. *Limnol Oceanogr* 16:906–920.
- Ballot A, Krienitz L, Kotut K, Wiegand C, Metcalf SJ, Codd AG, Pflugmacher S. (2004). Cyanobacteria and cyanobacterial toxins in three alkaline rift valley lakes of Kenya—Lakes Bogoria, Nakuru and Elmenteita. *J Plankton Res* 26:925–935.
- Ballot A, Krienitz L, Kotut K, Wiegand C, Pflugmacher S. (2005). Cyanobacteria and cyanobacterial toxins in the alkaline crater lakes Sonachi and Simbi. *Harmful Algae* 4:139–150.
- Brett MT, Muller-Navarra CD, Ballantyne PA, Ravet LJ, Goldman RC. (2006). *Daphnia* fatty acid composition reflects that of their diet. *Limnol Oceanogr* 51:2428–2437.
- Burian A, Schagerl M, Yasindi A (2012). Microzooplankton feeding behaviour: grazing on the microbial and the classical food web of African soda lakes. *Hydrobiologia* DOI 10.1007/s1050-012-1023-2.
- Ciferri O. (1981). Let them eat algae. *New Scientist* pp 810–812.
- Ciferri O. (1983). *Spirulina*, the Edible Microorganism. *Microbiol Rev* 4:551–578.
- Clarke KR, Gorley RN. (2006). PRIMER v6: User Manual/Tutorial. PRIMER-E, Plymouth.

Chapter 3

Dadheech KP, Ballot A, Casper P, Kotut K, Novelo E, Lemma B, et al. (2010). Phylogenetic relationship and divergence among planktonic strains of *Arthrospira* (Oscillatoriales, Cyanobacteria) of African, Asian and American origin deduced by 16S-23S ITS and phycocyanin operon sequences. *Phycologia* 49:361–372.

Dadheech KP, Mahmoud H., Kotut K & Krienitz L. (2012). *Haloleptolyngbya alcalis* gen. et. sp. nov., a new filamentous cyanobacterium from the soda lake Nakuru, Kenya. *Hydrobiologia* 691:269–283.

FAO Food and Aquaculture Circular. No. 1034. (2008). Habib BAM, Parvin M, Hantington CT, Hasan RM (eds). A review on culture, production and use of *Spirulina* as food for humans and feeds for domestic animals and fish. FAO: Rome.

Fott B, Karim AGA. (1973). *Spirulina* plankton community in a lake in Jebel Marra, Sudan. *Arch. Protistenkd.* 115:408–418.

Gao K, Li P, Watanabe T, Hebling WE. (2008). Combined effects of ultraviolet radiation and temperature on morphology, photosynthesis and DNA of *Arthrospira* (*Spirulina*) *platensis* (Cyanophyta). *J Phycol* 44:777–786.

Garcia CM, Garcia-Ruiz R; Rendon M, Neill FX, Lucena J. (1997). Hydrological cycle and interannual variability of the aquatic community in a temporary saline lake (Fuente de Piedra, Southern Spain). *Hydrobiologia* 345:131–141.

Hammer T. (1986). *Saline Lake Ecosystems of the World*. Dr W Junk Publishers: Dordrecht.

Harper DM, Childress BR, Harper MM, Boar RR, Hickley S, Mills C, et al. (2003). Aquatic biodiversity and saline lakes: Lake Bogoria National Reserve, Kenya. *Hydrobiologia* 500:259–276.

Helbing WE, Gao K, Ai H, Zengling M, Villafañe EV. (2006). Differential responses of *Nostoc sphaeroides* and *Arthrospira platensis* to solar ultraviolet radiation exposure. *J Appl Phycol* 18:57–66.

Hillebrand H, Durselen DC, Kirschtel D, Pollingher U, Zohary T. (1999). Biovolume calculation for pelagic and benthic microalgae. *J Appl Phycol* 35:403–424.

Chapter 3

Iltis A. (1969a). Phytoplankton des eaux natronees du Kanem (Tchad). I. Les lacs permanents a Spirulines. Cah. O.R.S.T.O.M. Ser. Hydrobiol. 3:29–44.

Iltis A. (1969b). Phytoplankton des eaux natronees du Kanem (Tchad). II. Les mares temporaires. Cah. O.R.S.T.O.M. Ser. Hydrobiol. 3:3–19.

Iltis A. (1971). Phytoplankton des eaux natronees du Kanem (Tchad). V. Les lacs mesohalins. Cah. O.R.S.T.O.M. Ser. Hydrobiol. 5:73–84.

Jacquet S, Zhong X, Parvathi A, Sriram A, Ram P. (2013). First description of a cyanophage infecting the cyanobacterium *Arthrospira platensis* (Spirulina). J Appl Phycol 25:195–203.

Jirsa F, Gruber M, Stojanovic A, Omondi OS, Mader D. (2012). Major and trace element geochemistry of Lake Bogoria and Lake Nakuru, Kenya, during extreme draught. Chemie Erde - Geochemistry <http://dx.doi.org/10.1016/j.chemer.2012.09.001>

Kaggwa NM, Gruber M, Oduor OS, Schagerl M. (2012). A detailed time series assessment of the diet of Lesser Flamingos: further explanation for their itinerant behaviour. Hydrobiologia DOI 10.1007/s10750-012-1105-1.

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.

Kociolek, PJ, Spaulding SA. (2003). Symmetrical naviculoid diatoms. In: Wehr, DJ, Sheath GR (eds). Freshwater Algae of North America: Ecology and Classification. Academic Press: San Diego, pp 637–639.

Komárek, J, Kling H, Komárková J. (2003). Filamentous cyanobacteria. In: Wehr, DJ, Sheath GR (eds). Freshwater Algae of North America: Ecology and Classification. Academic Press: San Diego, pp 117–191.

Komárek, J. (2003). Coccoid and colonial cyanobacteria. In: Wehr, DJ, Sheath GR (eds). Freshwater Algae of North America: Ecology and Classification. Academic Press: San Diego, pp 39–110.

Krienitz L, Ballot A, Kotut K, Wiegand C, Pütz S, Metcalf SJ, et al. (2003). Contribution of hot spring cyanobacteria to the mysterious deaths of Lesser Flamingos at Lake Bogoria, Kenya. FEMS Microbiol Ecol 43:141–148.

Chapter 3

Krienitz L, Bock C, Kotut K, Wei L. (2012). *Picocystis salinarum* (Chlorophyta) in saline lakes and hot springs of East Africa. *Phycologia* 51:22–32.

Krienitz L, Dadheech KP, Kotut K. (2013). Mass developments of the cyanobacteria *Anabaenopsis* and *Cyanospira* (Nostocales) in the soda lakes of Kenya: ecological and systematic implications. *Hydrobiologia* 703:79–93.

Krienitz L, Kotut K. (2010). Fluctuating algal food populations and the occurrence of Lesser Flamingos (*Phoeniconaias minor*) in three Kenyan Rift Valley lakes. *J Phycol* 46:1088–1096.

López-Archilla IA, Moreira D, López-García P, Guerrero C. (2004). Phytoplankton diversity and cyanobacterial dominance in a hypereutrophic shallow lake with biologically produced alkaline pH. *Extremophiles* 8:109–115.

Luo W, Kiplagat K, Krienitz K. (2013). Hidden diversity of eukaryotic plankton in the soda lake Nakuru, Kenya, during a phase of low salinity revealed by SSU rRNA gene clone library. *Hydrobiologia* 702:95–103.

MacIntyre S, Melack MJ. (1982). Meromixis in an Equatorial African Soda Lake. *Limnol Oceanogr* 27:595–609.

Melack JM. (1976). Limnology and dynamics of phytoplankton in equatorial African lakes. Dissertation, Duke University, Durham, North Carolina.

Melack MJ, Kilham P. (1974). Photosynthetic rates of phytoplankton in East African alkaline, saline lakes. *Limnol Oceanogr* 5:743–755.

Melack MJ. (1979). Photosynthesis and growth of *Spirulina platensis* (Cyanophyta) in an equatorial lake (Lake Simbi, Kenya). *Limnol Oceanogr* 4:753–760.

Melack MJ. (1981). Photosynthetic activity of phytoplankton in tropical African soda lakes. *Hydrobiologia* 81:71–85.

Melack MJ. (1988). Primary producer dynamics associated with evaporative concentration in a shallow, equatorial soda lake (Lake Elmenteita, Kenya). *Hydrobiologia*. 158:1–14.

Melack MJ. (1996). Saline and Freshwater lakes of the Kenyan Rift Valley. In: McClanahan, RT, Young PT (eds). *East African Ecosystems and Their Conservation*. Oxford University Press: New York, pp 171–182.

Chapter 3

Melack MJ. (2009). Diel variability and community metabolism in African soda lakes. *Nat Resour Env Iss* 15:28.

Montagnes, DJS, Lynn HD. (1993). A quantitative protargol stain (QPS) for ciliates and other protists. In: Kemp et al. (eds). *Handbook of Methods in Aquatic Microbial Ecology*. Lewis Publishers: Boca Raton, pp 229–240.

Mühling M, FullerJN, Millard A, Somerfield JP, Marie D, Wilson HW et al. (2005). Genetic diversity of marine *Synechococcus* and co-occurring cyanophage communities: evidence for viral control of phytoplankton. *Environ Microbiol* 7:499–508.

Mungoma S. (1990). The alkaline, saline lakes of Uganda: a review. *Hydrobiologia* 208:75–80.

Oduor OS, Schagerl M. (2007a). Phytoplankton primary productivity characteristics in response to photosynthetically active radiation in three Kenyan Rift Valley saline-alkaline lakes. *J Plankton Res* 29:1041–1050.

Oduor OS, Schagerl M. (2007b). Temporal trends of ion contents and nutrients in three Kenyan Rift Valley saline-alkaline lakes and their influence on phytoplankton biomass. *Hydrobiologia* 584:59–68.

Peduzzi P, Gruber M, Gruber M, Schagerl M. (2013). The virus's tooth - cyanophages affect the flamingo population of soda lakes in a bottom up cascade. *Nature* (in review).

Reynolds CS. (1984). Phytoplankton periodicity: the interactions of form, function and environmental variability. *Freshwater Biol* 14:111–142.

Reynolds CS. (2006). *Ecology, Biodiversity and Conservation. Ecology of Phytoplankton*. Cambridge University Press: Cambridge.

Salmaso N, Padisák J. (2007). Morpho-functional groups and phytoplankton development in two deep lakes (Lake Garda, Italy and Lake Stechlin, Germany). *Hydrobiologia* 578:97–112.

Schagerl M, Oduor OS. (2008). Phytoplankton community relationship to environmental variables in three Kenyan Rift Valley saline-alkaline lakes. *Mar Freshwater Res* 59:129–136.

Chapter 3

- Shubert, E. (2003). Nonmotile coccoid and colonial green algae. In: Wehr, DJ, Sheath GR (eds). *Freshwater Algae of North America: Ecology and Classification*. Academic Press: San Diego, pp 253–308.
- Sondergaard M, Jeppesen E, Kristensen P, Sortkjaer O. (1990). Interactions between sediment and water in a shallow and hypertrophic lake: a study on phytoplankton collapses in Lake Søbygard, Denmark. *Hydrobiologia* 191:139–148.
- Stoyneva PM, Descy J-P, Vyverman W. (2007). Green algae in Lake Tanganyika: is morphological variation a response to seasonal changes? *Hydrobiologia* 578:7–16.
- Suttle AC, Chan MA. (1994). Dynamics and distribution of cyanophages and their effect on marine *Synechococcus* spp. *Appl Environ Microbiol* 60:3167–3174.
- Talling JF, Wood BR, Prosser VM, Baxter MR. (1973). The upper limit of photosynthetic productivity by phytoplankton: evidence from Ethiopian soda lakes. *Freshwater Biol* 3:53–76.
- Tuite CH. (1981). Standing crop densities and distribution of *Spirulina* and benthic diatoms in East African alkaline saline lakes. *Freshwater Biol* 11:345–360.
- Tuite HT. (2000). The Distribution and Density of Lesser Flamingos in East Africa in Relation to Food Availability and Productivity. *Waterbirds: The International Journal of Waterbird Biology*, 23:52–63.
- Tweed S, Grace M, Leblanc M, Cartwright I, Smithyman D. (2011). The individual response of saline lakes to a severe drought. *Sci Total Environ* 409:3919–3933.
- Utermöhl H. (1958). Zur Vervollkommnung der quantitativen Phytoplankton-Methodik. *Mitt Int Ver Theor Ang Limn* 9:1–38.
- Vareschi E, Jacobs J. (1985). The Ecology of Lake Nakuru. VI. Synopsis of Production and Energy Flow. *Oecologia* 65:412–424.
- Vareschi E, Melack MJ, Kilham P. (1981). Saline waters. In: Symoens, JJ, Burgis, M & Gaudet, J. (eds). *The Ecology and Utilisation of African Inland Waters*. UNEP: Nairobi.

Chapter 3

Vareschi E, Vareschi A. (1984.) Ecology of Lake Nakuru (Kenya). IV. Biomass and Distribution of Consumer Organisms. *Oecologia* 61:70–82.

Vareschi E. (1978). Ecology of Lake Nakuru (Kenya). I. Abundance and feeding of Lesser Flamingo. *Oecologia* 32:11–35.

Vareschi E. (1982). Ecology of Lake Nakuru (Kenya). III. Abiotic factors and primary production. *Oecologia* 55:81–101.

Vareschi E. (1982). Ecology of Lake Nakuru (Kenya). III. Abiotic factors and primary production. *Oecologia* 55:81–101.

Vonshak A, Novoplansky N. (2008). Acclimation to low temperature of two *Arthrospira platensis* (Cyanobacteria) strains involves down-regulation of PSII and improved resistance to photoinhibition. *J Phycol* 44:1071–1079.

Vonshak A, Tomaselli L. (2000). *Arthrospira (Spirulina)*: Sytematics and Ecophysiology. In: Whitton, AB & Potts M. (eds). *The Ecology of Cyanobacteria*. Kluwer Academic Publishers: The Netherlands, pp 518.

Wang K, Chen F. (2004). Genetic diversity and population dynamics of cyanophage communities in the Chesapeake Bay. *Aquatic Microbial Ecology* 34:105–116.

Wei L, Kotut K, Krienitz L. (2013). Hidden diversity of eukaryotic plankton in the soda lake Nakuru, Kenya, during a phase of low salinity revealed by a SSU rRNA gene clone library. *Hydrobiologia* 702:95–103.

Weisse, T. (2003). Pelagic microbes: protozoa and the microbial food web. In: O’Sullivan PE, Reynolds CS (eds). *The Lakes Handbook Vol. 1*. Blackwell Science: Oxford, pp 417–460.

Wetzel GR, Likens EG. (1991). *Limnological Analyses*. Springer Verlag: New York.

Wetzel GR. (2001). *Limnology Lake and River Ecosystems*. Academic Press: San Diego.

Williams DW. (1981). Inland salt lakes: An introduction. *Hydrobiologia* 81:1–14.

Williams DW. (1998). Salinity as a determinant of the structure of biological communities in salt lakes. *Hydrobiologia* 381:191–201.

Chapter 3

Wu H, Gao K, Villafañe EV, Watanabe T, Helbling WE. (2005). Effects of Solar UV Radiation on Morphology and Photosynthesis of Filamentous Cyanobacterium *Arthrospira platensis*. Appl Environ Microb 71:5004–5013.

Conclusions and Outlook

The present PhD research work provided a much needed in-depth understanding in ecological and physiological changes of the predominant phytoplankton species, *Arthrospira fusiformis* in soda lakes. This original research work involved areas that were yet to be undertaken and the short sampling periods over an extended period of sixteen months enhanced the ability to capture the dynamic occurrences in the soda lakes. In addition, multivariate methods for result analysis made it possible to discover the underlying environmental and biological variables influencing *Arthrospira*'s prevalence and interactions with other components in these ecosystems.

- In all the three papers there was a common pointer of the phytoplankton community in shallow L. Nakuru being more dynamic than the comparably deeper L. Bogoria in response to the environmental, physico-chemical and biological variables.
- The nutritional composition and quality indeed had an effect on the prevalence of Lesser Flamingos at these lakes even though the degree of its influence may also be governed by other prevailing environmental conditions (Chapter 1). This study revealed therefore, that it is not only the phytoplankton biomass and composition that is important in regulating the Lesser Flamingo populations at the soda lakes but also the its nutritional value should be also be sufficient to meet the energy requirements of the birds which is important for them to sustain them during their long flights.
- Morphological changes of *Arthrospira* are highly subjective and therefore indicative of the existing environmental and biological variables as was indicated by the shifts in filament dimensions. *Arthrospira* shapes and sizes therefore would be a cheap and easy to obtain monitoring tool for the biological study in soda lakes (Chapter 2).
- There is a high possibility that factors controlling the phytoplankton community composition and more especially *Arthrospira* will be altered as driven by the changing climatic conditions over extended time periods (Chapter 3). This calls for continuous detailed ecosystem monitoring in order to track these changes which will be useful for long-term effective management of the soda lakes in turn preserving their economic potential.

Conclusions and Outlook

Recommendations

Based on the findings of the present study, the following are areas that could be proposed for further investigation.

- It was observed during the period of the second *Arthrospira* crash in L. Nakuru (see Chapter 1) *Haloleptolyngbya alcaris* pre-dominated the phytoplankton community which had not appeared previously in the community. This happened at the end of study period therefore we were unable to know what happened in the following months. Longer term consistent research studies would be useful in addressing such gaps and to assess whether the algal community shifts are similar and follow a predictable pattern or not.
- There is need to develop further a more robust method that enables one to determine the source of the nutritional component when using organic matter obtained from a raw sample. This will make it possible to determine which portion is contributed specifically by the phytoplankton community.
- Turbulence regime induced by changes in wind speed emerged as an important factor in regulating shifts in *Arthrospira* shapes (see Chapter 2). An in-depth study on the flow of mechanical energy in soda lakes and its effect on structuring the phytoplankton communities would give an insight in transfer of wind driven energy in addition to solar energy.
- Regarding the aspect of *Arthrospira* morphology to be used as a simple and easy tool for monitoring biological stability in soda lakes, I recommend similar morphological studies on *Arthrospira* to be carried out in other soda lakes as well for comparative analysis. This will be useful in determining whether such changes are consistent before they can be applied as a biological monitoring tool.

Curriculum Vitae

Curriculum Vitae

Personal data

Name	Mary Nakabungo Kaggwa
Date of birth	24 March 1976
Contact	kaggwateddy@yahoo.com ; mary.kaggwa@yahoo.com
Gender	Female

Academic qualifications

2004–2005	UNESCO-IHE Institute for Water Education, Netherlands. Master of Science in Environmental Science
2000– 2003	Makerere University – Kampala. Bachelor of Science in Fisheries & Aquaculture
1995–1998	Fisheries Training Institute – Entebbe. National Diploma in Fisheries Management & Technology
1993–1995	St. M. Kalemba Senior Secondary School. Advanced Level Certificate of Education
1989 – 1992	St. M. Kalemba Senior Secondary School. Ordinary Level Certificate of Education

Research involvements and Teaching

2008 (Oct)-To date	Austrian Science Fund Project No. P19911 <i>Factors controlling abundance of Arthrospira fusiformis</i> ''.
2007 (Jan)-2008(Jun)	EU Project, Contract No. 032103 <i>Integrating BOMOSA fish cage farming system in reservoirs, ponds and temporary water bodies in Eastern Africa</i>

Curriculum Vitae

2006 Teaching Fellow. Kyambogo University, Faculty of Science,
Department of Environmental Science

Conferences and seminars

July 2-4, 2010 SIL-AUSTRIA Fresh Blood for Fresh Water. WasserCluster
Lunz, Austria.

April 24-28, 2011 7th International Shallow Lakes Conference. Wuxi, China.

Fields of Interest

Environmental Science: Conservation and sustainable development, Industrialization and
the Environment, Aquatic resource management

Phycology: Algal ecology, water quality assessment, nutrient dynamics,
Algal isolation and culture, Aquatic ecology

Publications records

Peer-reviewed Journals

Kaggwa M.N., Gruber M., Omondi O. S. and Schagerl M., 2012. A detailed time series
assessment of the diet of Lesser Flamingos: further explanation for
their intenerant behaviour. *Hydrobiologia*. DOI 10.1007/s 10750-
012-1105-1

Kaggwa M.N., Liti M. D. and Schagerl M., 2011. Small tropical reservoirs and fish cage
culture: a pilot study conducted in Machakos district, Kenya.
Aquaculture International 19: 839-853

Proceedings

Kaggwa M.N., Gruber M., Omondi O. S. and Schagerl M., 2011. Nutritional value of algal
communities and its implication to the occurrence of Lesser

Curriculum Vitae

Flamingos (*Phoeniconais minor*) in African saline lakes.

Proceedings of the 7th International Shallow Lake Conference. 24-28 April, 2011, Shangai, China.

Kaggwa M.N., Liti M. D. and Schagerl M., 2011. Pioneering small-scale cage fish farming in reservoirs ponds and temporary water bodies in Eastern Africa, BOMOSA Specialized Conference. 2nd-4th September, 2009, Machakos, Kenya.