Contribution of toxic cyanobacteria to massive deaths of Lesser Flamingos at saline-alkaline lakes of Kenya

L. Krienitz, A. Ballot, P. Casper, G.A. Codd, K. Kotut, J.S. Metcalf, L.F. Morrison, S. Pflugmacher, C. Wiegand

Introduction

The saline-alkaline lakes of the African Rift Valley are home to a large population of the Lesser Flamingo (*Phoeniconaias minor* Geoffrey). With 1–3 million individuals, these lakes are established as one of the most fascinating "bird spectacles" of the world (Brown 1959). The population density of the flamingos at the different lakes is subject to wide fluctuations depending on changes in food quantity, breeding activity and episodic mass deaths (Owino et al. 2001; Fig. 1). The main causes of the high flamingo mortalities are thought to be infections by mycobacteria (Sileo et al. 1979, Kock et al. 1999), poisoning

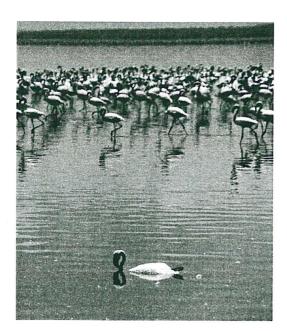


Fig. 1. Flamingos at Lake Nakuru, Kenya in November 2001. In the foreground is a dying Lesser Flamingo.

by heavy metals (KAIRU 1996, NELSON et al. 1998) and pesticides (GREICHUS et al. 1978). Additionally, cyanotoxins are reported to contribute to flamingo die-offs (MOTELIN et al. 2000, NDETEI & MUHANDIKI 2005). BALLOT et al. (2002) and KRIENITZ et al. (2003) detected microcystins and anatoxin-a in dead Lesser Flamingos collected at the Kenyan lakes. ALONSO-ANDICOBERRY et al. (2002) identified microcystins as having caused the death of hundreds of Greater Flamingo (Phoenicopterus ruber L.) chicks in a lagoon of the Donaña National Park, southwest Spain. CHITTICK et al. (2002) reported mortality induced by cyanotoxins of 10 adult captive Chilean Flamingos (Phoenicopterus chilensis MOLINA) at Sea World, Florida, USA. Summarizing the results of a 3year study (2001-2003), we report here three possible major sources of cyanotoxins, which may contribute to the mass deaths of the flamingos at Kenya's Lakes Bogoria and Nakuru.

Key words: African Rift Valley, saline-alkaline lakes, hot springs, Lesser Flamingo, flamingo mass mortality, cyanobacteria, cyanotoxins, *Arthrospira*

Study lakes and methods

The saline-alkaline Lakes Nakuru and Bogoria are situated in depressions of the Eastern (Gregory) Rift Valley. Their morphometric and physico-chemical characteristics are given by BALLOT et al. (2004). Sampling design, microscopy and determination of cyanotoxin concentrations are described by KRIENITZ et al. (2003) and BALLOT et al. (2004).

Results

The phytoplankton of Lake Bogoria is dominated by *Arthrospira fusiformis* (VORONICHIN) KOMÁREK (> 95% of total phytoplankton biomass), a cyanobacterium that constitutes the main food resource of the Lesser Flamingo. In

Table 1. Cyanotoxins in cyanobacteria and Lesser Flamingos from Lakes Nakuru and Bogoria, Kenya (in brackets = numbers of samples collected and analysed in 2001-2003). Toxin concentrations are given in $\mu g \, g^{-1}$ DW for the cyanobacteria and in $\mu g \, g^{-1}$ FW for the flamingo tissues. Where more than two samples were analysed, the toxin data represent minimum, median and maximum concentrations.

Source	Microcystin +)	Anatoxin-a
Cyanobacterial blooms (Phytoplankton)		
Lake Bogoria (9)	9.0-145.0-1164.4	0-3.2-104.1
Lake Nakuru (11)	148.9-748.2-4593.1	5.2-30.6-222.7
Cyanobacterial mats		
Hot Springs Bogoria (18)	0-6.1-836.1	0-0.06-20.2
Cultures of Arthrospira fusiformis		
*)Strain AB2002/10 Bogoria	15.02	10.38
*)Strain AB2002/04 Nakuru	n.d.	0.14
Lesser Flamingo liver		
Lake Bogoria (9)	0-0.2-0.9	0-0.1-4.6
Lake Nakuru (3)	0-0.2-0.4	0-0.03-5.8
Lesser Flamingo stomach/intestine		
Lake Bogoria (2)	0.04-0.2	0.8-4.3
Lake Nakuru (3)	0.03 - 0.05 - 0.2	0.04 - 0.1 - 0.2

⁺⁾ total of microcystin variants calculated as microcystin-LR equivalents; *) according to BALLOT et al. (2004); n.d. = not detectable

addition to A. fusiformis (25-70% of the biomass), heterocystous cyanobacteria (Anabaena and Anabaenopsis spp.) and coccoid picoplanktonic cyanobacteria (Synechocystis and Synechococcus spp.) were observed in Lake Nakuru. Anabaena sp., Anabaenopsis abijatae KEBEDE et WILLÉN and Anabaenopsis arnoldii APTEKAIS produced over 50% of the phytoplankton biomass (BALLOT et al. 2004) throughout several months of the investigation. The mats in the hot springs at Bogoria were dominated by Phormidium terebriformis (AGARDH ex GOMONT) ANAGNOSTIDIS et KOMÁREK, Spirulina subsalsa OERSTED, Oscillatoria willei GARDNER and Synechococus bigranulatus SKUJA. Cyanobacterial hepatotoxins (five structural microcystin variants: MC-LR, -RR, -LA, -LF, -YR) and a neurotoxin (anatoxin-a) were identified in cyanobacterial blooms in Lakes Nakuru and Bogoria, and in cyanobacterial mats growing in hot springs at Bogoria. Toxic strains of Arthrospira fusiformis were isolated from the plankton samples of both lakes. The cyanotoxins were detected in samples of the liver and stomach/intestine of carcases of Lesser Flamingos collected at both lakes (Table 1). In the dying phase, the flamingos showed an opisthotonus behaviour, characterized by the convulsed positions of extremities and neck.

Discussion

The hepatotoxic microcystins and the neurotoxic anatoxin-a are acutely poisonous substances that can kill waterfowl (CARMICHAEL 1994, MATSUNAGA et al. 1999). Investigations on dead Lesser Flamingos from Kenyan lakes revealed microcystins and anatoxin-a in different body tissues in such concentrations that a major contribution to the bird deaths can be expected. The intake of the cyanotoxins by the flamingos is largely via feeding (CODD et al. 2003). By means of its filter-equipped bill, an adult Lesser Flamingo consumes about 72 g dry weight of cyanobacterial food daily (VARESCHI 1978). We found three possible sources in the cyanobacterial food of the flamingos: 1) toxic strains of Arthrospira fusiformis; 2) invading populations of potentially toxic Anabaena and Anabaenopsis in the natural phytoplankton communities; and 3) cyanobacterial mats in hot springs, dominated by potentially toxic Oscillatoriales and Synechococcus.

The main food resources of Lesser Flamingos are dense, almost monocyanobacterial blooms of Arthrospira fusiformis (syn. Spirulina fusiformis VORONICHIN). Variations in the population structure of this highly productive phytoplankton directly impact the flamingos because of their immediate juxtaposition as primary producers and primary consumers. Customarily, dense field populations ("quasimonocultures") and mass cultures of Arthrospira are used as food sources with high nutritional and potential pharmaceutical value (CIFFERI 1983, Belay et al. 1993). Arthrospira is regarded as nontoxic (Fox 1996, SALAZAR et al. 1998). Nevertheless, Iwasa et al. (2002) found hepatotoxic symptoms in a patient after treatment with Arthrospira-based tablets. We isolated two toxic strains of Arthrospira from Lakes Nakuru and Bogoria, which supports the hypothesis that the main food source can also act as the cause of toxicosis.

A widely-held assumption is that *Arthrospira* forms monocyanobacterial mass developments in the East African saline-alkaline lakes. However, our microscopic analyses support the findings of VARESCHI (1982) that the phytoplankton community in Lake Nakuru is subject to wide fluctuations in composition. During 2001–2003 the phytoplankton composition shifted between *Arthrospira*-dominated and *Anabaena/Anabaenopsis*-dominated phases. The latter two genera include toxigenic species that could pose an additional poisoning hazard to the flamingos.

A third source of microcystins and anatoxina is the cyanobacterial mats in hot springs on the Lake Bogoria shoreline (KRIENITZ et al. 2003). The mats covering the ground of rivulets flowing into the lake include genera with potentially toxic species (e.g. Oscillatoria, Phormidium). The flamingos come to these nonsaline rivulets daily to drink and to wash their feathers. Intoxication could occur by ingestion of detached cyanobacterial cells from the mats and/or extracellular cyanotoxin intake during drinking.

Studies in pathology, toxicology, ecology and ethology are in progress to evaluate the multiple stresses on the health and population dynamics of the Lesser Flamingo. Our investigations indicate that cyanotoxins should be included among the major agents contributing to the bird deaths.

Acknowledgements

This study was supported in the frame of the BI-OLOG (Biodiversity and Global Change) initiative of the German Federal Ministry of Education and Research (Grant No. 01LC0001). We thank the Government of Kenya for providing research permission (No. MOEST 13/001/31 C 90). Our sincere appreciation is due to the authorities of the Kenya Wildlife Service and the County Council of Koibotek for granting free access to the sampling sites. We thank the specialists from the WWF for valuable information and discussion.

References

- ALONSO-ANDICOBERRY, C., GARCIA-VILLADA, L., LOPEZ-RODAS, L. & COSTAS, E., 2002: Catastrophic mortality of flamingos in a Spanish national park caused by cyanobacteria. Vet. Rec. 151: 706–707.
- BALLOT, A., PFLUGMACHER, S., WIEGAND, C., KOTUT, K., KRAUSE, E., METCALF, J.S., MORRISON, L.F., CODD, G.A. & KRIENITZ, L., 2002: Cyanobacterial toxins, a further contributory cause of mass deaths of flamingos at Kenyan Rift Valley lakes. Proc. Xth Int. Conf. on Harmful Algae, St. Pete Beach, Florida, 21.-25.10. 2002, 20 pp.
- BALLOT, A., KRIENITZ, L., KOTUT, K., WIEGAND, C., METCALF, J.S., CODD, G.A. & 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.
- BELAY, A., OTA, Y., MIYAKAWA, K. & SHIMAMATSU, H., 1993: Current knowledge on potential health benefits of *Spirulina*. J. Appl. Phycol. 5: 235–241.
- Brown, L.A., 1959: The mystery of the flamingos. Country Life Ltd., London, 116 pp.
- CARMICHAEL, W.W., 1994: The toxins of cyanobacteria. Scientific American 270: 78–86.
- CHITTICK, E., PUSCHNER, B., WALSH, M., GEARHART, S., St. LEGER, J., SKOCELAS, E. & BRANCH, S., 2002: Blue-green algae microcystin toxicosis in captive Chilean flamingos. Proc. Am. Assoc. Zoo Vet. Milwaukee, USA, 115–116.
- CIFERRI, O., 1983: Spirulina, the edible microorganism.
 Microbiol. Rev. 47: 551–578.
- CODD, G.A., METCALF, J.S., MORRISON, L.F., KRIENITZ, L., BALLOT, A., PFLUGMACHER, S., WIEGAND, C. & KOTUT, K., 2003: Susceptibility of flamingos to cyanobacterial toxins via feeding. Vet. Rec. 152: 722–723.
- Fox, R.D., 1996: *Spirulina*: Production & Potential. EDISUD, Aix-en-Provence, 232 pp.

- Greichus, Y.A., Greichus, A., Ammann, B.B. & Hopcraft, J., 1978: Insecticides, polychlorinated biphenyls and metals in African lake ecosystems. III. Lake Nakuru, Kenya. Bull. Environ. Contam. Toxicol. 19: 454–461.
- IWASA, M., YAMAMOTO, M., TANAKA, Y., KAITO, M. & ADACH, Y., 2002: Spirulina-associated hepatotoxicity. – Am. J. Gastroenterol. 97: 3212–3213.
- KAIRU, J.K., 1996: Heavy metals residues in birds of Lake Nakuru, Kenya. Afr. J. Ecol. 34: 397–400.
- KOCK, N.D., KOCK, R.A., WAMBUA, J., KAMAU, G.J. & MOHAN, K., 1999: Mycobacterium avium-related epizootic in free-ranging Lesser Flamingos in Kenya. – J. Wildl. Dis. 35: 297–300.
- KRIENITZ, L., BALLOT, A., KOTUT, K., WIEGAND, C., PÜTZ, S., METCALF, J.S., CODD, G.A. & PFLUGMA-CHER, S., 2003: Contribution of hot spring cyanobacteria to the mysterious deaths of Lesser Flamingos at Lake Bogoria, Kenya. – FEMS Microbiol. Ecol. 43: 141–148.
- MATSUNAGA, H., HARADA, K.I., SENMA, M., ITO, Y., YASUDA, N., USHIDA, S. & KIMURA, Y., 1999: Possible cause of unnatural mass death of wild birds in a pond in Nishinomiya, Japan: sudden appearance of toxic cyanobacteria. Nat. Toxins 7: 81–84.
- MOTELIN, G., THAMPY, R. & DOROS, D., 2000: An ecotoxicological study of the potential roles of metals, pesticides and algal toxins on the 1993/5 Lesser Flamingo mass die-offs in Lake Bogoria and Nakuru, Kenya; and the health status of the same species of birds in the Rift Valley Lakes during the 1990s. Proc. East African Environmental Forum, Nairobi, May 2000, 11–12.
- NDETEI, R. & MUHANDIKI, V.S., 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.
- Nelson, Y.M., Thampy, R.J., Motelin, K., Raini, J.A., Disante, C.J. & Lion, L.W., 1998: Model for trace metal exposure in filter-feeding flamingos at alkaline Rift Valley lakes, Kenya. Environ. Toxicol. Chem. 17: 2302–2309.
- OWINO, A.O., OYUGI, J.O., NASIRWA, O. & BENNUN, L.A., 2001: Patterns of variation in waterbird numbers on four Rift Valley lakes in Kenya, 1991–1999. Hydrobiologia **458**: 45–53.
- SALAZAR, M., MARTINEZ, E., MADRIGAL, E., RUIZ, L.E. & CHAMORRO, G.A., 1998: Subchronic toxicity study in mice fed *Spirulina maxima*. – J. Ethnopharmacol. 62: 235–241.
- SILEO, L., GROOTENHUIS, J.G., TUITE, C.H. & HOPCRAFT, B.D., 1979: Mycobacteriosis in the Lesser Flamingos of Lake Nakuru, Kenya. J. Wildl. Dis. 15: 387–389.
- VARESCHI, E., 1978: The ecology of Lake Nakuru (Kenya). I. Abundance and feeding of the Lesser Flamingo. Oecologia 32: 11–35.
- VARESCHI, E., 1982: The ecology of Lake Nakuru (Kenya). II. Abiotic factors and primary production. Oecologia 55: 81–101.

Author's addresses:

- L. KRIENITZ, A. BALLOT, P. CASPER, S. PFLUGMACHER, C. WIEGAND, Leibniz-Institute of Freshwater Ecology and Inland Fisheries, D-12587 Berlin, Germany. Email: krie@igb-berlin.de
- G.A. CODD, J.S. METCALF, L.F. MORRISON, School of Life Sciences, University of Dundee, Dundee DD1 4HN, UK.
- K. KOTUT, Botany Department, Kenyatta University, P.O. Box 43844. Nairobi, Kenya.