Amer et al. Flamingo 2018, pages: 71-75

SHORT REPORT

Towards understanding lesser flamingo unpredictability in East Africa; what might cause crashes of their major food item at a lake?

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Abstract

Very little is known about the factors which can cause collapse of the major food of lesser flamingos (*Phoeniconaias minor*) in East Africa, the cyanobacterium phytoplankton *Arthrospira* spp. (usually *A. fusiformis*), although it is known that collapses frequently occur. One possibility is infection by virus - cyanophages. We examined water samples from Lake Bogoria and found many different viruses, which could be infectious. We studied the density of *Arthrospira* spp. over a two-year period and found a crash in July 2016 at the same time as the maximum density of Virus-Like Particles (VLP) in the lake water; we achieved break-up of laboratory cultures grown in July 2016 lake water. We were not able to identify the virus with genetic analysis, but this is the first time that population collapse through cyanophage infection has been indicated.

Resumen

Se sabe muy poco acerca de los factores que pueden causar el colapso del alimento principal de los flamencos menores *(Phoeniconaias minor)* en África oriental, el fitoplancton de cianobacterias *Arthrospira spp.* (usualmente *A. fusiformis*), aunque se sabe que frecuentemente ocurren colapsos. Una posibilidad es la infección por virus - cianófagos. Examinamos muestras de agua del lago Bogoria y encontramos muchos virus diferentes, que podrían ser infecciosos. Se estudió la densidad de *Arthrospira spp.* durante un período de dos años y encontró un choque en julio de 2016 al mismo tiempo que la densidad máxima de partículas similares a virus (VLP) en el agua del lago; Logramos la ruptura de cultivos de laboratorio cultivados en julio de 2016 en el agua del lago. No pudimos identificar el virus con el análisis genético, pero esta es la primera vez que se indica el colapso de la población a través de la infección por cianófagos.

Résumé

On sait très peu de choses sur les facteurs susceptibles de provoquer l'effondrement de l'aliment principal des flamants nain (*Phoeniconaias minor*) en Afrique de l'Est, le phytoplancton à cyanobactérie *Arthrospira spp*. (généralement *A. fusiformis*), bien qu'il soit connu que des effondrements se produisent fréquemment. Une possibilité est

l'infection par desvirus - les cyanophages. Nous avons examiné des échantillons d'eau du lac Bogoria et découvert de nombreux virus pouvant être infectieux. Nous avons étudié la densité d'*Arthrospira spp*. sur une période de deux ans et nous avons trouvé un crash en juillet 2016 en même temps que la densité maximale de particules pseudo-virales (VLP) dans l'eau du lac; nous avons réussi à reproduire des effondrement dans les cultures au laboratoire de souches cultivées en juillet 2016 de l'eau du lac. Nous n'avons pas été en mesure d'identifier le virus par analyse génétique, mais c'est la première fois qu'un effondrement de population dû à une infection à cyanophage est mise en évidence.

Introduction

The population of lesser flamingos (*Phoeniconaias minor*) in East Africa is believed to be about 1.5 million (Childress et al., 2007), but there has never been a concurrent census in all of the main countries where the species occurs (Ethiopia, Kenya, Tanzania). In the one country where regular counts are undertaken, Kenya, through the annual Nature Kenya/Kenya Wildlife Services water bird count, there have been major differences in the numbers counted on the accessible alkaline-soda lakes (Lakes Bogoria, Nakuru, Elementeita and Magadi) from one year to the next, between a few thousands and over a million (Owino et al., 2001). These dramatic differences have led some ornithologists to believe that the population is under decline (Harper et al., 2016). So too have irregular occurrences of population mortalities (Kock et al., 1999; Ndetei and Muhandiki, 2005; Straubinger-Gansberger et al., 2014).

Those that occurred in August through mid-November 1993 (Kock et al., 1999) and with several hundred thousand deaths in 2000-1 (Harper et al., 2003), were caused by infectious disease at the high densities of birds that can be stressed after flying in from a lake with declining food (Oaks et el., 2006; Harper et al., 2016). Irregular reproductive events in this species, at a single location - Lake Natron, Tanzania - are enough to replace such mortalities and, most recently (July 2018), over 1.1 million birds were counted at Lake Bogoria alone, so the likelihood of a serious population decline is low.

One main reason the birds are unpredictable in their occurrences is that the number of lakes where they can feed is few and their main food, the cyanobacteria *Arthrospira* spp (commonly called Spirulina) that dominates the plankton can wax and wane unpredictably (Kaggwa et al., 2013). Some of the fluctuations in *Arthrospira* abundance are caused by hydrology, because many of the alkaline-soda lakes are shallow, fluctuating in area and in water chemistry considerably according to the rains (Krienitz and Kotut, 2010; Kaggwa et al., 2013). In others, such as Lake Bogoria, a moderately deep lake whose water chemistry is very consistent, crashes in the *Arthrospira* population cannot, at present, be explained by environmental factors (Harper et al., 2003). Large crashes have occurred in the past with locally-dramatic results; a crash in August-September 2004 (Tebbs et al., 2013), for example resulted in the complete de-oygenation of the lake through decomposition of the cyanobacterial mass, producing an odour that was detected up to 10 miles away and resulting in a total departure of lesser flamingoes (William Kimosop, pers comm.).

Evaluation

Cyanophage causes of *Arthrospira* bloom collapse

We have been examining the possibility that *Arthrospira* crashes are caused by infection of viruses, cyanophages, because it has no grazers other than lesser flamingoes. It is known that cyanophages can control populations of marine cyanobacteria, but freshwater species have rarely been investigated before. We found that potential agents were abundant in appropriately

filtered water, examined under the Transmission Electron Microscope, TEM, (Figure 1). The dominant types of phage were of the Siphoviridae, Myoviridae and Podoviridae morphotype, however a large proportion of indistinguishable phage types mean further anatomical study is necessary.

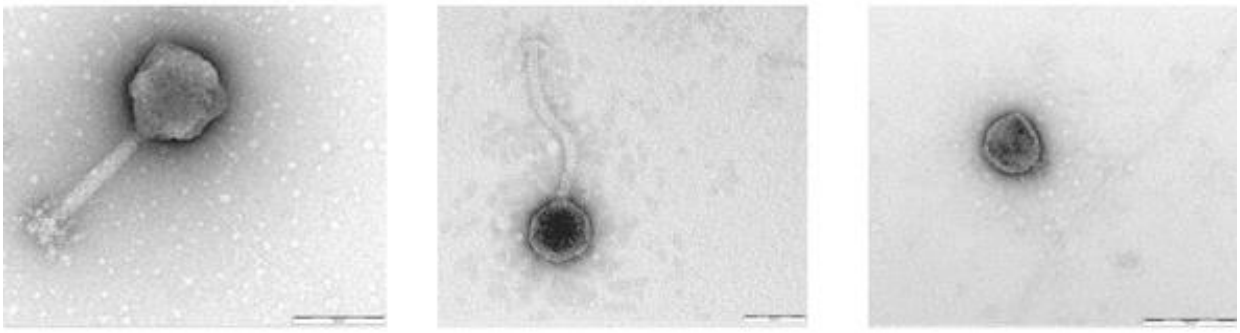

Figure 1: TEM photographs of virus from Lake Bogoria water. Scale bar = 100 nm; a) Myoviridae morphotype, b) Siphoviridae morphotype, c) Podoviridae morphotype

We collected weekly samples of water from the lake between May 2015 and May 2017, examining *Arthrospira* density and biomass as chlorophyll 'a'. The lake was very high during this period, due to heavy rains that have been experienced in this central part of the Eastern Rift Valley since 2010, and its conductivity was initially about half the 'normal' level due to dilution but rising from 41 - 66 $mScm^{-1}$ over this period, compared with the usual concentration of 72-77 $mScm^{-1}$. The biomass of *Arthrospira* was also lower than it had been recorded by several authors during the late 20th-early 21st Century, fluctuating between 180 and 350 μgL^{-1}, but gradually increasing with over the two years (Figure 2). We found that a population crash occurred in July 2016, which was coincident with Virus Like Particles (VLPs) resembling phages in the lake water detected at their highest density (1.755×10^{8} mL^{-1}) over the study period, using a 'NanoSight' instrument. VLPs were also detected within *Arthrospira* sections under Electron Microscopy. Laboratory cultures of *Arthrospira* in lake water with VLPs showed symptoms of phage infection within 5 days of incubation, as appearance of visible fragments of filaments, which indicate of host cell lysis.

Conclusions

We have tried to identify the viruses using genetic sequencing, but metagenomic results showed no hit with any in the Genebank Database, most likely because the database does not contain enough virus genomes at present. The greatest probability is that the cyanophages may be RNA viruses ((+) ssRNA virus), based on their size. A natural progression of our study would thus be to perform RNA sequencing, as this would provide definitive evidence as to whether the particles observed in the lake water are phages, also those seen within *Arthrospira,* or just natural cell inclusions. Nevertheless, we provide here and will shortly publish in full, the first evidence that an *Arthrospira* population crash in one lake could well have been caused by virus infection.

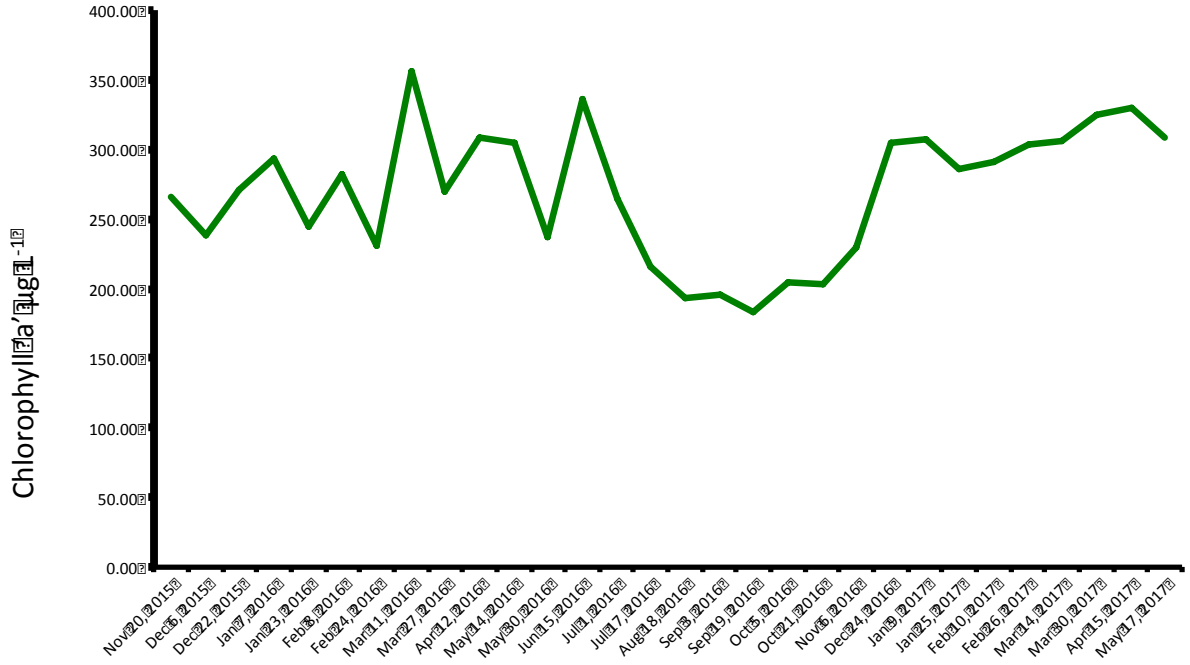

Figure 2: Arthrospira *density at Lake Bogoria 2015-7 as Chlorophyll 'a' biomass calculated from Landsat satellite images using the formula of Tebbs et al. (2013).*

Acknowledgements

This work was carried out whilst DMH & NP were affiliated to the National Museums of Kenya and with a NACOSTI Research Permit issued to DMH. We thank the Senior Warden at Lake Bogoria National Reserve and the Local Community for access permission to collect samples and process them in the laboratory at Reserve HQ. We thank the Libyan Government for funding the PhD of ASMA, which made all this work possible.

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