Species-specific separation of lake plankton reveals divergent food assimilation patterns in rotifers

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SUMMARY

1. The analysis of functional groups with a resolution to the individual species level is a basic requirement to better understand complex interactions in aquatic food webs. Species-specific stable isotope analyses are currently applied to analyse the trophic role of large zooplankton or fish species, but technical constraints complicate their application to smaller-sized plankton.

2. We investigated rotifer food assimilation during a short-term microzooplankton bloom in the East African soda lake Nakuru by developing a method for species-specific sampling of rotifers.

3. The two dominant rotifers, Brachionus plicatilis and Brachionus dimidiatus, were separated to single-species samples (purity >95%) and significantly differed in their isotopic values (4.1‰ in δ¹³C and 1.5‰ in δ¹⁵N). Bayesian mixing models indicated that isotopic differences were caused by different assimilation of filamentous cyanobacteria and particles <2 μm and underlined the importance of species-specific sampling of smaller plankton compartments.

4. A main difference was that the filamentous cyanobacterium Arthrospira fusiformis, which frequently forms blooms in African soda lakes, was an important food source for the larger-sized B. plicatilis (48%), whereas it was hardly ingested by B. dimidiatus. Overall, A. fusiformis was, relative to its biomass, assimilated to small extents, demonstrating a high grazing resistance of this species.

5. In combination with high population densities, these results demonstrate a strong potential of rotifer blooms to shape phytoplankton communities and are the first in situ demonstration of a quantitatively important direct trophic link between rotifers and filamentous cyanobacteria.

Keywords: Brachionus plicatilis, cyanobacteria, dietary sources, Lake Nakuru, size fractionation, stable isotopes, zooplankton

Introduction

Microzooplankton are the main consumers in marine systems, ingesting more than half of the daily primary production across all major oceanic biomes (Calbet and Landry, 2004). Similarly, consumption and production of microzooplankton in inland waters can easily surpass that of larger crustacean zooplankton with higher community biomass (Bogdan and Gilbert, 1982; Vareschi and Jacobs, 1985). Microzooplankton also affects the quantity and quality of energy flows to higher trophic levels: repacking of small particles by microzooplankton increases the amount of available food, while upgrading of biochemical composition can lead to higher food quality for secondary consumers (Klein Breteler et al., 1999).

In freshwater systems, rotifers are often a substantial part of microzooplankton. Despite their ecological importance, they are rarely incorporated into in situ food-web studies (Chick et al., 2010), and rotifer grazing rates and feeding behaviour are typically studied only under laboratory conditions (e.g. Starkweather and Kel lar, 1983; Ka et al., 2012). In natural systems, examining the broad variety of available food types, ranging from algae and heterotrophic protists to bacteria and detritus, remains a fundamental challenge for food-web ecologists.
There are several methodological problems impeding in situ studies of rotifers. Gut content analyses usually cannot be performed since grinding mouthparts homogenise food particles. Biomarkers such as stable isotopes or fatty acids, on the other hand, are limited by minimum mass requirements and the difficulties of isolating rotifers, whose size frequently overlaps with other plankton groups (Fernando, 1994; Work et al., 2005). Together, this commonly results in size fractionation of seston samples and pooling of different functional groups when lower levels and microbial components of food webs are examined.

East African soda lakes have several times been proposed as ideal model systems for food-web studies (e.g. Vareschi and Jacobs, 1985) due to high productivity, but low diversity at most trophic levels (Melack and Kilham, 1974; Vareschi and Jacobs, 1984). Accordingly, also metazoan zooplankton are frequently dominated by only two rotifers (Ilitís and Riou-Duwart, 1971; Vareschi and Vareschi, 1984), which can form blooms with staggering densities of over $6 \times 10^5$ ind. L$^{-1}$ (Ilitís and Riou-Duwart, 1971). Such short-term peaks of extreme biomass do not only seem very suitable for studying microzooplankton food assimilation at a single-species level, but are also an important ecological feature of tropical soda lakes, affecting their long-term plankton community structure.

The phytoplankton of Lake Nakuru, a well-studied African soda lake, is either dominated by the filamentous cyanobacterium *Arthrospira* *fusiformis* or by a more diverse community of micro- and nanoalgae (Vareschi, 1982; Ballot et al., 2004; Schagerl and Oduor, 2008), and grazing of the two dominant rotifers, *Brachionus* *dimidiatus* and *Brachionus* *plicatilis*, has been suggested as one of the potential factors leading to a transition between these alternative phytoplankton communities (Melack, 1988). Such transitions alter the food-web structure of the entire lake and can critically affect the food availability of the plankton-filtering lesser flamingo (Harper et al., 2003; Krienitz and Kotut, 2010), one of the most important umbrella species of East Africa. However, in situ grazing behaviour of rotifers is currently poorly understood, which makes assumptions highly speculative and underpins the need for techniques that facilitate the investigation of single functional plankton groups, ideally at a single-species level.

We examined interspecific differences in food assimilation of rotifers during a microzooplankton biomass outbreak in Lake Nakuru. Such events occur only occasionally and last for a short period of time, but can potentially cause critical transitions in plankton communities of African soda lakes. Therefore, we restricted our study to a snapshot analysis of micro- and mesoplankton communities and focused instead on the development of effective separation methods for lake plankton to explore the resource dependence and selection for rotifer blooms.

**Methods**

**Sampling design and methods**

The sampling site was a central off-shore station of Lake Nakuru, a shallow saline-alkaline lake in the East African Rift Valley (Fig. 1). All required permits were obtained for entry into the park and sampling in the lake. At the time of sampling, the maximum water level of Lake Nakuru, an endorheic system with large interannual lake-level fluctuations, was 1.2 m. Surface water samples were collected on 7 April 2009 using a Schindler trap (10 L). Temperature, specific conductivity, salinity, pH, dissolved oxygen and Secchi depth were measured on site. Water samples were analysed for dissolved organic carbon (DOC; TOC-VCPH analyser; Schimadzu, Kyoto, Japan), nutrient concentrations (modified spectrophotometric standard procedures), chlorophyll *a* (cold acetone extraction), bacteria (CYBR green stain), protozoa (quantitative protargol stain) and phytoplankton and zooplankton abundance (for details, see supporting Fig. 1 Map of East Africa and the catchment of Lake Nakuru, Kenya. The sampling station of this study ($500°21'12'', E036°05'00''$) is plotted in the map.

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information Data S1). Total carbon content of different groups was calculated from species abundance, average species biovolumes and specific bioconversion factors (Table S1).

At the time of sampling, plankton groups widely overlapped in size. To obtain species-specific samples of phyto- and microzooplankton for stable isotope analysis, we first removed mesozooplankton with a 250 μm sieve, concentrated rotifers and filamentous cyanobacteria with a 40 μm sieve and used phototaxis to separate phyto- from zooplankton: the concentrated 40 to 250 μm fraction was set up in petri dishes (20 cm diameter) in a dark room with small cold light spots fixed directly above one edge of each petri dish. Rotifers were attracted by light of intermediate intensity, whereas cyanobacteria were equally distributed across petri dishes. This allowed us to pipette dense swarms of rotifers into new petri dishes. Repetition of this procedure led to pure rotifer samples.

B. dimidiatus and B. plicatilis. These two species could be further separated by filtration through 150 and 100 μm sieves. Fractions <100 and >150 μm contained species-specific rotifer samples.

Pre-filtered (250 μm) and concentrated (40 μm) samples were allowed to settle in glass cylinders (1 L each). Buoyancy caused an accumulation of cyanobacteria in top layers, which were pipetted off and repeatedly filtered through a 100 μm sieve to remove protozoans. This yielded a purified cyanobacteria sample (>40 μm). Particles <40 μm were size-fractionated through filters (nylon, Millipore, Darmstadt, Germany) to obtain different plankton size classes: (i) 20–40 μm, (ii) 2–20 μm and (iii) <2 μm. All plankton samples were collected on precombusted glass-fibre filters (Whatman GF/F) and dried (60 °C; 24 h). Rotifers were kept without food for 2 h to facilitate gut evacuation. Subsamples were taken for direct counts and biomass quantification to verify taxonomic purity.

For dissolved organic matter (DOM) samples, GF/F filtered lake water (100 mL) was acidified to pH 2, dried at 60 °C and the residue homogenised. This method was applied successfully due to extremely high DOM concentrations. Sediment samples were repeatedly rinsed with HCl (1%) to remove carbonates and subsequently dried (60 °C).

Stable isotope values of other aquatic animals were investigated to provide points of reference for microzooplankton samples. Mesozooplankton were collected using a plankton net (250 μm), and species-specific samples were picked with forceps. Muscle tissue of the only fish species of Lake Nakuru, Oreochromis alcalicus grahami, was taken from behind the pectoral fin of adult individuals, solvent-rinsed (chloroform) for 12 h to remove lipids, dried and homogenised for stable isotope analysis.

Samples of the dominating macrophyte of the littoral zone (Cyperus laevigatus) and allochthonous plant detritus particles from the main river inflow (River Njoro) were collected for stable isotope analysis to evaluate the contribution of non-pelagic sources to the planktonic food web.

**Stable isotope and data analysis**

C and N isotopic analyses were performed by combusting samples (triplicates) using a Flash EA 1112 series elemental analyzer (Thermo Finnigan, Milano, Italy) coupled to a Delta Plus XP IRMS (Thermo Electron, Karlsruhe, Germany), at the University of Cape Town. All stable isotope values are reported in the δ notation where δ\(^{13}\)C or δ\(^{15}\)N = [(Rsample/Rstandard) – 1]·1000, where R is \(^{13}\)C/\(^{12}\)C or \(^{15}\)N/\(^{14}\)N. Global standard for δ\(^{13}\)C is PeeDee Belemnite and for δ\(^{15}\)N is atmospheric nitrogen, whereas ‘Evian’ water and Cape Town Mountain precipitation water were used as internal standards. Measurement errors were <0.10‰ for δ\(^{13}\)C and <0.18‰ for δ\(^{15}\)N. The plankton samples <40, 20–40 and 2–20 μm consisted of two cyanobacteria (together >99% total biomass), but the biomass ratio between the cyanobacteria changed in different size fractions (see results). We inverted a classical isotope mixing model and calculated the species-specific isotope signals of the two cyanobacteria based on their relative contributions to mixtures,

\[
as1 + bS2 = M1
\]

\[
cS1 + dS2 = M2,
\]

where a, b, c and d are the known relative contributions to mixtures 1 (M1) and 2 (M2), and S1 and S2 are the sources 1 and 2. We solved this system of two equations with two unknowns and obtained δ\(^{13}\)C values for each cyanobacterium species.

The contribution of potential food sources to the rotifer diet was assessed using SIAR, a Bayesian mixing model (Parnell et al., 2010), coded for the software package R, version 2.11 (R Development Core Team, 2010). We followed the general recommendation to use group-specific instead of general fractionation values for the configuration of mixing models (McCutchan et al., 2003; Vanderklift and Ponsard, 2003) and have listed the respective values and the assumed uncertainty of these values in the supplementary information (Section S2).
Results

Physiochemical and biological characteristics

During sampling, Lake Nakuru was saline (35\%\textsubscript{o}o), had a maximum depth of 1.2 m and was characterised by high P and N concentrations (Table 1). Accordingly, chlorophyll a levels reached 364 $\mu$g L$^{-1}$, reflecting hypereutrophic conditions. The two filamentous cyanobacteria *Arthrospira fusiformis* and *Anabaenopsis elenkinii* accounted for 96\% of the algal biomass (13.4 g C m$^{-3}$; Table 2). Biomass of *A. fusiformis* was 3.2 X higher than that of *A. elenkinii*. Both taxa were characterised by a widely varying filament size range, with a tendency of *A. fusiformis* to form larger filaments.

Zooplankton were dominated by rotifers (11.3 g Cm$^{-3}$) as crustacean densities were very low (Table 2). Rotifer biomass was equally divided between the larger *B. plicatilis* (276 ± 26 $\mu$m body length) and the smaller, more abundant *B. dimidiatus* (132 ± 9 $\mu$m). The large, omnivorous ciliates *Holophrya* and *Frontonia* contributed appreciably (0.9 g C m$^{-3}$) to total zooplankton biomass. Surprisingly, however, the biomass of small bacterivorous protozoans (2–60 $\mu$m) was very low, despite bacterial densities of over 2 × 10$^7$ cells mL$^{-1}$ (Table 2).

Isotope signatures and sample purification

The two filamentous cyanobacteria contributed >99\% of the biomass of three plankton size fractions (2–20, 20–40 $\mu$m, phytoplankton ≥40 $\mu$m). However, in the larger size classes, the relative biomass ratios of the two algae changed and the contribution of *A. elenkinii* decreased. This shift in the relative contribution of *A. elenkinii* was reflected in large changes in $\delta^{13}$C values with increasing mesh size. Based on these differences, we applied an inverted end-member mixing model to calculate the species-specific $\delta^{13}$C values of *A. elenkinii* and *A. fusiformis*, which revealed a difference of 7.5\%\textsubscript{o}o between the two cyanobacteria (Fig. 2). As there was no significant difference in $\delta^{15}$N of the three cyanobacteria-dominated samples (ANOVA; $P = 0.29$), a mean $\delta^{15}$N value of 4.2\%\textsubscript{o}o was assumed for both species.

The two species-specific rotifer samples (purity of >96\%) differed significantly, in both their $\delta^{13}$C and $\delta^{15}$N values (Table S2; $P < 0.05$; t-test). Surprisingly, the $\delta^{15}$N values of the smaller *B. dimidiatus* were 1.5\%\textsubscript{o}o higher than those of the larger *B. plicatilis*. Likewise, the $\delta^{13}$N value of the <2 $\mu$m plankton size fraction (6.7\%\textsubscript{o}o),

| Parameter | Value |
|-----------|-------|
| Temp [°C] | 22.1 |
| O$_2$ [mg L$^{-1}$] | 18.8 |
| pH | 10.21 |
| Conductivity [mS cm$^{-1}$] | 49.4 |
| Salinity [%o] | 34.9 |
| Coefficient of attenuation [m$^{-1}$] | 0.14 |
| Dissolved organic carbon [mg L$^{-1}$] | 344.30 |
| Soluble reactive phosphorus [µg L$^{-1}$] | 1450 |
| Ammonium-N [µg L$^{-1}$] | 711 |
| Nitrate-N [µg L$^{-1}$] | 705 |
| Nitrite-N [µg L$^{-1}$] | 77 |
| Secchi depth [cm] | 35 |

Table 1 Some physical and chemical characteristics at the central offshore station in Lake Nakuru at the time of sampling (7 April 2009)

| Group     | Taxon                        | [Ind L$^{-1}$] | [mg C m$^{-3}$] |
|-----------|------------------------------|----------------|-----------------|
| het. Bacteria | *A. fusiformis*             | 1.37E + 07     | 1.02E + 04     |
| Algae     | *A. elenkinii*              | 3.35E + 07     | 3.22E + 03     |
| Other micro- & nanaalgae |                  | 9.71E + 07     | 6.19E + 02     |
| het. Protozoa | *Holophrya*             | 5.80E + 03     | 3.99E + 02     |
|            | *Frontonia*               | 9.60E + 03     | 5.27E + 02     |
| Rotifera  | *B. dimidiatus*           | 9.84E + 04     | 5.05E + 03     |
|            | *B. plicatilis*           | 2.40E + 04     | 6.25E + 03     |
|            | *Hexarthra jenkinae*      | 5.33E + 02     | 2.72E + 01     |
| Crustacea | *Lovenula africana*       | <0.1           | –               |

Table 2 Abundance and carbon concentration of the major components of the planktonic food web of Lake Nakuru (7 April 2009)

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composed of flagellates, bacteria and small colloidal particles, was isotopically enriched compared to cyanobacteria, but close to the $\delta^{15}N$ of DOM (6.1‰). The $\delta^{13}C$ values of other aquatic herbivores (Ephydra larvae, tilapia) were similar to the $\delta^{13}C$ value of A. elenkinii, while crustacean biomass was too low for isotope analysis. Stable isotope signatures and C:N ratios of DOM, sediments and all biota are presented in Table S2 and Fig. 2.

**Isotope mixing models**

Macrophytes and allochthonous material had stable isotope values distinctly different from those of all planktonic samples, indicating, in accordance with earlier food-web studies (Vareschi and Jacobs, 1985), a strong zooplankton dependence on autochthonous dietary sources. We therefore excluded macrophytes and allochthonous sources from our mixing models, but considered all known available pelagic food sources, including A. fusiformis, the $<2 \mu m$ size fraction and A. elenkinii. These three dietary resources contributed estimated mean proportions of 48% (95% confidence interval = 30–68%), 37% (3–66%) and 15% (0–31%), respectively, to the nutrition of B. plicatilis (Fig. 3a). The mixing model for the smaller B. dimidiatus resulted in an estimated dietary contribution of 70% (36–93%) from A. elenkinii, 21% (0–46%) from the $<2 \mu m$ size fraction and 9% (0–25%) from A. fusiformis. The model predicted a high carbon fractionation value for B. dimidiatus ($\Delta^{13}C >2\%$), much higher than the values set for the single food sources as SIAR model input (0.3 ± 0.14%). For this reason, we established a second mixing model, which was based on A. elenkinii, A. fusiformis and a calculated isotopic value of heterotrophic bacteria (see discussion) as food sources. This second mixing model predicted a contribution of 60% (26–93%) from heterotrophic bacteria, 32% (3–63%) from A. elenkinii and 8% (0–17%) from A. fusiformis (Fig. 3b).

**Discussion**

**Methodological consideration**

The diversity of plankton communities and the complexity of pelagic trophic interactions call for powerful and reliable ecological tracers for their exploration. Stable isotopes are a commonly applied technique of high potential (Layman et al., 2012), but difficulties connected with species-specific sampling of microbial communities has led to an underrepresentation of lower trophic levels and an oversimplification of microbial food webs in many studies. Here, we show that light-based separation processes, which have been used before to isolate rotifer strains from in situ samples (May, 1987), can lead to high taxonomic purity (>95%) of microzooplankton field samples, especially in combination with classical size fractionation, sedimentation and buoyancy techniques successfully applied in other studies (Vuorio, Meili and Sarvala, 2006).

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**Fig. 3** Contributions of different potential food resources to diets of (a) B. plicatilis and (b) B. dimidiatus based on a Bayesian mixing model incorporating variation in isotope signatures and uncertainty in fractionation factors.

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A high starting biomass of rotifers was critical for successful separation as a substantial fraction of individuals was lost in the separation process. Nevertheless, we argue that this separation technique can be successful in systems with much lower microzooplankton densities because in oligo- and mesotrophic systems large numbers of rotifers can be collected by repeated net hauls. Certainly, separation techniques always need to be adapted according to system-specific size and community structures, but purified samples have a high potential and can also be used for additional analytic techniques such as investigation of RNA/DNA ratios, quantification of fatty acids or even compound-specific stable isotope analyses.

Species-specific differences in food assimilation

Large isotopic differences were identified between two closely related rotifer species and three-source mixing models were established to evaluate whether such differences were due to the assimilation of different food sources. Our models were based on the assumption that rotifers were not integrating variations in the isotopic signatures of algae over a longer period of time. Because of very high phytoplankton biomass, algal turnover times are surprisingly long in tropical soda lakes (Vareschi and Jacobs, 1985) and isotopic values are therefore expected to change relatively slowly. Rotifer populations, on the other hand, are very dynamic (average half-life times of <2 days; Vareschi and Jacobs, 1984) and no substantial time-lag effects should be expected.

The stable isotope mixing model for B. dimidiatus resulted in elevated carbon fractionation values, which seem improbably high compared to group-specific literature values (McCutchan et al., 2003). Although Bayesian mixing models are very powerful tools, it is necessary to test critically whether the model outputs concur with common physiological and ecological knowledge (Layman et al., 2012). In the case of B. dimidiatus, the observed fractionation values suggest either an additional food source or the selective retention of certain particles from a mixed food fraction. As we thoroughly sampled the plankton community of Lake Nakuru, we can exclude the presence of alternative food sources. The <2 μm fraction, on the other hand, was a mixture of various components and it seems likely that one of these components, which was enriched in 13C, was the main dietary source of B. dimidiatus along with A. elenkinii. We considered free-living heterotrophic bacteria as a likely candidate because (i) bacterial biomass and production in Lake Nakuru are high (Table 2; Kilham, 1981) and can potentially provide enough carbon to support the high B. dimidiatus densities and (ii) it has recently been demonstrated that bacterial isotope signatures are often closely related to isotopic ratios of DOM (McCallister et al., 2004), which in Lake Nakuru were relatively enriched in δ13C. We therefore re-ran our mixing model, replacing the <2 μm fraction with a DOM-based signal of heterotrophic bacteria (incorporating a bacteria-specific Δ13C of 1‰ (Coffin et al., 1989) and because of the lack of bacteria-specific literature values a Δ15N of 3.4‰ (Post, 2002)].

In spite of realistic fractionation factors (most likely model prediction: Δ13C = 0.53‰; Δ15N = 2.59‰), the results of the second B. dimidiatus mixing model suggesting bacteria to be the main dietary source of B. dimidiatus at the time of sampling (Fig. 3b) should still be treated with caution. Due to the lack of controlled laboratory experiments, the calculation of the bacterial isotope values has wide confidence limits and also the acidification during the sampling of the DOM could have altered isotopic values (Brodie et al., 2011). However, a robust and important finding of the mixing models was that B. dimidiatus ingested A. elenkinii, but very little or no A. fusiformis, which was the main food source of B. plicatilis. This suggests substantial differences in the food assimilation of these closely related species, although cyanobacteria were an essential food source for both rotifers.

Trophic coupling between rotifers and cyanobacteria

Suspension-feeding rotifers are often regarded as strictly size selective with an optimal prey size ranging between 5 and 10 μm (Rothhaupt, 1990; Hansen, Wernberg-Möller and Wittrup, 1997). Nevertheless, a number of laboratory experiments have demonstrated ingestion of various cyanobacterial filaments (Starkweather and Kellner, 1983; Rothhaupt, 1991; Soares, Lurling and Huszar, 2010; Burian, Schagerl and Yasindi, 2012; Ka et al., 2012), sometimes even overlapping in size with rotifer consumers. Additionally, several field studies in tropical and subtropical systems have recorded intermediate to high rotifer densities co-occurring with monospecies blooms of filamentous cyanobacteria (Vareschi and Vareschi, 1984; Bouvy, Pagano and Trousellier, 2001). Vareschi & Jacobs (1984) described how spiral Arthospira filaments can be ingested in a spaghetti-slushing-like manner, which makes the filament diameter a much more important parameter than the filament length. In fact, the range of filament diameter of Arthospira (4–9 μm in this study) lies within the optimal prey size of Brachionus species (Hansen et al., 1997), although the whole filament can match the rotifer body size. Besides
the diameter of the filament, the rigidity (Rothhaupt, 1991) and the shape of the filaments (Kaggwa et al., 2013), as well as the size of the consumer, seem to be key factors explaining the prey selection of rotifer species. Overall, we were not only able to demonstrate for the first time the in situ assimilation of filamentous cyanobacteria, but also revealed that the link between filamentous cyanobacteria and rotifers can be quantitatively important in aquatic ecosystems.

Cyanobacteria are mostly considered as low-quality food (Arnold, 1971), lacking several essential biochemical compounds, such as long-chain polyunsaturated fatty acids and sterols (Brett et al., 2006; Wacker and Martin-Creuzburg, 2012). High cyanobacteria assimilation rates by rotifers together with elevated rotifer population densities therefore seriously challenge our perception of food quality in phytoplankton–rotifer interactions and lead to two possible explanations: either the biochemical requirements of rotifers are substantially different from those of other zooplankton or the food quality of filamentous cyanobacteria for rotifers is better than commonly perceived.

Although the poor food quality of single-celled cyanobacteria, mostly Synechococcus strains, is well documented (Brett et al., 2006; Wacker and Martin-Creuzburg, 2012), a meta-analysis of experimental feeding studies (Wilson, Sarnelle and Tillmanns, 2006) surprisingly found no significant difference in rotifer growth comparing filamentous cyanobacteria and chlorophytes and/or flagellates as dietary algae. This indicates that dietary effects of filamentous cyanobacteria on other zooplankton groups like daphnids could rather result from mechanical interference (Hawkins and Lampert, 1989) and their biochemical food quality may be better than commonly assumed; especially in the face of the predicted increase in filamentous cyanobacteria blooms with climate warming (Parel and Huisman, 2008), these results call for a more thorough investigation of the biochemical food quality of filamentous cyanobacteria for rotifers.

Another explanation of high population densities of rotifers feeding on filamentous cyanobacteria is the possible de novo synthesis of biochemical compounds by rotifers. Wacker & Martin-Creuzburg (2012) outlined strong dietary mismatches between rotifers and their ingested cyanobacteria. Although Wacker & Martin-Creuzburg (2012) could not rule out maternal effects, they demonstrated that several structurally important sterols and polyunsaturated fatty acids were present in rotifers, but not in their food items. Also, a report of cholesterol de novo synthesis (Teshima et al., 1981) and the presence of the sterol substitute tetrahymanol in rotifers (Wacker and Martin-Creuzburg, 2012) support a high dietary flexibility of rotifers, even though the quantitative efficiency of such processes is still uncertain. But whether food quality of filamentous cyanobacteria is better than expected or trophic upgrading of biochemical compounds occurs in rotifers, in both cases secondary consumers would have the possibility to prey on high-quality food particles in an exclusively cyanobacteria-dominated system.

The observed assimilation rates of cyanobacteria also bear important functional implications for Lake Nakuru. Rotifer biomass outbreaks can mark a transition in the phytoplankton community structure from a microalgae and A. elenkinii-dominated state to a monospecies A. fusiformis bloom (authors’ unpublished data). At the sampling time, the biomass ratio between A. elenkinii and A. fusiformis was 1 : 2.6. Total assimilation rates [calculations based on rotifer densities, species-specific grazing rates (Burian et al., 2012) and an assumed equal assimilation efficiency for the two rotifer species] were, however, 50% higher for A. elenkinii than for A. fusiformis. This suggests a much higher grazing pressure on A. elenkinii, in accordance with recent grazing experiments (Burian et al., 2012). Therefore, rotifer selectivity and other indirect effects of rotifer blooms, such as improved underwater light supply and enhanced nutrient recycling, are likely to be factors facilitating the dominance of A. fusiformis, a state often described as typical for African soda lakes (Vareschi and Jacobs, 1985; Harper et al., 2003).

In conclusion, we successfully applied separation techniques to obtain species-specific rotifer samples and demonstrated their importance for the analysis of food-web structures. In the case of Lake Nakuru, even a mixed rotifer sample would have led to intermediate isotopic values (δ15N: 9.2‰; δ13C: −17.2‰) and to substantially different outputs from isotope mixing models. The distinct isotopic niches of two rotifer species suggest interspecific differences in feeding behaviour and hold important ecological implications for phytoplankton dynamics of East African soda lakes.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Data S1.** Plankton counts and biomass conversions.

**Table S1.** Conversion factors used to calculate C content of various biota.

**Data S2.** Calibration of mixing models: Stable isotope fractionation

**Table S2.** Isotopic signatures of δ¹³C, δ¹⁵N and C:N ratios of major food-web components of Lake Nakuru (April 7, 2009). Isotopic ratios are given as ‰, C:N ratios as %.

**Text S3.** Separation of cyanobacteria stable isotope signatures via an inverted end-member mixing model.

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