Journal of
Plankton Research

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J. Plankton Res. (2017) 39(2): 220–231. *First published online February 21, 2017 doi:10.1093/plankt/fbx007*

An isotopic analysis of the phytoplankton–zooplankton link in a highly eutrophic tropical reservoir dominated by cyanobacteria

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Received May 4, 2016; editorial decision February 1, 2017; accepted February 6, 2017

Corresponding Editor: Karl Havens

Lake eutrophication often results in the dominance of cyanobacteria, which are generally regarded as an unsuitable food source for zooplankton, though this is still under debate. Here we tested whether small sestonic particles are the dominant component of the diet of zooplankton during periods of cyanobacterial dominance. For this, the contribution of seston fractions (<20 μm and ≥20 < 100 μm) to zooplankton diets was assessed at the species level in a highly eutrophic tropical reservoir in Ethiopia using dual isotope ($\delta^{15}N$ and $\delta^{13}C$) SIAR and MixSIAR models. Posterior area estimates of both consumer species and potential food sources were also modeled. Results of the two models indicated that the general diet of the zooplankton was composed primarily of small seston particles. Analysis of isotopic niche breadth of the two major crustacean zooplankton species, *Ceriodaphnia cornuta* and *Thermocyclops decipiens*, indicated that it was generally larger for the former species indicating consumption of a different prey and/or change in feeding strategy of both species. Overall, our field study indicates that under cyanobacterial dominance, zooplankton feed on small seston particles and that grazing on colonial or filamentous cyanobacteria seems not to be a generalized pattern in tropical freshwater reservoirs.

KEYWORDS: eutrophication; reservoirs; stable isotopes; zooplankton; food web

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INTRODUCTION

Many water bodies all over the world support massive cyanobacterial blooms due to eutrophication and climate change (Paerl and Huisman, 2009). Although light, nutrients and temperature are often regarded as factors of overriding importance for the development of such blooms (Paerl and Otten, 2013), their subsequent success may also be influenced by the grazing pressure of herbivorous zooplankton (Boon *et al.*, 1994; Smayda, 2008; Urrutia-Cordero *et al.*, 2015). Several studies (Lampert, 1987; Boon *et al.*, 1994; Smayda, 2008; Kâ *et al.*, 2012) have assessed the effect of zooplankton on phytoplankton dominated by cyanobacteria. The top-down effects of zooplankton are usually considered negligible, particularly when there is dominance by large cladocerans. However, grazing experiments in a eutrophic lake in southern Sweden indicated that cyclopoid copepods and small cladocerans may suppress the growth of cyanobacteria, except for toxic genera such as *Microcystis* spp. (Urrutia-Cordero *et al.*, 2015). In tropical waters, Havens *et al.* (1996) argued that poor control of phytoplankton biomass by herbivorous macrozooplankton in lowland tropical and subtropical water bodies is due to the small size of the dominant zooplankton and their inefficient grazing on large algal forms (i.e. filaments and colonies). However, size-specific preferences and trophic niche width might be different for distinct zooplankton species and little is known about tropical ones. For example, Kâ *et al.* (2012) found that small cladocerans such as *Moina micrura* and *Ceriodaphnia cornuta* consume small filaments of cyanobacteria and argued that grazing by zooplankton can eventually be important in controlling filamentous cyanobacteria in tropical waters.

Some cyanobacteria can be unsuitable as a food source for herbivorous zooplankton and so adversely influence not only their feeding ability, but also their growth (Chen and Xie, 2003; Chen *et al.* 2005). This may be due to their poor nutritional value, interference with filter-feeding and toxin content of some species (Lampert, 1987; Haney *et al.*, 1994). Nevertheless, cyanobacteria-derived detritus may be a potential nutritional source for aquatic consumers (Yu *et al.*, 2013). Grazing on bacteria directly or on protists through the microbial food web is another energy source for zooplankton (Vaquè and Pace, 1992; Sommaruga, 1995).

To understand complex trophic interactions in aquatic food-webs, analysis of functional groups with a resolution to the species level is crucial. Although species-specific stable isotope analyses have recently been applied to analyze the trophic role of large zooplankton, information on small-sized zooplankton is scarce. Stable isotope analysis is a powerful and widely applied tool to study energy and mass flows in plankton food webs (Fry, 2006). The stable carbon isotope signature ($\delta^{13}C$) is used to infer consumer carbon source, while stable nitrogen isotope is used to infer the relative trophic position of biota. Thus, analysis of $\delta^{13}C$ and $\delta^{15}N$ values in biota can help determine how the basal carbon source of a food-web is transferred to higher trophic levels. Furthermore, recent progress in the development of Bayesian mixing models (Moore and Semmens, 2008), such as MixSIR, MixSIAR (Stock and Semmens, 2013) or SIAR (Parnell *et al.*, 2010), allows to incorporate the uncertainty of sources and can give definite proportions of source contributions, which was not possible with previous models. Independent from trophic sources, the comparison of isotopic niche widths of zooplankton taxa allows for the identification of the degree of omnivory (Jackson *et al.*, 2011).

Considering the dominance of potentially toxic colonial and filamentous cyanobacteria in tropical lakes and reservoirs (Mowe *et al.*, 2015), we hypothesized that zooplankton do not feed directly on cyanobacteria, but rather use alternative food sources. Thus, we determined the $\delta^{13}C$ and $\delta^{15}N$ isotopic fractionation between sestonic size fractions and the dominant zooplankton species in a tropical eutrophic reservoir. Furthermore, we modeled isotopic niche width of two dominant zooplankton species to test whether zooplankton species generally overlap in their food spectrum.

STUDY AREA

Koka Reservoir (also known as Lake Gelila) is a tropical (8°18′–8°28′N and 38°59′–39°09′E, Ethiopia) ecosystem created by the construction of a dam across the Awash River to produce hydroelectricity. At present, the reservoir is also used for fisheries, irrigation, drinking water supply, watering of livestock, sanitation and as disposal system for industrial wastes. The reservoir is located at 1660 m above sea level and has a large area (~200 km^2), but it is shallow (mean depth: 9 m) (Wood and Talling, 1988; Willén *et al.*, 2011). It is fed by the rivers Awash and Modjo (Mesfin *et al.*, 1988) and the mean surface water temperature is 19°C. The water column does not have any marked thermal stratification (Mesfin *et al.*, 1988). Rainy seasons usually occur between March and May (minor rainy period) and from June to September (major rainy period). The reservoir receives wastewaters originating from a tannery and runoff from conventional agricultural lands and floriculture farms, which have dramatically increased its nutrient load (Willén *et al.*, 2011). As a consequence, harmful

cyanobacterial blooms have been recurring annually for the last 10 years (Willén *et al.*, 2011). These cyanobacterial blooms are dominated by *Microcystis*, *Anabaena* and *Cylindrospermopsis* spp. (Kebede and Willén, 1998; Willén *et al.*, 2011).

METHOD

Sampling

Samples were collected during the months of quantitative importance of cyanobacteria and/or high total chlorophyll a (Chl-*a*) concentration, that is in March, April and December 2014, from an inshore station (8°20′N and 39°5′E, Supplementary Fig. S1). The sampling months also correspond with the dry (December) and wet (March and April) seasons during which a shift in dominance of phytoplankton taxa generally occurs. Triplicate water samples were collected from selected depths (0, 0.5 and 1 m) distributed within the euphotic zone with a Van Dorn bottle sampler and mixed in equal proportions to produce composite samples. The composite samples were used for the determination of inorganic nutrients, phytoplankton abundance and Chl-*a*.

For the identification and enumeration of phytoplankton species, composite water samples were placed in 125 mL amber glass bottles and fixed immediately with Lugol's iodine solution. For zooplankton, triplicate samples were collected using a 62 µm mesh plankton net towed vertically from 2 m depth to the surface. Immediately following collection, the samples were preserved in formalin to a final concentration of ~4%.

For stable isotope analysis of seston, triplicate water samples were collected from 0.5 m depth with a water sampler and placed in a 10 L plastic carboy. Immediately after sampling, each sample was first screened through a 100 µm net sieve to remove zooplankton that otherwise will falsify isotopic signatures of the seston fractions. The seston was separated into two size-fractions (<20 µm and ≥20 < 100 µm). We did not consider a fraction >100 µm (the diameter of *Microcystis* spp. colonies ranged between 53 and 192 µm) because in addition to the problem of obtaining a "clean" isotopic signature (i.e. interference by small zooplankton), we assumed that cyanobacteria >100 µm will not be ingested by zooplankton because this fraction will mainly correspond with large colonies of *Microcystis aeruginosa*, which are known to have high cyanotoxin levels in Koka Reservoir (Willén *et al.*, 2011; Major *et al.*, unpublished). Aliquots from each size-fraction were gently sieved through 20 µm nylon sieves and both the material retained on the sieves, as well as the filtrate were concentrated onto Whatman GF/F filters that were previously combusted for 4 h at 450°C. The same size fractions were inspected under an inverted microscope to establish the dominant phytoplankton composition (see later).

Zooplankton samples for stable isotope analysis were obtained from repeated vertical hauls through the whole water column using a 62 µm mesh net. Upon returning to the laboratory, zooplankton were maintained in GF/F-filtered lake water for 24 h to allow for gut evacuation. Live crustacean zooplankton were sorted out into the five different species following narcotization of the organisms with carbonated water and then organisms (between 30 and 45 individuals) were placed in pre-weighted dry tin capsules. Samples were dried at 60°C for 24 h and stored in desiccators until the analysis of $\delta^{13}C$ and $\delta^{15}N$.

Taxonomy of phytoplankton and zooplankton

Identification of phytoplankton taxa was done using various keys (Komárek and Kling, 1991; Komárek and Cronberg, 2001; Cronberg and Komárek, 2004; Komárek and Anagnostidis, 2005; Bellinger and Sigee, 2010), while estimation of their abundance was done following the procedures outlined in Hotzel and Croome (1999).

Formalin-fixed water samples were used for the identification of zooplankton species using relevant keys (Koste, 1978; Defay, 1988; Jeje, 1988; Fernando, 2002). Zooplankton enumeration was done for planktonic crustaceans and rotifers. Counting was done using a gridded counting chamber under an optical microscope. Then, the zooplankton abundance was expressed as number of individuals per liter.

Auxiliary parameters

The transparency of the water was measured with a 20 cm diameter black and white Secchi disc. Depth profiles of oxygen and temperature were made between 0 and 5 m depth using HANNA field meters, HI 9143. pH and conductivity were measured using HANNA field meters, HI 9024 and HI 8733, respectively.

In the laboratory, concentrations of inorganic nutrients were determined following the procedures outlined in APHA *et al.* (1999). The samples used for the analyses of all nutrients except ammonia + ammonium-nitrogen (NH_3 + NH_4^+-N, hereafter ammonia) and total phosphorus (TP) were filtered through glass fiber filters (Whatman GF/F). Nitrate (NO_3-N) was analyzed by the sodium salicylate method (APHA, 1995) while ammonia was determined by the phenate method. Soluble reactive phosphorus (SRP) and TP were analyzed after

persulfate digestion by the ascorbic acid method. Chl-*a* concentration in the composite samples was used as a proxy for phytoplankton biomass. Appropriate volumes (100–250 mL) of composite water samples were filtered in triplicate through Whatman GF/F filters (47 mm diameter) for the determination of total Chl-*a*. Pigments of cells retained on the filters were extracted in 90% acetone for 24 h in the dark at 4°C after homogenization. Chl-*a* concentration was determined following the recommendations and formula of Lorenzen (1967) as outlined in APHA *et al.* (1999).

Stable isotope analysis

Samples were analyzed by isotope-ratio mass spectrometry (DeltaPLUS, ThermoFinnigan) at the Stable Isotope Laboratory of the University of Vienna, Austria. All stable isotope values were calculated using the following equation:

$$\delta X = \left[\left(R_{\text{sample}} / R_{\text{standard}} \right) \right] \times 1000$$

where R is the ratio of heavy to light isotopes of the element (X) in samples (R_{sample}) and standards (R_{standard}). The isotope ratio is expressed in the conventional delta (δ) notation, defined as the per mil (‰) deviation from the isotope standard. Nitrogen was expressed in terms of its value relative to atmospheric nitrogen, while carbon was expressed in terms of its value relative to Pee-Dee Belemnite (PDB). The consumers C/N ratio exceeded 3.5 and ranged between 4.1 and 6.4, indicating a lipid content of >5% of total weight. As high lipid contents (>5 % for aquatic animals) are known to deplete isotopes $\delta^{13}C$ signals, we made lipid normalization of consumers' $\delta^{13}C$ signatures as suggested by Post *et al.* (2007):

$$\delta^{13}C_{\text{normalized}} = \delta^{13}C_{\text{untreated}} - 3.32 + 0.99 \, x \, C:N$$

For the following analyses of stable isotope data, we used trophic enrichment factors (TEFs) for $\delta^{15}N$ of 3.42 ± 0.99‰ and 0.4 ± 1.3 ‰ for $\delta^{13}C$ (Post, 2002).

Isotope food source models

The relative contribution of the different size classes of seston (<20 µm and ≥20 < 100 µm) to zooplankton diets was assessed using dual isotope ($\delta^{15}N$ and $\delta^{13}C$) SIAR (Parnell *et al.*, 2010) and MixSIAR models (Stock and Semmens, 2013). MixSIAR models incorporate seasonally different source signatures and include uncertainty (Moore and Semmens, 2008). Firstly, the mean proportion of both food sources was modeled by pooling carbon and nitrogen signatures from both sampling seasons using SIAR. Secondly, a season-specific model was established to determine the relative contribution of the two seston fractions during the study period. The MixSIAR model, a Bayesian stable isotope mixing model that employs a mixed effects framework to estimate the relative contributions of selected potential food items to the diet of zooplankton species, was applied (Stock and Semmens, 2013). Within MixSIAR, models priors (prior probability distribution of food preference based on literature) were set to "uninformative" as the diet composition of zooplankton species was unknown for our system. Markov chain Monte Carlo simulation was conducted by running three replicate chains on a "long" run length and confirmed model's convergence using Gelman–Rubin and Geweke diagnostics (Gelman and Rubin, 1992). Finally, modeled posterior density estimates (n = 3000) were extracted, and the estimated medians (50% quantiles) were used for comparisons.

Isotopic niche area

The isotopic niche breadth and its seasonal variability was analyzed for *C. cornuta* and *Thermocyclops decipiens* (i.e. the only species present in all samplings) using the area in the bi-dimensional isotopic space and evaluated using standard Bayesian ellipse analysis in R (SIBER). Such ellipse corrected isotopic niche area is less influenced by extreme values and thus, represents a more reliable niche extension, when compared with the use of convex hulls (Jackson *et al.*, 2011). Similar to bootstrapping techniques, Bayesian modeling provides a so-called "posterior" distribution of estimations of ellipse areas, corrected for smaller sample sizes (i.e., n < 50). Such posterior estimates of isotopic niche area were modeled for both consumer species and potential food sources (seston < 20 µm and ≥20 < 100 µm) and the estimated medians (50% quantiles) were used for comparison. Isotopic area estimates of potential food sources were used to determine isotopic variability within sources. Estimated mean values were compared between species and time periods using ANOVA followed by post-hoc comparison (Tukey test) and illustrated with Beeswarm package (Eklund, 2015) of R (R Development Core Team, 2013).

Statistical analyses

Two-way-ANOVAs were used to test whether the average $\delta^{13}C$ and $\delta^{15}N$ values were significantly different among zooplankton species and over time, and to examine whether there were significant differences between isotopic areas of zooplankton species *C. cornuta* and *T. decipiens*. A Welch two sample *t*-test was used to exclude

Table I: Ranges and means of physico-chemical parameters measured in Koka Reservoir during the three sampling dates

Parameters	Range	Mean ± SD
Temperature (°C)	22.6–26.2	23.9 ± 2.0
Secchi depth (m)	0.14–0.18	0.16 ± 0.02
DO (mg L^{-1})	6.69–8.12	7.66 ± 0.62
pH	8.82–9.04	8.96 ± 0.12
Conductivity 25°C (µS cm^{-1})	372.0–381.0	377.7 ± 4.9
NO_3-N (µg L^{-1})	36.6–83.6	63.8 ± 24.4
NH_3-N (µg L^{-1})	345.0–394.3	362.0 ± 27.9
PO_4-P (µg L^{-1})	62.6–193.2	115.5 ± 68.7
Total P (µg L^{-1})	218.7–276.3	254.6 ± 41.9

coincidental differences of posterior estimates of bulk zooplankton resource use. We ran these statistical comparisons in R (R Development Core Team, 2013).

RESULTS

Physico-chemical parameters

Water transparency had only small seasonal variations (Table I). The average surface and bottom water temperatures of the reservoir during the study period were 23.8 and 23.5°C, respectively. The reservoir was well-oxygenated down to 5 m depth with a minimum depth-averaged value of 6.69 mg L^{-1} in April and a maximum of 8.12 mg L^{-1} in December, 2014. Conductivity in the dry season (381 µS cm^{-1}) was similar to that of the rainy season (376 µS cm^{-1}). The lowest (8.82) and highest (9.04) pH values of surface water were recorded in April (wet season) and December (dry season), respectively. Inorganic nutrients were at high levels (Table I) during all sampling months with peaks in SRP and NO_3-N concentration observed in April, while those of TP and NH_3-N occurred in December, 2014.

Biological parameters

Throughout the sampling period, cyanobacteria were the most abundant phytoplankton accounting for more than 94% of total abundance (Fig. 1). *M. aeruginosa* was the dominant species in March and December, 2014, while *Cylindrospermopsis africana* dominated the cyanobacterial assemblage in April, 2014. Chlorophyll-*a* concentrations ranged from 155.1 µg L^{-1} in April to 222.8 µg L^{-1} in December with the latter value coinciding with the highest cyanobacterial abundance (Figs 1 and 2). Inspection of the <20 µm size fraction indicated the presence of mainly *Aulacoseira granulata*, followed by *Scenedesmus* spp., *Cryptomonas* spp., *Euglena* spp. and *Trachelomonas* spp.,

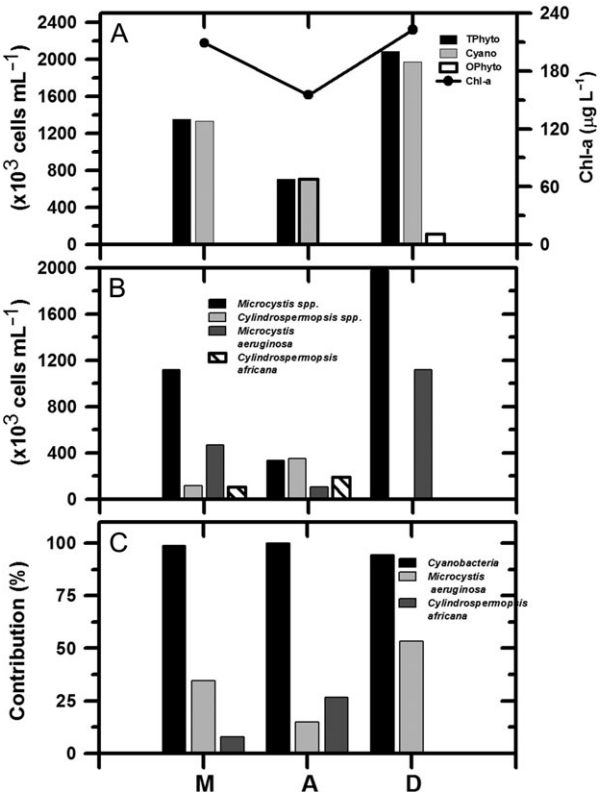

Fig. 1. Changes in the abundance (**A** and **B**) and percentage contribution (**C**) of major cyanobacterial genera and species in relation to the total abundance of phytoplankton (Tphyto), cyanobacteria (Cyano) and other algal groups (Ophyto), as well as total chlorophyll-*a* (Chl-*a*) (A) in March (M), April (A) and December (D) 2014.

whereas *Microcystis* and *Cylindrospermopsis* spp. were rarely observed. The most important taxa retained by the 20 µm sieve included *Cylindrospermopsis africana*, *Cylindrospermopsis raciborskii*, *Anabaena spiroides*, *Microcystis spp.* (*Microcystis aeruginosa*, *Microcystis botrys*, *Microcystis flos-aquae* and *Microcystis novacekii*), and some fragments of *Aulacoseira granulata*. Though, seston is assumed to comprise a mixture of phytoplankton, other planktonic organisms, organic detritus and inorganic particles, the high Chl-*a* values found in our samples suggest that an important fraction of the bulk sestonic fraction was phytoplankton.

Zooplankton abundance in the dry season was similar to that in the rainy season. Among zooplankton taxa, rotifers were the most abundant during the study period contributing to >58% of the total zooplankton abundance. This was followed by copepods (13%) (Fig. 2), mainly dominated by *Thermocyclops decipiens*. The cladoceran zooplankton was dominated by small body-sized species, including *C. cornuta* and *Diaphanosoma excisum*, whereas large cladocerans were absent except for the occurrence of *Daphnia barbata* in December, 2014.

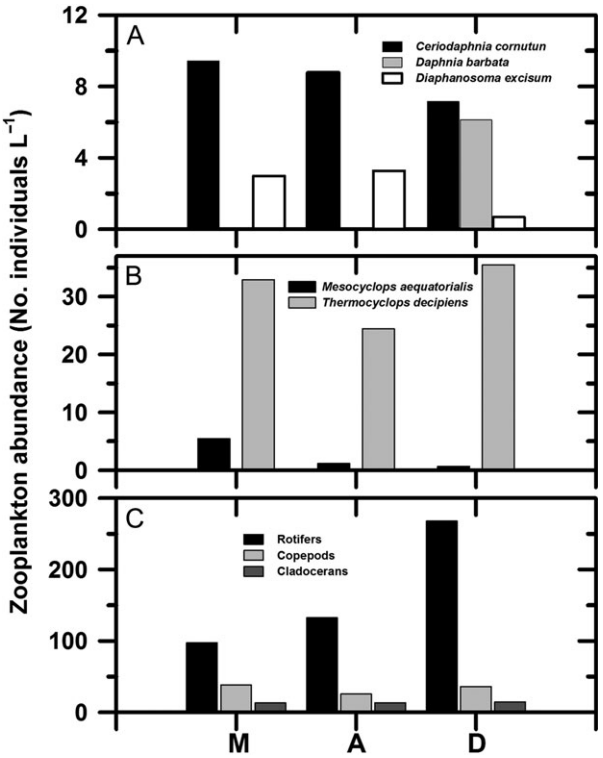

Fig. 2. Changes in the abundance of major cladoceran (**A**), and copepod (**B**) species in relation to the abundance of metazoan zooplankton groups (**C**) in March (M), April (A) and December (D) 2014.

Stable isotope composition of seston and crustacean zooplankton

Average values of δ^{15}N and δ^{13}C estimated for small seston particles (<20 μm) were −26.86 ± 2.06‰ and 13.05 ± 2.37‰ (n = 9), while signatures for larger particles (seston ≥ 20 < 100 μm) were −27.52 ± 0.52‰ and 13.31 ± 1.30‰; (n = 9), respectively. Significant seasonal differences were found for both seston particle size-classes (F = 138, P < 0.001) with particular separation of samples from December (Tukey test, P < 0.01). The δ^{13}C and δ^{15}N values of both seston fractions of the dry season (December) were lower than those of the rainy period (March–April). The mean δ^{13}C signatures of seston fractions were slightly different with higher values for the <20 μm fraction (mean: −26.86‰) than for the larger one (mean: −27.52‰). However, there was no significant difference between isotopic composition of both fractions (P = 0.199 and P = 0.201 for δ^{13}C and δ^{15}N, respectively). Isotopic signatures of zooplankton in the reservoir were on average −23.31 ± 1.10‰ for δ^{13}C and 15.47 ± 1.45‰ for δ^{15}N (n = 30). Signatures of the species *C. cornuta* and *T. decipiens* shifted to a lower mean δ^{13}C and δ^{15}N values in December. Trophic position varied significantly among zooplankton species (δ^{15}N:H = 15.9, P < 0.01), but no significant differences were found for carbon signatures. Zooplankton species were generally more δ^{15}N- and δ^{13}C-enriched relative to the <20 μm and ≥20 < 100 μm seston fractions. In December, 2014, *C. cornuta*, *D. barbata* and *T. decipiens* were, however, δ^{13}C-depleted relative to the <20 μm fraction, while *C. cornuta* and *D. excisum* were δ^{15}N-depleted relative to both seston size-fractions in April, 2014. The difference between mean δ^{15}N values for zooplankton species and those for seston fractions varied temporally with the greatest isotopic difference (~3.28‰) occurring in December (dry period) between zooplankton and < 20 μm fraction and the smallest (~0.11‰) in April (minor rainy period) between zooplankton and the ≥20 < 100 μm fraction.

The observed differences in the stable isotope signatures between the copepod and cladoceran species were not statistically significant (ANOVA tests for δ^{15}N, $F_{(2,\ 4)}$ = 4.325, P < 0.001). In December, the δ^{15}N of *C. cornuta* was about 1‰ lower than that of *D. barbata*, whereas there was a very small difference (0.37‰) for δ^{13}C between these species. On average, *T. decipiens* was more enriched in δ^{15}N relative to <20 μm and ≥20 < 100 μm seston fractions by +3.7 and +3.4‰, respectively, (i.e., one or nearly one trophic level above seston, Fig. 3).

On average, *C. cornuta* was more δ^{15}N-enriched than the <20-μm and ≥20 < 100 μm seston fractions by 1.18 and 0.92‰, respectively. Further, a strong and positive correlation was found between δ^{15}N values of *C. cornuta* and those of <20 μm (r = 0.958, P < 0.05) and ≥20 < 100 μm (r = 0.822, P < 0.05) size fractions. Likewise, this species was more δ^{13}C-enriched than the <20 μm and ≥20 < 100 μm seston fractions, by +1.07 and 1.73‰ respectively, but with a weak correlation between its δ^{13}C values and those of seston of <20 μm (r = 0.064, P < 0.095) and ≥20 < 100 μm (r = 0.367, P < 0.076) size fractions.

Proportions of seston in zooplankton diet

A global mixing SIAR model including TEFs revealed that mean signatures of both seston particle size-fraction and their deviations did not embed all zooplankton isotopic values (Fig. 3). Generally, all zooplankton species had less negative δ^{13}C signals compared to both potential food sources. The posterior distribution from MixSIAR indicated that the relative contribution of seston size-fractions to zooplankton diet differed significantly (t = 953, df = 5998, P < 0.001) (Fig. 4). The mean relative contribution of seston <20 μm was 95.6% when compared with the larger size-fraction. However, there were differences in the contribution of both seston size-fractions during the three sampling months (Fig. 5). Isotopic signatures for

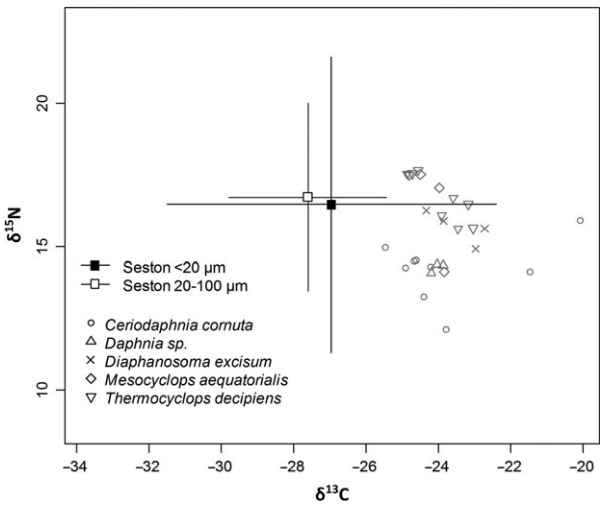

Fig. 3. Biplot of mean $\delta^{13}C$ and $\delta^{15}N$ signatures of seston fractions and major crustacean zooplankton species collected in Koka Reservoir. Lipid-corrected zooplankton consumer taxa (symbols) and mean source (black and white squares) isotope values ($\delta^{13}C$ and $\delta^{15}N$) in March, April and December. Trophic enrichment factors (TEFs) were considered in the stable isotope mixing models.

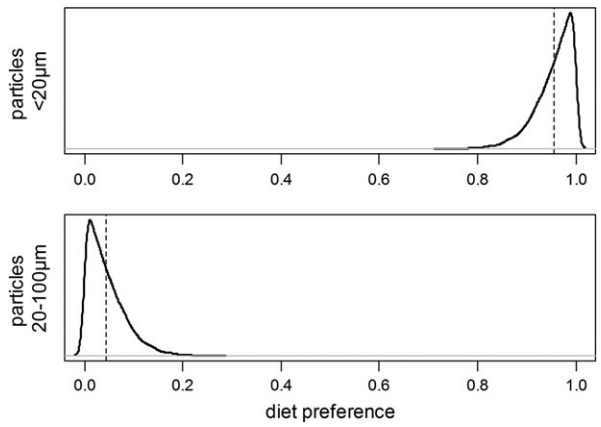

Fig. 4. Mean posterior density estimates of the proportions of both sestonic food-source particles (seston < 20 µm, ≥20 < 100 µm) of all sampled zooplankton species. Mean values of the proportions are shown in the vertical lines, *n* (number of simulations) = 3000.

April indicated a relatively low proportion of small seston as food for zooplankton, with mean estimates ranging between 55.3 and 58.2%. During March and December, the use of the small seston fraction was much more likely with a probability >83.4 and 95% when related to the larger seston fraction, respectively.

Trophic niche width

Isotopic niche area of the species *C. cornuta* and *T. decipiens* indicated significant seasonal variations. Different standard ellipse areas (i.e. Bayesian posterior estimates) indicated a larger isotopic niche area in March for both species (Fig. 6A and B). For example, the mean areas for *C. cornuta* and *T. decipiens* were 16 and 21 times higher in March than in April, respectively ($F = 3936$, $P < 0.001$). Generally, out of 10^4 model simulations, the trophic niches of *C. cornuta* and *T. decipiens* were larger in March than in April in over 99 and 100%, respectively. SIBER revealed small isotopic areas for potential food sources in March (Fig. 6C and D), with mean areas of 0.15 and 0.63 for small and larger seston fractions, respectively. In April, however, a larger isotopic ellipse area with a mean value of 2.3 was found for the small seston fraction (Fig. 6D).

DISCUSSION

Because phytoplankton, and particularly colonial and filamentous cyanobacteria, are difficult to separate from other similarly sized microplankton and detritus, seston is routinely used as a surrogate end-point with the implicit assumption that most of this material is composed of algae (Martineau *et al.*, 2004). In our study, phytoplankton was largely constituted by positively buoyant cyanobacteria, and the bulk seston particularly of the large size fraction collected from the shallow euphotic zone was largely of algal origin and dominated by cyanobacteria. Some variability in ambient $\delta^{13}C$ and $\delta^{15}N$ values of seston in reservoirs understandably stems from variations in the type and quantity of land-derived particulates of potentially different ^{13}C and ^{15}N content carried by the inflow rivers (Harding and Hart, 2013). Compared to natural lakes, reservoirs usually have greater changes in depth, greater flushing rates, and have larger phosphorus and nitrogen loads (Kalff, 2002), which were also evident in the measured ambient levels of Koka Reservoir. Such influences on ecosystem properties might affect phytoplankton growth and their distribution in the water column (Longhi and Beisner, 2009; Mellard *et al.*, 2011), which in turn might affect the feeding of zooplankton and thus explain considerable isotopic variation within sampled seston and zooplankton among reservoirs (Hou *et al.*, 2013).

The isotopic fractionation in zooplankton varies according to the types of consumed food (Minagawa and Wada, 1984; Adams and Sterner, 2000), the relative feeding rate (fraction) and taxa-specific metabolic turnover rate of different isotopes due to their slightly different mass (Fry, 2006). Furthermore, meta-analyses have identified other potential factors that affect trophic enrichments, such as starvation, diet quality and N excretion (McCutchan *et al.*, 2003; Spence and Rosenheim, 2005).

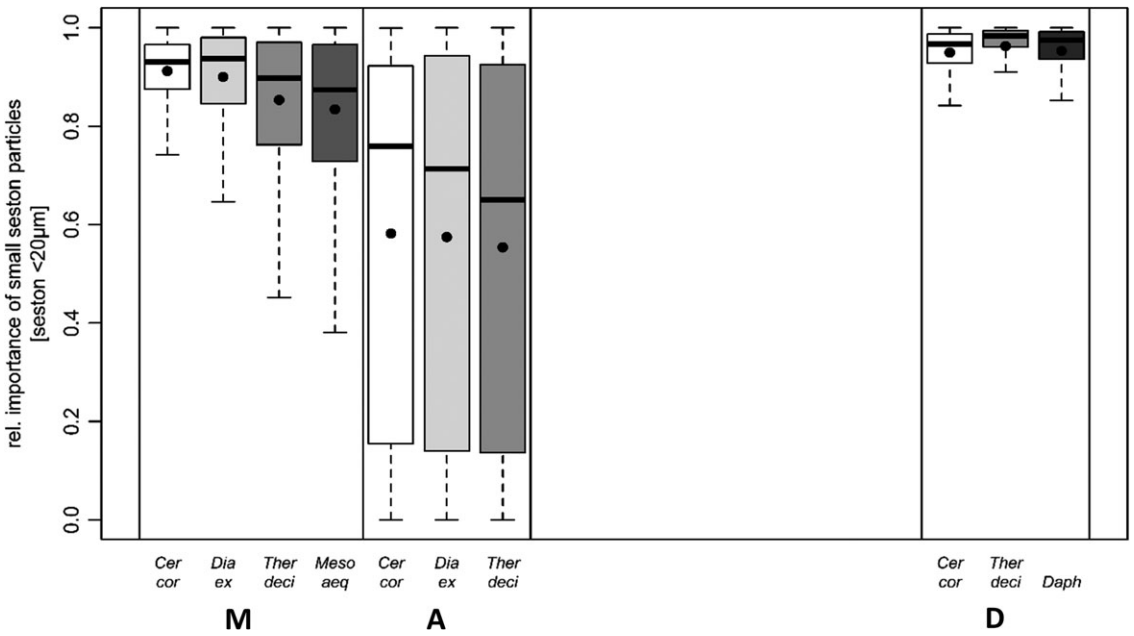

Fig. 5. Seasonal importance of small seston particles (seston size < 20 µm) as food source for zooplankton species (Cercor = *Ceriodaphnia cornuta*, Dia ex = *Diaphanosoma excisum*, Therdeci = *Thermocyclops decipiens*, Mesoaeq = *Mesocyclops aequatorialis*, Daph = *Daphnia barbata*). Bold lines indicate median, dots show mean values, *n* (number of simulations) = 3000.

Generally, the accuracy of the "trophic enrichment factors" (TEFs) are the subject of discussion (Galloway *et al.*, 2015), but its consideration in Bayesian modeling allows for the integration of this uncertainty when estimating the use of different food sources. The $\delta^{15}N$ signature of plankton is a good indicator of nitrogen sources (Vander Zanden *et al.*, 2005) and consumer trophic position because the nitrogen pools of aquatic animals have $\delta^{15}N$ signatures regularly enriched by a certain value (typically, 3.4‰) relative to their food sources (Vander Zanden and Rasmussen, 1999).

We found that when modeling the relative contribution of both size-classes of seston for zooplankton species, significant differences were detected. The SIAR illustration demonstrated that the feeding of zooplankton is generally related to seston particles. However, a slight ^{13}C-depletion of zooplankton samples indicated the use of additional food sources. This result contrasts with observations made in northern temperate lakes (Jones *et al.*, 1999; Grey *et al.*, 2000). For example, the ^{13}C-depletion of zooplankton relative to POM reported for temperate lakes is commonly attributed to either accumulation in zooplankton of ^{13}C-depleted lipids (Kling *et al.*, 1992) or spatial separation between the location where zooplankton was sampled and where it feeds (Zeng *et al.*, 2010). Further, such a ^{13}C-depletion can also be caused by selective feeding of zooplankton on isotopically light carbon sources that may be masked or diluted by a large detrital contribution to seston that is enriched in ^{13}C (del Giorgio and France, 1996). Moreover, phytoplankton collected during periods of high biomass such as the case of the present study tend to exhibit ^{13}C-enrichment (Zohary *et al.*, 1994; France *et al.*, 1997) due to reduced isotopic fractionation at high cell densities or growth rates (France *et al.*, 1997).

MixSIAR results indicated that the general diet of all zooplankton species in Koka Reservoir was primarily composed of small seston particles (<20 µm) and most probably complemented to a much lesser extent by larger particles. This result suggests that the zooplankton in this reservoir have a higher preference for smaller food particles and is coincident with those from feeding experiments done with tropical zooplanktonic species (Pagano, 2008). This author found that *Diaphanosoma excisum* ingests only very small particles. The <20 µm fraction was constituted by green algae, flagellates, diatoms (particularly *Aulacoseira granulata*), while small colonies of *Microcystis*, and fragments of *Cylindrospermopsis* filaments were rare. However, we cannot exclude the possibility that zooplankton used other potential food sources such as, for example, protists and POM-associated bacteria. In fact, the slight ^{13}C-depletion of zooplankton in relation to both seston size fractions and the variation of their isotopic niche area indicated that this maybe the case. Crustacean zooplankton is known to graze on a wide range of particulate matter, including phytoplankton, bacteria and detritus. The bulk seston for which we determined the stable isotope signature, can be considered the

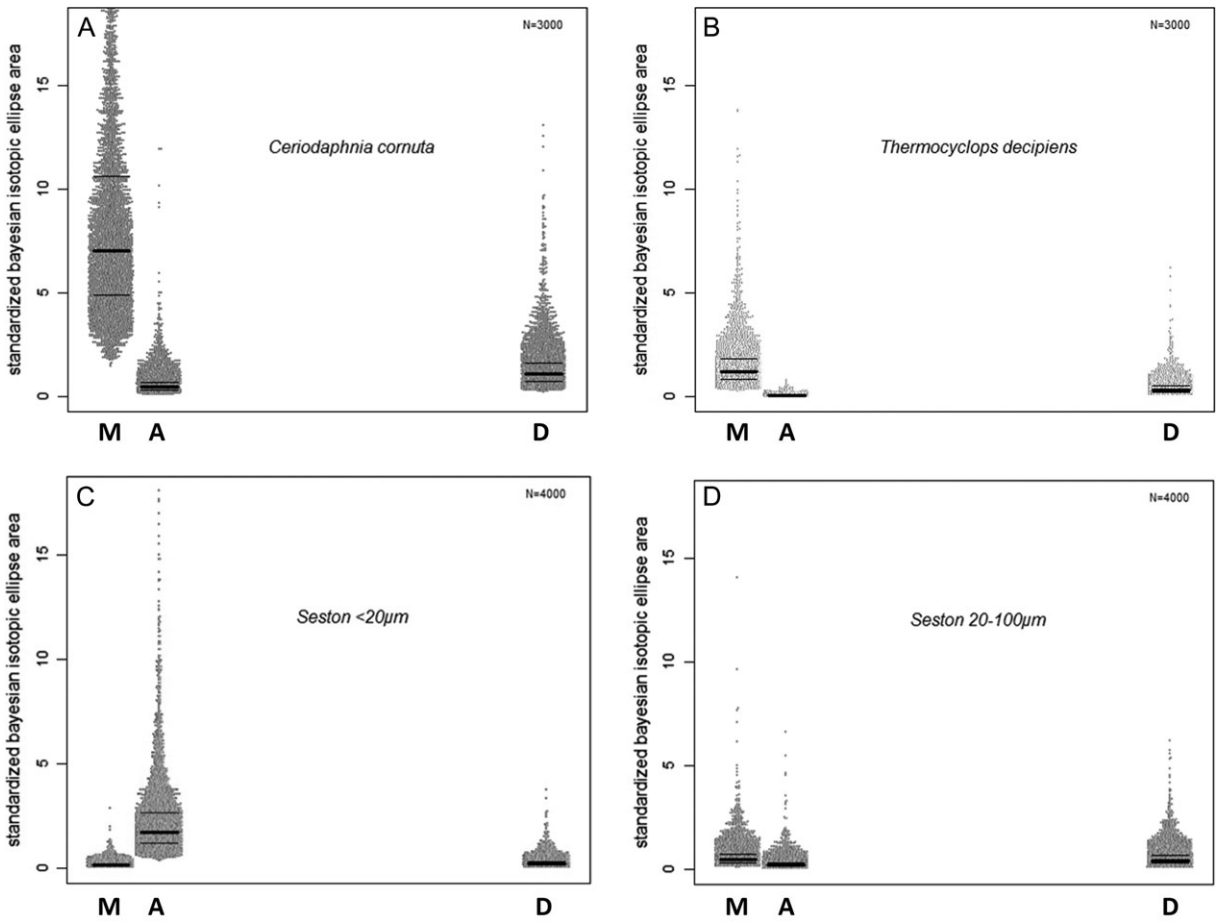

Fig. 6. Area estimates of Stable Isotope Bayesian Ellipses in R (SIBER) of (**A**) *Ceriodaphnia cornuta*, (**B**) *Thermocyclops decipiens*, (**C**) small seston particles < 20 µm, (**D**) seston particles ≥20 < 100 µm in March, April and December, 2014. Small dots represent single posterior estimates (single model simulations using the Bayesian approach), lines confine central 50% of the data and indicate the median (bold), *n* (number of simulations) = 3000 for zooplankton species (A and B) and 4000 for seston particles classes (C and D).

putative food source for zooplankton, although the degree of food selection from within the bulk seston may vary between species and lake type (Grey *et al.*, 2000).

Although all taxa exhibited a preference towards small seston particles, the importance of this fraction varied over time. In April, analyses indicated a nearly equivalent contribution of both seston particle size fractions, which might be related to general shifts within the phytoplankton community and thus isotopical drift (Lee *et al.*, 2013), which was also observed in Koka Reservoir as in this month *C. africana* dominated instead of *Microcystis* spp. (Fig. 1).

The isotopic ellipse corrected areas demonstrated a generally higher niche width for the species *C. cornuta* than for *T. decipiens*. This difference was particularly clear in March and indicates consumption of a different prey and/or feeding strategy of both species. However, both species followed the same seasonal patterns with increased range of stable isotope signatures in March. This suggests that both species are forced to feed on a broader range of food sources, collect more isotopically diverse prey items, and/or combine two feeding strategies (e.g., pelagic and benthic) in the early season, which is probably associated with the start of the minor rainy period. Generally, the larger isotopic niche areas in March suggest a higher degree of feeding plasticity when compared to the areas in April and December. When estimating the niche area of both seston fractions in the isotopic space, we found that the isotopic variability in consumers was not an after-effect of both sources' isotopic variability (Fig. 6C and D).

Kâ *et al.* (2012) found that none of the tropical zooplankton species studied, which also included *C. cornuta* has the capacity to ingest *M. aeruginosa*. This cyanobacterium dominated the phytoplankton of Koka Reservoir in December, 2014. *M. aeruginosa* can have negative impacts on *Daphnia* and other cladocerans (De Mott, 1999; Ferrão-Filho and Azevedo, 2003), copepods (DeMott and Moxter, 1991;

Kurmayer and Juettner, 1999) and rotifers (Rothhaupt, 1991) thereby deterring grazing on them. Consequently, cyanobacterial carbon is transferred inefficiently to herbivorous zooplankton, which might lead to a decoupling of primary and secondary production and to the subsequent accumulation of cyanobacterial biomass. This was also evident in Koka Reservoir in December 2014, despite the relatively high numerical abundance of zooplankton taxa (Fig. 2). Interestingly, there are some studies (Haney, 1987; Kâ *et al.*, 2012) that reported the consumption and/or size structure modification (cutting of filaments and mean size reduction) of filamentous cyanobacteria by copepods, cladocerans, or rotifers. Further, experimental studies have found that zooplankton from a subtropical lake have filamentous and colonial cyanobacteria in their guts (Work and Havens, 2003). Our results based on stable isotopes, however do not support the view that grazing on colonial cyanobacteria by zooplankton, particularly by copepods, is in general an important link in tropical lakes (Haney, 1987). This also agrees with the finding that the microbial pathway (though bacteria) is a dominant trophic link between zooplankton and *Microcystis*-dominated phytoplankton communities (de Kluijver *et al.*, 2012).

The disparity in size between phytoplankton and their grazers is accentuated in tropical shallow freshwater ecosystems by the scarcity of large cladocerans (*Daphnia*) and calanoid copepods, and the dominance of small grazers such as small cladocerans and rotifers (Fernando, 1994, 2002; Aka *et al.*, 2000). This explains the abundance, and sometimes proliferation, of large phytoplankton taxa, such as filamentous or colonial cyanobacteria in shallow tropical water bodies (Boon *et al.*, 1994; Lazzaro, 1997).

CONCLUSIONS

Overall, our results indicate that <20 µm seston particles are a major source of zooplankton nutrition during cyanobacterial dominance, particularly of *Microcystis* spp., which is a recurrent pattern in this reservoir (Mesfin *et al.*, 1988; Kebede and Willén, 1998; Willén *et al.*, 2011). In fact, *Microcystis* is the most frequently occurring bloom genus throughout tropical Asia, Africa and Central America (Mowe *et al.*, 2015). Though more research is needed to resolve other sources for zooplankton diets, our results indicate that grazing by small body-sized copepods and cladocerans, which are characteristic of tropical lakes and reservoirs (Fernando, 1980), on colonial or filamentous cyanobacteria seems not to be a generalized pattern. Further research may in turn also help to understand how zooplankton coexist with cyanobacteria (Ger *et al.*, 2014).

FUNDING

Addis Ababa University through a research fund granted to D.K. and through a grant from the University of Innsbruck, Vice-rectorate of Research to R.S.

SUPPLEMENTARY DATA

Supplementary data are available at *Journal of Plankton Research* online.

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