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Stable isotope and fatty acid markers in plankton assemblages of a saline lake: seasonal trends and future scenario

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Plankton fractions from a saline lake in Argentina were studied using a combined trophic marker approach. A strong seasonality of biomarkers was characteristic for the different fractions, particularly the variations in the 18:4($n - 3$) and 20:4($n - 3$) fatty acids and the $\delta^{13}\text{C}$ values. The primary production in the lake was mainly driven by diatoms, reflected by the close relation of $\delta^{13}\text{C}$, chlorophyll *a* and diatom fatty acid markers. The combined approach of $\delta^{13}\text{C}$ and 20:4($n - 3$) enabled processes in the lipid metabolism of the copepod *Boeckella poopoensis* to be inferred. The polyunsaturated fatty acid 22:6($n - 3$) and the $\delta^{15}\text{N}$ separated the trophic levels in this food web with copepods at higher trophic level. Nutritional stress and omnivory of *B. poopoensis* partially explained the $\delta^{15}\text{N}$ variations in mesozooplankton. The $\delta^{15}\text{N}$ signature was probably driven by cyanobacteria in the microplankton and by microbial processes in the nanoplankton fraction. Warmer temperatures may favour the saturation of microalgae fatty acids and the abundance of plankton groups richer in saturated fatty acids. The tendency to unsaturation in mesozooplankton at colder temperatures was probably influenced by diet and metabolic requirements. Future temperature increase and eutrophication-like processes may increase the importance of cyanobacterial and bacterial markers under climate change scenarios.

KEYWORDS: trophic markers; zooplankton; lipid; temperature; food web; climate change; Argentina

INTRODUCTION

Saline lakes are highly productive habitats and form large parts of inland aquatic systems. South America has many saline lakes principally in the Argentinian Pampa and in the Peruvian and Bolivian Altiplano. The drainage of the basins in many Pampean regions is endorheic or arheic, so that recurrent floods and droughts lead to strong variations in basin volume and salinity. Since these lakes are shallow and eutrophic, they experience strong seasonal changes in their plankton dynamics and are very vulnerable to climate-driven impacts (Kopprio *et al.*, 2010). The typical low biodiversity and simplicity of the food webs of saline lakes (Williams, 1981) make them exceptional models for the study of trophic markers. Stable isotopes and fatty acid trophic markers are complementary techniques and the combination of both provides an approach with higher accuracy and better resolution in trophic web studies (Nyssen *et al.*, 2005; El-Sabaawi *et al.*, 2009).

Fatty acid trophic markers indicate assimilated diets and have been used in ecological studies to determine trophic relationships because they are transferred conservatively through the food web (Dalsgaard *et al.*, 2003). The naturally occurring ratios of the $^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$ stable isotopes also offer integrated and conservative information about the diet of an organism. With trophic level a consumer is typically enriched in ^{15}N by 3.4‰ relative to its diet, while ^{13}C undergoes little fractionation (<1‰) (Peterson and Fry, 1987; Vander Zanden and Rasmussen, 2001; Post, 2002). Some fatty acids indicate the main dietary items in secondary producers: 16:1($n-7$) and 20:5($n-3$) are markers of diatoms, 18:4($n-3$) of flagellates, 18:2($n-6$) of terrestrial vegetation or chlorophytes and the 18:1($n-7$), odd-chain and branched fatty acids of bacteria and cyanobacteria (Dalsgaard *et al.*, 2003; Bec *et al.*, 2010). Cyanobacteria, chlorophytes and bacteria generally lack long-chain polyunsaturated fatty acids (Ahlgren *et al.*, 1990; Masclaux *et al.*, 2009; Ravet *et al.*, 2010). However, changes in environmental conditions, metabolic processes, and species or diet composition affect the isotopic and fatty acid profiles in aquatic organisms (e.g. Kattner *et al.*, 2007). For example, at colder temperatures the proportion of polyunsaturated fatty acids in membranes seems to increase or maintain their fluidity (Brett *et al.*, 2009; Gladyshev *et al.*, 2011).

Besides the value of fatty acids as trophic markers, some of them, such as 22:6($n-3$) (docosahexaenoic acid), 20:5($n-3$) (eicosapentaenoic acid) and 20:4($n-6$) (arachidonic acid), are required for maintaining the structure and function of cell membranes, are precursors of hormones and enhance zooplankton somatic growth and reproduction. These fatty acids, together with their precursors, 18:3($n-3$) (α -linolenic acid) and 18:2($n-6$)

(linoleic acid), are classified as essential because they are generally not biosynthesized by animals and therefore have to be ingested with the food. However, other potential precursors or intermediaries in the synthesis of the 22:6($n-3$) and 20:5($n-3$) fatty acids such as 18:4($n-3$) and 20:4($n-3$) are usually not considered essential. The 18:4($n-3$) is used as a flagellate marker and the 20:4($n-3$) is generally found only in trace amounts but has been proposed as an indicator of chain elongation (von Elert, 2002; Bec *et al.*, 2003; Kopprio *et al.*, 2012).

So far, the combination of stable isotope and fatty acid analyses has not been applied to investigate seasonal cycles of natural planktonic assemblages in South American lakes. Furthermore, information about the occurrence and distribution of fatty acids in pelagic limnological food webs is scarce (Kainz *et al.*, 2004; Persson and Vrede, 2006) and research on zooplankton fatty acids has usually focused on species of *Daphnia* from the northern hemisphere (Burns *et al.*, 2011). The aim of our study is to elucidate trophic relations, metabolic processes and the influence of plankton composition and temperature on the fatty acid and stable isotope signatures. We hypothesize strong seasonal differences of the major markers within the plankton fractions and a relatively stable trend of essential fatty acids in mesozooplankton. Furthermore, the low heat capacity of shallow lakes will enhance the effects of global warming on biota and consequently on their biomarkers. This study also assesses factors behind the dynamics of stable isotopes and fatty acids in natural plankton communities in Lake Chasicó and similar shallow systems under future climate change scenarios.

METHOD

Site description, field work and plankton survey

Limnological characteristics of Lake Chasicó are described in detail by Kopprio *et al.* (Kopprio *et al.*, 2010, 2014). Briefly, the lake (38°37'S; 63°05'W) is an endorheic basin of ~65 km² at 20 m b.s.l in the semiarid region of the Argentinian Pampa. Lake Chasicó is polymictic, shallow (mean depth 10 m) and eutrophic with annual mean concentrations of soluble reactive phosphorus and dissolved inorganic nitrogen of 3.6 and 6.7 μM , respectively. Three stations were surveyed in the lake: Station CV, near Chapalcó Village at 6 m depth; Station EE, "El Embudo" at 3 m and Station RM, near the "River Mouth" at 2.5 m (see map in Kopprio *et al.*, 2014). Physicochemical water parameters were measured monthly on the same day during daytime in 0.5 m intervals from the surface to the bottom with an electronic probe (U-10, Horiba Ltd.) from August 2007 to September 2008. Lake water for chlorophyll

a determination was also collected monthly at 1 m below the surface with a Van Dorn sampler. The chlorophyll *a* content was quantified after Lorenzen (Lorenzen, 1967).

Three plankton fractions were collected in addition to the water quality measurements. Net towing and filtration could not be performed in January at Station RM because of the strong runoff of River Chasicó with currents impeding vertical towing and large suspended particles clogging the 20 µm net. This sampling point was therefore excluded from the whole data set. Size limits among different fractions were considered after Sieburth *et al.* (Sieburth *et al.*, 1978). Mesozooplankton were collected by 20 vertical net tows of 200 µm mesh size (Nitex) from the lake bed to the surface. The filtered volume was calculated using a digital flowmeter (Hydro-Bios). Lake water was sampled with a Van Dorn sampler at 1 m depth and the fractions were separated by net fractionation: ~20 L for microplankton (seston between 200 and 20 µm) and ~1 L for nanoplankton (seston <20 µm). Live mesozooplankton and microplankton were transferred into 500 mL of water filtered through GF/F filters (precombusted at 500°C for 5 h, Whatman) and stored together with 20 µm sieved samples at 4°C for 3 h prior to being processed. The water with the nanoplankton fraction was directly filtered through GF/F filters in duplicate. The nanoplankton comprised partially the picoplankton (2–0.7 µm). Mesozooplankton and microplankton were homogenized with a small air pump and two aliquots of 100 mL were also filtered through GF/F filters in duplicate. Two filters of each fraction were conserved in dichloromethane–methanol (2:1 by vol.) under a nitrogen atmosphere in 4 mL precombusted glass vials at –30°C for later lipid determination. Two other filters of each fraction were dried overnight (50°C) and stored in a desiccator for later particulate organic matter and stable isotope analysis.

Mesozooplankton were preserved with buffered formaldehyde (4%), taxonomically identified and counted in a zooplankton counting chamber (Hydro-Bios) under a stereomicroscope (Wild M5). Total mesozooplankton (number of individuals per litre) was estimated from partial counting (10% of the total). Microplankton and nanoplankton were immediately fixed with Lugol's iodine solution and counted in duplicate in a combined plate chamber (Hydro-Bios) under an inverted microscope (Wild M40). The whole slide was scanned at lower magnification (×200) for microplankton counting. The minimum numbers of individuals counted was 2000 for mesozooplankton and 200 for microplankton. Nanoplankton were counted at high magnification (×400) along the main diameters of the plate chamber. Abundance was calculated considering the surface area counted, the total surface of the plate chamber and the volume of water sedimented. The biovolume was calculated after Hillebrand *et al.* (Hillebrand *et al.*, 1999)

using a standardized set of equations for volume calculations from microscopically measured linear dimensions of at least 15 cells.

Fatty acid extraction and determination

The samples in dichloromethane–methanol were homogenized with a high-performance disperser (Ultra-turrax) after addition of the fatty acid standards 19:0 and 23:0. Lipids were basically extracted after Folch *et al.* (Folch *et al.*, 1957). The lipid extracts were transesterified under nitrogen atmosphere with 3% concentrated sulphuric acid in methanol for 4 h at 80°C. The resulting fatty acid methyl esters (FAME) were extracted with hexane and analysed by gas–liquid chromatography (Hewlett Packard 6890 GC) on a 30 m wall-coated capillary column (inner diameter 0.25 mm, film thickness 0.25 µm; liquid phase DB-FFAP) according to Kattner and Fricke (Kattner and Fricke, 1986). Fatty acids were quantified with the internal standards and identified with standard mixtures and if necessary by mass spectrometry (GC-MS). FAME data were acquired with the software Chemstation Vers B04.01 (Agilent Technologies).

Organic matter and stable isotope survey

The dried samples were homogenized with a ball mill (PM 100, Retsch), placed in silver vials and acidified with 0.1 N HCl to remove inorganic carbon. Vials with samples were dried for 12 h at 50°C. Particulate organic carbon and nitrogen were completely oxidized by flash combustion at temperatures above 1000°C under pure O₂ and quantified with an elemental analyser (Carlo Erba NA 2100). Stable isotopes of carbon (¹³C) and nitrogen (¹⁵N) of each plankton fraction were analysed with a Thermo Finnigan Delta Plus mass spectrometer coupled with a Flash EA 1112 elemental analyser. Sample analysis was carried out without lipid extraction which is discussed later. Samples were analysed in duplicate, including peptone as standard and a blank every four samples. The amount of isotope per sample was within the analytical range. The relative standard deviation between the duplicates never exceeded 3%. Isotope ratios are reported in parts per million (‰), nitrogen relative to nitrogen in air and carbon to Pee Dee Belemnite (PDB) according to the formula:

$$R = {}^{13}\text{C}/{}^{12}\text{C} \text{ or } {}^{15}\text{N}/{}^{14}\text{N} \text{ and}$$

$$\delta(\text{‰}) = [(R_{\text{sample}} - R_{\text{standard}} - 1)] \times 1000$$

The isotope ratios were determined in accordance with reference standards of the International Atomic Energy Agency (IAEA), National Bureau of Standards (NBS) and

the United States Geological Survey (USGS). IAEA-N1 ($\delta^{15}\text{N} = 0.4\text{‰}$) and IAEA-N2 ($\delta^{15}\text{N} = 20.3\text{‰}$) were used to normalize the $\delta^{15}\text{N}$ and USGS-24 ($\delta^{13}\text{C} = -16.5\text{‰}$) and NBS 22 ($\delta^{13}\text{C} = -30.0\text{‰}$) to the $\delta^{13}\text{C}$.

Data analysis

From the proportions (Mass % of total fatty acids) of the 46 fatty acids detected those with $\geq 1\%$ were selected. The 14:0, 16:0 and 18:0 are ubiquitous among the microalgae and were therefore grouped together as even-chain saturated fatty acids (SFA even). The bacterial marker fatty acids 15:0 iso and anteiso and 17:0 were grouped as odd-chain saturated fatty acid (SFA odd) and the diatom indicators 16:2($n - 4$), 16:3($n - 4$) and 16:4($n - 1$) as polyunsaturated fatty acids with 16 carbon atoms (PUFA 16 C). The samples were ordinated by principal component analysis (PCA) to appreciate the relations between samples and markers. The PCA ordination was based on the correlation matrix of untransformed data and performed using the program XLSTAT 2013. We also analysed multivariate data of fatty acid proportions, stable isotopes of carbon and nitrogen (‰) using permutational multivariate analysis of variance (PERMANOVA) in PRIMER v6. Resemblance matrices of untransformed data were calculated using Euclidean. PERMANOVA with a significance threshold at $p_{\text{perm}} = 0.05$ was used to test significant differences among fractions, seasons and sampling sites. Linear correlations between plankton and markers and regressions between fatty acid content ($\mu\text{g FA mg C}^{-1}$) of the main polyunsaturated fatty acids were calculated with Xact version 7.21d.

RESULTS

Hydrography and plankton dynamics

The physicochemical values were almost homogenous throughout the water column because of mixing by strong winds and the polymictic nature of the lake. An intermediate salinity (~ 20) and an alkaline pH (~ 9) were typical for the lake. Salinity values were relatively constant, while temperature and chlorophyll *a* showed pronounced seasonal dynamics (Fig. 1). The water temperature ranged from 5°C in winter to 25°C in late summer. The winter of 2007 ($5.2 \pm 0.6^\circ\text{C}$) was colder than that of 2008 ($9.4 \pm 0.4^\circ\text{C}$). The lowest temperature was recorded in July 2007 ($4.7 \pm 0.1^\circ\text{C}$; data not shown).

Mesozooplankton were characterized by the abundant (Fig. 2A) large calanoid copepod *Boeckella poopoensis* (37 ± 18 organisms L^{-1}), which dominated the total zooplankton biomass (Fig. 2A). This copepod was exclusively

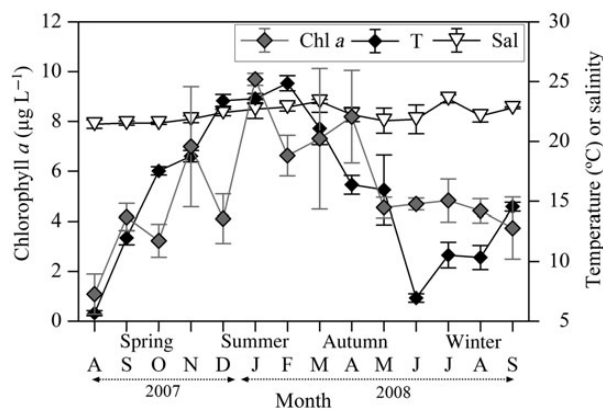


Fig. 1. Data show mean values \pm standard deviation (error bars) from the three sampling stations (CV, EE, RM) of chlorophyll *a* (Chl *a*), temperature (T) and salinity (Sal) in Lake Chasicó from August 2007 to September 2008.

found at the end of the colder winter in August and September 2007. Thereafter, the cladoceran *Moina eugeniae* emerged during spring and dominated in December, mainly at Station CV (84 organisms L^{-1}). In addition, the rotifer *Brachionus plicatilis* was detected from April to September 2008, showing its maximum abundance in March at Station RM (316 organisms L^{-1}). Small *B. plicatilis* in the microplankton peaked in March at Station RM (8350 organisms L^{-1}), together with their maximum abundance in the mesozooplankton. Microzooplankton were composed of small copepodids, nauplii, ciliates and rotifers dominating the biomass during winter. Diatoms (*Cyclotella* spp.) dominated the biovolume ($2.0 \pm 1.3 \text{ mm}^3 \text{ L}^{-1}$) of the microplankton fraction from late spring to early autumn (Fig. 2B). During the warmer months, non-flagellated chlorophytes (*Oocystis* spp., *Scenedesmus* spp.) and cyanobacteria (*Nodularia spumigena*, *Planktothrix* sp., *Oscillatoria* sp., *Anabaena* spp., *Microcystis* spp.) were particularly abundant ($300 \pm 210 \times 10^3$ cells L^{-1} and $470 \pm 600 \times 10^3$ cells L^{-1} , respectively). Throughout the sampling period the main proportion of the nanoplankton consisted of flagellates ($5.4 \pm 4.6 \times 10^6$ cells L^{-1}) which almost exclusively dominated the winter phytoplankton (Fig. 2C).

Seasonal variations in stable isotopes, fatty acids and correlations with plankton

The $\delta^{13}\text{C}$ values showed a strong seasonal trend in all plankton fractions (Fig. 3A). The biovolume of diatoms was positively correlated with the $\delta^{13}\text{C}$ in microplankton ($r = 0.55$, $P < 0.001$) and the cyanobacteria with the $\delta^{13}\text{C}$ in nanoplankton ($r = 0.40$, $P = 0.01$). Microplankton had mainly elevated $\delta^{13}\text{C}$ values, except in August and September 2007. During these months, mesozooplankton

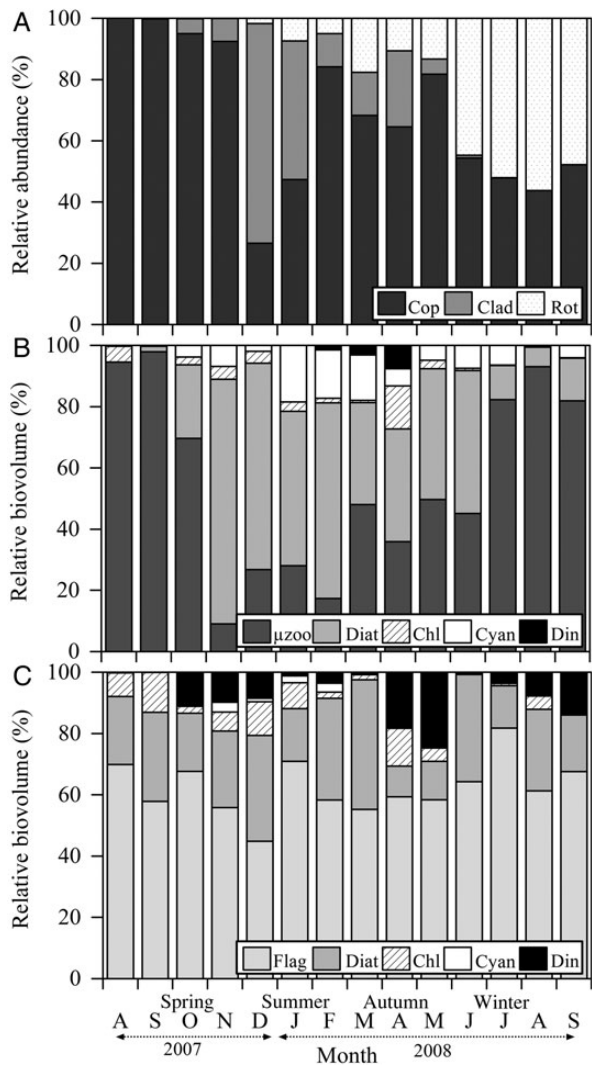


Fig. 2. Seasonal patterns in relative mean abundance of mesozooplankton (A) and in relative mean biovolume of microplankton (B) and nanoplankton (C) from August 2007 to September 2008. Mean values from the three sampling stations are presented. Cop, copepod *Boeckella poopoensis*; Clad, cladoceran *Moina eugeniae*; Rot, rotifer *Brachionus plicatilis*; μzoo, microzooplankton; Diat, diatoms; Chl: non-flagellated chlorophytes; Cyan, cyanobacteria; Din, dinoflagellates; Flag, flagellates.

had the minimum $\delta^{13}\text{C}$ signature. These values coincided with higher C/N ratios in mesozooplankton and nanoplankton (Fig. 3B), and higher values of $\delta^{15}\text{N}$ and total fatty acid content in mesozooplankton (Fig. 3C and D). The three plankton fractions were clearly separated by the $\delta^{15}\text{N}$ values (Table I and Fig. 3C). $\delta^{15}\text{N}$ of mesozooplankton did not follow a marked seasonal trend and the highest $\delta^{15}\text{N}$ signatures were detected in August and November 2007. The $\delta^{15}\text{N}$ values of the mesozooplankton were positively correlated with the *B. poopoensis* abundance ($r = 0.57, P < 0.001$) and negatively with the *B. plicatilis*

abundance ($r = 0.49, P < 0.001$). Microplankton and nanoplankton described a seasonal pattern but with opposite trends. Cyanobacteria were negatively correlated with the $\delta^{15}\text{N}$ values of the microplankton ($r = 0.47, P = 0.002$).

Mesozooplankton were characterized by higher total fatty acid content ($\mu\text{g mg C}^{-1}$), lower C/N ratios and higher percentages (mass % of total fatty acids) of 18:3 ($n - 3$), 18:4($n - 3$), 20:4($n - 6$), 20:4($n - 3$) and 22:6 ($n - 3$) (Table I). The relative abundance of *M. eugeniae* was mainly correlated with 16:1($n - 7$) ($r = 0.56, P < 0.001$), 18:2($n - 6$) ($r = 0.77, P < 0.001$) and 20:4($n - 6$) ($r = 0.50, P < 0.001$). The relative biovolume of microzooplankton was positively correlated with 18:4($n - 3$) ($r = 0.60, P < 0.001$). Although 18:4($n - 3$) is a flagellate marker, it did not follow the trend of flagellates and did not correlate with their relative biovolume. The content of 18:4($n - 3$) was negatively related with the water temperature and described a conspicuous seasonal trend in all plankton fractions (Fig. 4). The chain extension marker 20:4($n - 3$) showed the same pattern as the 18:4($n - 3$), but there was no significant correlation between temperature and this fatty acid in the nanoplankton fraction. The 20:4($n - 6$) in mesozooplankton and nanoplankton and the 20:5($n - 3$) in microplankton were linearly significantly correlated with water temperature. Conversely, the 22:6($n - 3$) showed a slightly negative slope only in mesozooplankton.

PERMANOVA and PCA analysis

PERMANOVA analysis revealed differences in size (pseudo- $F_{3, 122} = 11.2, p_{\text{perm}} < 0.001$), season (pseudo- $F_{2, 122} = 67.6, p_{\text{perm}} < 0.001$) and size-season interactions (pseudo- $F_{6, 122} = 5.2, p_{\text{perm}} < 0.001$). No significant differences were found among the sampling stations (pseudo- $F_{2, 122} = 0.5, p_{\text{perm}} = 0.788$). Mesozooplankton and microplankton of the four seasons were differentiated by pair-wise tests ($P < 0.05$) (Table II). For nanoplankton, autumn was the only season which was significantly separated from the others. The main relations between stable isotopes and fatty acids were summarized in their correlations with the principal components (Fig. 5A). The $\delta^{15}\text{N}$ values were positively related with the main polyunsaturated fatty acids, particularly with 22:6($n - 3$) ($r = 0.79, P < 0.001$) but generally negatively with the saturated and monounsaturated fatty acids, e.g. SFA even ($r = 0.74, P < 0.001$) and 16:1($n - 7$) ($r = 0.59, P < 0.001$). One of the highest correlation coefficients among fatty acids was found between the 18:4($n - 3$) and the 20:4($n - 3$) ($r = 0.77, P < 0.001$). The first three axes of the PCA analysis explained 70% of the variation: 37% the first, 19% the second and 14% the third component. The first

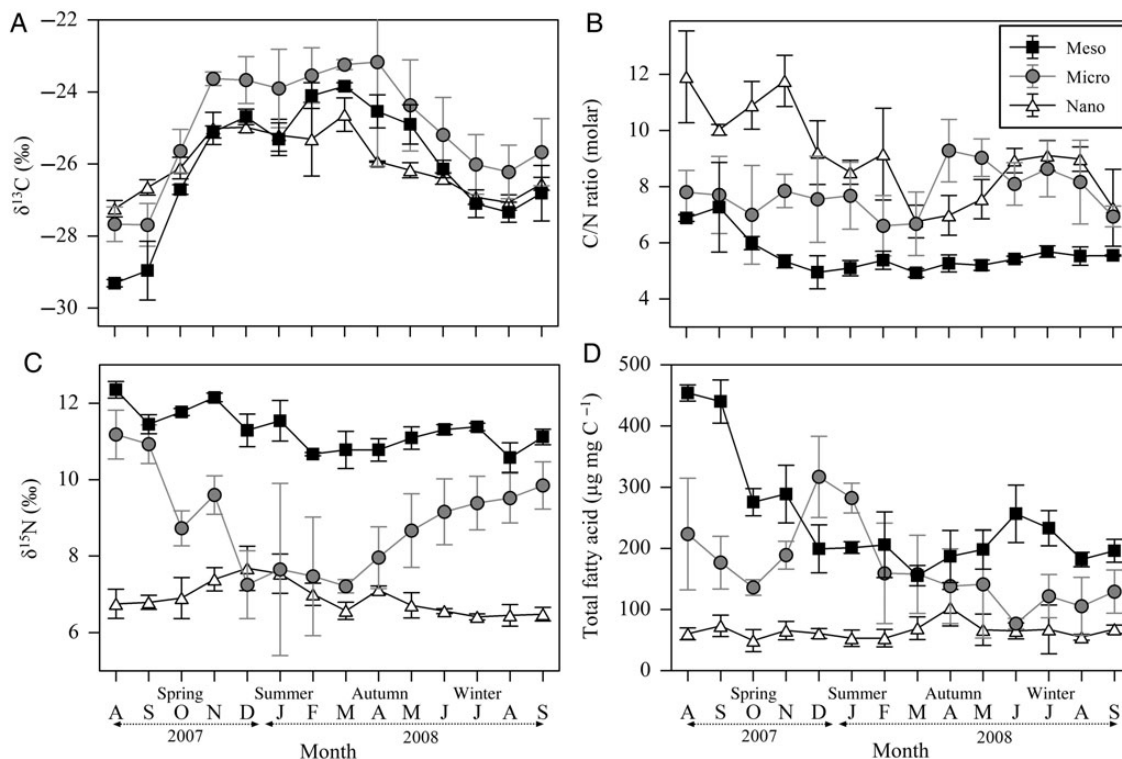


Fig. 3. Monthly dynamics of stable isotope ratios of carbon ($\delta^{13}\text{C}$) (A) and nitrogen ($\delta^{15}\text{N}$) (C), total fatty acid contents (D) and carbon (C)/nitrogen (N) ratios (B) from August 2007 to September 2008. Mean values \pm standard deviation (error bars) from the three sampling stations. Meso, mesozooplankton; Micro, microplankton; Nano, nanoplankton.

component could be associated with the trophic level and clearly separated the mesozooplankton from nanoplankton (Fig. 5C).

The mesozooplankton at the three stations from August 2007, with the exclusive dominance of *B. poopoen-sis*, were located at the positive extreme of the first component and negatively ordinated with respect to the second and third axis. The mesozooplankton of summer, late spring and early autumn exhibited higher values of $\delta^{13}\text{C}$ and saturated and monounsaturated fatty acids. The sample with the abundance peak of *M. eugeniae* in December at Station CV was arranged negatively with the first component, basically because of the highest percentage of 16:1($n-7$) and an elevated proportion of saturated fatty acids. The microplankton of the warmer months and nanoplankton were ordinated in general to the negative extreme due to their higher proportions of saturated and monounsaturated fatty acids and lower $\delta^{15}\text{N}$ signatures.

The second component (Fig. 5A) suggested a positive influence of diatoms by the markers, $\delta^{13}\text{C}$, 20:5($n-3$) and PUFA 16 C and a negative influence of bacteria as indicated by 18:1($n-7$). The third axis (Fig. 5D) positively ordinated the samples with generally higher proportions of ($n-6$) fatty acids. At the positive extreme of

this axis, the sample of microplankton with the highest abundance of *B. plicatilis* at Station RM (near the river) was located because of the highest 18:2($n-6$) proportion. The seasonality of the nanoplankton samples was less marked as was also shown by the PERMANOVA analysis. The PCA did not ordinate the samples in any clear pattern in relation to their location.

DISCUSSION

Stable isotope ratios and fatty acid markers

The relationship of $\delta^{13}\text{C}$ and the fatty acid markers suggested that the higher primary production in summer was mainly driven by diatoms. Consequently, these microalgae accounted for the elevated carbon fractionation in the microplankton fraction and were the main food source for the mesozooplankton during the warmer months. The $\delta^{13}\text{C}$ trend was a good indicator of primary production, as suggested by the strong correlation with chlorophyll *a* (Kopprio *et al.*, 2014). Diatoms show an enriched $\delta^{13}\text{C}$ (Fry and Wainright, 1991), and together with chlorophytes and cyanobacteria are capable of using bicarbonate (Moschen *et al.*, 2009). Bicarbonate is generally enriched by $\sim 8\%$ more than CO_2 (Ostrom *et al.*,

Table I: Mean values and standard deviations of stable isotopes (‰), C/N ratios (molar), total fatty acid contents (µg mg C⁻¹) and main fatty acids (mass % of total fatty acids) in the three fractions

Variables	Mesozooplankton	Microplankton	Nanoplankton
δ ¹⁵ N (‰)	11.3 ± 0.6	8.9 ± 1.5	6.9 ± 0.5
δ ¹³ C (‰)	-26.1 ± 1.7	-25.0 ± 1.8	-26.0 ± 0.9
C/N (M)	5.6 ± 0.8	7.8 ± 1.2	9.1 ± 1.8
Tot FA (µg mg C ⁻¹)	249 ± 140	165 ± 77	65 ± 20
FA (% mass)			
SFA even	25.1 ± 4.0	36.3 ± 1.6	38.4 ± 2.8
14:0	7.0 ± 2.1	6.9 ± 2.8	11.1 ± 3.7
16:0	15.6 ± 2.6	23.7 ± 2.7	19.7 ± 2.2
18:0	2.8 ± 1.1	5.7 ± 2.6	7.6 ± 2.7
SFA odd	4.0 ± 1.1	3.4 ± 1.9	5.8 ± 1.3
15:0	1.5 ± 0.6	1.7 ± 2.4	1.6 ± 0.4
16:1(n - 7)	12.6 ± 4.0	14.8 ± 7.6	18.7 ± 5.6
PUFA 16 C	2.4 ± 0.9	3.5 ± 2.4	3.4 ± 1.9
18:1(n - 9)	2.7 ± 1.1	4.2 ± 1.8	6.0 ± 2.5
18:1(n - 7)	5.5 ± 1.1	4.0 ± 1.6	6.9 ± 2.1
18:2(n - 6)	2.1 ± 0.7	2.2 ± 1.3	2.6 ± 0.8
18:3(n - 3)	5.0 ± 1.5	2.8 ± 1.2	2.7 ± 1.3
18:4(n - 3)	10.0 ± 5.8	4.3 ± 0.6	3.3 ± 2.0
20:4(n - 6)	1.5 ± 0.7	0.9 ± 0.6	0.5 ± 0.5
20:4(n - 3)	3.9 ± 4.2	2.7 ± 3.2	0.6 ± 0.6
20:5(n - 3)	8.6 ± 2.4	9.7 ± 3.4	3.8 ± 1.3
22:6(n - 3)	12.4 ± 4.0	7.2 ± 2.8	3.4 ± 1.6

Data obtained from August of 2007 to September of 2008 at the three sampling stations.

Tot FA, total fatty acid content; SFA, saturated fatty acids; PUFA 16 C, polyunsaturated acids of 16 carbon atoms.

1997), and the pH of Lake Chasicó (~9) favours the dominance of this anion. Small-sized cyanobacteria contributed to the δ¹³C enrichment of nanoplankton.

For the mesozooplankton, the combined marker approach allowed processes of the lipid metabolism to be evaluated. The major species *B. poopoensis* is a large-sized omnivorous species characteristic of saline lakes with low biodiversity, tolerating a wide range of salinities (De los Rios and Crespo, 2004; Vignatti *et al.*, 2007). The elevated accumulation of lipids in mesozooplankton during winter contributed to the seasonal dynamics of δ¹³C. Lipids have a lower δ¹³C signature (Vander Zanden and Rasmussen, 2001) and thus masked the carbon isotopic fractionation. Moreover, if elongation and desaturation processes are involved in lipid production and accumulation, the δ¹³C will be lighter because isotopic fractionation occurs during lipid synthesis. *Boeckella poopoensis* is probably able to elongate the 18:4(n - 3) fatty acid (marker of flagellate consumption) to 20:4(n - 3) during the biosynthesis of the storage lipids, wax esters and triacylglycerols (Kopprio *et al.*, 2012). If not only the carbon origin but also the dietary items consumed by a heterotrophic consumer are studied, it may be more appropriate to analyse the complete organisms without lipid extraction.

The close relationship between 22:6(n - 3) and δ¹⁵N indicated a planktonic food web dominated by copepods at higher trophic levels. The 22:6(n - 3) is typically accumulated in copepods (Scott *et al.*, 2002; Ravet *et al.*, 2010). Also the 20:4(n - 6) separated the trophic levels and may be evidence of metabolic processes such as the production of eicosanoids by mesozooplankton during summer. The amount and proportion of polyunsaturated fatty acids, generally necessary for growth and reproduction (e.g. Koski *et al.*, 2011), increase with trophic level (Copeman and Parrish, 2003; Persson and Vrede, 2006; Kim *et al.*, 2014). The highest value of δ¹⁵N in mesozooplankton during the coldest winter may also be related to nutritional stress because animals seem to catabolize their own proteins to enrich their bodies with proteins richer in ¹⁵N (Hobson *et al.*, 1993). The low food quality may be partially compensated by the lipid storage of *B. poopoensis* with elevated proportions of the 20:4(n - 3) fatty acid. Some zooplankton species are able to considerably elongate precursor fatty acids (Graeve *et al.*, 2005; Kattner *et al.*, 2012), and probably this also applies to *B. poopoensis* (Kopprio *et al.*, 2012).

Some other species partially affected the isotopic and fatty acid compositions directly through their small contribution to the total biomass or indirectly by input to the diet of *B. poopoensis*. The fatty acids 20:4(n - 6) and 20:5(n - 3) are typical of cladocerans (Ballantyne *et al.*, 2003; Persson and Vrede, 2006) but only 20:4(n - 6) was related to *M. eugeniae*. The 18:2(n - 6) and the 16:1(n - 7) indicated the herbivory of *M. eugeniae*. The high proportion of 18:2(n - 6) in the sample with a considerable contribution of *B. plicatilis* near the river seemed to reflect terrestrial input. The river input was characterized by higher δ¹³C and lower δ¹⁵N signatures (Kopprio *et al.*, 2014); nevertheless, no differences among stations were revealed. The seasonal variations of δ¹⁵N and the saturation of fatty acids in microplankton were likely related to the occurrence of cyanobacteria in this fraction in summer and to that of microzooplankton in winter. The depletion of δ¹⁵N is a strong indication of nitrogen fixation which may be caused by cyanobacteria with lower levels of polyunsaturated acids. Elevated values of δ¹⁵N in nanoplankton during summer have been explained by microbial processes and degradation of organic matter (Ostrom *et al.*, 1997; Gu, 2009; Reuss *et al.*, 2013).

Trophic markers: influence of seasonality and temperature

Our trophic marker study in Lake Chasicó demonstrated the strong seasonal variations of fatty acid and stable isotope composition in the plankton fractions, principally in mesozooplankton and microplankton. Typical

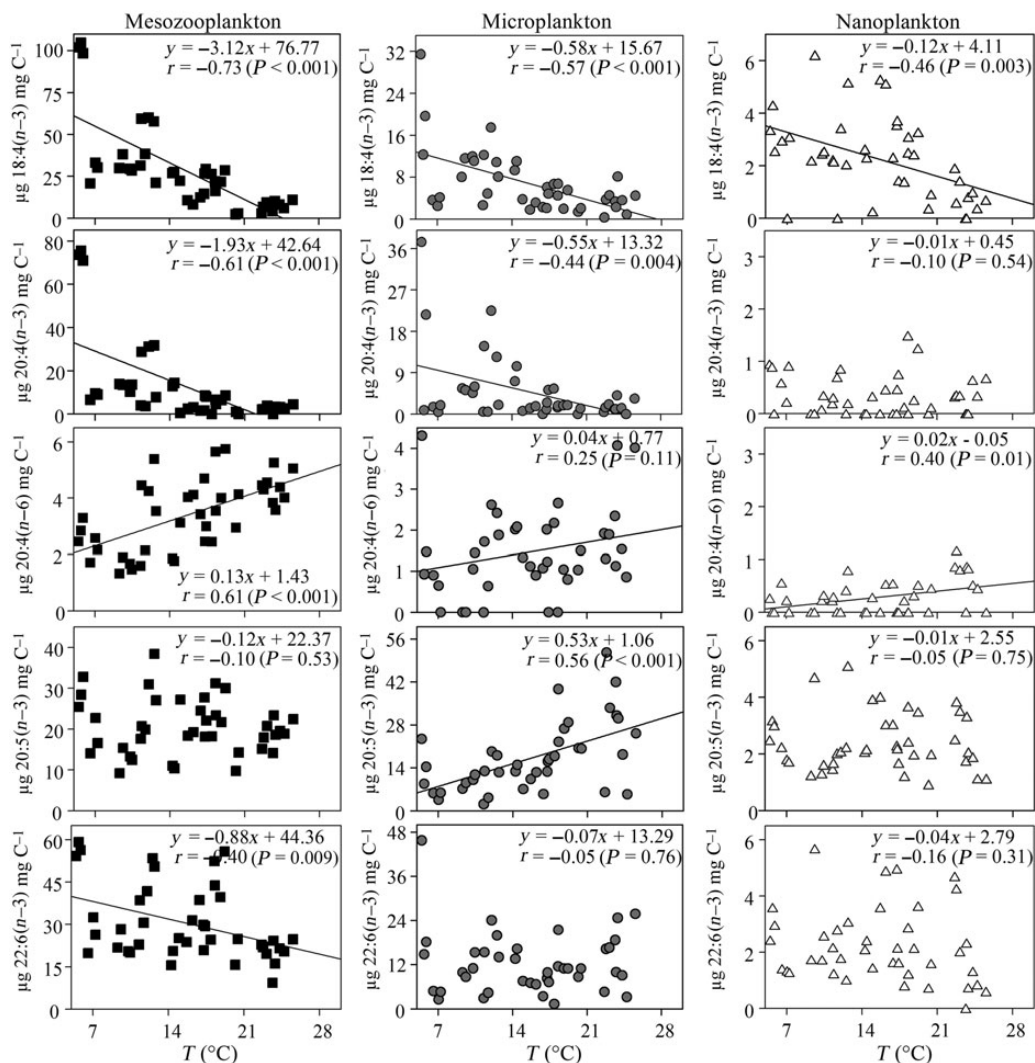


Fig. 4. Regressions between the contents of the main polyunsaturated fatty acids ($\mu\text{g mg C}^{-1}$) in the plankton fractions with water temperature at each sampling site from August 2007 to September 2008.

Table II: Pair-wise tests comparing seasons within plankton fractions derived from the permutational multivariate analysis of variance (PERMANOVA)

Comparisons	Den. d.f.	Mesozooplankton		Microplankton		Nanoplankton	
		<i>t</i>	ρ_{perm}	<i>t</i>	ρ_{perm}	<i>t</i>	ρ_{perm}
Winter and Spring	22	2.48	0.003	1.94	0.017	1.17	0.225
Winter and Summer	18	4.55	0.001	4.27	0.001	1.46	0.091
Winter and Autumn	19	3.84	0.001	3.04	0.001	2.06	0.014
Spring and Summer	18	3.23	0.001	2.73	0.002	1.01	0.341
Spring and Autumn	19	2.76	0.001	1.87	0.023	2.40	0.004
Summer and Autumn	15	2.30	0.016	1.76	0.049	2.36	0.003

Significance threshold at ρ_{perm} 0.05.

Den. d. f., denominator degrees of freedom.

mesozooplankton trophic markers were related to those in the seston fractions (microplankton and nanoplankton). There were also significant relationships between

the fatty acid trophic markers within the fractions and their plankton groups which allow identification and confirmation of food web interactions (Dalsgaard *et al.*, 2003;

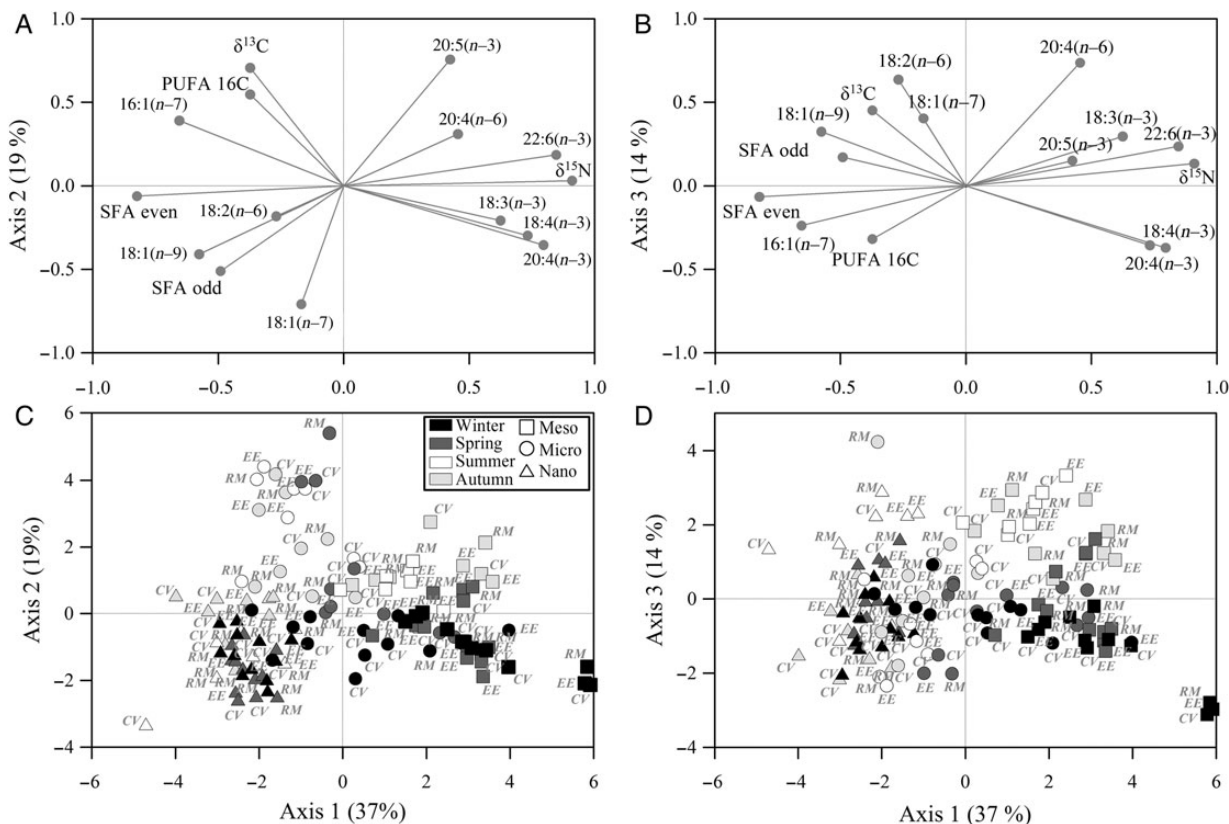


Fig. 5. Principal components analysis (PCA) of all samples based on a correlation matrix among fatty acid (mass % of total fatty acids) and stable isotopes (‰) markers. **(A–B)** Circle of correlations of the variables with the main components. **(C–D)** Ordination of samples according to the main components. Meso, mesozooplankton; Micro, microplankton; Nano, nanoplankton; CV, Station “Chapalcó Village”; EE, Station “El Embudo”; RM, Station “River Mouth”; SFA even, even-chain saturated fatty acids; SFA odd, odd-chain saturated fatty acid; PUFA 16 C, polyunsaturated fatty acids with 16 carbon atoms.

Ravet *et al.*, 2010; Peters *et al.*, 2013). However, some field studies also failed to find correlations between the fatty acid composition of zooplankton and seston (Persson and Vrede, 2006; Smyntek *et al.*, 2008).

The relation of nanoplankton with the other plankton fractions was less pronounced which was probably due to the highly variable composition of this group on the one hand and selective feeding of the copepods on the other hand. There were higher variations of fatty acids in this fraction, since it represents a mixture of phytoplankton species, bacteria and detritus, which undergoes a steady alteration owing to different abiotic and biotic conditions (Dalsgaard *et al.*, 2003; Bec *et al.*, 2010). The 18:1(*n* – 9) fatty acid, for example, was relevant for the nanoplankton fraction suggesting a clear contribution from detrital material (Fahl and Kattner, 1993), probably also derived from zooplankton (e.g. faecal detritus) because it is considered a marker of higher trophic levels (Kattner *et al.*, 2003; Dutto *et al.*, 2014).

Warmer temperatures may result in higher proportions of saturated fatty acids in the various plankton groups,

especially in microalgae (Guschina and Harwood, 2009) and thus may potentially influence zooplankton dietary lipids. The saturated fatty acids were typical of nanoplankton and microplankton, particularly during summer. These groups were more abundant during the hot months which may confirm that organisms, exposed to periods of elevated temperatures, are enriched in saturated and monounsaturated fatty acids. We therefore hypothesize that the strong seasonal variations of the flagellate marker 18:4(*n* – 3) in the nanoplankton fraction were not only due to the seasonal temperature change itself but also to the temperature-dependent change of the nanoplankton communities.

The flagellate marker 18:4(*n* – 3) and the indicator of chain elongation 20:4(*n* – 3), which were strongly associated with the temperature oscillations, occurred mainly in the wax esters and also but to a lesser extent in the triacylglycerols of *B. poopoensis* (Kopprio *et al.*, 2012), however not in the polar lipids characteristic of membranes. The seasonal variation in 20:4(*n* – 3) may be relevant to increase the fluidity of the reserve lipid in

winter. The 20:4($n - 3$) could also be easily extended to 20:5($n - 3$) or 22:6($n - 3$) to sustain growth and reproduction in winter, when the requirements of essential fatty acids for zooplankton are generally higher (Masclaux *et al.*, 2009; Sperfeld and Wacker, 2012; Hartwich *et al.*, 2013). Therefore, the homeoviscous adaptation of membranes (HVA) does not explain the high amounts and the turnover of 18:4($n - 3$) and 20:4($n - 3$) in the storage lipids of the copepods. Neither does the HVA model explain the positive correlation of 20:4($n - 6$) in mesozooplankton with the water temperature.

Concluding remarks and climate change outlooks

The combination of fatty acids and stable isotopes offers richer information about trophic position and links, metabolic processes and seston composition. Our study demonstrates the importance of seasonal variations, represented by temperature and plankton composition, on the fatty acid and stable isotope signature of the lake planktonic fractions. Sampling effort should be detailed enough to capture the conspicuous variation of markers in such temperate shallow lakes. The extrapolation of conclusions about trophic markers from laboratory work at a constant temperature or during one season (mainly summer)

to natural plankton communities should be carefully considered. However, in field studies it is often difficult to separate environmental factors from trophic interactions.

Lake Chasicó and similar shallow lacustrine ecosystems will be likely affected by climate change not only due to higher temperatures but also due to effects similar to eutrophication (Fig. 6). Cyanobacteria blooms, primary production changes, hypoxic or anoxic events, deterioration of benthic producers, enhanced liberation of greenhouse gases, reduction of zooplankton size and fish mortalities are amongst the expected events in shallow lakes (Jeppesen *et al.*, 2009; Kopprio *et al.*, 2010; Moss, 2012). A predicted dominance and higher primary production of cyanobacteria may increase the $\delta^{13}\text{C}$ values, the proportions of saturated fatty acids and may decrease the $\delta^{15}\text{N}$ and long-chain polyunsaturated fatty acids particularly in microplankton. The better competitive abilities of cyanobacteria are very likely to decrease the abundance of other microalgae (Paerl and Paul, 2012). The poor food quality of cyanobacteria will probably impact on the nutritional value of the zooplankton diet. An enhanced fish predation is also expected to decrease the size of zooplankton, and more frequent fish mortality events are expected to occur due to cyanobacteria toxins or anoxia.

Anoxia favours the liberation of nutrients and greenhouse gases and consequently stimulates phytoplankton

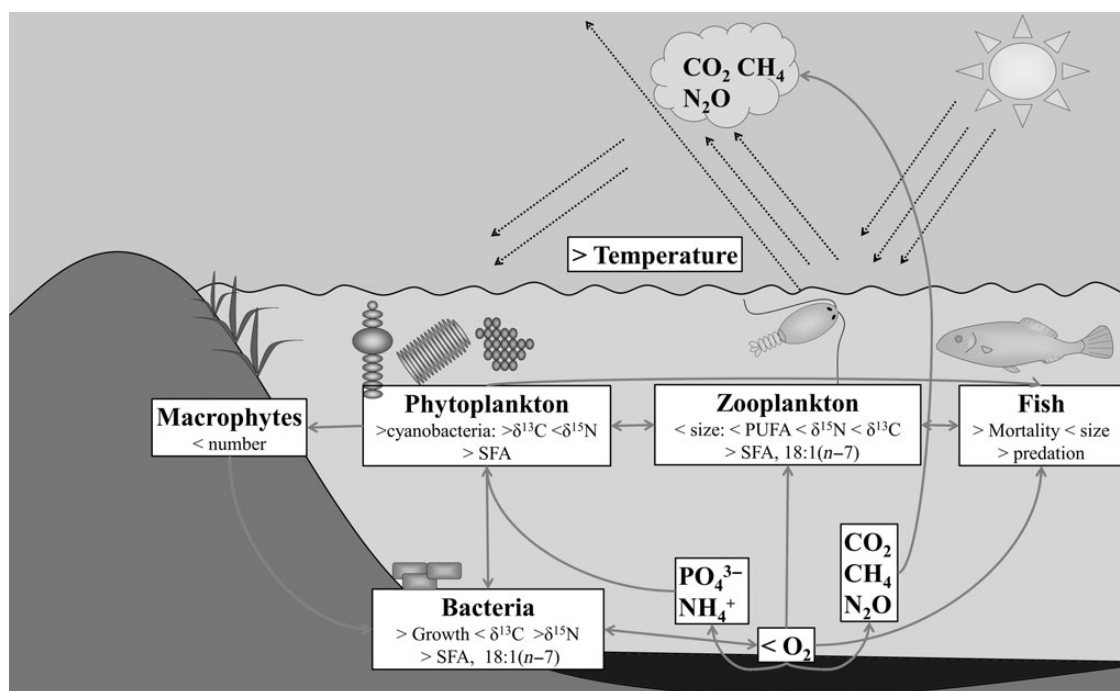


Fig. 6. Likely climate-driven changes in shallow lakes and perspectives on the development of biomarkers. The changes in the ecosystem are related to an increasing temperature and presented as an increase (>) or decrease (<) of the biological and environmental components.

blooms and more global warming, respectively (Moss, 2012; Kopprio *et al.*, 2015). Higher turbidity in combination with anoxia will decrease the biomass of macrophytes. The presence of methanotrophic bacteria is very likely to decrease the $\delta^{13}\text{C}$, while microbial degradation of organic matter may enhance the $\delta^{15}\text{N}$ values in nanoplankton and detritus as well as bacterial fatty acid markers. Smaller zooplankton species generally feed on bacterioplankton and consequently may increase their bacterial marker signature. The reduced size of zooplankton may result in a decline in their predation on heterotrophic organism richer in $\delta^{15}\text{N}$ and polyunsaturated fatty acids. Eutrophication-like changes will be affected by hydrological instabilities making future scenarios even more complex and uncertain.

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