Ann. Limnol. - Int. J. Lim. 50 (2014) 279–287 © EDP Sciences, 2014 DOI: 10.1051/limn/2014022

Available online at: www.limnology-journal.org

# Spatiotemporal dynamics of C and N isotopic signature of zooplankton: a seasonal study on a man-made lake in the Mediterranean region

Amedeo Fadda<sup>1\*</sup>, Ruth Rawcliffe<sup>2</sup>, Bachisio Mario Padedda<sup>1</sup>, Antonella Lugliè<sup>1</sup>, Nicola Sechi<sup>1</sup>, Federica Camin<sup>3</sup>, Luca Ziller<sup>3</sup> and Marina Manca<sup>2</sup>

<sup>1</sup> Department of Sciences for Nature and Environmental Resources (DipNET) of the University of Sassari (Italy), 4 07100 Sassari (SS), Italy

<sup>2</sup> CNR Institute of Ecosystem Study (CNR-ISE), Largo Tonolli 50 28922 Verbania (VB), Italy

<sup>3</sup> IASMA, Fondazione Edmund Mach, Research and Innovation Centre, Via Mach 1, San Michele all'Adige (TN), Italy

Received 13 April 2014; Accepted 19 August 2014

**Abstract** – Reservoirs are subject to severe fluctuations in the water level from seasonal and interannual climatic variations, as well as abstraction for irrigation, hydropower, and drinking water. This can affect the matter and energy transfer through the food web, of which zooplankton is of crucial importance. We traced seasonal changes in the carbon and nitrogen stable isotope signatures of suspended particulate matter and crustacean zooplankton from a small Mediterranean reservoir. The  $\delta^{13}$ C and  $\delta^{15}$ N isotopic baseline signature of the lake varied seasonally, becoming more <sup>13</sup>C-depleted and <sup>15</sup>N-enriched in winter and less <sup>13</sup>C-depleted and <sup>15</sup>N-enriched values in the drier summer months, when external water inputs were negligible. Seasonal changes in the  $\delta^{13}$ C and  $\delta^{15}$ N SPM isotopic signature were well reflected in the herbivorous cladocerans.  $\delta^{15}$ N of the calanoid and cyclopoid copepods were at least 3‰ greater than for the herbivorous cladocera, suggesting their potential use as a food resource.  $\delta^{13}$ C of predatory copepods were also consistent with seasonal fluctuations in the  $\delta^{13}$ C SPM baseline, except during the heavy rains in early spring, when they were observably rich in lipids with a higher C/N ratio, suggesting that they had entered dormancy and were not actively feeding in the water column. This indicates the importance of taking into account not only the seasonality, but the community dynamics and trophic level of zooplankton taxa when interpreting stable isotope studies.

**Key words:** Reservoir / water-level fluctuations / suspended particulate matter / zooplankton / stable isotope analysis

#### Introduction

Knowledge of the energy flow and trophic interactions in an ecosystem is important for understanding population dynamics and predicting the likely response of a community to a disturbance (Lindeman, 1942; Karlsson *et al.*, 2003; Finlay and Kendall, 2008; Marcarelli *et al.*, 2011; Thompson *et al.*, 2012). At its simplest level, a food chain describes a pathway for energy (biomass) transferral from a primary producer through a series of consumers on increasingly higher trophic levels. This is particularly effective if used in tandem with stable isotope analysis (SIA), which provides an independent means of assessing the pathways of energy flow within a community. SIA is increasingly being used to investigate the ecological structure and function of freshwater systems (Finlay and Kendall, 2008). The isotopic fractionation of <sup>13</sup>C and <sup>15</sup>N can be used to trace energy flow through ecological communities (Peterson and Fry, 1987; Cabana and Rasmussen, 1996) and provide time-integrated information of complex trophic interactions (Post, 2002) to complement dietary "snapshots" derived from gut content analysis.

Despite the growing literature describing food webs in lakes and reservoirs, there have been few studies on man-made waterbodies in the Mediterranean region. The biodiversity and conservation of such reservoirs are particularly sensitive to climate change (Moss *et al.*, 2009)

<sup>\*</sup>Corresponding author: amedeo.fadda@gmail.com

and are considered as stressed environments (Leira and Cantonati, 2008 and references therein; Naselli-Flores, 2003; Henry et al., 2011; Perbiche-Neves et al., 2011). Compared with natural lakes, artificial waterbodies are subject to severe fluctuations in the water level from seasonal and interannual climatic variations, as well as abstraction for irrigation, hydropower and drinking water. This high seasonal variability, combined with spatial heterogeneity, characterizes reservoirs as an intermediate waterbody between lakes and rivers (Tundisi, 1999). Receiving significant allochthonous inputs during the wet season and relying on internal cycling processes to sustain biota during the dry season (Zohary and Ostrovsky, 2011), the littoral zone is often only periodically submerged, which limits the persistence of the aquatic macrophyte community in favour of phytoplankton.

In lacustrine systems,  $\delta^{13}C$  and  $\delta^{15}N$  ratios of suspended particulate matter (SPM) vary over time (Zohary et al., 1994; Lehman et al., 2004). This variability is related to external loadings, phytoplankton species composition and primary productivity, as well as sources and concentrations of dissolved inorganic C and N (Grey and Jones, 2001; Caroni et al., 2012). Zooplankton are crucial in transferring matter and energy in pelagic food webs (Matthews and Mazumder, 2003). Vulnerability to fish predation (de Bernardi et al., 1987; Sprules and Bowerman, 1988; Einsle, 1996; Mauchline, 1998) and differential exploitation and availability of food resources can lead to seasonal variations in the dominance of different taxa and developmental stages (Smyntek et al., 2012). In this study, we investigate seasonal changes in the carbon ( $\delta^{13}$ C) and nitrogen ( $\delta^{15}$ N) isotopic signatures of crustacean zooplankton in a small reservoir, Sos Canales Reservoir, in Sardinia, Italy. By analysing the seasonal variation in  $\delta^{13}$ C and  $\delta^{15}$ N for dominant taxa with respect to an isotopic baseline determined from SPM, we consider how water-level fluctuations and changes to the hydrological regime affects resource availability, differential exploitation by consumers and trophic energy transfer in the pelagic food web of a man-made reservoir.

#### Material and methods

#### Study area

Sos Canales Reservoir (SCR; Fig. 1) was built in 1956 by the damming of the upper stream of the River Tirso (152 km), the largest river in Sardinia, Italy. The reservoir is small (0.33 km<sup>2</sup>, maximum depth 39 m) and lies on granite bedrock surrounded by cork oak (*Quercus suber*) woodlands in the Northeastern part of the island, 714 m above sea level. Water flow into the lake is mainly retained by the dam for future supply use. The watershed is small (16 km<sup>2</sup>) and minimally impacted by anthropogenic activity, except for that related to cork production and traditional, low-intensity sheep farming. The reservoir provides the drinking water supply for the resident population of 15000 inhabitants with an extraction of  $< 265 \text{ m}^3 \text{month}^{-1}$ . As is typical for Mediterranean manmade lakes (*i.e.*, Naselli-Flores, 2003; Zohary and Ostrovsky, 2011), SCR is subject to large water-level fluctuations. Rainfall events are concentrated between late-autumn and spring, often causing floods, while streams are almost completely dry in summer.

#### Sampling and analyses

Between October 2010 and September 2011 water and zooplankton samples were collected ca. monthly at two sampling stations:  $S_S$  near the river inflow with a water depth of < 10 m and D, the deepest point of the lake  $(d \sim 40 \text{ m})$  near the dam, as shown in Figure 1. A vertical profile of water temperature (Hydrolab multiprobe HdS5) and the water level were measured at the both stations, monthly. Water samples were collected with a 5 L Niskin bottle at 2.5 m intervals from the surface to 7.5 m depth at stations S and D<sub>S</sub>, with an additional vertical sample taken from 10 m depth to the bottom of the lake bed  $(D_D)$ , and pre-filtered in the field using a 76 µm nylon sieve to remove zooplankton and large detrital matter. SPM (size fraction  $1.2 \le \mu m \ge 0.76$ ) for  $\delta^{13}C$  and  $\delta^{15}N$  SIA was obtained by a secondary filtration onto pre-weighed GF/C glass-fibre filters (1.2 µm pore size). Zooplankton samples for quantification and biomass estimation were collected via integrated vertical hauls with an Apstein zooplankton net (mesh size 76  $\mu$ m), from the surface to 2 m above the lake bed, and fixed in 99% ethanol. The standing stock biomass (SSB) of zooplankton was estimated by length-weight regression equations (McCauley, 1984; Manca and Comoli, 2000). Planktonic crustacean zooplankton for SIA were collected using a 100 µm large-mouth plankton net and sorted into calanoid and cyclopoid copepods, with Daphnia and Ceriodaphnia spp. representative of the cladoceran fraction.

Duplicates of each specimen were oven dried for 24 h at 60 °C, homogenized and transferred into  $6 \times 4$  mm tin-capsules. If necessary, samples were pooled to obtain a minimum mass of 0.5 mg DW.  $\delta^{13}$ C and  $\delta^{15}$ N SIA were run on a Continuous Flow-Isotope Ratio Mass Spectrometer (Delta plus XP ThermoFinnigan, Bremen, Germany), after total combustion in an elemental analyser (EA Flash 1112 ThermoFinnigan). The isotope ratios with respect to international standards (Pee Dee Belemnite for  $\delta^{13}$ C and Atmospheric N<sub>2</sub> for  $\delta^{15}$ N) were calculated from acetanilide, calibrated against the international standards MRIET-ISO-64 and MRI-ET-ISO-63 (Camin *et al.*, 2008), and expressed in  $\delta$  notation per mille (‰):

$$\delta I = \{ (R_{\text{sample}} / R_{\text{standard}}) - 1 \} \times 1000 \tag{1}$$

where *I* is the isotope of interest and *R* is the ratio of heavy-to-light isotope (*i.e.*,  ${}^{13}C/{}^{12}C$ ). Repeat analyses of the internal standards gave a precision of less than  $\pm 0.3\%$  for both  $\delta^{13}C$  and  $\delta^{15}N$ . Statistical analyses, such as Spearman–Rank correlations, were performed with Statistica for Windows (StatSoft, 2001). Non-parametric



Fig. 1. Map showing the locality of the sampling stations, D and S, in Sos Canales Reservoir, Sardinia, Italy.

two independent groups Mann–Whitney U tests were undertaken, but did not show any significant correlation, so significance was also tested using more than two groups comparison, Kruskal–Wallace test.

#### Results

#### **Environmental factors**

The water column was mixed at all stations during winter, December 2010 to February 2011, with the temperature dropping from 7.3 to  $5.5 \,^{\circ}$ C. Thermal stratification started in April and lasted until October, with a surface water temperature of  $20 \,^{\circ}$ C in July. The water level fluctuated throughout the year ( $\pm 10 \,$ m), with at least 265 m<sup>3</sup> abstracted for human consumption each month. The reservoir was at its shallowest in October 2010 (27 m), but the water level rapidly increased by 10 m in its refilling period, due to heavy rainfall of 263.2 mm in February and March. From then on, water levels decreased steadily, especially during the summer months of July–September, when only 25.8 mm rain fell. The

rainfall followed a similar trend to that of previous years (2006–2011), as is evident in Figure 2. Secchi disc transparency was at its lowest of 1.2 m in February, from the increased turbidity, gradually increasing, until stabilizing at 3.5 m during the dry season.

### Changes in the composition of SPM, phytoplankton and zooplankton

The important contribution of phytoplankton to SPM in the surface waters of station  $D_S$  is indicated by the significant correlations between SPM dry weight and Chl-*a* (Spearman R = 0.883, n = 9, P = 0.001) and total phytoplankton biomass (Spearman R = 0.636, n = 11, P = 0.03). Chl-*a* was highest in surficial waters, increasing to a maximum of 21.1 mg.m<sup>-3</sup> in February, despite the photic zone (PZ = 2.5 SD) being at a minimum of 2.9 m, before peaking again at 12.09 mg.m<sup>-3</sup> in August when the PZ was 8.75. Phytoplankton cell density and biomass were also higher in the surficial waters, the latter ranging from 32.74 mg.L<sup>-1</sup> in March to 1.64 mg.L<sup>-1</sup>in September, decreasing with the increase in temperature.



**Fig. 2.** Bar chart of the monthly rainfall in 2010–2011 and averaged monthly rainfall in 2006–2011, with the water level and temperature of the surficial (dark grey) and deep waters (light grey) plotted in the background.

The species composition and biomass varied seasonally, with species density more diverse than biomass. Species density was dominated by Chlorophyceae, mainly *Chlorella* sp., from October to December, Cryptophyceae, mainly *Plagioselmis lacustris* (Pascher & Rutter) and the Dinophyceae *Gymondinium uberrimum* (G. J. Allman, Kofoid & Swezy) from January to May, and Cyanobacteria such as *Aphanothece minutissima* (W. West) Komárková-Legnerováet & Cronberg) in August and September when cell density was highest. However, phytoplankton biomass was dominated by the larger dinoflagellate *G. uberrimum*, which exhibited a strong seasonal dynamic preferring a depth range of 0–2.5 m during the wet season and 5–7.5 m in the epilimnion during the summer months (Fadda et. al, submitted).

When the lake level was lowest, from October to December (and in September 2011), the dry weight of SPM was similar at both sampling sites and there was no depth variation. In February, when the lake level was at its highest, the dry weight of SPM at Station D, by the dam wall, was nearly double that of Station  $S_S$ , by the inflow. After the onset of stratification there was a decrease in the mass of SPM at Station S consistent with the increased rainfall, except for an isolated precipitation event in June when 58.8 mm fell. As the lower water level had exposed the shoreline, this could be a direct result of erosion. At Station D, the highest SPM mass was observed in the wet season when the lake water levels were highest and the concentration of Chl-a and biomass of G. uberrimum peaked in the surficial waters (0-2.5 m).

The calanoid copepods *Copidodiaptomus numidicus* (Gurney, 1909), and the cyclopoid copepods *Cyclops abyssorum* gr. (Sars, 1863) and, to a minor extent, *Macrocyclops albidus* (Jurine, 1820) were found. In the cladoceran fraction, two *Daphnia* species, *D. pulex* (Leydig, 1860; Fadda *et al.*, 2011) and *D. longispina* gr. (as defined in Petrusek *et al.*, 2008; Thielsh *et al.*, 2009), two *Ceriodaphnia* species, *C. pulchella* (G.O. Sars, 1862)



**Fig. 3.** The seasonal variation of the percentage SSB of planktonic crustaceans and rotifers in the deep (D) and shallow (S) sampling stations.



**Fig. 4.** Isotopic bi-plot illustrating the seasonal changes in  $\delta^{13}$ C and  $\delta^{15}$ N of the SPM baseline in (a) D and (b) S stations, at water depths (solid line) D<sub>S</sub> and (dotted line) D<sub>D</sub> in Sos Canales Reservoir.

and *C. reticulata* (Jurine, 1820) were identified. The seasonal SSB of zooplankton is shown in Figure 3.

## Seasonal changes in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic signatures

The  $\delta^{13}$ C and  $\delta^{15}$ N isotopic signatures of SPM fluctuated seasonally ( $\delta^{13}$ C Kruskal–Wallis test: H = 18.05 (n = 26) P = 0.0208;  $\delta^{15}$ N Kruskal–Wallis



Fig. 5. Isotopic bi-plot illustrating the seasonal changes in  $\delta^{13}$ C and  $\delta^{15}$ N of (a) *Copidodiaptomus numidicus*, (b) cyclopoid copepods, (c) *Ceriodaphnia* spp. and (d) *Daphnia* spp. at (solid line) D<sub>D</sub> and (dashed line) D<sub>S</sub> sampling stations.

test: H = 20.67 (n = 26) P = 0.0081), becoming more  $\delta^{13}$ Cdepleted between December and May, with the exception of February, and more enriched in summer, with a reversal of the trend towards increasingly  $\delta^{13}$ C-depleted isotopic signatures after June (Fig. 4). The  $\delta^{15}$ N isotopic signatures were more enriched in the wet season, becoming increasing depleted in spring and summer (Fig. 4). Variations were least pronounced in the deeper waters (>10 m) of Station D, with total carbon (CR) and nitrogen (NR) ranges of CR = 5.88, 6.03, 5.19 and NR = 2.98, 2.53, 0.35 for S, D<sub>S</sub> and D<sub>D</sub>, respectively.

The seasonal  $\delta^{13}$ C and  $\delta^{15}$ N isotopic signatures of all zooplankton taxa shifted from being <sup>13</sup>C-depleted and <sup>15</sup>N-enriched in winter to being less <sup>13</sup>C-depleted and <sup>15</sup>N-enriched in summer (Fig. 5), as was observed for SPM. Kruskal–Wallis tests of the  $\delta^{13}$ C and  $\delta^{15}$ N isotopic signatures of zooplankton with respect to seasonality gave H (63, n = 100, P = 0.037 for  $\delta^{13}$ C and H (63, n = 100, P = 0.005) for  $\delta^{15}$ N.  $\delta^{13}$ C and  $\delta^{15}$ N were similar for Ceriodaphnia at both stations and were on the whole enriched with respect to the  $\delta^{13}$ C and  $\delta^{15}$ N SPM signal. Daphnia followed a similar trend, although were typically more <sup>13</sup>C-depleted at station D than from the shallower sites. The calanoid copepod C. numidicus was the only species of zooplankton present all year round.  $\delta^{15}N$  of Copidodiaptomus and the cyclopoid copepods was at least 3‰ greater than that of the herbivorous cladocera, suggesting their potential use as a food resource.  $\delta^{13}C$  of C. numidicus was consistent with seasonal fluctuations

in the  $\delta^{13}$ C SPM baseline, except for a deviation during the heavy rains in February, when they were less enriched (Fig. 6). This was also observed for the cyclopoid copepods. The C:N ratio of copepods was highest in February at 5.5, being 3.4 in December. Quantification of zooplankton biomass and abundance indicated that the copepod fraction in February was mostly composed of copepodites and adults that were observably rich in lipids (Fig. 7).

#### Discussion

The  $\delta^{13}$ C and  $\delta^{15}$ N isotopic baseline signature of temperate lakes varies seasonally (Zohary *et al.*, 1994; Lehman *et al.*, 2004), due to changes in external loading (Gu *et al.*, 2006), phytoplankton species composition (Peterson and Fry, 1987), sources of primary production (Grey *et al.*, 2000) and changes in the concentration of dissolved inorganic C and N (Grey and Jones, 2001; Caroni *et al.*, 2012). Seasonal changes in the hydrodynamic regime caused  $\delta^{13}$ C and  $\delta^{15}$ N of SPM in SCR to fluctuate, following the expected trend observed in natural lakes of being more <sup>13</sup>C-depleted and <sup>15</sup>N-enriched in winter to less <sup>13</sup>C-depleted and <sup>15</sup>N-enriched values in the drier summer months (Perga and Gerdeaux, 2006; Visconti and Manca, 2011; Woodland *et al.*, 2012).

In fact, two phases could be distinguished in the lake. During the drier season in summer, external water inputs



Fig. 6. Cluster analysis of  $\delta^{13}$ C and  $\delta^{15}$ N of SPM and zooplankton with rainfall and water-level fluctuations.

were negligible, and as abstraction levels remained constant to meet the needs of the drinking water supply, the water level was at a minimum in this "still-water phase". A second "dynamic-water phase" could be differentiated during the wetter months from a rise in water level after heavy rainfall. Allochthonous (terrestrial) input is usually more enriched in  $\delta^{13}$ C and depleted in  $\delta^{15}$ N (Finlay and Kendall, 2008), which was evident from the isotopic signatures of SPM in the water column.

As well as hydrological variability, other physical variables, such as temperature, are important determinants of seasonal change in  $\delta^{13}$ C (Rau *et al.*, 1989, 1992). In monomic lakes a positive correlation between  $\delta^{13}$ C and temperature is often observed (Zohary, 1994; Perga and Gerdeaux, 2006; Visconti and Manca, 2011; Caroni et al., 2012), as increasing temperature decreases the atmospheric dissolution and solubility of CO<sub>2</sub>, influencing the isotopic fractionation of carbon during phytoplankton uptake (Rau et al., 1989, 1992).  $\delta^{13}$ C of SPM in SCR became more enriched (less negative) from the onset of thermal stratification, peaking when the surface temperature reached its maximum. This also explained why variations in the  $\delta^{13}C$  and  $\delta^{15}N$  signal with respect to water-level fluctuations were similar in the surficial waters of stations S and D, but less pronounced at depths > 10 m.  $\delta^{13}$ C was more depleted in the hypolimnion in late summer, as a consequence of increased respiration activity (Cattaneo et al., 2004) in deeper waters (Vander Zanden and Rasmussen, 1999; Grey et al., 2000; Cattaneo et al., 2004). Also,  $\delta^{15}$ N was more enriched in D<sub>D</sub>, as in stratified lakes and reservoirs, biological activity in the sediments decreases the hypolimnetic dissolved oxygen concentration and pH, reducing the redox potential at the sedimentwater interface (Nowlin et al., 2005). Stagnation during a period of prolonged drought can remobilize nutrients sequestered in the sediments (Zohary et al., 1994).

It is likely that the  $\delta^{13}$ C and  $\delta^{15}$ N isotopic signature of SPM was influenced by the phytoplankton composition and uptake activity (Zohary *et al.*, 1994; Lehmann *et al.*,



**Fig. 7.** Percentage of *C. numidicus* (a) and cyclopoids (b) adults (AD) and copepodites (CO) stages at DEEP (D) and SHALLOW (S) stations in Sos Canales Lake.

2004; Gu *et al.*, 2006). The biomass of phytoplankton was dominated by *Gymnodinium uberrimum*, a naked dino-flagellate that preferentially inhabits nutrient-rich surficial waters when the water column is fully mixed during the dynamic phase (Niesel *et al.*, 2007), but moves to deeper water in summer when the reservoir becomes thermally stratified (Tilzer, 1973). Also, a decrease of  $\delta^{15}$ N in spring and summer is a common trait of stratified lakes (Visconti and Manca, 2011; Visconti *et al.*, 2013), which can be attributed to nutrient recycling by zooplankton in the epilimnion.

Seasonal changes in the  $\delta^{13}$ C and  $\delta^{15}$ N SPM isotopic signature were reflected in the zooplankton by a shift from being <sup>13</sup>C-depleted and <sup>15</sup>N-enriched in winter to being less <sup>13</sup>C-depleted and <sup>15</sup>N-enriched in summer. As is commonly observed in Mediterranean reservoirs (Geraldes and Boavida, 2004), *C. pulchella* was the dominant taxon in the dry season at both stations, due to increased water temperature (Fadda *et al.*, 2011).  $\delta^{13}$ C and  $\delta^{15}$ N were similar for *Ceriodaphnia* at both stations, and although populations did not persist throughout the year, herbivorous cladocerans reflected the patterns and mechanisms of matter and energy flow through the lake food web and were on the whole enriched with respect to the  $\delta^{13}$ C and  $\delta^{15}$ N SPM signal. However, *Daphnia* sampled from the deeper waters of station D were typically more <sup>13</sup>C-depleted than the shallower sites, which could indicate between-population differences in feeding strategy (Strørm, 1946) or temperature-related body size differences (Power *et al.*, 2003; Matthews and Mazumder, 2008).

The calanoid copepod C. numidicus was the only species of zooplankton present all year round.  $\delta^{13}C$  of C. numidicus was consistent with seasonal fluctuations in the  $\delta^{13}$ C SPM baseline, except for a deviation during the heavy rains in February. That this was also detected in the isotopic signature of cyclopoid copepods, suggests that copepods may not have been actively feeding in the water column. Quantification of abundance of zooplankton indicated that the copepod fraction in February was mostly composed of copepodites and adults that were observably rich in lipids, with a higher C/N ratio (El-Sabaawi et al., 2009) from <sup>15</sup>N-enrichment. Lipids are stored by copepods entering dormancy (Lee et al., 2006) in harsh environmental conditions (Gyllström and Hansson, 2004), when they tend to migrate downwards into cooler and deeper waters and become less motile (Vanderploeg et al., 1998). The depletion in  $^{13}$ C could be a result of the upwelling and mixing of the water column transporting dormant copepods into the surface waters. We were not able to capture any resting stages, as we could only sample zooplankton to a depth of 2 m above the lake bed. This could also be related to the tissue turnover time of larger zooplankton (Shurin et al., 2006 and references therein) being longer than for phytoplankton, whose  $\delta^{13}C$  and  $\delta^{15}$ N isotopic signatures more rapidly reflect changes in the dissolved carbon pool.

Between-station differences in spring are likely to be attributed to exploitation of differential food sources and differences in the size structure and developmental stages of the zooplankton population (Marcarelli et al., 2011). Whereas for large cyclopoid species, such as C. abyssoroum gr. and M. albidus, predation on cladocerans is well documented (Dussart and DeFaye, 2001), little is known on feeding behaviour of C. numidicus. Adults and large copepodites of calanoid copepods are often referred as selective detrital feeders (Demott, 1986, 1995), although a recent study by Gonçalves reported C. numidicus as omnivorous (Gonçalves et al., 2012). Interpretation of  $\delta^{13}$ C is complicated because of the dramatic seasonal fluctuation, but  $\delta^{15}N$  of *Copidodiaptomus* and the cyclopoid copepods was at least 3‰ greater than the herbivorous cladocera, suggesting their potential use as a food resource.

#### Conclusions

The  $\delta^{13}$ C and  $\delta^{15}$ N isotopic baseline signature of SCR varied seasonally in response to fluctuations in water level and thermal stratification. Following the trend observed in similar lakes,  $\delta^{13}$ C and  $\delta^{15}$ N of the SPM became more <sup>13</sup>C-depleted and <sup>15</sup>N-enriched in winter and less <sup>13</sup>C-depleted and <sup>15</sup>N-enriched values in the drier summer months. The composition of SPM was influenced by the biomass of phytoplankton, which was dominated by *G. uberrimum.*  $\delta^{13}$ C and  $\delta^{15}$ N of SPM were

well-represented by the herbivorous cladocerans, although *Daphnia* sampled from deeper waters were typically more <sup>13</sup>C-depleted than the shallower sites.  $\delta^{13}$ C of calanoid and cyclopoid copepods were also consistent with seasonal fluctuations in the  $\delta^{13}$ C SPM baseline, except during the heavy rains in early spring, when they were observably rich in lipids and depleted in <sup>13</sup>C with a higher C/N ratio. This suggested that they had entered in dormancy and were not actively feeding in the water column.  $\delta^{15}$ N of the calanoid and cyclopoid copepods were at least 3‰ greater than for the herbivorous cladocera, confirming their potential use as a food resource.

This study indicates the importance of taking into account the seasonal variation in  $\delta^{13}$ C and  $\delta^{15}$ N of isotopic baseline in lakes, especially reservoirs that are subject to severe water-level fluctuations and thermal stratification. Although the seasonal fluctuation in  $\delta^{13}$ C and  $\delta^{15}$ N of SPM is evident in zooplankton, other factors (*i.e.*, dormancy, diel migration) may confuse the signal. Primary consumers, such as *Daphnia*, track  $\delta^{13}$ C and  $\delta^{15}$ N more closely than secondary consumers, such as *Copidodiaptomus*, so the size and age distribution of the population should be carefully considered in the design and interpretation stable isotope studies.

Acknowledgements. The study was supported by the Master & Back grant to AF from Regione Autonoma della Sardegna and by Ente Acque della Sardegna.

#### References

- Cabana G. and Rasmussen J.B., 1996. Comparison of aquatic food chains using nitrogen isotopes. *Proc. Natl. Acad. Sci.* USA, 93, 10844–10847.
- Camin F., Perini M., Colombari G., Bontempo L., Versini G., 2008. Influence of dietary composition on the carbon, nitrogen, oxygen and hydrogen stable isotope rations of milk. *Rapid. Comm. Mass. Spectrom.*, 22, 1690–1696.
- Caroni R., Free G., Visconti A. and Manca M., 2012. Phytoplankton functional traits and seston stable isotopes ratio: a functional-based approach in a deep, subalpine lake, Lake Maggiore (N. Italy). *J. Limnol.*, 71, 84–94.
- Cattaneo A., Manca M. and Rasmussen J.B., 2004. Peculiarities in the stable isotope composition of organisms from an alpine lake. *Aquat. Sci.* 66, 440–445.
- de Bernardi R., Giussani G. and Manca M., 1987. Cladocera: predators and prey. *Hydrobiology*, *145*, 225–243.
- DeMott W.R., 1986. The role of taste in food selection by freshwater zooplankton. *Oecologia*, 69, 334–340.
- DeMott W.R., 1995. Optimal foraging by a suspension-feeding copepod: responses to short-term and seasonal variation in food resources. *Oecologia*, 103, 230–240.
- Dussart B.H. and Defaye D., 2001. "Introduction to the Copepoda." Guides to the Identification of the Microinvertebrates of the Continental Waters of the World (Netherlands).
- Einsle U., 1996. Copepoda: Cyclopoida: Genera Cyclops, Megacyclops, Acanthocyclops. In: Dumont, H.J.F. (ed.),

Guides to the Identification of the Microinvertebrates of Continental Waters of the World, Vol. 10, SPB Publisching, Amsterdam.

- El-Sabaawi R., Dower J.F., Kainz M. and Mazumder A., 2009. Characterizing dietary variability and trophic positions of coastal calanoid copepods: insight from stable isotopes and fatty acids. *Mar. Biol.*, *156*, 225–237.
- Fadda A., Marková S., Kotlík P., Lugliè A., Padedda B., Buscarinu P., Sechi N. and Manca M., 2011. First record of planktonic crustaceans in Sardinian reservoirs. *Biologia*, 66, 856–865.
- Fadda A., Manca M., Camin F., Ziller L., Mariani A.M., Padedda B.M., Sechi N., Virdis T. and Lugliè A., 2014. "Study on the suspended particulate matter of a Mediterranean artificial lake (Sos Canales Lake) using Stable Isotope Analysis of carbon and nitrogen". Submitted.
- Finlay J.C. and Kendall C., 2008. Stable Isotopes in Ecology and Environmental Science. Stable Isotope tracing of temporal and spatial variability in organic matter sources to freshwater ecosystems, pp. 283–324.
- Geraldes A.M. and Boavida M.J., 2004. What factors affect the pelagic cladocerans of the meso-eutrophic Azibo Reservoirs?. *Ann. Limnol. Int. J. Lim.*, 40, 101–111.
- Gonçalves A.M., Pardal M.Â., Marques S.C., Mendes S., Fernández-Gómez M.J., Galindo-Villardón M.P. and Azeiteiro, U.M., 2012. Responses of Copepoda life-history stages to climatic variability in a Southern-European temperate estuary. *Zoof. Stud.*, 51, 321–335.
- Grey J., Jones R.I. and Sleep D., 2000. Stable isotope analysis of the origins of zooplankton carbon in lakes of differing trophic state. *Oecologia*, 123, 232–240.
- Grey J. and Jones R.I., 2001. Seasonal changes in the importance of the source of organic matter to the diet of zooplankton in Loch Ness, as indicated by stable isotope analysis. *Limnol. Oceanogr.*, 46, 505–513.
- Gu B., Chapman A.D. and Schelske C.L., 2006. Factors controlling seasonal variations in stable isotope composition of particulate organic matter in a soft water eutrophic lake. *Limnol. Oceanogr.* 51, 2837–2848.
- Gyllström M. and Hansson L.A., 2004. Dormancy in freshwater zooplankton: induction, termination and the importance of benthic-pelagic coupling. *Aquat. Sci.*, 66, 274–295.
- Henry R., Panarelli E.A., Caglierani S.M. and Casanova D.C., 2011. Plankton richness and abundance in several different hydrological situation in lakes later to a river: case a study in the mouth zone of a tributary into a tropical reservoir. *Oecol. Aust.*, 15, 537–558.
- Karlsson J., Jonsson A., Meili M. and Jansson M., 2003. Control of zooplankton dependence on allochthonous organic carbon in humic clear water lakes in Northern Sweden. *Limnol. Oceanogr.*, 48, 269–276.
- Lee R.F., Hagen W. and Kattner G., 2006. Lipid storage in marine zooplankton. *Mar. Ecol. Progr. Ser.*, 307, 273–306.
- Lehman M.F., Bernasconi S.M. and McKenzie J.A., 2004. Seasonal variation of the  $\delta^{13}$ C and  $\delta^{15}$ N of particulate and dissolved carbon and nitrogen in Lake Lugano: constraints on biogeochemical cycling in a eutrophic lake. *Limnol. Oceanogr.*, 49, 415–429.
- Leira M. and Cantonati M., 2008. Effects of water-level fluctuations on lakes: an annotated bibliography. *Hydrobiology*, *612*, 171–184.

- Lindeman R.L., 1942. The trophic-dynamic aspect of ecology. *Ecology*, 23, 399–417.
- Manca M. and Comoli P., 2000. Biomass estimates of freshwater zooplankton from length-carbon regression equations. J. Limnol., 59, 15–18.
- Marcarelli A.M., Colden V.B., Mineau M.M. and Hall R.O., 2011. Quantitiy and quality: unifying food web and ecosystem perspectives on the role of resource subsidies in freshwaters. *Ecology*, 92, 1215–1225.
- Matthews B. and Mazumder A., 2003. Consequences of large temporal variability of zooplankton  $\delta^{15}N$  for modeling fish trophic position and variation. *Limnol. Oceanogr.*, 50, 1404–1414.
- Mauchline J., 1998. The Biology of Calanoid Copepods, Academic Press, London.
- McCauley E., (1984). The estimation of abundance and biomass of zooplankton in samples. *In:* Downing J.A. and Rigler F.H. (eds.), A Manual of Methods for the Assessment of Secondary Productivity in Freshwater, Blackwell Scientific Publication, 228–265.
- Moss B., Hering D., Green A.J., Aidoud A., Becares E., Beklioglu M., Bennion H., Boix D., Brucet S., Carvalho L., Clement B., Davidson T., Declerck, S., Dobson M., van Donk E., Dudley B., Feuchtmayr H., Friberg N., Grenouillet G., Hillebran H., Hobaek A., Irvine K., Jeppesen E., Johnson R., Jones I., Kernan M., Lauridsen T.L., Manca M., Meerhoff M., Olafsson J., Ormerod S., Papastergiadou E., Penning W.E., Ptacnik R., Quintana X., Sandin L., Seferlis M., Simpson G., Trigal C., Verdonschot P., Verschoor A.M. and Weyhenmeyer G.A., 2009. Climate change and the future of freshwater biodiversity in Europe: a primer for policy-makers. *Freshwat. Rev.*, 2, 103–130.
- Naselli-Flores N., 2003. Man-made lakes in Mediterranean semiarid climate: the strange case of Dr. Deep and Mr Shallow Lake. *Hydrobiology*, 506, 13–21.
- Niesel V., Hehn E., Sudbrack R., Willmitzer H. and Chorus I., 2007. The occurrence of the Dynophyte species *Gymnodinium uberrimum* and *Peridinium willei* in German reservoirs. *J. Plank. Res.*, 29, 347–357.
- Nowlin W.H., Evarts J.L. and Vanni M.J., 2005. Release rates and potential fates of nitrogen and phosphorus from sediments in a eutrophic reservoir. *Fresh. Biol.* 50, 301–322.
- Perbiche-Neves G.R., Romero Ferreira R. and Gomes Nogueira M., 2011. Phytoplankton structure in two contrasting cascade reservoirs (Paranapanema River, Southeast Brazil). *Biologia*, 66, 967–976.
- Perga M.E. and Gerdeaux, D., 2006. Seasonal variability in the δ13C and δ15N values of the zooplankton taxa in two alpine lakes. *Acta Ecol.*, 30, 69–77.
- Peterson B.J. and Fry B., 1987. Stable isotopes in ecosystem studies. Annu. Rev. Ecol. Syst., 18, 293-320.
- Petrusek A., Hobæk A., Nilssen J.P., Skage M., Černý M., Brede N. and Schwenk K., 2008. A taxonomic reappraisal of the European *Daphnia longispina* complex (Crustacea, Cladocera, Anomopoda). *Zool. Scr.*, 37, 507–519.
- Post D.M., 2002. Using stable isotopes to estimate trophic position: models, methods, and assumption. *Ecology*, *83*, 703–718.
- Power M., Guiguer K.R.R.A. and Barton D.R., 2003. Effects of temperature on isotopic enrichment in Daphnia

magna: implications for aquatic food-web studies. *Rapid.* Commun. Mass Spectrom., 17, 1619–1625.

- Rau G.H., Takahashi T. and Des Marais D.J., 1989. Latitudinal variations in plankton C: implications for CO and productivity in past oceans. *Nature*, 341, 165.
- Rau G.H., Takahashi T., Des Marais D.J., Repeta D.J. and Martin, J.H., 1992. The relationship between  $\delta^{13}$ C of organic matter and [CO<sup>2</sup> (aq)] in ocean surface water: Data from a JGOFS site in the northeast Atlantic Ocean and a model. *Geochim. et Cosmochim. Acta*, 56, 1413–1419.
- Shurin J.B., Gruner D.S. and Hillebrand H., 2006. All wet or dried up? Real differences between aquatic and terrestrial food webs. *Proc. R. Soc. B.*, 273, 1–9.
- Smyntek P.M., Teece M.A., Schulz K.L. and Storch A.J., 2008. Taxonomic differences in the essential fatty acid composition of groups of freshwater zooplankton relate to reproductive demands and generation time. *Freshwat. Biol.*, 53, 1768–1782.
- Sprules W.G. and Bowerman J.E., 1988. Omnivory and Food Chain Length in Zooplankton *Food Webs. Ecology*, *69*, 418–426.
- StatSoft Inc., 2001. STATISTICA for Windows (Data Analysis Software System), Version 6. StatSoft, Tulsa, 1098 pp.
- Strørm K.M., 1946. The ecological niche. Nature 157, 375.
- Thielsh A., Brede N., Petrusek A., De Meesteer L. and Schewnk K., 2009. Contribution of cyclic parthenogenesis and colonization history to population structure in Daphnia. *Mol. Ecol.*, 18, 1616–1628.
- Thompson R.M., Dunne J.A. and Woodward G., 2012. Freshwater food webs: towards a more fundamental understanding of biodiversity and community dynamics. *Freshwat*. *Biol.*, 57, 1329–1341.

- Tilzer, Max M. 1973. "Diurnal periodicity in the phytoplankton assemblage of a high mountain lake." *Limnol. Oceanogr. 18*, 15–30.
- Tundisi J.G., 1999. Theoretical basis for reservoir management. *In:* Tundisi, J.G. and Straškraba, M. (eds.), Theoretical Reservoir Ecology and Its Applications s.l.: IIE. BAS, Backhuys Publishers, 505.
- Vanderploeg H.A., Cavaletto J.F., Liebig J.R. and Gardner W.S., 1998. *Limnocalanus macrurus* (Copepoda: Calanoida) retains a marine arctic lipid and life cycle strategy in Lake Michigan. J. Plank. Res., 20, 1581–1597.
- Visconti A. and Manca M., 2011. Seasonal changes in the  $\delta^{13}$ C and  $\delta^{15}$ N signatures of the Lago Maggiore pelagic food web. *J. Limnol.*, 70, 263–271.
- Visconti A., Volta P., Fadda A., Di Guardo A. and Manca M., 2013. Seasonality, littoral vs. pelagic carbon sources and stepwise 15<sup>N</sup>-enrichment of pelagic food web in a deep subalpine lake: the role of planktivorous fish. *Can. J. Fish. Aquat. Sci.*, 71, 436–446.
- Woodland R.J., Rodriguez M.A., Magnan P., Glèmet H. and Cabana G., 2012. Incorporating temporally dynamic baselines in isotopic mixing models. *Ecology*, 93, 131–144.
- Zanden M., Vander J. and Rasmussen J.B., 1999. Primary consumer  $\delta^{13}$ C and  $\delta^{15}$ N and the trophic position of aquatic consumers. *Ecology* 80, 1395–1404.
- Zohary T., Erez J., Gophen M., Bermanfrank I. and Stiller M., 1994. Seasonality of stable carbon isotopes within the pelagic food web of Lake Kinneret. *Limnol. Oceanogr. 39*, 1030–1043.
- Zohary T. and Ostrovsky I., 2011. Ecologica impacts of excessive water level fluctuations in stratified freshwater lakes. *Inland Water*, *1*, 47–59.