

Zooplankton predators and prey: body size and stable isotope to investigate the pelagic food web in a deep lake (Lake Iseo, Northern Italy)

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ABSTRACT

Seasonal changes in trophic position and food sources of deep subalpine lake (Lake Iseo, Northern Italy) zooplankton taxa were investigated during the year 2011. Furthermore, it's combined carbon and nitrogen Stable Isotope Analysis (SIA) with size-specific analyses of both, the major predatory cladoceran (*Leptodora kindtii*, Focke) and two potential preys (*Daphnia longispina* complex and *Eubosmina longicornis*). SIA studies have been extremely useful to track the energy flow through complex trophic network, however, if it is applied to analyze relation between two/few species may lead to misleading interpretations. In fact, integrating size-specificity allowed for understanding why *L. kindtii* nitrogen isotopic fingerprint fully overlapped with *Daphnia*, in spring. By investigating changes in *L. kindtii*'s feeding basket, we found that in spring, *L. kindtii* mainly relied upon *E. longicornis* as prey, *Daphnia* being of too large body size for being captured by *L. kindtii*. Among preys encountered directly in front by a free-swimming *Leptodora*, only those able to fit into basket opening can be captured. As basket diameter increases with animal body length, size selection of prey depends on *L. kindtii* body length. As in other deep, subalpine lakes, *E. longicornis* was less ¹⁵N-enriched than *Daphnia*, most likely because of exploiting nitrogen fixing, cyanobacteria colonies, commonly detected in Lake Iseo with the onset of thermal stratification. Cyclopoid adults were at the top of zooplankton food chain and they could

potentially be feeding on *Daphnia*. They, however, likely fed in a different habitat (>20 m deep water), as suggested by a rather than negligible carbon fractionation.

The results overall suggest that size-specificity is crucial for addressing space and time changes in trophic links between organisms composing the two hierarchical levels within open water zooplankton community.

INTRODUCTION

Ecological studies are increasingly devoted to quantifying functional roles of species through direct approaches, allowing for a better definition of food webs (Fink *et al.*, 2012). As for aquatic environments, temporal and spatial changes in biological and physicochemical variables are relevant (Visconti and Manca, 2011). Zooplankton is a critical link in lake pelagic food webs: energy and matter accumulated through primary production and photosynthetic activity are early incorporated into primary consumers; and conveyed to planktivorous fish through zooplankton (primary and secondary consumers). Relative trophic position in pelagic food web of primary and secondary zooplankton consumers vary seasonally, thus affecting transfer of matter and energy, eventually pollutants, through the ecosystem (Bettinetti *et al.*, 2012; Bettinetti and Manca, 2013). The entire process is regulated by size-specific interactions (*e.g.*, SHE; Brooks and Dodson, 1965): size selectivity influencing grazing (Carpenter *et al.*, 1985) of filter-feeding primary consumers, infra-zooplankton and fish predation (de Bernardi *et al.*, 1987).

Quantifying taxa-specific trophic positions and how they vary seasonally is also an important step towards an evaluation of functional diversity, which is crucial for *e.g.* defining taxa vicariance and predicting impact of perturbations on ecosystem operational properties (*e.g.* Moss *et al.*, 2009).

Among different approaches (*e.g.*, gut contents, analysis lipid biomarkers) for addressing seasonal changes in zooplankton trophic position and food sources, carbon and nitrogen stable isotope analysis (SIA) is increasingly applied due to their ability to simultaneously summarize complex trophic networks and track the energy flow through them (see Matthews and Mazumder, 2007 and references therein). The use of SIA has improved food-web descriptions due to their ability to simultaneously summarize complex trophic networks and track the energy flow through ecological communities. Organism carbon isotopic signatures are mainly related to habitat and lake typology and the ratio of $\delta^{13}C$ changes only little as carbon flows through food-web (Smyntek *et al.*, 2012; Rinta *et al.*, 2015). In contrast, nitrogen isotopic

signatures are strongly influenced by trophic position because the $\delta^{15}N$ of a consumer is typically enriched by 3-4‰ relative to its diet (Cattaneo *et al.*, 2004; Perga and Gerdeaux, 2006; Visconti and Manca, 2011). Therefore, feeding histories of zooplankton taxa can be traced and quantified by comparing their carbon and nitrogen stable isotope signatures with those of potential food sources (Matthews and Mazumder, 2008; Wada, 2009).

In the present paper, it is investigate the link between the seasonality of size structure of *L. kindtii kindtii* population and of two potential preys, along with isotopic variation in the plankton food web. It was expected to make inferences about seasonal changes in the trophic position of a range of zooplankton species. A deep subalpine lake, Lake Iseo, well-studied for both plankton and hydrochemistry (Salmaso *et al.*, 2010, 2014; Leoni *et al.*, 2014a, 2014b) offered a good opportunity for applying this approach and for comparing results with those obtained on other lakes in the region (*i.e.*, Lake Maggiore, Manca *et al.*, 2009; Visconti and Manca, 2011; Visconti *et al.*, 2014). I hypothesized and tested: i) the diet shift of *L. kindtii* from feeding on small to large zooplankters depending on its seasonal size structure; ii) the space and time changes of few hierarchical levels within the zooplankton community.

METHODS

Study site

Lake Iseo (also known as Sebino) is the fourth largest Italian lake, after Garda, Maggiore and Como. It lies in the foothills of the Alps, at the end of a southalpine valley (Val Camonica). The inflow and outflow is the River Oglio, which forms the border between the provinces of Bergamo and Brescia. Like all subalpine lakes, Iseo has been classified by Hutchinson (1965) as 'warm monomictic', water temperatures do not drop below 4°C and water mixing occurring during or close to winter. Being deep, however, water overturn over the last 40 years has happened only occasionally (in 2005 and 2006, Pilotti *et al.*, 2013; Leoni *et al.*, 2014a), so the lake can presently be regarded as olo-oligomictic with anoxic conditions at the bottom in the deepest area (Garibaldi *et al.*, 2003, Leoni *et al.*, 2014a).

Sample collection and laboratory analysis

Zooplankton samples were collected seasonally in 2011 from a sampling station at the point of maximum lake depth (45°43'11"N; 10°03'46"E), traditionally sampled for long-term limnological studies (Fig. 1). Live zooplankton for isotopic analysis and body size was collected with large (58-cm opening mouth diameter), 450-µm mesh nylon net through

vertical hauls at 0-20 m depth (the water layer used for long-term lake monitoring; Leoni *et al.*, 2014a). It was planned sampling in March, May, August and November to capture major seasonal changes in carbon and nitrogen zooplankton isotopic signatures (Visconti and Manca, 2011; Visconti *et al.*, 2014) and in zooplankton population structure (Leoni *et al.*, 2014a) and to estimate body size structure of zooplankton main predators and potential preys. Use of a large net mesh-size ensured that large phytoplankton colonies were avoided while allowing for capturing zooplankton components eligible as food for zooplanktivorous fish, which preferentially capture large-bodied and well-visible zooplankters (de Bernardi *et al.*, 1988). Water temperature vertical profiles were also measured on each sampling date with a multiprobe (WTW Multi 3410).

Organisms sorted by taxa were kept overnight in filtered (1.2 μm GF/C filters) lake water for gut clearance. Analyses included *Daphnia longispina-galeata-cucullata* complex (hereafter *Daphnia*), *Eubosmina longicornis* Schoedler 1866, *Bythotrephes longimanus* (Leydig 1860; Onychopoda) and *Leptodora kindtii* (Focke 1844; Haplopoda) among the Cladocera; *Copidodiaptomus steueri*, Brehm & Zederbauer, 1904, cyclopoids (*Mesocyclops leuckarti* Claus 1857 + *Thermocyclops dybowskii* Lande 1890+ *Cyclops abyssorum* Sars 1863) among copepods. Early developmental stages of copepods and plankton rotifers were not included.

Depending on individual body mass, between 100 individuals/taxon (for *B. longimanus*) and 800 individuals/taxon (for *E. longicornis* and the cyclopoids) were processed, to reach optimal weight requested per sample, two replicates of which were prepared. Fourteen zooplankton samples were analyzed for their $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic signatures.

Samples were oven-dried at 60°C for 48 h, finely powdered and then transferred into 5x9 mm tin capsules (Manca *et al.*, 1994). They were sent to the G.G. Hatch Stable Isotope Laboratory (University of Ottawa, Canada, www.isotope.uottawa.ca) where isotopic composition of organic carbon and nitrogen was determined by analysis of CO_2 and N_2 produced by combustion followed by gas chromatograph separation on a Vario EL III Elemental Analyser. Samples were sent into the DeltaPlus Advantage CF-IRMS via a ConFlo II interface. The analytical precision of the analyses (S.D.), based on the laboratory internal standards, was <0.2‰ for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$.

Isotope ratios were expressed as parts per thousand (‰) differences from a standard reference (PeeDee Belemnite for carbon; atmospheric N_2 for nitrogen), according to the following equation:

$$\delta^{13}\text{C}, \delta^{15}\text{N} = [(R_{\text{sample}} / R_{\text{standard}}) - 1] \times 1000 \quad (\text{eq. 1})$$

where R is the isotopic ratio: $^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$.

Being conservative between consumer and food source, carbon isotopic ratios ($\delta^{13}\text{C}$) were used to assess the origin of carbon sources fuelling the pelagic food web and to detect seasonal changes in taxon-specific feeding behaviour. According to de Niro and Eipstein (1978), a common carbon source was attributed when fractionation ($F = \delta^{13}\text{C}_{\text{cons}} - \delta^{13}\text{C}_{\text{diet}}$) was $\leq 0.8\text{‰}$ ($\pm 1.1\text{‰}$ S.D.). Nitrogen isotopic signatures ($\delta^{15}\text{N}$), being enriched of $\approx 3.4\text{‰} \pm 1.0\text{‰}$ (Post, 2002) in consumers with respect to their direct diets (Enrichment, $E = \delta^{15}\text{N}_{\text{cons}} - \delta^{15}\text{N}_{\text{diet}}$), were used to assess seasonal changes in taxon-specific trophic positions. When a consumer carbon signature was related solely to the pelagic baseline, its trophic position (T) was assessed by the simple linear equation:

$$T = (E/\lambda) + 2 \quad (\text{eq. 2})$$

Where: λ is the stepwise $E = 3.4 \text{‰}$ (Matthews and Mazumder, 2003); and 2 is the value commonly assigned to the primary consumer used as isotopic baseline. *Daphnia* was used for tracing the pelagic baseline (Matthews and Mazumder, 2003; Marty and Planas, 2008), for its persistence in non-negligible abundance along the seasons and for its well-known ability of incorporating seston particles without taste selection (differently from e.g. copepods, Greene, 1988; Šorf and Brandl, 2012). At $T=3$, a consumer feeds directly on the primary consumer used as pelagic baseline; at $T=4$ an intermediate prey is between the two. At $T=2$ (and $F \leq 0.8 \pm 1.1$) the same trophic level as the baseline indicate that the zooplankton is an alternative prey to *Daphnia* for secondary consumers.

Body size of zooplankton taxa

Individual body length was estimated in each fixed sample, by using a PC image analysis software (AxioVision Rel. 4.6; Leoni and Garibaldi, 2009) connected with a microscope (Zeiss Axioskop 40, AxioCam MRc 5; 5x magnification eyepiece). Depending on population density, the number of analyzed individuals (representative of each population) varied between 100/sample (for *Daphnia*) and 40/sample (for *E. longicornis*). *L. kindtii* body length was measured from top of head to caudal portion of the body, furca excluded, on 251 individuals. Individual feeding basket diameter was estimated from Manca and Comoli (1995), to assess *L. kindtii* edible prey body length spectra along the seasons (Abrùsan, 2003).

Replicate samples, collected and fixed in buffered formaldehyde (4% by volume), were used for estimating zooplankton taxon abundance. Samples were collected by vertical tows from the 20 m depth to the surface with a Wisconsin type net of 25 cm diameter and 200 μm mesh, each sample was obtained by pooling three replicate hauls (3 hauls corresponding to 2.94 m^3 of total filtered water). In the laboratory, Cladocera were identified and counted mostly to the species level. For details of procedures used in the field and laboratory see in Leoni *et al.* (2007).

RESULTS

Seasonal changes in zooplankton population density

In late Spring, *Daphnia* and *E. longicornis* reached their annual population density peaks. *Diaphanosoma* was found only in winter and autumn, in the latter season at its annual population density peak. The two predatory cladocerans, *L. kindtii* and *B. longimanus*, were found in spring-autumn and in summer-autumn, respectively (Fig. 1).

Carbon and nitrogen isotopic signatures in the different seasons

Daphnia carbon signature was least ^{13}C -depleted in summer ($-28.51\% \pm 0.2$ SD) and most ^{13}C -depleted in winter ($-40.35\% \pm 0.2$ SD), with intermediate and similar values in spring and autumn, ($-33.98\% \pm 0.2$ SD, $-31.81\% \pm 0.2$ SD, respectively; Fig. 2). The seasonal pattern developed along with lake warming and progressive lake thermal vertical stratification, as observed in deep lakes (Perga and Gerdaux, 2006; Caroni *et al.*, 2012).

Seasonal changes in carbon signatures of cladoceran taxa were consistent with those of *Daphnia*, suggesting exploitation of pelagic food sources. The cyclopoids relied upon pelagic food sources in winter, while being, later on, clearly more ^{13}C -depleted (-41.8 ± 0.2 SD), than the other taxa, thus suggesting they might feed in deeper ($>20\text{m}$ depth) waters, likely on preys other than those analyzed in this study ($\delta^{15}\text{N}$ of $9.0\% \pm 0.2$ SD; Fig. 3). Cyclopoids became progressively more ^{15}N -enriched towards summer ($10.04\% \pm 0.08$ SD, when they were at the same level as the predatory cladocerans *L. kindtii* ($9.09\% \pm 0.2$ SD) and *B. longimanus* ($10.14\% \pm 0.17$ SD), although by exploiting different preys.

In spring, *E. longicornis* fully overlapped with *Daphnia* for carbon isotopic signature (respectively 34.6 ± 0.1 SD and -33.95 ± 0.02 SD), while being nitrogen isotopic signature lower than *Daphnia*'s (respectively 3.34 ± 0.14 S.D. and 5.76 ± 0.8 S.D.). At this time of year, *Leptodora*'s carbon and nitrogen signatures overlapped with *Daphnia* (^{13}C : -33.32 ± 0.06 SD

and -33.95 ± 0.02 SD; ^{15}N : 5.76 ± 0.8 SD and 5.76 ± 0.8 SD, respectively). *Leptodora*'s enrichment was poor (0.3) and T level low (2.6), both similar to those of *Copidodiaptomus* ($E=0.5$).

In summer, *B. longimanus* shared carbon signature with *L. kindtii*, with respect to which, however, was more ^{15}N -enriched ($E=6.5$ with respect to 5.5; Fig. 3). At this time of the year, however, *Leptodora*'s carbon fractionation ($F=1.6$), might suggest contribution of other than solely pelagic food sources exploited by *B. longimanus*. Also in autumn, *B. longimanus* increased carbon fractionation with respect to *L. kindtii* also suggests exploitation of different sources than those exploited by *L. kindtii*, the latter being more tightly linked to *Daphnia* carbon isotopic baseline.

Seasonal changes in *L. kindtii* feeding basket diameter (Fig. 4) suggest that the largest part of *E. longicornis*, and only a small fraction of *Daphnia* population, were eligible prey for *L. kindtii* in spring. In summer, *L. kindtii* basket range of sizes included *E. longicornis*, the latter being on average larger than in spring, along with a large fraction of *Daphnia* population (of body size between 0.6 and 1.2 mm). In autumn, the entire range of *E. longicornis* body size and a fraction between 0.7 and 1.3 mm of *Daphnia* population were eligible prey, according to *Leptodora*'s feeding basket size range. Overall, the results suggest that *L. kindtii* was able to prey on a large part of *E. longicornis* population, only marginally on *Daphnia*, the latter being mostly of body length exceeding the upper limit of *L. kindtii* basket diameter.

DISCUSSION

Carbon and nitrogen stable isotope analyses are increasingly used to trace conveyance of carbon sources through lake food webs and identifying zooplankton taxa roles and relationships (Matthews and Mazumder, 2005, 2006; Persaud *et al.*, 2012). A major concern is that I cannot address prey available for a given predator, as I do not actually know all actors in the scene of the lake theatre, nor I know all possible roles they might play (Hutchinson 1965). Yet, stable isotopes analyses revealed unexpected trophic links in food web structure, also related to predator and prey body sizes (Matthews and Mazumder, 2007; Kuwae *et al.*, 2012).

Size-specificity is a major trait governing biotic and abiotic interactions within and between the zooplankton (Geller and Müller 1981; Peters, 1983; Porter *et al.*, 1983, Obertegger and Manca, 2011). Size-specificity in prey selection characterizes the predatory cladoceran *L. kindtii*. This carnivorous cladoceran swims continuously through the water column ('cruise

predator'; Greene, 1988), capturing prey by means of five pairs of thoracic appendages spread to form a feeding basket (Herzig and Auer, 1990; Manca and Comoli, 1995; Vogt *et al.*, 2013). Among preys encountered directly in front by a free-swimming *L. kindtii*, only those able to fit into basket opening can be captured. As basket diameter increases with animal body length, size selection of prey depends on *Leptodora's* body length.

In this study, *Daphnia* has been used as pelagic baseline, as a short-lived organism, it is better suited for finer scale temporal integration of pelagic d13C or d15N signatures; furthermore, due to its unselective mode to feeding it constitutes a good estimate for base of base of the food chain (Matthews and Mazumder, 2003). It has been found that *L. kindtii* overlapped with *Daphnia* in Spring, not only for carbon, which is indicative of common sources, but also for nitrogen isotopic signature. The lack of enrichment with respect to *Daphnia* suggested that *L. kindtii* did not prey on *Daphnia* during Spring. Analysis of *L. kindtii* feeding basket size suggested that one possible alternative prey, namely *E. longicornis*, well fitted *Leptodora's* size-specific predation in Spring. A close predator-prey seasonal dynamics between *L. kindtii* and *E. longicornis* were found in other deep, large lakes (*e.g.*, Lake Michigan, Branstrator and Lehman, 1991). I cannot, however, exclude contribution by other preys to *Leptodora's* predation, such as *Conochilus* sp., Rotifera (Edmondson and Litt, 1987). The latter, however, were not among the major components of zooplankton in Lake Iseo, only occasionally being found in high densities (*e.g.*, in June 1999, July 2003-2005; Garibaldi *et al.*, 2003; Leoni *et al.* 2014a).

Rotifera were not included in this study focused on size fraction of crustacean zooplankton potentially exploited by planktivorous fish of Lake Iseo, such as *Leuciscus cephalus*, *L. souffia*, *Alosa fallax lacustris* and *Coregone lavaretus* (de Bernardi *et al.*, 1988).

Unique interpretations cannot be achieved, particularly when I face complexity of the natural environment. On the other side, extrapolation from the laboratory to the field is complicated by the temporal and spatial heterogeneity of the natural environment (Kareiva *et al.*, 1996), particularly because short generation times allow zooplankton to respond rapidly to changing abiotic conditions or biotic interactions (Adrian *et al.*, 2006). Stable isotopes analyses have, with respect to *e.g.*, laboratory feeding experiments, the potential of combining field and laboratory approaches, tracing at fine, molecular level (Strayer, 2010) time-specific, step-wise functional role of organisms in the ecosystem.

In spring, when the pelagic food web was most diversified, *Daphnia* was not the less ¹⁵N-enriched open-water zooplankter. In fact, *E. longicornis* showed a lowest nitrogen isotopic

signature; this trait is a common of deep lakes in the subalpine region (Visconti *et al.*, 2014). When *Daphnia* and *E. longicornis* carbon signatures overlap, thus suggesting habitat sharing (Visconti *et al.*, *ibidem*), differences in nitrogen isotopic signatures can be reasonably explained by taking into account the feeding behaviour. Food particles are retained by *Daphnia* through filter combs bared by thoracic limbs, in a range of sizes constrained by the distance between the setae forming the combs (Geller and Müller, 1981). Such distance tends to increase with body size (Geller and Müller, 1981), thus allowing larger species/specimens for retaining increasingly larger food particles, and smaller ones, to exploit also small food particles, such as bacteria. Using *Daphnia* as tracer, seasonal changes in size of seston particles fuelling pelagic food web are accounted for *E. longicornis* feeding behaviour is quite different from *Daphnia*'s (DeMott and Kerfoot, 1982). *E. longicornis* is able to feed on filamentous colonies, including those of Cyanobacteria. Cyanobacteria are usually lower in nitrogen isotopic signature than other phytoplankton taxa; nitrogen-fixing cyanobacteria in particular, are poor in heavy isotope, being almost un-enriched with respect to standard samples (Carpenter *et al.*, 1999). Ability to exploit also poorly ^{15}N -enriched food sources is a reasonable explanation for *Eubosmina*'s low nitrogen isotopic signature. A predator will be relatively less enriched when selecting *E. longicornis* than *Daphnia* as prey, enrichment being related to predator's body size. Such was the case for *L. kindtii* in Lake Iseo in spring, as *E. longicornis* falls within size range of *Leptodora*'s potential prey (Fig. 4).

As commonly observed in deep subalpine lakes, pelagic baseline signatures were, respectively, least ^{13}C -depleted and ^{15}N -enriched in summer.

Carbon isotopic signature is affected by water depth (Zohary *et al.*, 1994; Gu *et al.*, 1996; Lehmann *et al.*, 2004; Manca *et al.*, 2009; Visconti and Manca, 2011). In the epilimnion of deep lakes, $\delta^{13}\text{C}$ signature increases with warming and onset of thermal stratification (Perga and Gerdeaux, 2006; Manca *et al.*, 2009; Visconti and Manca, 2011; Caroni *et al.*, 2012). Temperature controls the solubility of CO_2 and in turn influences the fractionation of carbon isotopes during phytoplankton uptake (Rau *et al.*, 1989, 1992, Grey *et al.*, 2001). Particularly in late spring and summer, carbon baseline signature reflects contribution by phytoplankton (Pel *et al.*, 2003; Matthews and Mazumder, 2005; Perga and Gerdeaux, 2006). Changes in phytoplankton community structure and functional groups are also related to carbon isotopic signature (Caroni *et al.*, 2012).

A decrease in nitrogen baseline isotopic signature in spring and summer is common for deep lakes: it is attributed to recycling by the zooplankton in the epilimnion. In spring, the rapid

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increase in primary consumers led to excretion of N/NH₄, depleted in heavy isotope (Peterson and Fry, 1987) and quickly assimilated by primary producers. Moreover, different algal groups along the seasons contributing to phytoplankton biomass also differ in nitrogen fractionation (Adams and Sterner, 2000; Lehman *et al.*, 2004). Lake Iseo seasonally exhibits concentrations of cyanobacteria (mostly *Planktothrix rubescens*), green algae (mainly *Sphaerocystis Schroeteri*), Conjugatophyceae (mostly *Mougeotia* spp.) and diatoms (mainly *Fragilaria* sp. and *Diatoma elongatum*) (Leoni *et al.*, 2014a, 2014b; Marti *et al.*, 2016).

As in Lake Maggiore, the most ¹⁵N enriched crustacean zooplankton in Lake Iseo was *B. longimanus longimanus*, in summer sharing the same *E* as the cyclopoids. Thus, cyclopoid adults were at the top of zooplankton food chain and they could potentially be feeding on *Daphnia*. They, however, likely fed in a different habitat (>20 m deep water), as suggested by a rather than negligible carbon fractionation. In Lake Iseo, *B. longimanus*' presence in the open-water was restricted to summer and autumn. The enrichment was close but slightly higher than *Leptodora*'s, suggesting exploitation of intermediate preys in addition to *Daphnia*.

CONCLUSIONS

The novel aspect of this work is the combination of zooplankton body size structure and isotopic variation of carbon and nitrogen. It was analyzed the stable isotopic composition (C and N) of several cladocerans and copepods taxa of Lake Iseo, in four seasons. At the same time, it was measured the feeding basket size of predator and body size of potential prey to better investigate predator-prey interactions in zooplankton community. Combining seasonal changes in the size structure with carbon and nitrogen stable isotopes analyses of zooplankton taxa from a deep subalpine lake, I attempted to take into account size-specificity, an important trait governing seasonal changes in zooplankton population structure.

It was found that the apparent anomaly of being an important invertebrate predator, *Leptodora*, almost un-enriched with respect to *Daphnia*, was reasonably consequent to ability to exploit alternative, other-than-*Daphnia* prey, e.g. *Eubosmina*, whose nitrogen signature was less ¹⁵N-enriched than *Daphnia*'s. The results overall suggest that size-specificity is crucial for addressing space and time changes in trophic links between organisms composing the two hierarchical levels within open water zooplankton community. This study, however, cautions against universal uncritical application of general rules of isotopic fractionation to all aquatic food webs. This research also underlines the need to extend stable isotope studies to

components, which might have been overlooked, in view of a better understanding of roles zooplankton taxa may play in pelagic food web.

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REFERENCES

- Abrùsan G, 2003. Morphological variation of the predatory cladoceran *L. kindtii kindtii* in relation to prey characteristics. *Oecologia* 134:278-283.
- Adams TS, Sterner RW, 2000. The effect of dietary nitrogen content on trophic level ¹⁵N enrichment. *Limnol. Oceanogr.* 45:601-607.
- Adrian R, Wilhelm S, Gerten D, 2006. Life-history traits of lake plankton species may govern their phenological response to climate warming. *Glob. Change Biol.* 12:652-661.
- Bettinetti R, Garibaldi L, Leoni B, Quadroni S, Galassi S., 2012. Zooplankton as an early warning system of persistent organic pollutants contamination in a deep lake (Lake Iseo, Northern Italy). *J. Limnol.* 71:335-338.
- Bettinetti R, Manca M, 2013. Understanding the role of zooplankton in transfer of pollutants through trophic food webs, p. 1-18. In: G. Kehayias (ed.), *Zooplankton: species diversity, distribution and seasonal dynamics*. Nova Science Publishers, Inc., Hauppauge.
- Branstrator DK, Lehman JT, 1991. Invertebrate predation in Lake Michigan: Regulation of *Bosmina longirostris* by *L. kindtii kindtii*. *Limnol. Oceanogr.* 36:483-495.
- Brooks JL, Dodson SI, 1965. Predation, body size and composition of plankton. *Science* 150:28-35.
- Caroni R, Gary F, Visconti A, Manca M, 2012. Phytoplankton functional traits and seston stable isotopes signature: a functional-based approach in a deep, subalpine lake, Lake Maggiore (N. Italy). *J. Limnol.* 71:e8.
- Carpenter SR, Kitchell JF, Hodgson JR, 1985. Cascading trophic interactions and lake productivity. *BioScience* 35:634-639.
- Carpenter EJ, Montoya JP, Burns J, Mulholland MR, Subramaniam A, Capone DG, 1999. Extensive bloom of a N-fixing diatom/cyanobacterial association in the tropical Atlantic Ocean. *Mar. Ecol. Prog. Ser.* 185:273-283.
- Cattaneo A, Manca M, Rasmussen JB, 2004. Peculiarities in the stable isotope composition of organisms from an alpine lake. *Aquat. Sci.* 66: 440-445.
- de Bernardi R, Giussani G, Manca M, 1987. Cladocera: predators and prey. *Hydrobiologia* 145:225-243.
- de Bernardi R, Giussani G, Manca M, 1988. Seasonal evolution of Cladocera in Lago Maggiore (N. Italy) as influenced by environmental parameters. *Verh. Internat. Verein. Limnol.* 23:530-534.
- DeMott WR, Kerfoot WC, 1982. Competition among cladocerans: nature of the interaction between *Bosmina* and *Daphnia*. *Ecology* 63:1949-1966.

- De Niro MJ, Epstein S, 1978. Influence of diet on the distribution of carbon isotopes in animals. *Geochim. Cosmochim. Ac.* 42:495-506.
- Edmondson WT, Litt AH, 1987. *Conochilus* in Lake Washington. *Hydrobiologia* 147:157-162.
- Fink P, Reichwaldt ES, Harrod C, Rossberg AG, 2012. Determining trophic niche width: an experimental test of the stable isotope approach. *Oikos* 121:1985-1994.
- Garibaldi L, Anzani A, Marieni A, Leoni B, Mosello R, 2003. Studies on the phytoplankton of the deep subalpine Lake Iseo. *J. Limnol.* 62:177-189.
- Geller W, Müller H, 1981. The filtration apparatus of Cladocera: filter mesh-sizes and their implications on food selectivity. *Oecologia* 49:316-321.
- Greene CH, 1988. Foraging tactics and prey-selection patterns of omnivorous and carnivorous calanoid copepods. *Hydrobiologia* 167:295-302.
- Grey J, Jones RI, Sleep D, 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, Schelske CL, Hoyer MV, 1996. Stable isotope of carbon and nitrogen as indicators of diet and trophic structure of the fish community in a shallow hypereutrophic lake. *J. Fish Biol.* 49:1233-1243.
- Herzig A, Auer B, 1990. The feeding behaviour of *L. kindtii kindtii* and its impact on the zooplankton community of Neusiedler See (Austria). *Hydrobiologia* 198:107-117.
- Hutchinson GE, 1965. *The ecological theatre and the evolutionary play*. Yale University Press, New Haven: 139 pp.
- Kareiva P, Parker MP, Pascual M, 1996. Can we use experiment and models in predicting the invasiveness of genetically engineered organisms? *Ecology* 77:1670-1675.
- Kuwae T, Miyoshi E, Hosokawa S, Ichimi K, Hosoya J, Amano T, Moriya T, Kondoh M, Ydenberg RC, Elnor RW, 2012. Variable and complex food web structures revealed by exploring missing trophic links between birds and biofilm. *Ecol. Lett.* 15:347-56
- Lehmann MF, Bernasconi SM, McKenzie JA, 2004. Seasonal variation of the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of particulate and dissolved carbon and nitrogen in Lake Lugano: constraints on biogeochemical cycling in a eutrophic lake. *Limnol. Oceanogr.* 49:415-429.
- Leoni B, Garibaldi L, 2009. Population dynamics of *Chaoborus flavicans* and *Daphnia* spp.: effects on a zooplankton community in a volcanic eutrophic lake with naturally high metal concentrations (L. Monticchio Grande, Southern Italy). *J. Limnol.* 68:37-45.
- Leoni B, Garibaldi L, Gulati RD, 2014a. How does interannual trophic variability caused by vertical water mixing affect reproduction and population density of the *Daphnia longispina* group in Lake Iseo, a deep stratified lake in Italy? *Inland Waters* 4:193-203.
- Leoni B, Marti C, Imberger J, Garibaldi L, 2014b. Summer spatial variations in phytoplankton composition and biomass in surface waters of a warm-temperate, deep and oligo-holomictic lake: Lake Iseo, Italy. *Inland Waters* 4:303-310.
- Leoni B, Morabito G, Rogora M, Pollastro D, Mosello R, Arisci S, Forasacco E, Garibaldi L, 2007. Response of planktonic communities to calcium hydroxide addition in a hardwater eutrophic lake: results from a mesocosm experiment. *Limnology* 8:121-130.
- Manca M, Comoli P, 1995. Seasonal changes in size of the feeding basket of *L. kindtii kindtii* (Focke) in Lago Maggiore as related to variations in prey size selection. *Limnol. Oceanogr.* 40:834-838.
- Manca M, Spagnuolo T, Comoli P, 1994. Variations in carbon and nitrogen content with body length of *Daphnia hyalina-galeata* s.l. from laboratory and field observations. *J. Plankton Res.* 16:1303-1314.

- Manca M, Visconti A, Fadda A, Caroni R, Cerutti I, Volta P, 2009. [Indagini sullo zooplancton e sulla rete trofica pelagica attraverso analisi d'isotopi stabili di carbonio e azoto], p. 34-52. In: CNR-ISE (ed.), [Ricerche sull'evoluzione del Lago Maggiore. Aspetti limnologici. Programma quinquennale 2008-2012. Campagna 2008]. [Book in Italian] Commissione Internazionale per la protezione delle acque italo-svizzere.
- Marti CL, Imberger J, Garibaldi L, Leoni B, 2016. Using time scales to characterize phytoplankton assemblages in a deep subalpine lake during the thermal stratification period: Lake Iseo, Italy. *Water Resour. Res.* 52:1762-1780.
- Marty J, Planas D, 2008. Comparison of methods to determine algal $\delta^{13}\text{C}$ in freshwater. *Limnol. Oceanogr.-Meth.* 6:51-63.
- Matthews B, Mazumder A, 2003. Compositional and interlake variability of zooplankton affect baseline stable isotope signature. *Limnol. Oceanogr.* 48:1977-1987.
- Matthews B, Mazumder A, 2005. Temporal variation in body composition (C:N) helps explain seasonal patterns of zooplankton $\delta^{13}\text{C}$. *Freshwater Biol.* 50:502-515.
- Matthews B, Mazumder A, 2006. Habitat speciation and the exploitation of allochthonous carbon by zooplankton. *Ecology* 87:2800-2812.
- Matthews B, Mazumder A, 2007. Distinguishing trophic variation from seasonal and size-based isotopic ($\delta^{15}\text{N}$) variation of zooplankton. *Can. J. Fish. Aquat. Sci.* 64:74-83.
- Matthews B, Mazumder A, 2008. Detecting trophic-level variation in consumer assemblages. *Freshwater Biol.* 53:1942-1953.
- Moss BD, Hering AJ, Green AA, 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, Hillebrand H, Hobaek A, Irvine K, Jeppesen E, Johnson R, Jones I, Kernan M, Lauridsen TL, Manca M, Meerhoff M, Olafsson J, Ormerod S, Papastergiadou E, Penning WE, Ptacnik R, Quintana X, Sandin, L, Seferlis M, Simpson G, Triga C, Verdonschot P, Verschoor AM, Weyhenmeyer GA, 2009. Climate change and the future of freshwater biodiversity in Europe: a primer for policy-makers. *Freshwater Rev.* 2:103-130.
- Obertegger U, Manca M, 2011. Response of rotifer functional groups to changing trophic state and crustacean community. *J. Limnol.* 70:231-238.
- Pel R, Hoogveld H, Floris V, 2003. Using the hidden isotopic heterogeneity in phyto- and zooplankton to unmask disparity in trophic carbon transfer. *Limnol. Oceanogr.* 48:2200-2207.
- Perga ME, Gerdeaux D, 2006. Seasonal variability in the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the zooplankton taxa in two alpine lakes. *Acta Oecol.* 30:69-77.
- Persaud AD, Dillon PJ, Molot LA, Hargan KE, 2012. Relationships between body size and trophic position of consumers in temperate freshwater lakes. *Aquat. Sci.* 74:203-212.
- Peters RH, 1983. Size structure of the plankton community along the trophic gradient of Lake Memphremagog. *Can. J. Fish. Aquat. Sci.* 40:1770-1778.
- Peterson BJ, Fry B, 1987. Stable isotopes in ecosystem studies. *Annu. Rev. Ecol. Syst.* 18:293-320.
- Pilotti M, Valerio G, Leoni B, 2013. Data set for hydrodynamic lake model calibration: A deep prealpine case. *Water Resour. Res.* 49:1-5.
- Porter KG, Feig Y, Vetter E, 1983. Morphology, flow regimes, and filtering rates of *Daphnia*, *Ceriodaphnia* and *Bosmina* fed natural bacterioplankton. *Oecologia* 58:156-163.
- Rau GH, Takahashi T, Des Marais DJ, Repeta DJ, Martin JH, 1992. The relationship between $\delta^{13}\text{C}$ of organic matter and $[\text{CO}_2(\text{aq})]$ in ocean surface water: data from a JGOFS site in the northeast Atlantic Ocean and a model. *Geochim. Cosmochim. Ac.* 56:1413-1419.

- Rau GH, Takahashi T, Des Marais DJ, 1989. Latitudinal variations in plankton ^{13}C : implications for CO_2 and productivity in past oceans. *Nature* 341:516-518.
- Salmaso N, Buzzi F, Cerasino L, Garibaldi L, Leoni B, Morabito G, Logora M, Simona M, 2014. Influence of atmospheric modes of variability on the limnological characteristics of large lakes south of the Alps: a new emerging paradigm. *Hydrobiologia* 731:31-48.
- Salmaso N, Buzzi F, Garibaldi L, Morabito G, Simona M, 2010. Effects of nutrient availability and temperature on phytoplankton development: a case study from large lakes south of the Alps. *Aquat. Sci.* 74:555-570.
- Rinta P, Bastviken D, van Hardenbroek M, Kankaala P, Leuenberger M, Schilder J, Stötter T, Heiri O, 2015. An inter-regional assessment of concentrations and $\delta^{13}\text{C}$ values of methane and dissolved inorganic carbon in small European lakes. *Aquat. Sci.* 77:667-680.
- Smyntek PM, Maberly SC, Grey J, 2012. Dissolved carbon dioxide concentration controls baseline stable carbon isotope signatures of a lake food web. *Limnol. Oceanogr.* 57:1292-1302.
- Šorf M, Brandl Z, 2012. The rotifer contribution to the diet of *Eudiaptomus gracilis* (G. O. Sars, 1863) (Copepoda, Calanoida). *Crustaceana* 85:1421-1429.
- Strayer DL, 2010. Alien species in fresh waters: ecological effects, interactions with other stressors, and prospects for the future. *Freshwater Biol.* 55:152-174.
- Visconti A, Manca M, 2011. Seasonal changes in the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures of the Lago Maggiore pelagic food web. *J. Limnol.* 70:263-271.
- Visconti A, Volta P, Fadda A, Di Guardo A, Manca M, 2014. Seasonality, littoral vs. pelagic carbon sources, and stepwise ^{15}N -enrichment of pelagic food web in a deep subalpine lake: the role of planktivorous fish. *Can. J. Fish. Aquat. Sci.* 71:436-446.
- Vogt RA, Ignoffo TR, Sullivan J., Herndon J, Stillman JH, 2013. Feeding capabilities and limitations in the nauplii of two pelagic estuarine copepods, *Pseudodiaptomus marinus* and *Oithona davisae*. *Limnol. Oceanogr.* 58:2145-2157.
- Wada E, 2009. Stable $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ isotope ratios in aquatic ecosystems. *Proc. Jpn. Acad. B* 85:98-107.
- Zohary T, Erez J, Gophen M, Berman-Frank I, Stiller M, 1994. Seasonality of stable carbon isotopes within the pelagic food web of Lake Kinneret. *Limnol. Oceanogr.* 39:1030-1043.

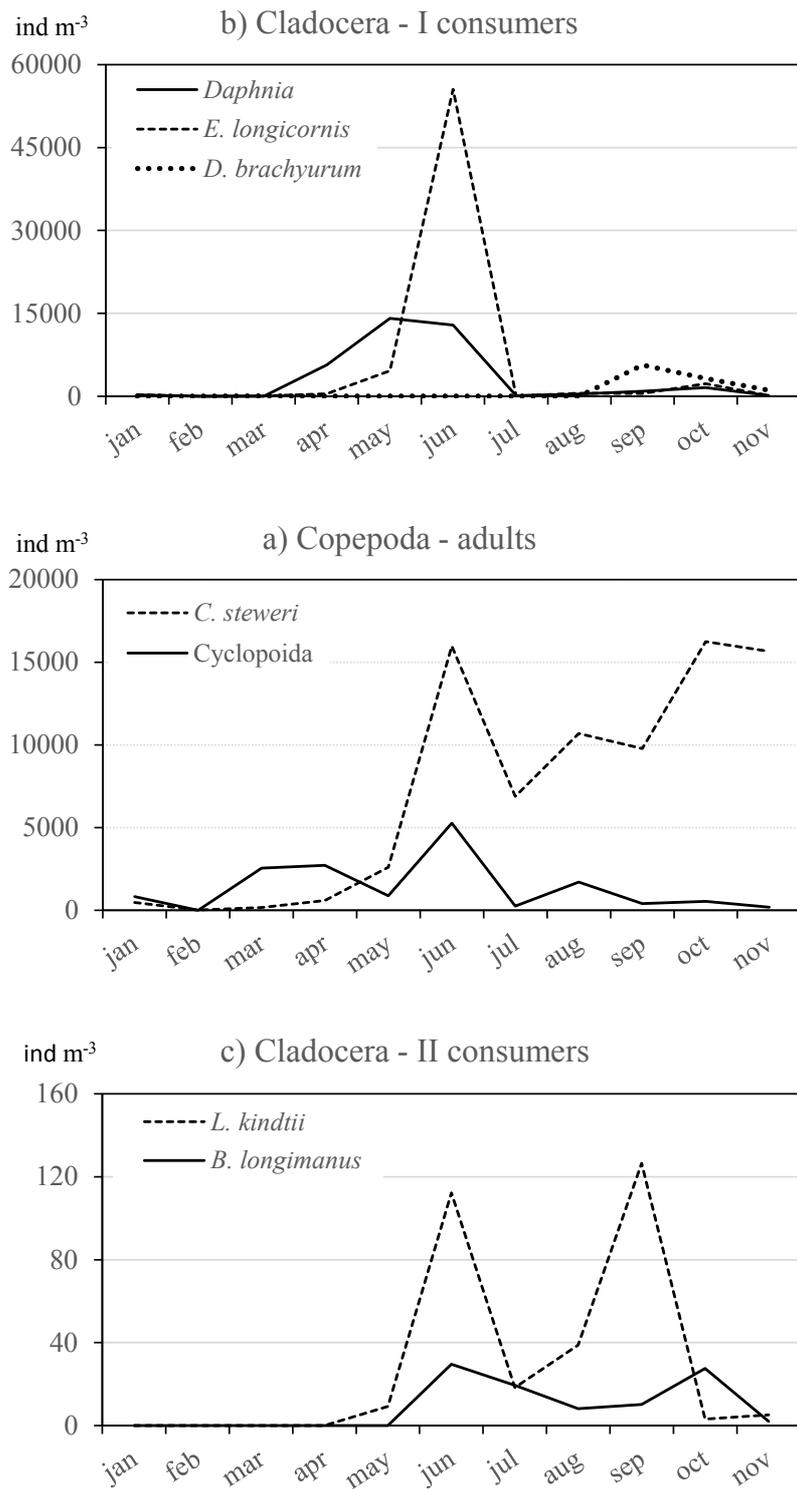


Fig. 1. Seasonal dynamics of zooplankton densities in Lake Iseo. a) Adults of Copepoda. b) Cladocera primary consumers. c) Cladocera secondary consumers.

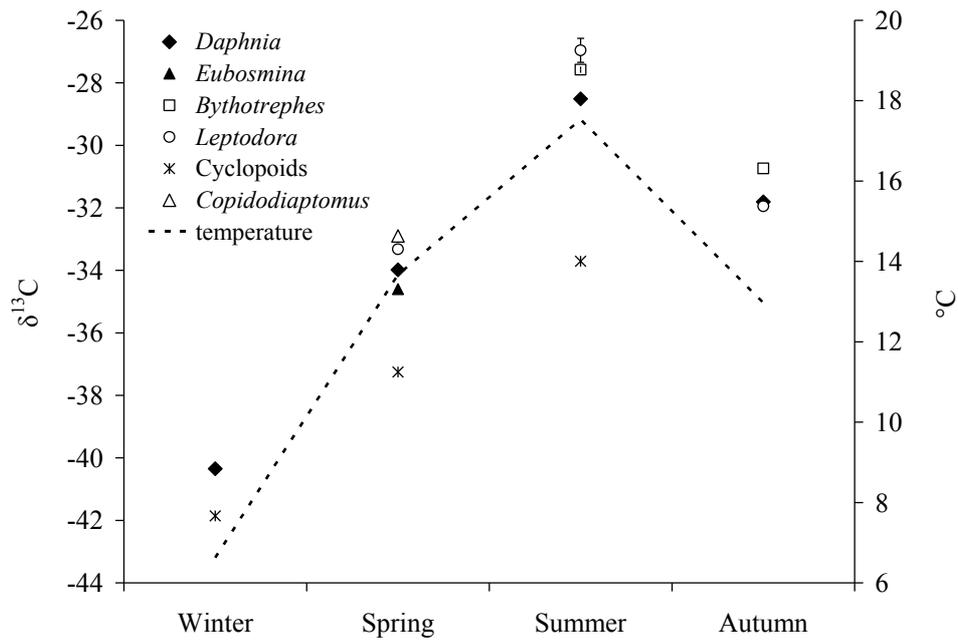


Fig. 2. Seasonal changes in carbon isotopic signatures of zooplankton taxa from Lake Iseo, 2011. Dotted line refers to water temperature (average) in the water layer from which zooplankton was sampled (0-20 m).

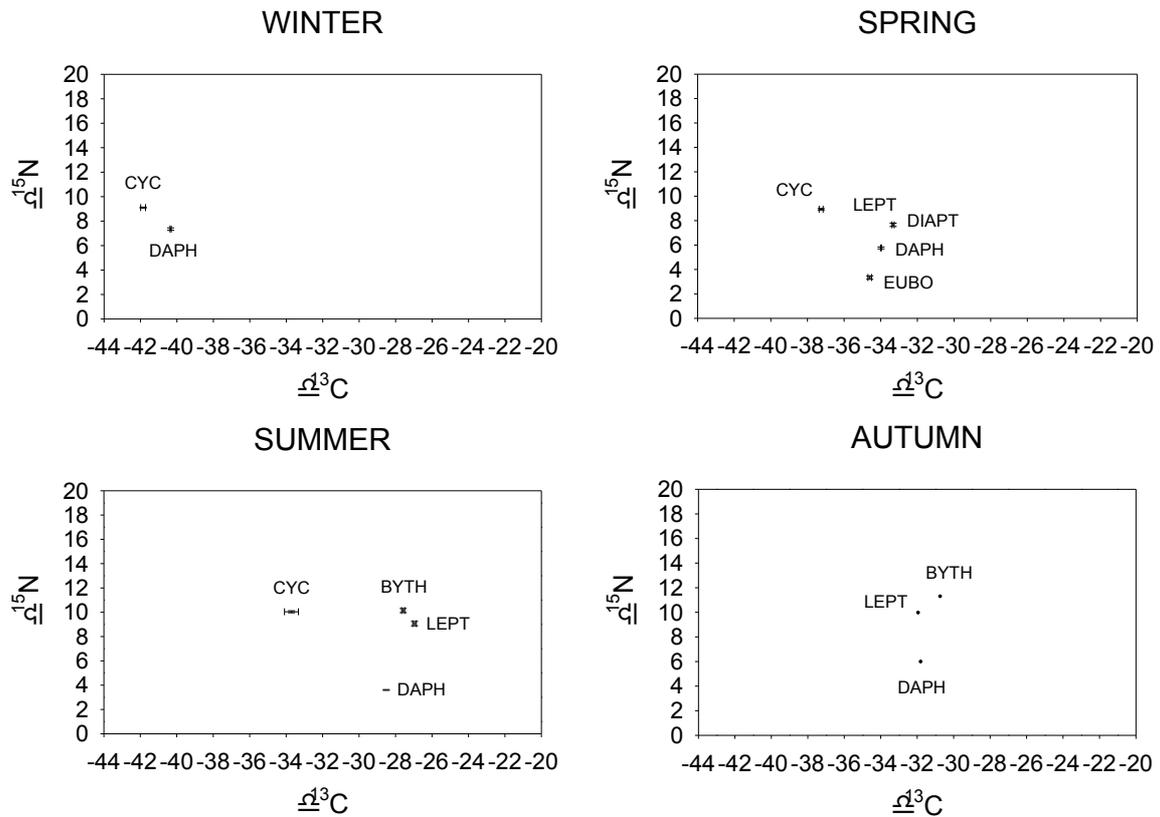


Fig. 3. Trophochemical graphs showing seasonal carbon and nitrogen isotopic signatures (average \pm SD) of zooplankton taxa from Lake Iseo, 2011. Byth, *B. longimanus*; Cyc, Cyclopoids; Diapt, *Copidodiaptomus stewarti*; Dap, *Daphnia longispina* group; Eubo, *E. longicornis*; Lept, *L. kindtii*. In spring, *L. kindtii* and *Copidodiaptomus stewarti* show similar signatures.

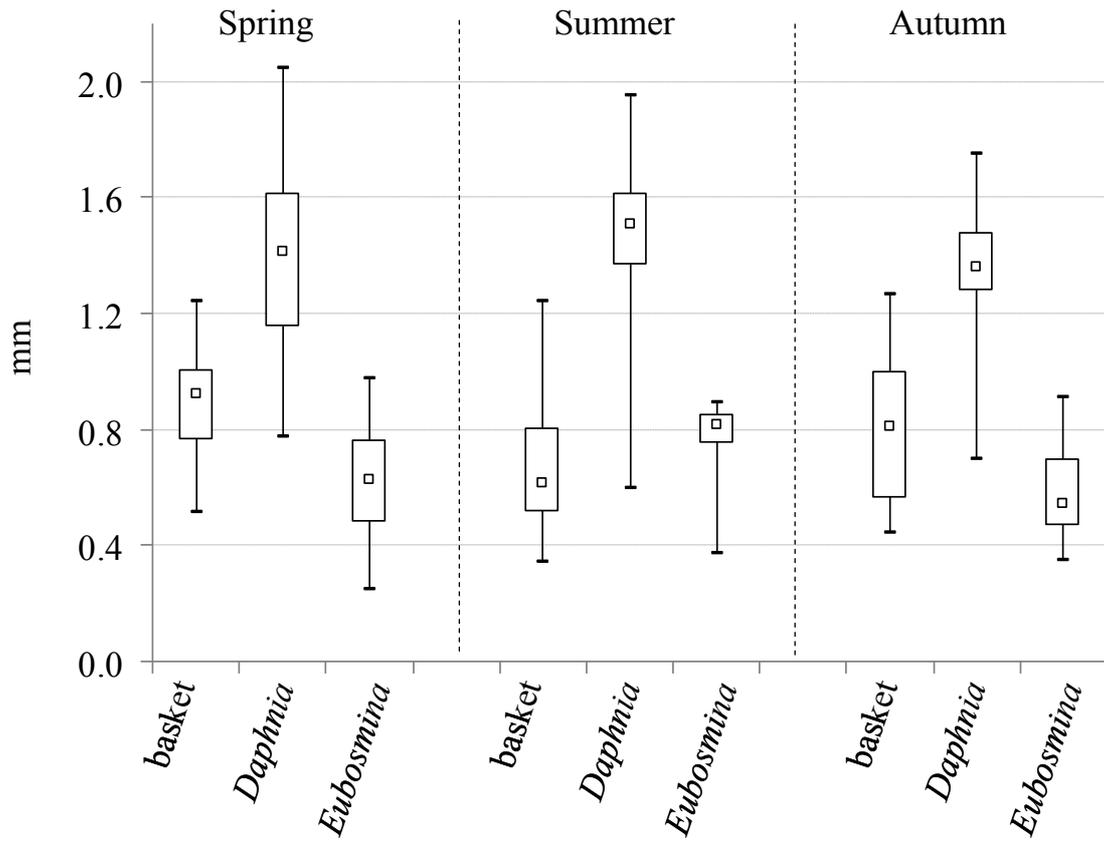


Fig. 4. Box-Whisker plots (boxes: median, upper and lower quartile; whiskers: minimum and maximum value) of seasonal changes in *L. kindtii* feeding basket (diameter) and body size (length) of potential cladoceran prey.