Pseudo-diel vertical migration in zooplankton: a whole-lake ^{15}N tracer experiment

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Diel vertical migration (DVM) of zooplankton is commonly considered an adaptation for feeding in food-rich and warm surface waters at night and avoiding visual predators during the day. However, the critical assessment of migration patterns frequently suggests that: (i) zooplankton may leave deeper waters with rich, deep-chlorophyll layers and move into the epilimnion where food resources are lower and/or (ii) the night-time increase in epilimnetic plankton abundance is not matched by a density decrease in deeper strata. To study these discrepancies, we measured DVM of zooplankton in a 1.3-ha Spanish karst lake (Laguna del Tejo) where the phytoplankton in the deep chlorophyll layer had been labelled with the isotopic tracer ^{15}N. There were only limited changes in the vertical distribution patterns of rotifers and copepods over the diel cycle, but night-time biomass (mg L^{-1}) of the cladoceran, *Diaphanosoma brachyurum* in the water column of the oxic zone increased 168% in the central area of the lake but there was not a parallel reduction in their density in the metalimnion or hypolimnion. Additionally, the isotopic signatures of the zooplankton in the epilimnion stayed constant from day to night, suggesting that there was little vertical migration. The results suggest that horizontal movements rather than vertical migration explain the increasing abundance of zooplankton in the central epilimnion of Laguna del Tejo during the night and that low food resources in this layer provide no incentive for DVM.

KEYWORDS: zooplankton; diel vertical migration; nutrient; horizontal movements; deep chlorophyll maxima; stable isotope; ^{15}N

INTRODUCTION

Zooplankton play a key role in the transfer of matter and energy in aquatic food webs, usually connecting primary producers and higher consumers. Zooplankton have low swimming capacity, but many planktonic organisms perform pronounced diel vertical migrations (DVMs). This phenomenon has been a major subject of study during recent decades (Worthington, 1931; Lampert, 1989; Ringelberg, 2010). The normal pattern of DVM consists of an upward movement around dusk to spend the night in surface waters, with organisms returning to deeper waters at dawn. Plankton from different taxa have different vertical migrations that are often related to their size and swimming capacity. Marine zooplankters (e.g. euphausiids) are able to migrate more than 200 m (Brinton, 1979). Freshwater crustaceans, such as copepods and cladocerans, can

migrate tens of metres (Stich and Lampert, 1981). For rotifers, such migrations are usually measured in metres (George and Fernando, 1970; Armengol and Miracle, 2000). Light-dependent predation risk plays an important role in crustacean migrations (Zaret and Suffern, 1976; Lampert, 1989; Loose, 1993) but other hypotheses based on metabolic advantages or different feeding strategies have been proposed (McLaren, 1974; Lampert and Taylor, 1985; Geller, 1986; Wurtsbaugh and Neverman, 1988; Williamson et al., 1996; Armengol and Miracle, 2000; Lampert and Grey, 2003) to explain migrations. Many authors have suggested that DVM allows zooplankton to feed in food-rich surface waters at night, and escape predation during the night by descending into deep strata with insufficient light for fishes to prey on them (Lampert, 1989). However, in many lakes and in the oceans, these deep strata often contain deep chlorophyll layers where food levels are actually higher than in the surface waters, so under these circumstances, it is not clear why zooplankton would bother to migrate into surface waters to feed (Williamson et al., 1996). There is also conflicting evidence as to the food value of seston in the deep chlorophyll layers (Cole et al., 2002; Winder et al., 2003). DVM can transport nutrients upwards (Haupt et al., 2010), but epilimnetic grazing may also increase sedimentation rates and strip the upper layer of organic matter and nutrients (Pilati and Wurtsbaugh, 2003). Lampert and Grey (Lampert and Grey, 2003) found that when seston levels are higher in deep, cold strata, organisms do feed in the deep chlorophyll layer, but limit their time there.

An important unresolved issue is whether the day–night differences in zooplankton vertical distribution are really driven solely by vertical migration. Most studies of the diel changes in lakes demonstrate that the total integrated catch in the pelagic zone of many zooplankton taxa is considerably higher at night than during the day (Worthington, 1931; Cushing, 1951; Bast and Seitz, 1993; Armengol and Miracle, 2000). This "diurnal deficit" (De Stasio, 1993) is often ignored and researchers frequently sidestep this issue by presenting catch rates at each depth as percentages of the total captured in the water column during daylight, and a separate percent distribution at night. However, if vertical movement from the hypolimnion to the epilimnion is the sole mechanism driving the diel patterns, night-time increases in the epilimnion should be matched by equivalent decreases in deeper strata. The pioneering work of Worthington (Worthington, 1931) addressed the concept of the diurnal deficit, and more recent research has also documented significant discrepancies between the total number of plankton found in the water column during the day and night (Bast and Seitz, 1993;

Haney, 1993; Armengol and Miracle, 2000). To account for the diurnal deficit, four main explanations have been proposed: (i) daytime concentration of organisms near the sediment or in fine or undetected layers (De Stasio, 1993), (ii) daytime residence in anoxic layers (Worthington, 1931; De Meester and Vyverman, 1997), (iii) better escape avoidance of collection devices during the day (Omori and Hamner, 1982; Ianson and Jackson, 2004) or (iv) horizontal movements (Carrillo et al., 1989; Masson et al., 2001; Pinel-Alloul et al., 2004).

The original intent of our study was to determine whether zooplankton transported isotopically labelled nutrients from the metalimnion into the surface strata. Here, however, we use the field data on the temporal changes in abundance and isotopic composition of the zooplankton to address the following hypotheses: (i) Is the nocturnal increase in zooplankton in the epilimnion driven by migrations from deeper strata? and (ii) What is the relevance of a deep chlorophyll layer for the feeding of zooplankton? We assessed these questions in Laguna El Tejo, a 1.3-ha karst lake, where an experiment was in progress that had labelled phytoplankton in the metalimnion with ^{15}N tracer. The small size and great relative depth of the lake, together with a deep chlorophyll layer with labelled phytoplankton made it ideal for assessing zooplankton DVM.

STUDY SITE

Laguna El Tejo is a 1.3-ha circular flooded doline located at $39°59'20''$N, $1°52'25''$W near the city of Cuenca (central Spain). This small lake (145 m mean diameter) is inside a sink hole with steep banks; at the time of the study, mean and maximum depths were 11.9 and 28 m, respectively, and the lake volume was around 196 000 m^3. The lake lacks superficial water inflows or outflows, and it is protected from wind due to the steep walls of the sink hole rising $30–50$ m above the lake level. Laguna El Tejo is oligomesotrophic with summer epilimnetic chlorophyll levels of 2.0 μg L^{-1} and a mean Secchi depth of 7.1 m. On the date when DVM was measured the photic zone (1% of surface light; PAR) extended to 17.5 m. Chlorophytes, diatoms and dinophytes were dominant through the epi- and metalimnion (Morata et al., 2003), with cryptophytes and particularly autotrophic picoplankton increasing in importance below 14 m (Camacho et al., 2003a). Chlorophyll levels in the metalimnion were 70% higher than those in the epilimnion. Additionally, there was a deep chlorophyll maximum near 19 m at the boundary of the anoxic hypolimnion where a dense population of

autotrophic picoplankton resulted in chlorophyll levels raised to 380% of those in the surface strata (Fig. 1). In summer, the growth of phytoplankton is nitrogen limited throughout the water column (Camacho *et al.*, 2003a). Because of its steep shores, macrophytes (*Myriophyllum spicatum* and *Chara aspera*) are not abundant, occurring only in $\sim 1-2$ m diameter sparse dispersed patches around the littoral zone. Introduced fish (*Luciobarbus guiraonis* and *Achondrostoma arcasii*) were abundant in the lake at the time of our sampling. Young stages of both species and *A. arcasii* adults are omnivorous and feed on zooplankton. Additional information on the limnology of doline lakes in the area is available (Miracle *et al.*, 1992; Armengol and Miracle, 1999; Camacho *et al.*, 2003b).

METHODS

To characterize limnological conditions of the lake, Secchi disk depth and vertical profiles of oxygen, pH, temperature, light and chlorophyll *a* were measured using the methods detailed in Camacho *et al.* (Camacho *et al.*, 2003a).

On 17 September 1997, we began an experiment to follow the movement of nitrogen from deep layers of the lake. Ammonium ^{15}N (14.5 g), mixed with rhodamine solution as a water mass tracer, was pumped into the metalimnion of the lake at a depth of 15.5 m at nine stations distributed around the lake. Initially the tracers formed a layer only $0.5-1$ m thick (Fig. 1), but at the time of our DVM analyses 2 weeks later, the ^{15}N and rhodamine had dispersed slightly vertically (Fig. 2). The nitrogen tracer in the seston and in sediment traps was followed for 12 months, but those results will be presented elsewhere. Rhodamine, temperature and conductivity vertical profiles were measured at $\sim$5-cm accuracy with a WET Labs, Inc., FlashPak fluorometer attached to a SeaBird, Inc., SBE19 conductivity−temperature−depth profiler.

On 30 September−1 October, 14 days after the ^{15}N addition, we used three approaches to measure temporal changes in vertical distribution of zooplankton and seston.

(i) At midnight and mid-day (0100 and 1300 h), we collected zooplankton at eight different depths (0.5, 3, 8, 11, 14, 15.5, 18 and 19.5 m) at one station located near the centre of the lake with a transparent 25-L Schindler−Patalas trap with 100-μm nylon mesh. To characterize the fraction of organisms between 30 and 100 μm and to address the importance of smaller zooplankton (mainly rotifers), we filtered 1 L of the previously 100-μm filtered water with a 30-μm Nytex net. All samples were preserved using 4% formalin and counted using an inverted microscope at $100-400$ magnification. For dominant crustacean species, over 100 individuals from the remaining 24 L in the trap were also measured to estimate their abundance and biomass utilizing the length−weight regressions of Lawrence *et al.* (Lawrence *et al.*, 1987).
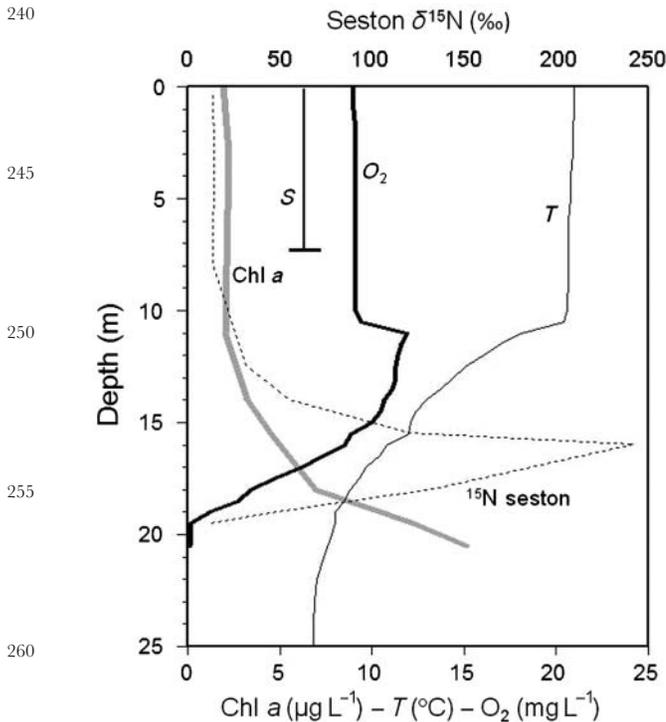
Fig. 1. Temperature (*T*), oxygen (O$_2$), chlorophyll *a* and seston δ^{15}N profiles in Laguna El Tejo on 30 September 1997. The Secchi (*S*) depth transparency is also shown.
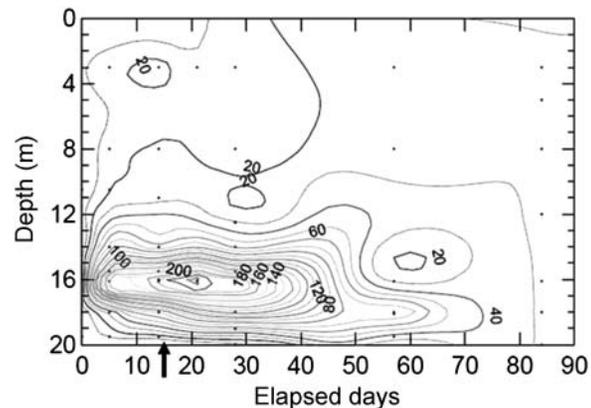
Fig. 2. Isopleths of seston δ^{15}N (above background) sampled at four stations in Laguna El Tejo from 17 September to 10 December 1997. The isotope injection at 15.5 m occurred after background measurements were made on 17 September. The arrow on the *x*-axis shows the date when DVM measurements were made (30 September to 1 October).

Estimates of biovolume and then biomass were also made for rotifers (Ruttner-Kolisko, 1977).

(ii) We also collected samples to assess the nitrogen mass and ^{15}N enrichment of the zooplankton at the same depths as those used for the density estimates. These vertical profiles of the plankton were taken 1 h before the previously described samples for numerical counts. These were collected at four stations (including the above-mentioned station) at the corners of a square (25-m side) near the centre of the lake where the depth was near 24 m. Because of logistic constraints, we used four stations for the day samples but only two during the night. At each station, two Schindler–Patalas trap catches (50 L) of zooplankton from each depth were filtered through a 100-μm mesh. We also collected seston for isotopic analysis at four stations and seven depths with a 2.7-L Ruttner bottle.

(iii) We collected a third set of samples from only the epilimnion (0–9 m) for carbon and nitrogen mass and ^{15}N analysis. This was done six times (21:00; 01:00; 6:30; 8:30; 15:00 and 18:00) over the diel period. We collected these samples with vertical hauls of a 100-μm mesh zooplankton net with a 25-cm diameter mouth. These collections were made at two diagonally opposed stations used for the Schindler–Patalas trap samples.

Zooplankton for isotopic analyses were filtered within 8 h at a field laboratory on pre-combusted (550°C, 12 h), 25-mm glass fibre GF/F filters. These were subsequently dried for 24–48 h at 45°C and finally the entire filters were encapsulated for isotopic processing. Seston samples were processed by filtering 0.5–1.25 L aliquots of lake water on pre-combusted 47-mm glass fibre GF/F filters until clogging occurred. The filters were rolled slightly, placed in plastic vials, dried for 24 h at 45°C and then frozen. To encapsulate the seston material in tin capsules, a 36-mm diameter punch was used to sub-sample 81% of the seston on the filters. Seston and zooplankton samples were analysed for particulate organic nitrogen and particulate organic carbon and ^{15}N with a Europa Scientific ANCA 2020 mass spectrometer linked to a CN analyzer. All enrichments are expressed relative to that of atmospheric N_2 but corrected by subtracting background delta values measured at each depth prior to the addition of the tracer to the lake.

The mean residence depth (MRD), a weighted average depth, was calculated for the different zooplankton groups and species as:

$$MRD = \frac{\sum(N_i \times d_i)}{\sum N_i}$$

where N_i is the concentration of individuals at depth i and d_i is the depth of the ith sample.

We also estimated the total zooplankton mass in the oxic zone (above 20 m) of the whole lake for each depth stratum. For this, we assumed a homogeneous horizontal distribution of the zooplankton across the lake, and multiplied day and night zooplankton concentrations and biomass at each depth times the water volume in that stratum (obtained from a hypsographic curve). This calculation is useful because even if zooplankton are dense in deep stratum, they may not contribute much to total mass because deep strata have relatively less volume because of the funnel shape of the lake.

Two-way ANOVA was used to test for statistical differences ($P < 0.05$) in zooplankton $\delta^{15}N$ between sampling time (day or night) and between depths. To compare the zooplankton taken with vertical tows in the epilimnion the samples were grouped in two categories: day (samples taken at 8:30, 12:00, 15:00 and 18:00) and night (21:00, 0:00, 1:00 and 6:00), and statistical differences among these groups for the zooplankton content in C, N and $\delta^{15}N$ were tested using one-way ANOVA. Statistical calculations were made using the statistical package PAST 1.92 (Hammer et al., 2001).

RESULTS

Limnological characterization

Near the end of the stratification period when the DVM analyses were made (30 September to 1 October) the epilimnion extended to 10.5 m, with a metalimnion located between 10.5 and 18 m (Fig. 1). Temperatures ranged from 20°C at 0.5 m to 8°C at 20 m. The water was clear, with a Secchi depth of 7.1 m. An oxygen peak (137% of saturation) was present in the upper metalimnion. The lake was anoxic below 20 m. Chlorophyll a concentrations in the epilimnion were 2 μg L^{-1}, increased to 3 μg L^{-1} in the upper metalimnion and reached maximum values 13.1 mg L^{-1} just above the oxic-anoxic interface at 19.5 m, where picocyanobacteria were quite abundant (Camacho et al., 2003a).

The addition of $^{15}NH_4^+$ to the metaliminion greatly enriched the heavy isotope concentration in the seston (Fig. 1). The $\delta^{15}N$ (‰) of seston in the epilimnion increased from $\delta^{15}N$ +2 before the isotopic addition to +20 afterwards. However, the isotopic enrichment of nearly all of the metalimnion increased considerably more, with a peak of +125 at 15.5 m. Subsequent measurements through mid-December indicated that the highest point of seston enrichment occurred slightly lower at 16.1 m (Fig. 2), however the sampling depth during the DVM study was the depth of the addition
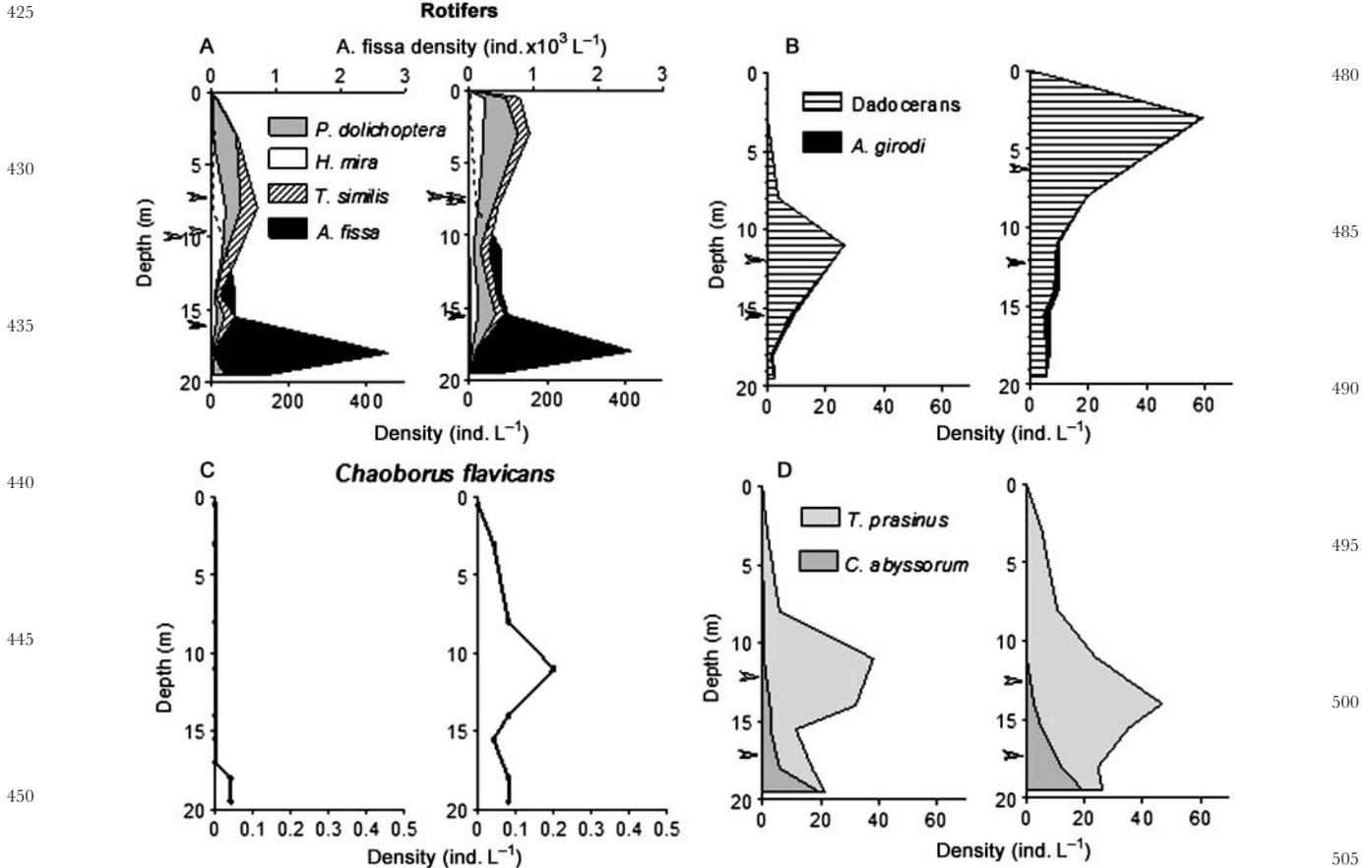
Fig. 3. Vertical distribution patterns of zooplankton in Laguna El Tejo on 1 October 1997. In each pair of figures, the daytime distribution is shown on the left and the night-time distribution on the right. Arrows show the MRD of each taxon (not shown for *Chaoborus*).

(15.5 m). The strong stratification of the seston $\delta^{15}N$ was maintained through mid-October. By mid-November the lake had mixed to 16 m and by December 20th the ^{15}N in the seston was evenly dispersed to 21 m.

The zooplankton community was dominated numerically by rotifers but in terms of biomass by crustaceans (Figs 3 and 4). *Diaphanosoma brachyurum* was the most abundant crustacean, while *Ceriodaphnia dubia* and *Daphnia longispina* had densities <1% of *D. brachyurum*. The cladocerans were located primarily in the epi- and metalimnion. The copepods *Tropocyclops. prasinus* and *Cyclops abyssorum* were also abundant but located mainly in the hypo- and metalimnion. Most species of rotifers had broad distributions throughout the water column, with the exception of *Anuraeopsis fissa*, which peaked at the hypolimnion. *Chaoborus flavicans* larvae were also present.

Diel changes in zooplankton abundance and isotopic composition

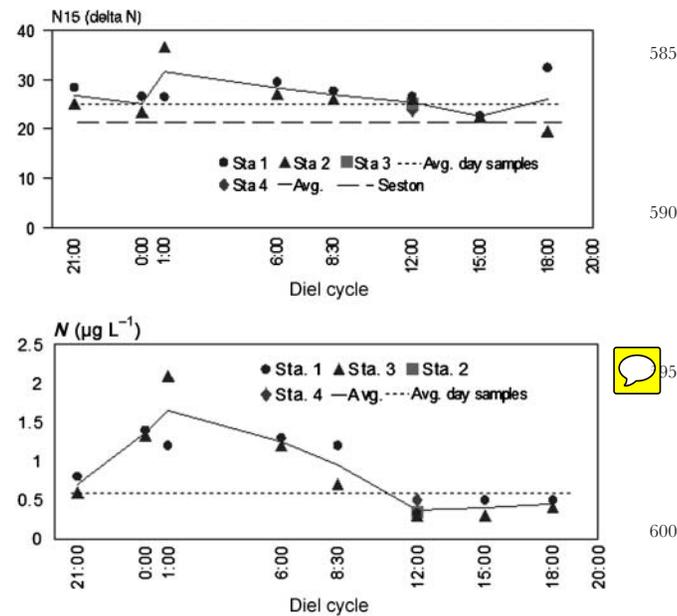There were distinct differences in zooplankton abundance in the water column during the day and night (Fig. 3). The median depth of the rotifers *Asplanchna girodi*, *Hexarthra mira* and *Trichocerca similis* became ~2.5 m shallower at night, but despite the migration, all of these species still resided in the epilimnion or metalimnion over the diel period. The distribution of both *Polyarthra dolichoptera* and *A. fissa* changed little from day to night (Fig. 3A). The cladocerans (98% *D. brachyurum*) showed the greatest difference in distribution between the day and night, with their maximum density at 11 m during midday and 3 m at midnight. *C. flavicans* larvae were found in the hypolimnion during the day, but they moved throughout the water column during the night (Fig. 3C). The vertical distribution of the two copepod species (nauplii and copepodites included) was very similar between day and night. *T. prasinus* was abundant in the metalimnion with peaks at 11 m during the day and at 14 m during the night. *C. abyssorum* were in the hypolimnion, with the nocturnal maximum located slightly above the day maximum (Fig. 3D).

The comparison of vertical profiles of cumulative zooplankton biomass per litre showed that at night
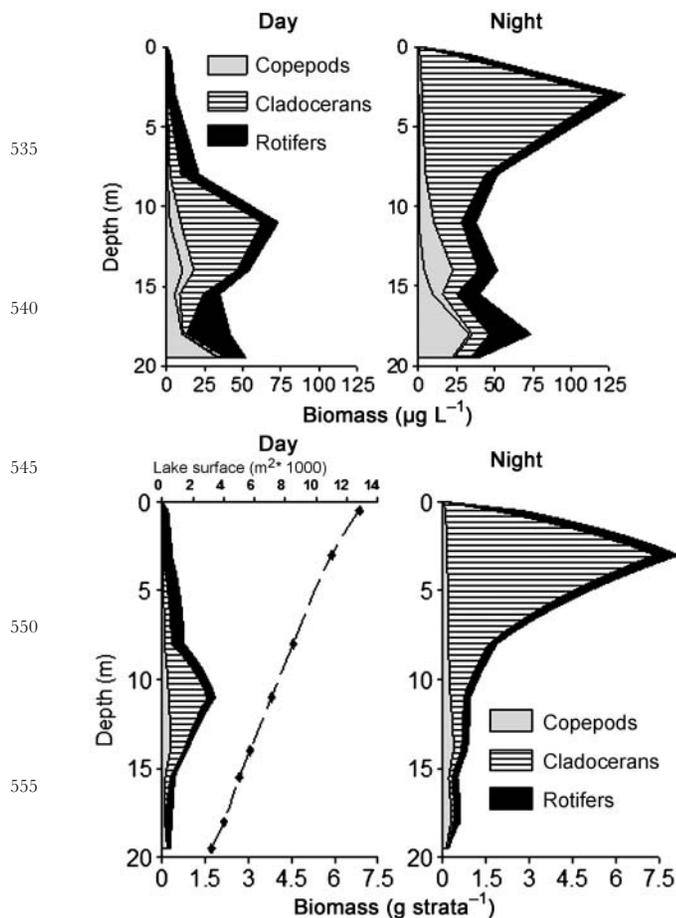
Fig. 4. (Upper) The vertical profile of the biomasses of different taxonomic groups in Laguna El Tejo on 1 October 1997. (Lower, left) The hypsographic curve of Laguna El Tejo showing the cross-sectional area at different depths, as well as the daytime whole lake estimates of integrated zooplankton biomass within different depth strata. (Lower, right) Night-time whole-lake distributions of different zooplankton taxa (g/strata). Distributions in the lower two figures are corrected for the relative lake volumes in each depth strata.


Fig. 5. (Upper) Diel changes in the isotopic signatures of zooplankton captured with vertical hauls (0–9 m) at different stations in the epilimnion of Laguna El Tejo on 30 September–1 October 1997. The bold dashed line shows the mean isotopic signature of seston in the epilimnion on this date. The light dashed line shows the mean isotopic signature of the day samples. (Lower) Changes in the nitrogen mass of zooplankton in the epilimnion of the lake over the diel cycle, light dashed line shows the mean nitrogen mass of the zooplankton day samples. Represented values at 0:00 h and 12:00 came from the series of samples taken with plankton traps. (delta N, δ^{15}N), mean isotopic signatures are expressed in ‰.

there was a pronounced increase of biomass in the epilimnion and also slight *increase* in the meta- and hypolimnion (Fig. 4). An increase in *D. brachyurum* density in the epilimnion was primarily responsible for the nocturnal increase of zooplankton biomass in surface waters. When the cumulative biomass distribution was corrected for the relative volume of each stratum using the hypsographic curve, the day-night change in cladoceran abundance was even more pronounced (Fig. 4, lower). The hypsographically-adjusted cladoceran biomass indicated that during the night there was a 2-fold decrease in cladoceran abundance in the metalimnion and a 10-fold increase in the epilimnion (day, 0.60 g; night 6.60 g dry weight). Biomass of copepods increased somewhat in the epilimnion at night, but a commensurate decrease did not occur in the meta- or hypolimnion

(Fig. 4). The distribution of rotifer biomass also showed a slight increase in surface waters during the night.

The nocturnal epilimnetic increase of zooplankton nitrogen and carbon mass estimated from net hauls was similar to that estimated by counts and length-weight relationships. During the day, the N mass of zooplankton averaged 0.6 µg L^{-1} but increased 2-fold at night to 1.2 µg L^{-1} (Fig. 5). Changes in zooplankton carbon in the epilimnion were similar, increasing from 3.3 µg C L^{-1} during the day to 6.2 µg C L^{-1} at night (data not shown). The isotopic enrichment of nitrogen in the epilimnetic zooplankton did not, however, increase significantly at night (Fig. 5). Enrichments were δ^{15}N of 25.2 ‰ during the day, and 28.8 ‰ during the night but there was no significant change between day and night samples over the 24-h cycle (ANOVA, $F_{1,10} = 2.197$; $P = 0.169$). Nevertheless, significant differences were found over the same cycle for the mass of zooplankton N ($F_{1,10} = 5.716$; $P = 0.038$) and C ($F_{1,10} = 6.265$; $P = 0.031$), both of which increased markedly at night in the epilimnion.

Vertical distribution in the mass of N and C in the zooplankton taken with Schindler-Patalas trap followed
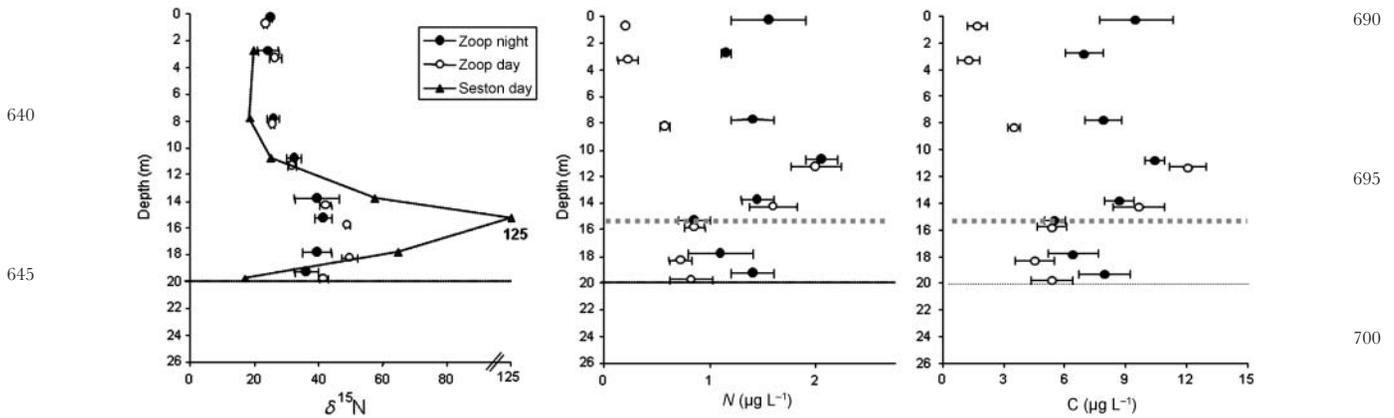
Fig. 6. (Left) Vertical patterns in the isotopic signatures of zooplankton during the night and the day and in the seston during the day. The bold arrow shows the depth where $^{15}NH_4Cl$ was injected into the metalimnion. (Middle and right) Vertical distribution of nitrogen and carbon mass in the zooplankton during the night and the day. Note the modest "increase" in zooplankton biomass in the upper hypolimnion at night, and the very large increase in the epilimnion. The arrow in the left frame and dotted line indicates the depth at which the $\delta^{15}N$ (expressed in ‰) tracer was injected. The dashed line at 20 m shows the depth below which the water column was anoxic (<0.1 mg O_2 L^{-1}). Mean ± s.e.

a similar pattern (Fig. 6). There was a notable increase for both elements at midnight in the epilimnetic (0.5, 3 and 8 m) samples, but the hypolimnetic N mass of zooplankton also increased at night. In the metalimnion (11, 14 and 15.5 m) day and night samples of zooplankton had nearly identical masses of N (Fig. 6B).

The vertical profiles indicated that zooplankton in the lower metalimnion and upper hypolimnion was highly labelled with ^{15}N, reaching levels of +50 in daytime samples (Fig. 6A). However, in spite of the large biomass differences in the epilimnion between day and night (Fig. 6B), $\delta^{15}N$ of the zooplankton in the vertical profile did not change significantly in epilimnetic samples. Surprisingly, the observed night-time enrichments of zooplankton in the lower metalimnion and hypolimnion were also lower at night than during the day (Fig. 6A). A two-way ANOVA comparing day and night $\delta^{15}N$ of zooplankton along the vertical profile indicated that there were significant isotopic differences with depth ($F_{7,30} = 34.9$; $P < 0.001$) and time ($F_{1,30} = 11.86$; $P = 0.002$), but there was no significant interaction between depth and time ($F_{7,30} = 1.628$; $P = 0.166$). The latter suggests that ^{15}N-labelled zooplankton underwent minimal DVM.

DISCUSSION

DVM has been commonly described through the comparison of day/night vertical distributions of the different zooplankton species or groups and changes in MRD over a diel cycle have been commonly employed to quantify such vertical movements (e.g. Nauwerck,

1993; Ringelberg, 2010). Although our study was done in a small lake where the littoral zone may exert a large influence, the importance of DVM appeared to be very limited.

In many studies on zooplankton vertical migration a recurrent difficulty is that more individuals are usually caught during the night than during the day and we observed the same phenomenon during our study in El Tejo. When we compare day/night vertical distributions using MRD variations, a preliminary inspection of our results suggested large vertical movements of *D. brachyurum*. However, we found a general increase of this species at night in samples from almost every depth. The estimated whole-lake population of this species during the night was much higher than during the day. Assuming a homogeneous distribution, when the total number of organisms in the water column was integrated, the night time estimate was 268% of the biomass estimated during the day. Our numerical counts and the N and C biomass results suggest that there was a marked increase of zooplankton in the epilimnion which was not matched by a decrease in the meta- and hypolimnion. Consequently, it is difficult to explain where the organisms come from by only considering vertical migration.

Four other possibilities to explain the perceived nocturnal increase in abundance include: (i) zooplankton avoided our samplers more during the day than at night; (ii) zooplankton concentrating in fine layers or close to the sediment during the day were missed with our sampling methods; (iii) zooplankton in the anoxic hypolimnion were not sampled during the day, but migrated into the epilimnion at night and (iv) nocturnal

horizontal migrations of the zooplankton increased our catch rates in the pelagic zone. We consider each of these possibilities in turn.

(i) Different sampling methods can influence catch rates of zooplankton (Schindler, 1969; Masson et al., 2004), and Gliwicz and Pijanowska (Gliwicz and Pijanowska, 1989) indicated that *Diaphanosoma* sp. has a strong escape response. However, we doubt that the 10-fold day-night difference we found in the epilimnion could be due to avoidance alone because we used both a transparent 25-L Schindler-Patalas trap, which is believed to be one of the most efficient zooplankton sampling methods (Schindler, 1969), and a 25-cm diameter plankton net, and both devices provided similar results. Furthermore, Drenner and McComas (Drenner and McComas, 1980) showed that *Diaphanosoma* has intermediate escape probabilities relative to *Daphnia* and copepods, but it was still captured with 100% success rate at a distance of 1.5 cm from a simulated suction-feeding fish. This suggests that avoidance of the mouth of the large Schindler-Patalas trap would have been minimal and cannot explain the large day-night differences in densities in the epilimnion. (ii) Concentrations of organisms (mostly rotifers or nauplii) in fine layers, which we may have missed, have been described in other karst lakes (Miracle and Armengol, 1995) and because we lack a complete series of samples at each depth we cannot completely reject such an explanation. Nevertheless, if organisms were missed from fine layers they would have to have been below 9 m, as we took vertical net hauls from 9 m and thus would have encountered fine-scale concentrations in this stratum. We also cannot reject the hypothesis that zooplankton were concentrated at the oxic-anoxic interface during the day and migrated into the water column at night and dispersed. It does, however, seem improbable that they migrated from strata below 9 m because the plankton there should be highly labelled with ^{15}N and we did not measure a night-time increase of δ^{15}N in zooplankton in the epilimnion. (iii) Zooplankton utilization of deep anoxic layers during the day is common for *Chaoborus* larvae (LaRow, 1970), and some Daphnids can also use low oxygen layers as a refuge (Luecke and Teuscher, 1994; Larsson and Lampert, 2011). However, in Laguna El Tejo, *D. brachyurum* was the zooplankter showing strongest migration, and this species is a warm stenothermic filter feeder (Alonso, 1996). Their distribution is generally epilimnetic but they can also carry out vertical migrations (Worthington, 1931; Miracle, 1976; Matsumura-Tundisi et al., 1984; Thys and Hoffmann, 2005) although is often only between the epilimnion and metalimnion (Nauwerck, 1993). In our analysis their daytime densities were maximal at 11 m and

declined markedly in deeper strata, suggesting they were not approaching or entering the anoxic hypolimnion where we did not sample. (iv) Diel horizontal migrations (DHM) of zooplankton can increase the night-time abundances of some species in the pelagic zone and several studies in shallow lakes have demonstrated that these migrations are an important mechanism allowing zooplankton to avoid visual predation (e.g. Burks et al., 2002). In a shallow lake in Denmark *D. brachyurum* migrated horizontally and occupied open waters at night (Lauridsen et al., 1996). Consequently, DHM of *D. brachyurum* in Laguna El Tejo is a likely possibility for explaining the large increase in this species in the pelagic zone during the night. El Tejo is a small lake (the distance of sampling stations to the shore was ca. 40 m), and movements from or to the shore could be possible fast-swimming cladocera such as *Diaphanosoma*. Vertical movements covering these distances have been described for cladocerans (Worthington, 1931). However, DHM are most probably favoured by water movements, thus these "migrations" may have been aided by currents promoted by the wind or by the differential cooling of the shores during the night. At that time of the year when the lake was sharply stratified and day-night thermal fluctuations were large, convection currents likely occurred in the epilimnion-upper metalimnion and therefore may have helped redistribute the zooplankton (mainly *Diaphanosoma*) occurring in this stratum. The convection currents may have also transported zooplankton to the pycnocline or even into the hypolimnion since we also observed night time changes in the overall abundance and isotopic composition in those layers. Nevertheless in all cases, active movement may also have been involved in these migrations.

Carrillo et al. (Carrillo et al., 1989) found that a combination of DVM and DHM explained the complex diurnal movements of a rotifer species in a shallow mountain lake. It is likely that some vertical migration occurs in Laguna El Tejo. Armengol and Miracle (Armengol and Miracle, 2000), studying an adjoining karst lake, found vertical movements into upward- and downward-facing funnel migration traps that were consistent with a DVM pattern. Nevertheless, they found discrepancies between upwards and downwards catches in the migration traps, which could also be explained by a combination of vertical and horizontal movements. Indeed, several studies also suggest that vertical and horizontal movements should be studied together (e. g. De Stasio, 1993; Masson et al., 2001; Pinel-Alloul et al., 2004).

Zooplankton species from different sizes and taxonomic groups can have different migratory behaviour. In our samples *D. brachyurum* was by far the most

abundant cladoceran species, representing 43% of the total zooplankton biomass measured during day and 60% during night, thus accounting for most of the day/ night differences observed. *Diaphanosoma brachyurum* is a fast-swimming cladoceran that is mostly epilimnetic (Gliwicz and Pijanowska, 1989; Nauwerck, 1993; Alonso, 1996), and consequently our results may have been influenced by its dominance in the community. Movements of rotifers and copepods were much less important for two reasons: they did not show marked day/night differences and they represented a lower biomass; around $21-27\%$ for copepods and $19-30\%$ for rotifers. For rotifers, the most frequent species were *A. girodi*, *H. mira*, *T. similis*, *P. dolichoptera* and *A. fissa*, and although these species do migrate vertically, their migrations are relatively short (Armengol and Miracle, 2000), similar to what we observed in our study. Only two cyclopoid copepod species were abundant during our study, *T. prasinus* and *C. abyssorum*. The first is a small copepod, widely distributed and omnivorous, while the second one is larger and characteristic of the hypolimnion (Armengol and Miracle, 1999, 2000). Nauwerck (Nauwerck, 1993) suggested that *C. abyssorum* has remarkable vertical migrations in Lake Mondsee, but in our study *C. abyssorum* showed minimal vertical migration.

Our initial interpretation of the data collected in the pelagic zone was that most of the *Diaphanosoma* population resided during the day time between 11 and 15 m, where the $\delta^{15}N$ of seston and zooplankton $\delta^{15}N$ was high. If this population had significantly migrated upwards we should have seen an increase of $\delta^{15}N$ in the pooled zooplankton collected in the surface waters during the night. However, we did not find this expected increase in either the detailed day/night profile of $\delta^{15}N$ taken with Schindler-Patalas trap, nor with the more frequent analysis of zooplankton in the epilimnion captured with the zooplankton net. In contrast, there was clearly a night-time epilimnetic increase in zooplankton as shown in the species counts, and the increase in C and N mass. The increased night time zooplankton biomass in the hypolimnion was accompanied by an actual decrease in the $\delta^{15}N$, indicating that non-labelled individuals were migrating into this zone, perhaps carried there on cold density currents originating near the shore.

Pearre (Pearre, 2003) argued that complex factors related to food, satiation and hunger interact to influence DVM, which is the summation of individual behaviours (Pearre, 2003; Ringelberg, 2010). Some studies have shown than when phytoplankton is abundant in deep waters it can be used by cladocerans as a food source (Williamson *et al.*, 1996; Lampert and Grey,

2003), and that the normal DVM pattern can be moderated (Gliwicz and Pijanowska, 1988; Ringelberg, 2010). Our results suggest that the observed decrease in abundance in the upper metalimnion at night may indicate some weak vertical migration of these *D. brachyurum*. Seston nitrogen content in the metalimnion where *D. brachyurum* was abundant $(12.5-14\,\text{m})$ was 70% higher than in the epilimnion. Consequently, the higher food levels in deeper waters may have weakened the diel migration. Nevertheless, the very large increase of cladocerans in the epilimnion at night suggests that this was primarily due to a combination of both DVM and DHM, and not only to DVM.

From a bioenergetic perspective horizontal migration might be a much better strategy for *D. brachyurum*. Dawidowicz and Loose (Dawidowicz and Loose, 1992), Cole *et al.* (Cole *et al.*, 2002) and Pangle and Peacor (Pangle and Peacor, 2010) have indicated that higher metabolic rates and filtering rates in the epilimnion allow faster growth rates of cladocerans, even if food levels are higher in the metalimnion. Although we did not sample in the littoral zone at that time, we know from shore sampling on other occasions and from similar lakes (Boronat, 2003) that *D. brachyurum* is also abundant there. In this zone warm daytime temperatures allow for high grazing and digestion rates and there is decreased predation risk in the complex cover afforded by the shoreline and macrophytes.

An original objective of our research was to use ^{15}N labelling to determine if zooplankton transported nitrogen from the deep strata to the surface by feeding in the metalimnion and excreting nutrients in the surface after DVM. The lack of significant vertical migration precluded a vigorous test of this hypothesis. However, after the initial tracer addition we did not see any increase in ^{15}N labelling in the epilimnetic seston until lake turnover in November distributed the nitrogen tracer upwards suggesting that few nutrients may be transported upward by zooplankton. Subsequent work has shown that even if there is strong DVM of cladocerans, the net effect of this is to transport nutrients downward via increased sedimentation rates induced by zooplankton grazing in the epilimnion and faecal matter production (Pilati and Wurtsbaugh, 2003).

In conclusion, the increased densities of zooplankton in the epilimnion during the night in deep lakes are usually explained as a consequence of DVM. Although our data showed such an increase for the cladoceran *D. brachyurum* in Laguna El Tejo two lines of evidence suggest that the increase was due primarily to other mechanisms such as horizontal migration rather than only to DVM. The strongest evidence was from the observation that the large night-time increase in densities

and in zooplankton C and N mass in the epilimnion was not matched by a similar decrease from deeper strata. Secondly, zooplankton labelled with ^{15}N in the metalimnion did not appear in the epilimnion at night. Our work and other studies indicate that diel migrations are clearly a complex phenomenon affected by many factors (e.g. fish, macrophytes, trophic degree, depth, water currents, etc.). The migration pattern can be different for different species and for the particular conditions in a lake. Our work shows that detailed estimation of diel changes in distribution should be done by using methods other than mere comparisons of day and night vertical profiles.

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