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ORIGINAL ARTICLE

Spatial and seasonal variability of the carbon isotopic signature of *Daphnia* and their ephippia in four French lakes: Implications for the study of carbon transfers in lake food webs

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Abstract

1. Carbon isotope analysis ($\delta^{13}C$) of *Daphnia* resting-eggs (ephippia) in sedimentary records can be used to reconstruct past carbon transfers in pelagic food webs in lakes. However, there may be seasonal variability of cladoceran $\delta^{13}C$ and ephippia production that could affect their use as palaeoecological indicators of pelagic carbon transfers. This is particularly likely in stratified lakes where availability of different pelagic carbon sources is seasonal. In addition, there are currently no studies on spatial variability of ephippia $\delta^{13}C$ in deep areas of lakes and its implications for sampling strategies in paleolimnological studies.
2. Four French lakes were sampled for over a year to evaluate seasonal variation of the carbon sources consumed by *Daphnia* using analyses of the *Daphnia* carbon isotope signature ($\delta^{13}C_{Daphnia}$) and suspended organic matter signature ($\delta^{13}C_{seston}$). *Daphnia* ephippia were also collected from surface sediments at maximum depth in the four lakes. Ephippia signatures ($\delta^{13}C_{ephippia}$) were compared to the $\delta^{13}C_{Daphnia}$ to evaluate ephippia production periods and the implications for the use of $\delta^{13}C_{ephippia}$ in trophic functioning studies. In addition, spatial variability of $\delta^{13}C_{Daphnia}$ among the four lakes and the variability of $\delta^{13}C_{ephippia}$ in the deep area of one lake were assessed to determine the relevance of a single sampling point in the context of paleolimnological studies.
3. A similar pattern was observed in all four lakes: $\delta^{13}C_{Daphnia}$ was close to $\delta^{13}C_{seston}$ during the summer stratification period, but $\delta^{13}C_{Daphnia}$ became much lower than $\delta^{13}C_{seston}$ after the autumnal turnover. Ephippia seem to be produced either during the summer stratification period or after autumnal turnover depending on the lake. No spatial variability was observed among the sampling points in $\delta^{13}C_{Daphnia}$ in the four lakes, and $\delta^{13}C_{ephippia}$ seems to be spatially randomly distributed in the deep zone of the studied lake.
4. In the four lakes, summer $\delta^{13}C_{Daphnia}$ values were never below −40‰, reflecting mainly the consumption of phytoplankton, which in turn uses varying amounts of CO_2 from respiration (depending on the lake). After autumnal turnover, the $\delta^{13}C_{Daphnia}$ values reached values far below −40‰, reflecting the transfer of a non-negligible part of C-CH_4 for three of the lakes. Seasonal stratification and

turnover mechanisms seem to influence the availability of carbon sources in the pelagic compartment of the four lakes.

5. This study shows that the timing of ephippia production affects the information provided by $\delta^{13}C_{ephippia}$ due to seasonal differences in the mechanisms (stratification, autumnal turnover) that determine which carbon sources are available in the pelagic compartment. Therefore, using $\delta^{13}C_{ephippia}$ to study past pelagic transfers of carbon in stratified lakes may entail uncertainty if used alone and requires multi-proxy studies. Finally, $\delta^{13}C_{ephippia}$ appear to be homogeneously distributed in the deepest area of a medium-sized, single basin. A single core retrieved from the deepest part of the lake should therefore provide a representative sample of the ephippia produced in a lake.

KEYWORDS
carbon cycle, Cladocera, methane, pelagic food web, stable carbon isotope

1 | INTRODUCTION

Stable isotope analysis of carbon is a method that has been widely used in recent decades for studying food webs and carbon pathways in aquatic ecosystems, especially in lakes (Frossard et al., 2014; Fry, 2006; Grey et al., 2001; Taipale et al., 2007). This approach is built on the principle that the isotopic carbon signature of an organism ($\delta^{13}C$) closely reflects the isotopic signature of its diet (DeNiro & Epstein, 1978). Delta ^{13}C analysis of different trophic components of lake food webs thus allows the significance of the different carbon pathways to be assessed. Two carbon pathways that commonly occur in lakes are the fixation of CO_2 by autochthonous and allochthonous photosynthetic organisms and oxidation of biogenic CH_4 by methane-oxidising bacteria (MOB). Numerous studies have reported the $\delta^{13}C$ value of phytoplankton in lakes to range from −25‰ to −40‰ (France, 1995; Masclaux et al., 2013; Peterson & Fry, 1987; Vuorio et al., 2006; Wang et al., 2013) depending on CO_2 origin and fractionation mechanisms (Bade et al., 2004, 2006; Gu et al., 2006). Other studies have reported that the $\delta^{13}C$ of terrestrial derived materials from catchment is similar to the $\delta^{13}C$ value of C3 plants, ranging from −25‰ to −29‰ (France, 1995; Grey et al., 2001; O'Leary, 1988; Peterson & Fry, 1987). In contrast, biogenic methane is highly depleted in ^{13}C, resulting in $\delta^{13}C$ values ranging from −80‰ to −50‰ (Jedrysek, 2005). Fractionation by MOB further reduces the signature (Templeton et al., 2006), making the biogenic CH_4 signature highly characteristic and distinct from the signature resulting from CO_2 fixation by phytoplankton or catchment vegetation.

Stable isotope analysis is therefore a widely used tool in paleolimnological studies to investigate past changes in the trophic functioning of lakes. Delta ^{13}C signatures of chitinous invertebrate remains retrieved from lake sediments are increasingly used to reconstruct past changes in carbon sources and pathways in food webs. Some studies have focused on benthic invertebrate remains, particularly chironomid larvae remains, to reconstruct these past changes in carbon pathways (Belle et al., 2014, 2017; Belle, Millet, et al., 2016; Frossard et al., 2014; van Hardenbroek et al., 2010). Other recent studies have suggested that additional information can be obtained from analyses of the isotopic signatures of zooplankton remains (Frossard et al., 2014; Schilder, Bastviken, et al., 2015; Schilder, Tellenbach, et al., 2015; van Hardenbroek et al., 2013, 2014; Wooller et al., 2012). One of the most commonly used types of zooplankton remains are ephippia, the resting-eggs produced by *Daphnia* and other cladocerans. *Daphnia* often dominate zooplankton communities in lakes (Lampert & Kinne, 2011), so their chitinous ephippia are therefore found abundantly in most sedimentary records and they remain well preserved with time (Szeroczyńska & Sarmaja-Korjonen, 2007). Moreover, the carbon isotopic signature of *Daphnia* resting eggs reflects the signature of *Daphnia* at the time of egg production (Perga, 2011; Schilder, Bastviken, et al., 2015a; Schilder, Tellenbach, et al., 2015). As a result, $\delta^{13}C$ analysis of *Daphnia* ephippia is a valuable tool for reconstructing past carbon pathways in pelagic food webs in lakes.

Daphnia is a filter-feeding cladoceran whose diet is mainly based on phytoplankton and detritus (Lampert & Kinne, 2011; Perga et al., 2008). In some lakes, a significant part of the carbon consumed by *Daphnia* (and zooplankton in general) may come from methane (CH_4) via the consumption of MOB (Bastviken et al., 2003; Kankaala, Taipale, et al., 2006; Taipale et al., 2007, 2008, 2009) and can be transferred to ephippia (Schilder, Bastviken, et al., 2015; van Hardenbroek et al., 2013). Trends of ephippia $\delta^{13}C$ in sedimentary records can then be interpreted as the result of changes in the contribution of different carbon sources in the *Daphnia* diet or changes of phytoplankton $\delta^{13}C$ (van Hardenbroek et al., 2014), and to some extent interpreted as a proxy for methane transfers in pelagic food webs (Morlock et al., 2017; Rinta et al., 2016; van Hardenbroek et al., 2013; Wooller et al., 2012). However, few studies have questioned the implications of seasonal variability of cladoceran $\delta^{13}C$ and the implications of the timing of the period when ephippia are produced for their use as paleoecological indicators of pelagic carbon transfers (Morlock et al., 2017; Schilder et al., 2017; van Hardenbroek et al., 2018). These questions seem crucial to consider, particularly for stratified lakes where methane availability is seasonal (Taipale et al., 2008, 2009) and fluctuations of *Daphnia* δ^{13}C may be substantial (Morlock et al., 2017; Perga & Gerdeaux, 2006; Smyntek et al., 2012) as a result of variation in both phytoplankton δ^{13}C and the availability of different carbon sources. In addition, there are currently no studies of the spatial variability of ephippia δ^{13}C in deep areas of lakes or on the implications of this variability for sampling strategies in paleolimnological studies.

This study aims to assess the seasonal and spatial variability of the carbon sources consumed by *Daphnia* to improve our understanding of the information provided by the δ^{13}C of *Daphnia* and their ephippia in neo- and paleolimnological approaches. For this purpose, we first sampled four French lakes for more than a year to study the seasonal variability of *Daphnia* signatures ($\delta^{13}C_{Daphnia}$) and signatures of suspended organic matter ($\delta^{13}C_{seston}$) to evaluate the carbon sources consumed by *Daphnia*. Sediment was also sampled at the deepest point of each lake to collect ephippia and measure their δ^{13}C signatures, allowing us to evaluate their period of maximal production during the year. Seasonal variations in the carbon isotopic signature of *Daphnia* are expected due to the seasonal availability of the different carbon sources for pelagic consumers in stratified lakes (Morlock et al., 2017; Taipale et al., 2008, 2009). As a result of the point-in-time nature of ephippia production by *Daphnia* (Conde-Porcuna et al., 2014), the ephippia δ^{13}C may then potentially reflect the δ^{13}C values of the carbon sources consumed by the *Daphnia* source population over a limited time period. The implication of the timing of ephippia production by *Daphnia* during the year on the information provided by their δ^{13}C was then assessed. In a second step, we studied the spatial variability of $\delta^{13}C_{Daphnia}$ in the four lakes as well as the variability of $\delta^{13}C_{ephippia}$ in the deep zone of one of the four lakes to determine the relevance of a single sampling point in the context of paleolimnological studies.

2 | MATERIAL AND METHODS

2.1 | Study sites

Four lakes (Figure 1) that covered different morphological properties (water depth and area), elevations (Table 1), and catchment area characteristics (land cover and geological substratum) were chosen for this study. Lakes Remoray and Bonlieu are in the Jura Mountains in eastern France (Figure 1). The bedrock in this region is composed of carbonate. Lake Remoray is a medium-sized dimictic lake with an area of 95 ha, and the water depth reaches 28 m. Forests (46.7%) and agricultural areas (42.5%) dominate the catchment basin of this lake. The rest of the catchment is divided into 5.5‰ peat bogs, 2.6% wetlands, and 2.7% urban areas (Corine Land Cover, 2006). Lake Bonlieu is a smaller lake (22 ha) with a maximum depth of 10 m and a catchment area covered by forests (96.9%) and wetlands (3.1%; CLC, 2006).

Lakes Longemer and Retournemer are located in the Vosges Mountains in north-eastern France (Figure 1). The bedrock in this region is mostly sandstone and granite. Lake Longemer is a medium-sized lake with an area of 76 ha, and the water depth reaches 30 m. The catchment area of the lake is mainly covered by forests (91.8%). A total of 6.3% of the catchment is covered by urban areas and 1.8% is covered by agricultural land (CLC, 2006). Lake Retournemer is a small lake of 5.25 ha with a maximum depth of 11.5 m. The catchment area of this lake is almost exclusively forested (96%; CLC, 2006).

2.2 | Sample collection

Eight to 12 field campaigns were carried out from March 2018 to August 2019. For each field campaign, vertical profiles of temperature, and dissolved oxygen ($[O_2]$) and chlorophyll *a* concentrations ([Chl*a*]) were performed along the whole water column using a multi-sensor probe (EXO1 probe, YSI, Yellow Springs USA) at the point of maximum depth (Z_{max}; Figure 1). Lake transparency was measured using a Secchi disk at Z_{max} and the depth of the euphotic zone was determined as 2.5 × the Secchi transparency.

For sampling of seston and *Daphnia*, multiple sampling points were made for each lake. Three sampling points were defined for lakes Remoray and Longemer (Figure 1), all located in the profundal zone; one point was located at Zmax of each lake. For lakes Bonlieu and Retournemer, which are smaller, two sampling points were defined (Figure 1), also located in the deep areas of the lakes, one of which was located at Zmax. A vertically integrated sample of zooplankton was taken across the euphotic zone at each sampling point with a 200-µm mesh plankton net. The samples were frozen after each field campaign and stored at −20°C. Seston were also collected across the euphotic zone at each sampling point with a 5-L integrating water sampler (IWS, Hydro-bios Apparatebau).

In lakes Bonlieu, Retournemer, and Longemer, a surface sediment sample was collected at the deepest point using a 90-mm-diameter gravity corer (UWITECH, Mondsee Austria) to collect *Daphnia* ephippia. Only the first 2 cm were sampled to collect only recent ephippia, and two cores were performed to obtain enough ephippia for isotope analysis. In Lake Remoray, 30 sediment samples were collected in the deep area following the same method to study the spatial variability of $\delta^{13}C_{ephippia}$. A random strategy was used to generate the coordinates of the 30 sampling points.

2.3 | Sample preparation and δ^{13}C analyses

In the laboratory, the zooplankton samples were thawed, and the *Daphnia* spp. were manually sorted under a binocular microscope without species differentiation. For each sample, a minimum of 20 *Daphnia* were picked up to obtain a minimum dry weight of 0.07 mg required for isotope analysis.

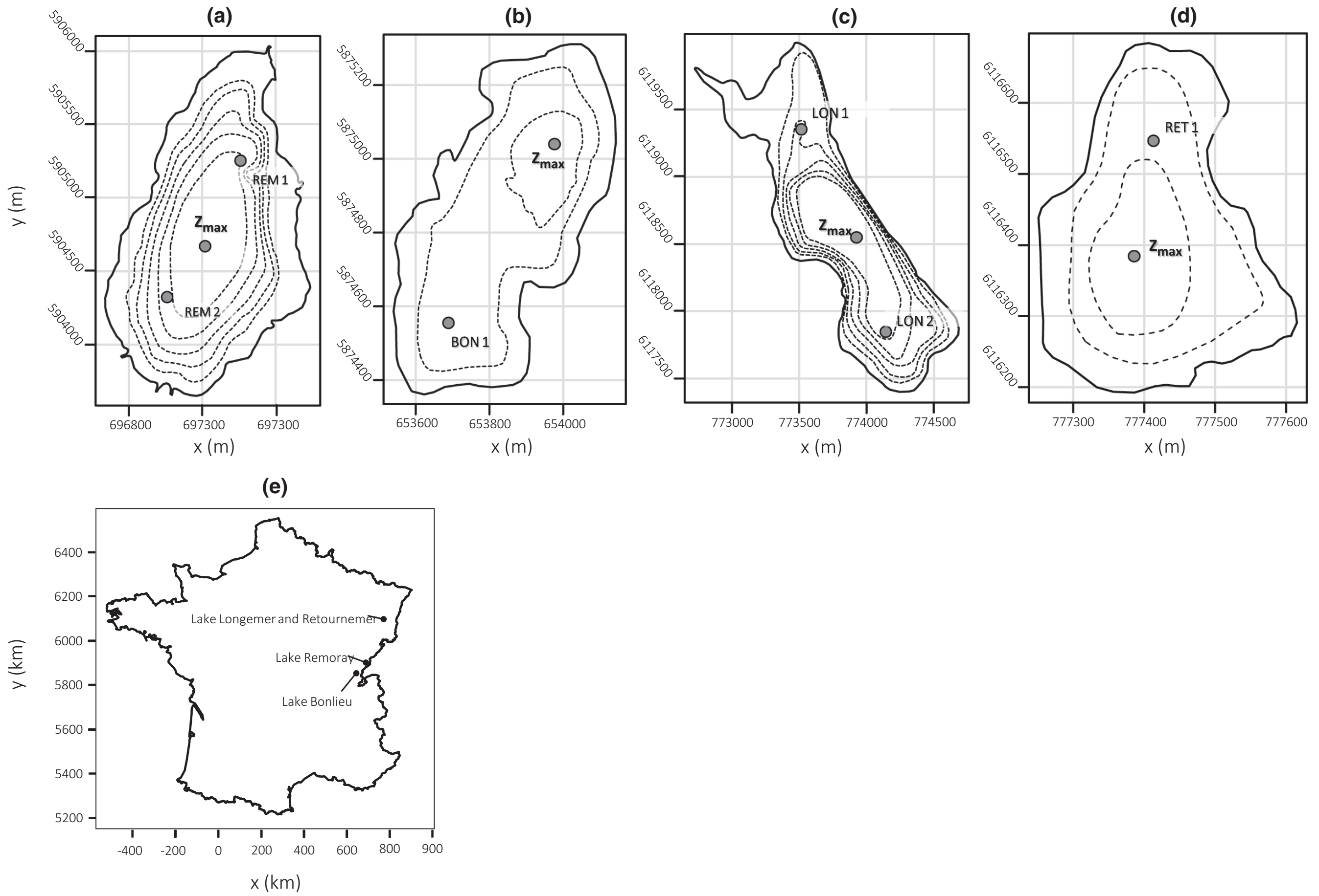


FIGURE 1 Location, bathymetry and sampling points of Lake Remoray (a), Lake Bonlieu (b), Lake Longemer (c), and Lake Retournemer (d), and location of the lakes in France (e). The isobaths of bathymetry are plotted on a 5-m pitch. Sampling points are represented by grey dots. Maps are projected according to WGS84/pseudo-Mercator system

TABLE 1 Characteristics of the studied lakes

Lake	*x* (m)	*y* (m)	Depth (m)	Lake area (ha)	Altitude (m)	[Chl*a*]mean	[Chl*a*]max
Remoray	697,335	5,904,779	28	85	850	2.5	4
Bonlieu	653,804	5,874,864	10	17	790	4.8	25
Longemer	773,800	6,118,688	30	5	735	4.4	15.6
Retournemer	777,412	6,116,424	11	76	780	8.2	58.2

Note: x and *y* are based on the WGS84 coordinate system. Chlorophyll *a* concentrations ([Chl*a*]mean and [Chl*a*]max) are expressed in μg/L and are calculated based on summer stratification data of [Chl*a*].

The integrated water samples were prefiltered with a 200-μm mesh filter to remove large particles and most of the zooplankton. The seston samples were filtered onto pre-combusted GF/F filters (Whatman™, 0.7-μm mesh) immediately after sampling.

The sediments were passed through a 120-μm mesh sieve, and ephippia were thereafter carefully handpicked under a stereomicroscope. The ephippia of *Daphnia* were selected according to the description provided by Vandekerkhove et al. (2004). The number of ephippia analysed per sample varied between four and 33 depending on their abundance in the sediment of each lake.

All the samples were exposed to 10% HCl solution for carbonate removal, rinsed three times with demineralised water, and put into ultra-clean tin capsules. The samples were then dried at 60°C in an oven for 2 days.

The stable isotope analyses of carbon were performed using an isotope ratio mass spectrometer interfaced with an elemental analyser (EA-IRMS) at INRA Nancy (Champenoux, France). The isotope ratios were expressed in the delta notation with Vienna Pee Dee Belemnite as the standard: $\delta^{13}C$ (‰) = (Rsample/Rstandard − 1) × 1,000, where R = $^{13}C/^{12}C$. The replication of samples measured on an internal standard gave analytical errors of ±0.13‰ (n = 11) for the *Daphnia* samples, ±0.06‰ (n = 11) for the seston samples, and ±0.10‰ (n = 16) for the ephippia of lakes Bonlieu, Retournemer, and Longemer. The analytical error for the study of the spatial variability in $\delta^{13}C_{ephippia}$ in Lake Remoray was ±0.06‰ (n = 7).

2.4 | Data analyses

Spearman's correlation method was used for each lake to assess the relationship between $\delta^{13}C_{seston}$ and $\delta^{13}C_{Daphnia}$ without differentiating among the different sampling points. The normality of the variables was tested prior to the analysis using the Shapiro–Wilk test. As the data did not fit a normal distribution, Spearman's non-parametric rank correlation test was used, and Spearman's correlation coefficient (ρ) and the p-value were calculated.

The $\delta^{13}C$ data were then separated into two distinct groups for each of the four lakes: data obtained during the summer stratification period and data obtained during the winter period. The summer stratification period is characterised by total or partial stratification of the water column, while the winter period is defined as the period when the water column is not stratified, that is the period lasting from the complete mixing of the water column in late autumn/early winter to the stratification of the water column in spring. These two periods were determined based on the temperatures and oxygen saturation profiles measured during the monitoring of the four lakes. Non-parametric tests were then used to assess the correlations and differences between $\delta^{13}C_{Daphnia}$ and $\delta^{13}C_{seston}$ according to the sampling seasons.

Non-parametric tests for paired samples were performed to compare the $\delta^{13}C$ values obtained at the different sampling points on each lake. Normality tests of residuals and homoscedasticity tests of the variances were previously performed with the Shapiro–Wilk test and Bartlett's test, respectively. As the variables did not satisfy the conditions, non-parametric tests were used. Friedman variance analysis followed by the associated multiple comparison test was used for lakes Remoray and Longemer, which each had three sampling points. For lakes Bonlieu and Retournemer, which had only two sampling points each, the Mann–Whitney–Wilcoxon non-parametric rank correlation test for paired samples was used.

The spatial distribution of $\delta^{13}C_{ephippia}$ was analysed with spatial correlograms to detect spatial structures. A spatial correlogram tests the null hypothesis of spatial randomness, meaning that the values observed at one location do not depend on the values observed at different distance classes. Spatial correlograms are plots showing spatial autocorrelation coefficients against distance classes. This type of plot allows the examination of the shape of the spatial structure of data. The simulated spatial patterns produced by Kraan et al. (2009) were used to interpret the spatial correlogram obtained in this study. Moran's index (I; Moran, 1950) was used to quantify spatial autocorrelation. Moran's I was computed for all samples for each class interval. The optimal number of class intervals was determined with the Sturges method (Sturges, 1926; class interval = $R/(1 + 3.322 \times \log[n])$, where R is the range of Euclidean geographic distances between samples and n is the number of samples). Moran's I varies from −1 to 1, with a value of 0 indicating the absence of spatial autocorrelation, a negative value indicating a negative autocorrelation and a positive value indicating a positive spatial autocorrelation. The statistical significance of Moran's I was tested to highlight the distance classes that showed significant differences against the randomisation assumption.

The Automap package (Hiemstra et al., 2009) was used to perform automatic ordinary kriging of the spatial variability of $\delta^{13}C_{ephippia}$ in the deep area of Lake Remoray. Different models were tested during automatic variogram fitting, and the best model was automatically used for kriging. In this case, the selected model was *Matern, M. Stein's parameterisation*. This variogram model was used to make predictions at the different locations at which ordinary kriging was performed. The results of this spatial interpolation are data of predicted values, variances and standard deviations at different locations in the deep area of the lake. Kriging maps showing the predictions and standard deviations were edited with these data.

All statistical analyses and figure creations were performed using R 3.5.1 statistical software (R Core Team, 2019).

3 | RESULTS

3.1 | Physico-chemical monitoring

3.1.1 | Thermal regimes

Clear summer stratification was observed in the four lakes. Even though reverse winter stratification is not apparent in Figure 2 due to the lack of monitoring data, all four lakes were frozen in winter. Based on the temperature and oxygen concentration profiles, the waters of lakes Bonlieu and Retournemer mixed at the end of autumn in November, whereas the water columns of lakes Remoray and Longemer started to mix in early December. The water columns of the four lakes mixed again at the beginning of spring when the surface waters warmed up in April. This mixing pattern is typical of dimictic lakes. The maximum surface water temperatures measured were 23.3°C for Lake Remoray in July 2018, 20°C in June 2018 for Lake Bonlieu, 21.4°C in August 2018 for Lake Longemer, and 19.2°C in June 2018 for Lake Retournemer. The bottom temperatures varied from 4 to 5.6°C in Lake Remoray, 4.8 to 5.9°C in Lake Bonlieu, 4.2 to 4.8°C in Lake Longemer, and 4.3 to 6.4°C in Lake Retournemer.

3.1.2 | Seasonal variations of the dissolved oxygen concentration

Strong seasonal variations in the O_2 saturation of the water column were observed (Figure 3). In the four lakes, the deep waters were O_2-depleted during the summer stratification period, with varying thicknesses of the anoxic layers (Figure 3). The hypolimnetic

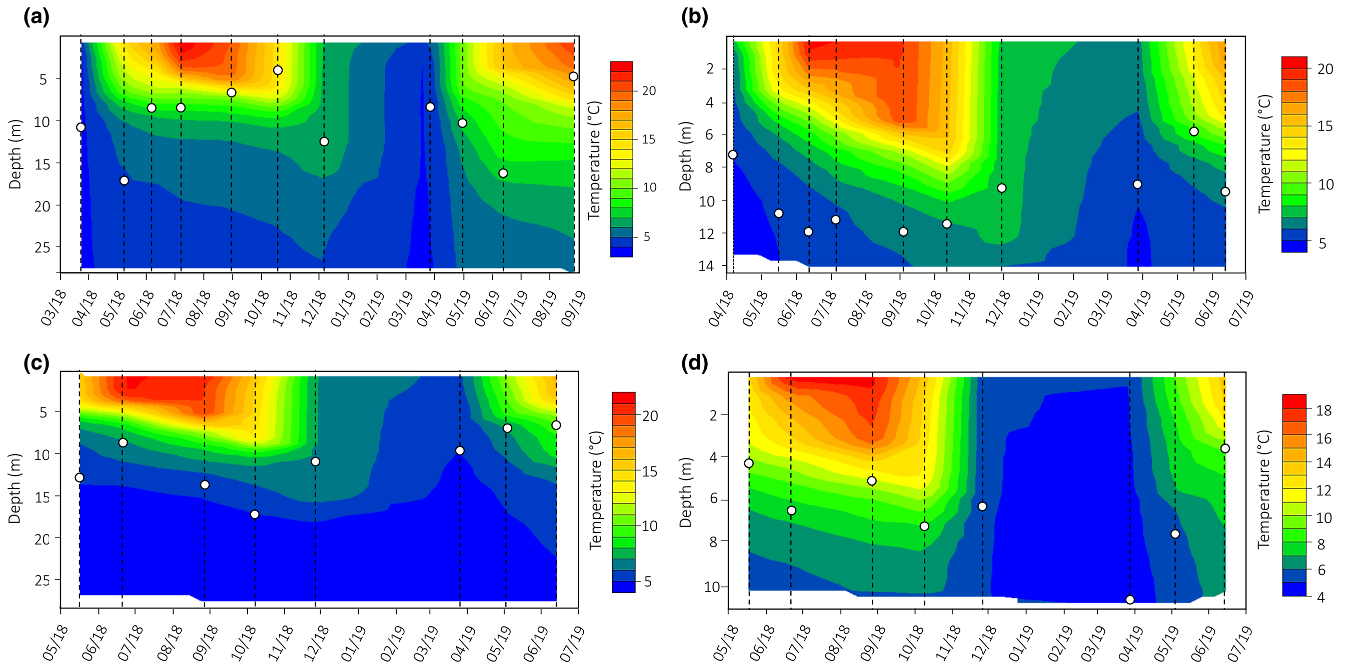


FIGURE 2 Heatmap of water temperature measured on Zmax location in water column of (a) Lake Remoray, (b) Lake Bonlieu, (c) Lake Longemer, and (d) Lake Retournemer over the monitoring period in 2018–2019. The dotted lines indicate the monitoring dates. White dots refer to the depth of the euphotic zone (Secchi depth ×2.5)

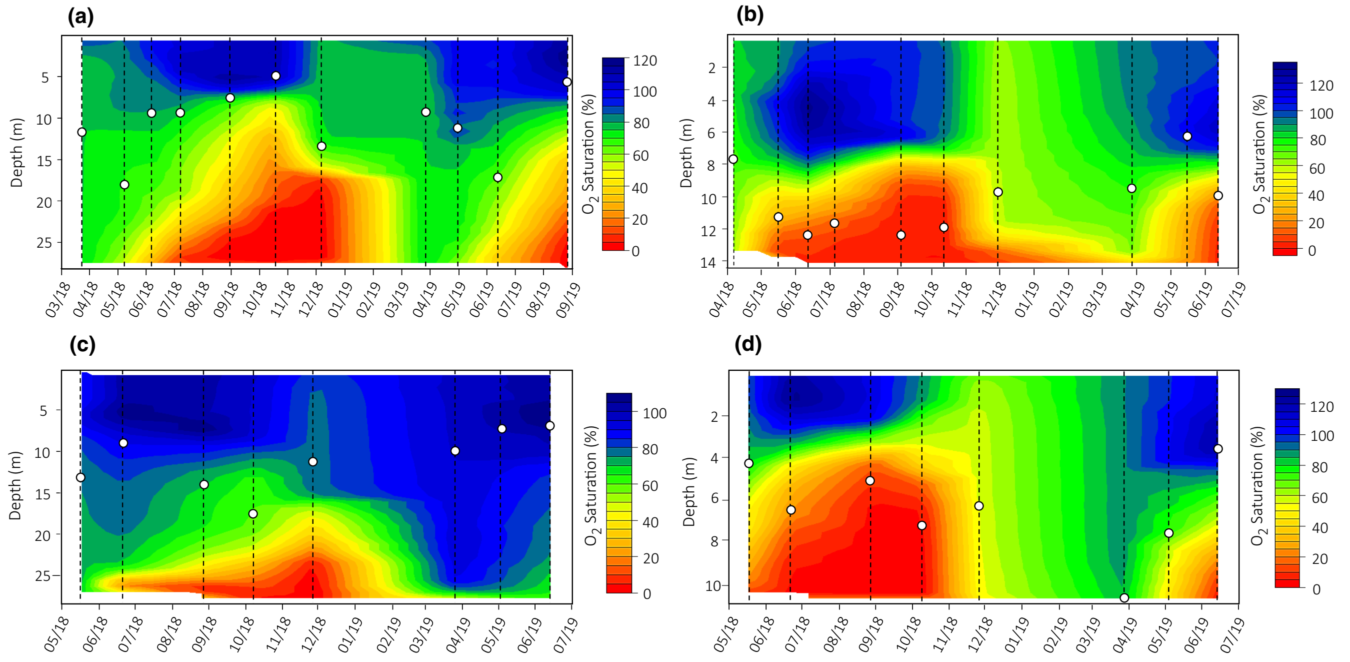


FIGURE 3 Heatmap of O_2 saturation (%) in water column measured on Z_{max} of (a) Lake Remoray, (b) Lake Bonlieu, (c) Lake Longemer, and (d) Lake Retournemer over the monitoring period in 2018–2019. The dotted lines indicate the monitoring dates. White dots refer to the depth of the euphotic zone (Secchi depth ×2.5)

waters were then re-oxygenated owing to the autumnal turnover. For Lake Remoray, complete anoxia was observed in deep waters as early as July. This anoxic layer spread throughout the summer season. The oxygen saturation dropped under 10% below 16.5 m depth at the end of the summer stratification period. Constraining oxygen conditions were also observed at Lake Bonlieu, where the deepest layers became anoxic as early as May. In late summer, O_2 saturation in Lake Bonlieu had dropped under 10% below a depth of 9 m. Lake Longemer had the least developed anoxic layer among the four lakes. Oxygen saturation at the end of the stratification period was below 10% at depths below 23 m. Lake Retournemer was the lake with the most oxygen depleted conditions. Indeed, during the summer period, the hypoxic layer extended to more than half of the water column, with the oxygen saturation dropping below 10% under 4.5 m in August.

The water columns of lakes Remoray and Longemer appeared to be mixed very late in autumn (Figure 3a,c). Indeed, during the last 2018 campaigns for these two lakes, conducted in early December and late November, the water columns were barely beginning to mix. Mixing was therefore achieved for these two lakes in December. The absence of other surveys during the 2018–2019 winter season makes the interpolation plot imprecise during this period, and the stretching of the stratification period to January in these two lakes as shown in Figure 2 is an artefact of the plotting method.

3.1.3 | Seasonality of chlorophyll *a* concentrations and trophic status

The chlorophyll *a* concentrations ([Chl*a*]) measured with the multi-sensor probe appeared to be heterogeneous among the lakes (Figure 4). In all four lakes, phytoplankton blooms were observed in spring, and in lakes Remoray and Bonlieu a second phytoplankton bloom occurring during autumn. However, the autumnal bloom at Lake Remoray came later (December 2019) than that of Lake Bonlieu (October 2019) and was considerably smaller. Considering the results of $[\mathrm{Chl}a]_{\mathrm{mean}}$ and $[\mathrm{Chl}a]_{\mathrm{maximum}}$ (Table 1), Lakes Remoray, Bonlieu, and Longemer can be categorised as mesotrophic, while Lake Retournemer can be categorised as eutrophic according to the OECD lake trophic classification (1982).

3.2 | Spatial variability in carbon stable isotope signatures

3.2.1 | Spatial variability of $\delta^{13}C_{Daphnia}$ and $\delta^{13}C_{\mathrm{seston}}$

We did not observe any variability in $\delta^{13}C_{Daphnia}$ among the different sampling points in any of the four studied lakes (Friedman test: *p*-value = 0.529 and 0.311 for lakes Remoray and Longemer, respectively; Wilcoxon test: *p*-value = 0.625 and 0.218 for lakes Bonlieu and Retournemer, respectively). Very weak or no spatial variability in the measured $\delta^{13}C_{\mathrm{seston}}$ values was observed (depending on the lake). The Friedman test indicated significant differences in $\delta^{13}C_{\mathrm{seston}}$ among the three sampling points in Lake Remoray (*p*-value = 0.030). Nevertheless, when paired comparisons were performed using the *friedmanmc* multiple comparison test and the *p*-values were adjusted accordingly, there was no significant difference. The Friedman test performed for Lake Longemer indicated no significant differences in $\delta^{13}C_{\mathrm{seston}}$ among the three sampling points (*p*-value = 0.606). Wilcoxon's test for paired samples, when performed on the measured $\delta^{13}C_{\mathrm{seston}}$ in Lake Bonlieu, suggested that significant differences in $\delta^{13}C_{\mathrm{seston}}$ existed between the two sampling points (*p*-value = 0.013). However, the deviation was small, and the mean $\delta^{13}C_{\mathrm{seston}}$ was 0.73‰ lower at the Zmax point than at the other point. Finally, in Lake Retournemer, no significant differences in $\delta^{13}C_{\mathrm{seston}}$ were found between the two sampling points (*p*-value = 0.468) according to Wilcoxon's test.

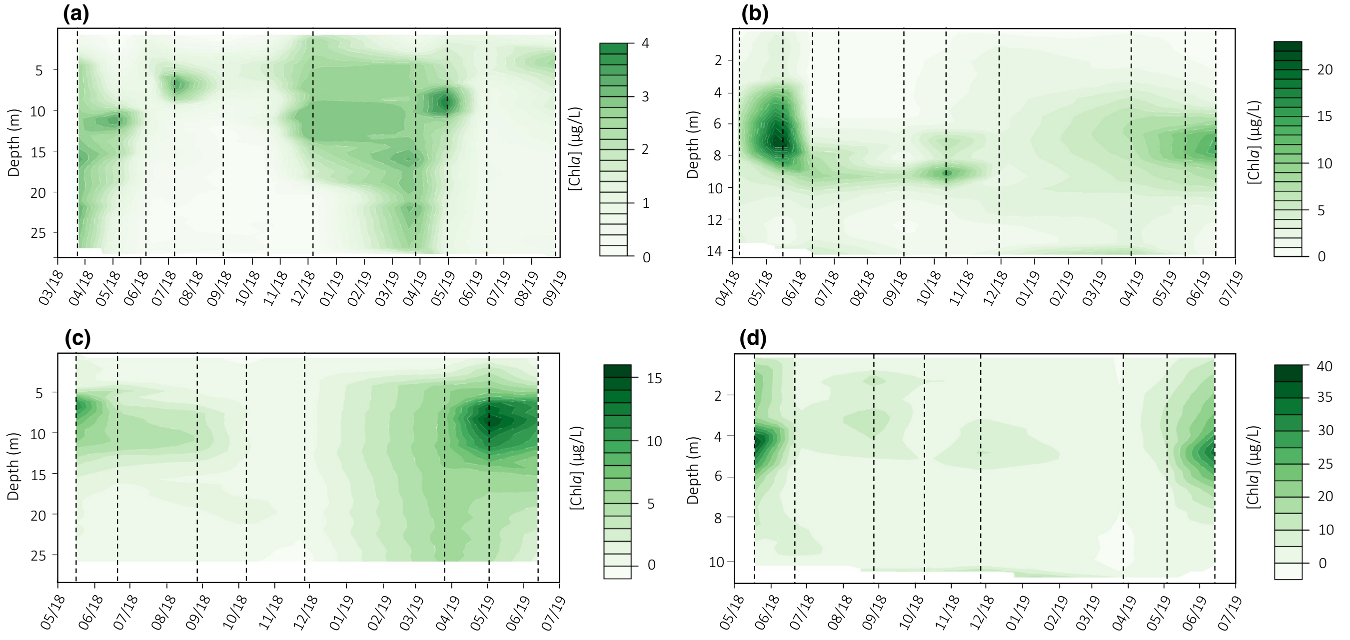


FIGURE 4 Heatmap of chlorophyll *a* concentrations ([Chla]; µg/L) measured on Z_{max} location in water column of (a) Lake Remoray, (b) Lake Bonlieu, (c) Lake Longemer, and (d) Lake Retournemer over the monitoring period in 2018–2019. The dotted lines indicate the monitoring dates

3.2.2 | Spatial variability of $\delta^{13}C_{ephippia}$

The $\delta^{13}C_{ephippia}$ in the 30 surface sediment samples of Lake Remoray ranged from −38.6‰ to −34.9‰ and averaged −37.1 ± 0.8‰. A very weak spatial gradient was observed on the prediction map of the kriging model (Figure 5). This small gradient must be placed in the context of the large standard error associated with the kriging model (0.9‰), reflecting a rather random distribution of $\delta^{13}C_{ephippia}$ values in space and low spatial autocorrelation. Moran's correlogram showed fluctuating Moran's I values for different distance classes, ranging from −0.44‰ to 0.16‰ (Figure 6), and weak or no spatial autocorrelation for the different distance classes. However, for points with distances greater than 500 m, the Moran's I values were more negative than those obtained for pairs of points with shorter distances. This observation confirmed the very low gradient observed on the kriging prediction map (Kraan et al., 2009). Nevertheless, the p-values associated with the indices did not reveal significant autocorrelation for each distance class (p-value > 0.05), indicating a random spatial distribution of the $\delta^{13}C_{ephippia}$ values observed in the deep area of Lake Remoray.

3.3 | Seasonal variability in carbon stable isotope signatures

3.3.1 | Seasonal variability of $\delta^{13}C_{seston}$ and $\delta^{13}C_{Daphnia}$

Daphnia and seston $\delta^{13}C$ showed strong seasonal variation in the four lakes. The $\delta^{13}C_{seston}$ may vary up to 10 ‰ during the seasonal cycle depending on the lakes (Figure 7) with values ranging from −30‰ to −41‰ for lakes Remoray, Bonlieu, and Retournemer (Figure 7a,b,d).

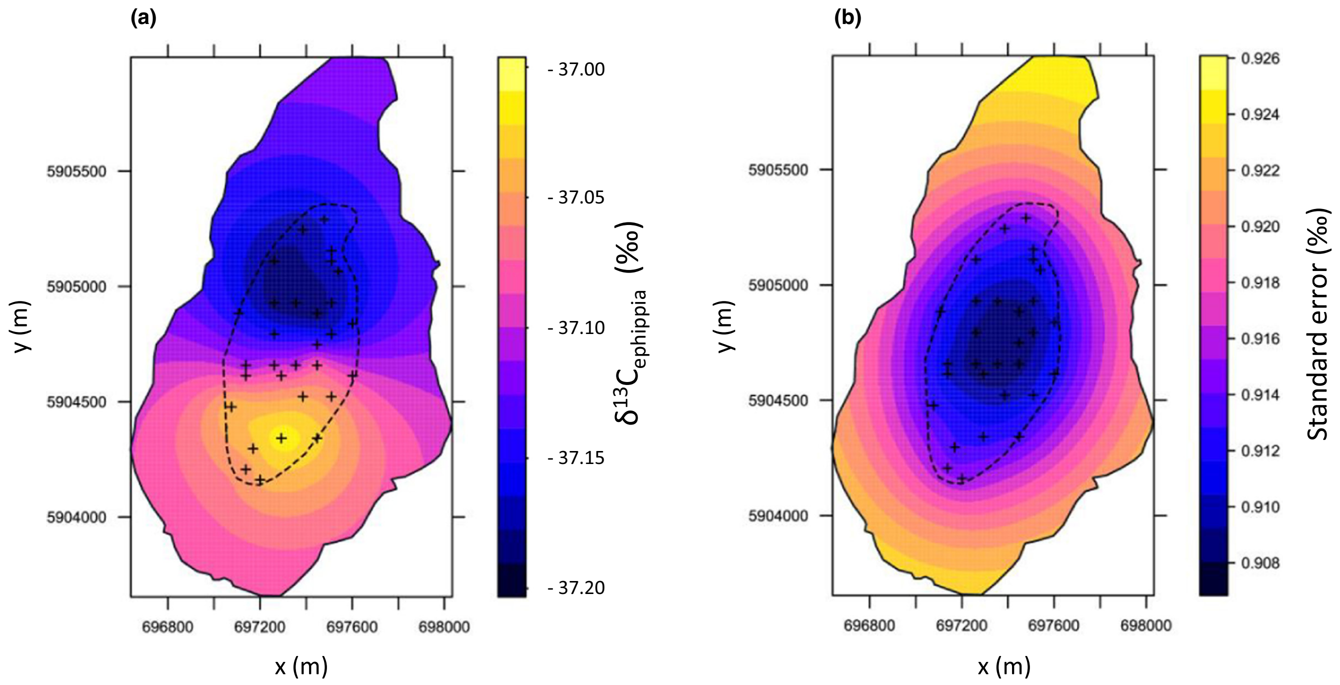


FIGURE 5 (a) Map of Lake Remoray showing $\delta^{13}C_{ephippia}$ spatial data predicted by the kriging model. (b) Map of the standard error of predicted values of $\delta^{13}C_{ephippia}$ calculated by the kriging model. For both maps, lake is represented by the solid outline, the deep zone of the lake is represented by the dashed line, and each sampling point is marked by a cross

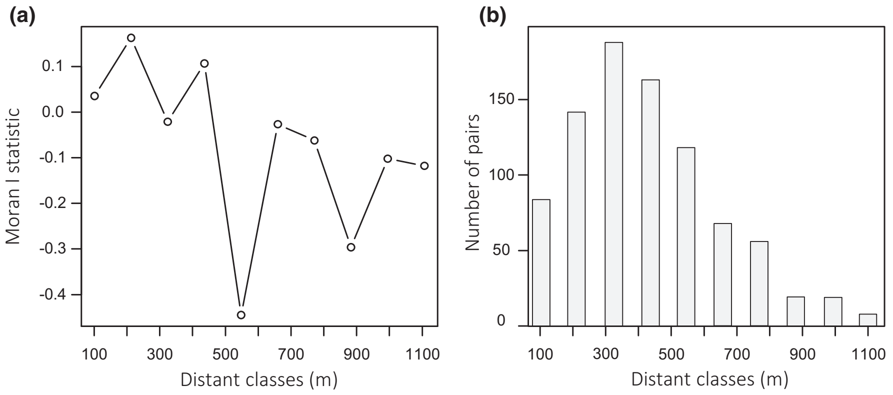


FIGURE 6 (a) Spatial correlogram based on Moran's I for $\delta^{13}C_{ephippia}$ of Lake Remoray at the different distance classes (in m). Open symbols indicate non-significant spatial autocorrelation ($p < 0.05$). (b) Histogram of number of pairs per distance classes (units expressed in m)

Lake Longemer presented the least ^{13}C-depleted $\delta^{13}C_{seston}$ values among the four lakes studied during the whole sampling period, with value ranging from −27.6‰ to −36.4‰. The $\delta^{13}C_{Daphnia}$ measured in the four lakes also varied considerably during the annual survey, with a marked decrease of up to more than 10‰ between summer stratification and winter periods (Figure 7). The *Daphnia* sampled in winter are particularly ^{13}C-depleted in lakes Remoray, Bonlieu, and Retournemer, with minimum $\delta^{13}C_{Daphnia}$ values lower than or equal to −42‰ (Figure 7a,b,d). Lake Longemer had the least ^{13}C-depleted *Daphnia* samples among the four lakes studied during the whole sampling period, especially in winter with minimal $\delta^{13}C_{Daphnia}$ value of only −39.1‰ (Figure 7c).

3.3.2 | Relationship between $\delta^{13}C_{seston}$ and $\delta^{13}C_{Daphnia}$

When considering the δ^{13}C data of all sampling points and dates, Spearman's correlation showed significant positive correlations between $\delta^{13}C_{seston}$ and $\delta^{13}C_{Daphnia}$ in Lake Remoray ($\rho = 0.37$, *p*-value = 0.039, $n = 33$), Lake Bonlieu ($\rho = 0.64$, *p*-value = 0.002, $n = 20$), Lake Longemer ($\rho = 0.53$, *p*-value = 0.011, $n = 22$) and Lake Retournemer ($\rho = 0.6$, *p*-value = 0.026, $n = 14$). Despite these strong correlations, the mean difference between $\delta^{13}C_{seston}$ and $\delta^{13}C_{Daphnia}$ ($\Delta^{13}C_{ses\text{-}daph} = \delta^{13}C_{seston} - \delta^{13}C_{Daphnia}$) strongly varied with the season. The $\delta^{13}C_{Daphnia}$ values were close to the $\delta^{13}C_{seston}$ values during the summer stratification period, with signatures enriched in ^{13}C, whereas after the autumnal turnover, the $\delta^{13}C_{Daphnia}$ values moved away from the $\delta^{13}C_{seston}$ values to reach much lower values. During the summer stratification period, the $\Delta^{13}C_{ses\text{-}daph}$ values were, on average, 1‰ for Lake Remoray, −0.4‰ for Lake Bonlieu, −0.2‰ for Lake Longemer, and 1.7‰ for Lake Retournemer, whereas in winter, these values reached 5.6‰ for Lake Remoray, 4.9‰ for Lake Bonlieu, 2.6‰ for Lake Longemer, and 11.3‰ for Lake Retournemer.

The limited availability of winter data prevented us from assessing the correlations between $\delta^{13}C_{daphnia}$ and $\delta^{13}C_{seston}$ according to the sampling season (i.e., the summer stratification period and winter period) for each lake individually. Consequently, these analyses were performed on the whole dataset without differentiation among the lakes (Figure 8). A positive correlation was observed between $\delta^{13}C_{daphnia}$ and $\delta^{13}C_{seston}$ during summer (Spearman's correlation test, $\rho = 0.78$, *p*-value <0.001, $n = 70$). In winter, we also found a significant positive correlation between $\delta^{13}C_{daphnia}$ and $\delta^{13}C_{seston}$ (Spearman, $\rho = 0.59$, *p*-value = 0.017, $n = 16$) but with a lower correlation coefficient than that found for the summer period. Furthermore, in summer, the pairs of values were distributed along a 1:1 line, in contrast to the winter values, in which the $\delta^{13}C_{Daphnia}$ values were clearly lower than the $\delta^{13}C_{seston}$ values (Figure 8). Indeed, according to the Wilcoxon test for paired samples, the summer signatures of *Daphnia* were not significantly different from the signatures of seston (*p*-value = 0.154, $n = 70$), as opposed to the winter values, for which the Wilcoxon test for paired samples indicated that the signatures of seston were significantly different from the those of *Daphnia* (*p*-value <0.001, $n = 16$).

3.3.3 | Production period and $\delta^{13}C_{ephippia}$

In the recent sediments collected in Lake Remoray, the $\delta^{13}C_{ephippia}$ values ranged from −38.6‰ and − 34.9‰ and averaged −37.1 ± 0.8‰. For the three other lakes, the δ^{13}C values of ephippia ($\delta^{13}C_{ephippia}$) collected at the surface sediment of the deepest points were − 46.0‰ for Lake Bonlieu, −36.8‰ for Lake Retournemer, and − 32.9‰ for Lake Longemer.

As $\delta^{13}C_{ephippia}$ reflects the $\delta^{13}C_{Daphnia}$ value at the time the ephippia were produced (Morlock et al., 2017; Perga, 2011; Schilder, Bastviken, et al., 2015), we can deduce the most likely period of ephippia production for each lake based on the $\delta^{13}C_{Daphnia}$ measured during monitoring. Ephippia sampled on Lake Remoray seemed to be produced between August and November at the end of the stratification period. Ephippia collected on Lake Retournemer seemed to be produced before the autumnal turnover, between September and October. Ephippia sampled on Lake Longemer seemed to be produced at the late end of the stratification period, around December. In contrast to the other lakes studied, ephippia from Lake Bonlieu seemed to be produced from late autumn to early winter (after the autumnal turnover).

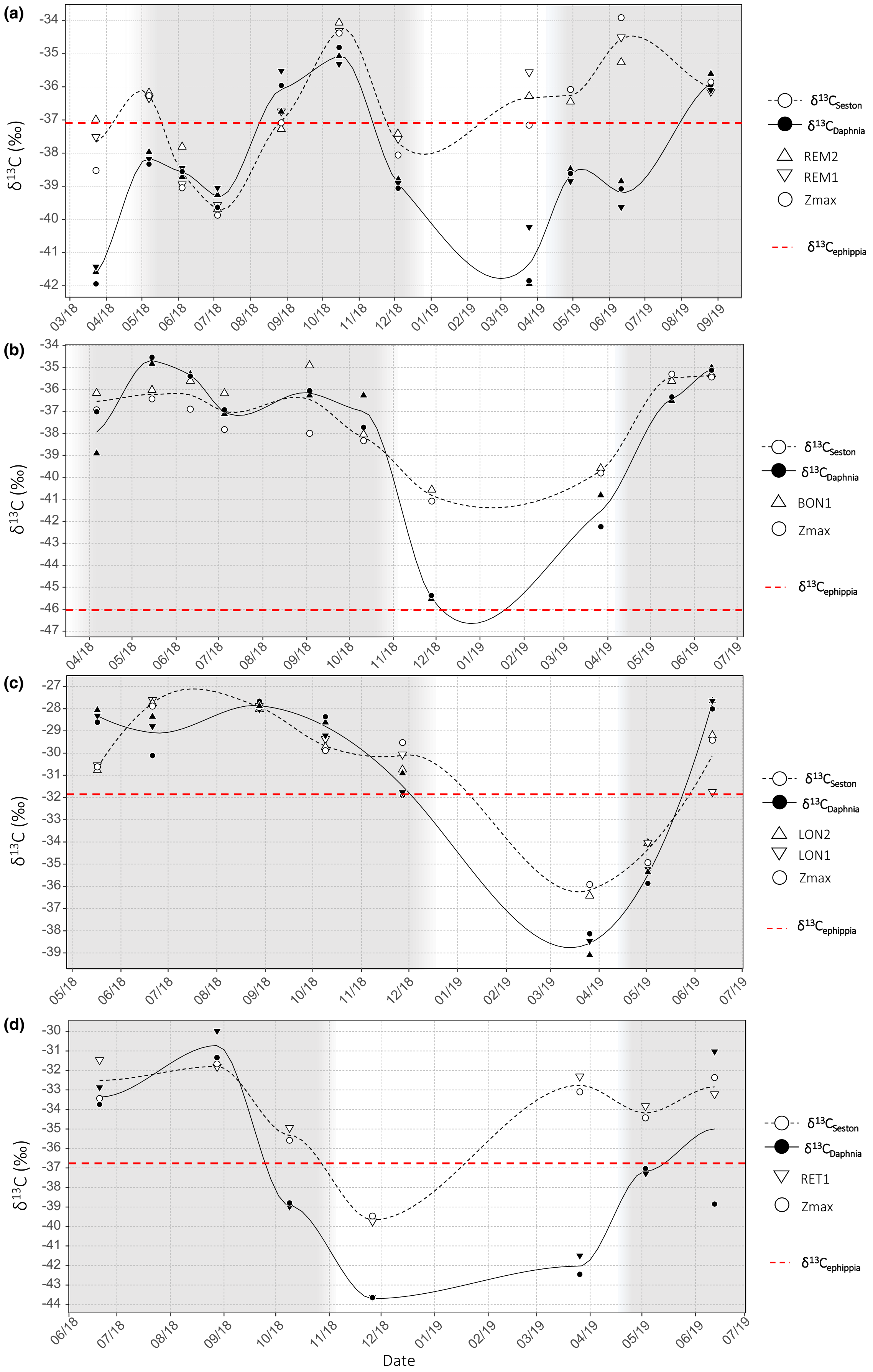
4 | DISCUSSION

Based on the physico-chemical monitoring and surveys of the δ^{13}C values of *Daphnia* and seston conducted in the four lakes, we identified two distinct periods characterised by contrasting pelagic trophic functioning. During the summer stratification period, the $\delta^{13}C_{Daphnia}$ values were close to the $\delta^{13}C_{seston}$ values. During winter, after the autumnal turnover, $\delta^{13}C_{Daphnia}$ moved away from $\delta^{13}C_{seston}$ to become more negative. More ^{13}C-enriched $\delta^{13}C_{Daphnia}$ values were always observed during the summer stratification period, while more ^{13}C-depleted values were always observed during the winter period.

4.1 | Summer $\delta^{13}C_{seston}$ and $\delta^{13}C_{Daphnia}$

4.1.1 | Controlling factors of the δ^{13}C signature of seston during the summer stratification period

During summer stratification, the $\delta^{13}C_{seston}$ values ranged from −27.6‰ to −39.8‰ in the four lakes. These observed signatures correspond with those expected for phytoplankton according to the literature, which usually reports values between −25‰ and − 40‰ (France, 1995; Masclaux et al., 2013; Peterson & Fry, 1987; Vuorio et al., 2006; Wang et al., 2013). Furthermore, our [Chl*a*] results reflects significant phytoplankton biomass during spring and summer. We can thus conclude that in summer, the δ^{13}C of seston largely reflected the δ^{13}C of phytoplankton, even though other sources undoubtedly contribute to seston, such as allochthonous detritus, which has overlapping δ^{13}C ranging from −25‰ to


(a)
δ13C (‰)
-34
-35
-36
-37
-38
-39
-40
-41
-42
03/18
04/18
05/18
06/18
07/18
08/18
09/18
10/18
11/18
12/18
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04/19
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06/19
07/19
08/19
09/19
δ13CSeston
δ13CDaphnia
REM2
REM1
Zmax
δ13Cephippia
(b)
δ13C (‰)
-34
-35
-36
-37
-38
-39
-40
-41
-42
-43
-44
-45
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04/18
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δ13CSeston
δ13CDaphnia
BON1
Zmax
δ13Cephippia
(c)
δ13C (‰)
-27
-28
-29
-30
-31
-32
-33
-34
-35
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δ13CSeston
δ13CDaphnia
LON2
LON1
Zmax
δ13Cephippia
(d)
δ13C (‰)
-30
-31
-32
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FIGURE 7 The $\delta^{13}C$ values of *Daphnia* ($\delta^{13}C_{Daphnia}$) and seston ($\delta^{13}C_{seston}$) for the different sample locations over the monitoring period of (a) Lake Remoray, (b) Lake Bonlieu, (c) Lake Longemer, and (d) Lake Retournemer. The $\delta^{13}C_{Daphnia}$ values are represented by full symbols, the smoothed curve adjusted by local regressions associated with $\delta^{13}C_{Daphnia}$ values is represented by a full line; $\delta^{13}C_{seston}$ values are represented by open symbols, the smoothed curve associated is represented by a dashed line; the horizontal dashed red line represents the value of $\delta^{13}C_{ephippia}$ collected in the deep zone. The grey background symbolises summer stratification periods

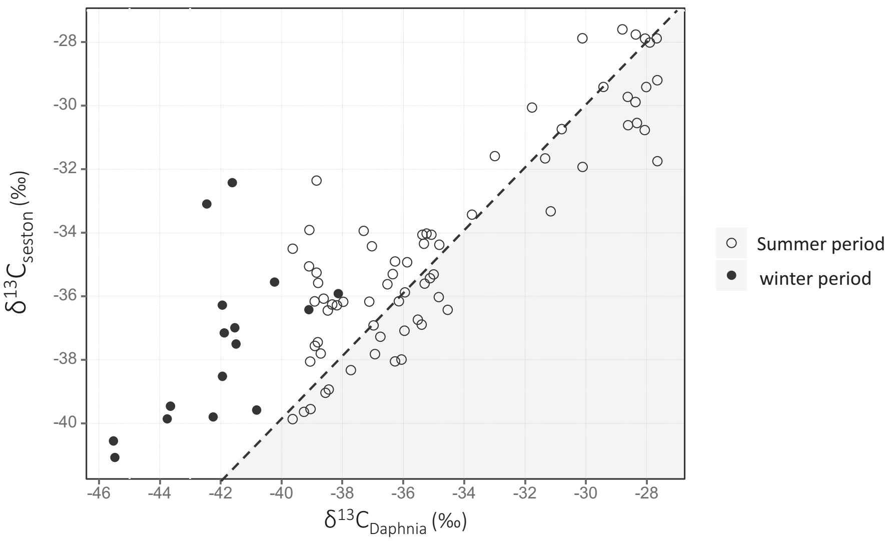

FIGURE 8 Distribution all data of $\delta^{13}C_{Daphnia}$ as a function of $\delta^{13}C_{seston}$ for summer and winter period. Summer values are represented by open symbols. Winter values are represented by full symbols. The dotted line represents $f(\delta^{13}C_{Daphnia}) = 1 \times \delta^{13}C_{seston}$, all dots to the right of this line in the grey area have $\delta^{13}C_{Daphnia}$ greater than $\delta^{13}C_{seston}$, all dots to the left in the white area have $\delta^{13}C_{Daphnia}$ lower than $\delta^{13}C_{seston}$

−29‰ (France, 1995; Grey et al., 2001; O'Leary, 1988; Peterson & Fry, 1987) or bacterial biomass, such as MOB, which can be highly ^{13}C depleted (Templeton et al., 2006). However, differences were apparent among the studied lakes. Lakes Remoray, Bonlieu, and Retournemer had lower summer mean $\delta^{13}C_{seston}$ (−36.7‰, −36.4‰, and −33‰, respectively) compared to Lake Longemer (−29.7‰). Disparities between lakes in the proportion of phytoplankton, bacterial biomass, and allochtonous detritus constituting the seston may explain in part the differences of $\delta^{13}C_{seston}$ between the four lakes. It is also likely that these inter-lake variations in $\delta^{13}C_{seston}$ are the result of differences in the isotopic signature of phytoplankton. The two main factors influencing the signature of phytoplankton are: (1) the intensity of isotopic fractionation performed by phytoplankton, which depends on DIC availability and, indirectly, the growth rate of the phytoplankton (Bade et al., 2004, 2006; Gu et al., 2006; Laws et al., 1995; Lehmann et al., 2004; Smyntek et al., 2012); and (2) the dissolved inorganic carbon (DIC) signature. An increase in phytoplankton production may lead to an increase in phytoplankton signatures (Gu et al., 2006; Lehmann et al., 2004). Indeed, in the case of low DIC availability in the water column, the ^{13}C discrimination performed by phytoplankton is less important, leading to a ^{13}C enrichment of the phytoplankton signature (Farquhar et al., 1989; Fogel et al., 1992). The isotopic signature of phytoplankton is also strongly dependent on the relative contributions of atmospheric CO_2 dissolution, calcium carbonate dissolution (for the Jura lakes), and CO_2 released by respiration to DIC. High respiration rates in the water column led to a ^{13}C-depleted DIC signature (Fry, 2006). Due to the negligible fractionation process during respiration (Degens, 1969), the DIC resulting from respiration has a $\delta^{13}C$ value equivalent to the degraded organic matter (OM). However, due to the fractionation process occurring during photosynthesis, the $\delta^{13}C$ of OM is lower than the $\delta^{13}C$ of the fixed DIC (Raven, 1996). DIC resulting from OM respiration has then a $\delta^{13}C$ value substantially lower than DIC resulting from atmospheric CO_2 and carbonate dissolution (DeNiro & Epstein, 1978; Keough et al., 1996). Therefore, the more that phytoplankton use respiration-derived CO_2 over atmospheric and carbonate origin DIC, the more negative its $\delta^{13}C$ value will be. The particularly low summer $\delta^{13}C$ measured in lakes Bonlieu, Remoray, and Retournemer could therefore indicate strong respiratory activity in the epilimnion in summer. The important respiratory activities in these three lakes may be supported by high phytoplankton production linked to eutrophication and/or significant allochthonous inputs (Frossard et al., 2014; Leigh & del Giorgio, 2008). Significantly higher $\delta^{13}C_{seston}$ values measured in Lake Longemer compared to the other three lakes may indicate the use of less ^{13}C-depleted DIC by phytoplankton for photosynthesis and/or a higher proportion of allochthonous detritus in seston, which has a $\delta^{13}C$ range close to those observed in this lake (−25‰ to −29 ‰; O'Leary, 1988; France, 1995; Grey et al., 2001; O'Leary, 1988; Peterson & Fry, 1987).

4.2 | Origin of carbon consumed by *Daphnia* in summer

Throughout the summer stratification period, the $\delta^{13}C_{Daphnia}$ values were very close to the $\delta^{13}C_{seston}$ values in all four lakes studied. The slight differences found between the signatures of *Daphnia* and seston (averaging 0.4‰ during the summer stratification period) are similar to those already reported in the literature (del Giorgio & France, 1996; Grey et al., 2000; Masclaux et al., 2014; Morlock et al., 2017; Schilder et al., 2017). At that time of the year, seston is mainly composed of phytoplankton, and it is likely that most of the carbon assimilated by *Daphnia* comes from phytoplankton during this period, even if we cannot totally exclude other food sources.

In lakes Remoray, Bonlieu, and Retournemer, *Daphnia* seems to consume a non-negligible portion of carbon derived from respiration. In contrast, the higher $\delta^{13}C$ measured on Lake Longemer during the summer stratification period potentially reflects the use by *Daphnia* of a lower proportion of respiration derived carbon and a higher proportion of atmospheric carbon fixed by phytoplankton and/or catchment vegetation compared to the other three lakes.

4.3 | Winter shift of the carbon source in the *Daphnia* diet

After water column mixing, the $\delta^{13}C_{Daphnia}$ values in lakes Remoray, Bonlieu, and Retournemer are substantially lower than the signatures of allochthonous detritus and phytoplankton (−25‰ to −40‰) reported in the literature (France, 1995; Grey et al., 2001; Masclaux et al., 2013; O'Leary, 1988; Peterson & Fry, 1987; Vuorio et al., 2006; Wang et al., 2013). Moreover, the differences between $\delta^{13}C_{Daphnia}$ and $\delta^{13}C_{\text{seston}}$ increased in winter. At this time of year, *Daphnia* would assimilate a carbon source not or only partially sampled in our study.

Pronounced summer hypolimnetic anoxia was observed in lakes Remoray, Bonlie,u and Retournemer, reflecting important OM degradation activities. Under such anoxic conditions, a major part of organic matter degradation is carried out by methanogenic Archaea, leading to the production of methane (Capone & Kiene, 1988; Rudd & Hamilton, 1978). A large quantity of CH_4 can therefore potentially be produced in these three lakes. In such stratified lakes, CH_4 produced in the sediments accumulates in the hypolimnion during summer and is released into the water column during the autumnal turnover (Utsumi et al., 1998). When O_2 and CH_4 become available in the water column, CH_4 can be oxidised by MOB (Rudd & Hamilton, 1978; Bastviken et al., 2002; Kankaala, Huotari, et al., 2006; Rudd & Hamilton, 1978). The release of this methane results in a high production of MOB, which can then oxidise a large proportion of CH_4 (Kankaala et al., 2007; Schubert et al., 2012; Utsumi et al., 1998). During this period, the proportion of MOB among the bacteria suspended in the water column is at its maximum, and phytoplankton are absent or rare. The diet of the *Daphnia* was therefore probably composed largely of bacteria during this period, among which MOB are abundant. This methanogenic carbon (C–CH_4) transfer probably explains the decrease in $\delta^{13}C_{Daphnia}$ observed after the autumnal mixing. Indeed, C–CH_4 has a highly depleted signature ranging from −80‰ to −50‰ (Jedrysek, 2005), and the isotopic fractionation that occurs during the oxidation of CH_4 by MOB leads to further depletion of the signature (Templeton et al., 2006). Similar signatures ranging between −40‰ and −47‰ reflecting C-CH_4 transfers have already been reported in winter in several papers studying stratified lakes (Harrod & Grey, 2006; Morlock et al., 2017; Taipale et al., 2008). Methane can therefore be an important carbon source for lake zooplankton, especially in winter after the autumnal turnover (Bastviken et al., 2003; Kankaala, Taipale, et al., 2006; Taipale et al., 2007; Taipale et al., 2008). Based on these highly ^{13}C-depleted values, methanogenic carbon appears to support pelagic food webs in lakes Remoray, Bonlieu, and Retournemer.

Winter $\delta^{13}C_{Daphnia}$ values measured in Lake Longemer (averaging −38.5‰) do not allow us to confidently conclude that methanogenic carbon contributed to the pelagic food web in this lake during winter. Even though $\delta^{13}C_{Daphnia}$ decreased after the autumnal turnover, $\delta^{13}C_{Daphnia}$ remained within the range of values already reported for phytoplankton in lakes (−25‰ and −40‰; Masclaux et al., 2013; Peterson & Fry, 1987; France, 1995; Peterson & Fry, 1987; Vuorio et al., 2006; Wang et al., 2013).

4.4 | Delta $^{13}C_{\text{ephippia}}$ and implications for the use of ephippia in paleolimnological studies

4.4.1 | Spatial variability of δ13Cephippia

Analysis of the $\delta^{13}C_{\text{ephippia}}$ values measured at 30 sampling points in the profundal zone of Lake Remoray indicated the absence of spatial autocorrelation and that the values were randomly distributed in space. Such results were expected considering the observed absence of spatial $\delta^{13}C_{Daphnia}$ variability during the sampling of Lake Remoray and the three other lakes. In addition, ephippia may drift for a year or more in the water column (Morlock et al., 2017). Ephippia settling at one point thus potentially constitute a representative sample of ephippia produced in the whole lake. For these reasons, single sediment samples collected from the deep zones of the other lakes to obtain ephippia should be representative of the whole deep zone.

4.4.2 | Factors controlling $\delta^{13}C_{\text{ephippia}}$

The carbon isotopic signature of *Daphnia* resting eggs reflects the $\delta^{13}C$ signature of *Daphnia* at the time of egg production (Perga, 2011; Schilder, Bastviken, et al., 2015; Schilder, Tellenbach, et al., 2015). Based on the ephippia and *Daphnia* signatures recorded during sampling, we were able to approximate the period of maximum production of these ephippia in each lake.

Ephippia collected from the sediments of lakes Remoray, Longemer, and Retournemer ($\delta^{13}C$ = −37.1‰, −32.9‰ and −36.8‰, respectively) seemed to be produced at the end of the summer stratification period. These signatures therefore mainly reflected the consumption of phytoplankton with varying degrees of ^{13}C depletion, probably resulting from the variable intensities of the respiration processes among the lakes in summer. These ephippial $\delta^{13}C$ values measured in lakes Remoray, Retournemer, and Longemer thus reflected the carbon transfers that occurred during the summer stratification period, and did not provide information on the origin of the carbon transferred in the pelagic food web after the autumnal turnover, when methane is more available. In contrast, ephippia from Lake Bonlieu surface sediment appeared to be produced in late autumn/early winter ($\delta^{13}C_{ephippia}$ = −46‰; after the autumnal turnover). As previously discussed, methane-derived carbon probably constituted an important part of the *Daphnia* diet during this period in this lake. Consequently, the carbon isotopic signatures of ephippia reflected the strong transfer of C-CH_4 into the pelagic food web in winter.

Two main stressors may control resting egg production by *Daphnia*: (1) reduced photoperiod and (2) limited quantity and/or quality of food resources (Kleiven et al., 1992; Koch et al., 2009; Stross & Hill, 1965). Due to the geographical proximity of the studied lakes, photoperiod probably does not play a role in determining the observed differences in the timing of ephippia production. In contrast, the seasonal availability of food resources may cause the observed differences in the timing of production in the four studied lakes. Indeed, according to the [Chl*a*] data, Lake Bonlieu had a sustained peak in phytoplankton biomass in autumn. In the other lakes, phytoplankton production seemed to be limited during this period. The stress response that is linked to food availability and induces ephippia production may therefore occur later in Lake Bonlieu than in the three other lakes.

Both the production period of ephippia and the origin of carbon used by *Daphnia* at the corresponding time seem to condition the carbon isotope signature of ephippia and therefore influence the information provided in paleolimnological approach. From our dataset, $\delta^{13}C_{ephippia}$ may reflect either the consumption of phytoplankton by *Daphnia* during the summer stratification period or the consumption of methanogenic carbon if *Daphnia* produce ephippia after the autumnal turnover.

4.4.3 | Implications for the interpretations of the $\delta^{13}C$ of *Daphnia* ephippia in paleolimnology

In our study, the measured $\delta^{13}C_{ephippia}$ appeared to be homogeneously distributed in the deep area in this type of relatively small lake with a single basin. A single core retrieved from the deepest part of the lake would provide a representative sample of the ephippia produced in the lake.

Moreover, our results suggest that the period of production of *Daphnia* ephippia differs from one lake to another depending on the availability and/or quality of the food resource. Consequently, the production period of ephippia and the origin of the carbon used by *Daphnia* at the corresponding time influenced the carbon isotope signature of the ephippia and therefore the information provided by the paleolimnological approach. The use of ephippia $\delta^{13}C$ values in paleolimnological studies to assess carbon transfers in the pelagic compartment has to be conducted cautiously in stratified lakes, where availability of different pelagic carbon sources varies seasonally (Taipale et al., 2008, 2009; Utsumi et al., 1998). Due to these seasonal mechanisms, the information provided by ephippia $\delta^{13}C$ depends on their periods of production. Signatures below −40‰ leave few doubts about the use of C-CH_4 by *Daphnia* at the time of ephippia production, as these values are outside the range of reported values of phytoplankton and particulate organic matter from the catchment (−25‰ to −40‰; France, 1995; Grey et al., 2001; Masclaux et al., 2013; O'Leary, 1988; Peterson & Fry, 1987; Vuorio et al., 2006; Wang et al., 2013). However, ^{13}C depleted signatures above −40‰ are more difficult to interpret, as they can reflect the transfer of different ^{13}C depleted carbon sources (i.e. respiration and methane derived carbon) in proportions that may vary according to the period of ephippia production. Nonetheless, whatever the production period of these ephippia, ^{13}C depletion over time may reflect a dynamic of dystrophication/accelerated eutrophication marked by the intensification of respiratory processes, increasing hypoxia, and potential intensification of methanogenesis processes, which finally intensifies the incorporation of ^{13}C-depleted carbon from respiration and/or methanogenesis by invertebrates (Frossard et al., 2014). Delta $^{13}C_{ephippia}$ may be used to follow the general evolution of trophic functioning in lakes but it may prove to be insufficient to follow specific carbon pathways. Moreover, potential shifts in the production period of ephippia over time induced by modifications of environmental conditions may lead to the misinterpretation of $\delta^{13}C_{ephippia}$ trends in sediment records.

Lake sediments offer a wide variety of other markers that can be used in association with ephippia $\delta^{13}C$ to provide a better understanding of the evolution of past carbon transfers in lakes. The signatures of *Daphnia* exoskeletons retrieved from sedimentary records are an example of markers that can also be measured (Perga, 2010). Unlike *Daphnia* ephippia, the isotopic compositions of these exoskeletons are assumed to reflect a time-integrated average signature of the *Daphnia* source population (Davidson et al., 2007). As these two *Daphnia*-related remains provide differently time-integrated information, combined analyses and comparisons of *Daphnia* ephippia and exoskeleton signatures should thus allow a more accurate assessment of these past transfers of carbon in pelagic food webs. Other consumer remains can also be used in combination with *Daphnia* ephippia to study past carbon transfers, such as ephippia from *Ceriodaphnia*, another cladoceran (Morlock et al., 2017), or Chironomid cephalic capsules (Belle et al., 2014, 2017; Belle, Millet, et al., 2016; Frossard et al., 2014; van Hardenbroek et al., 2010) to reconstruct benthic transfers. Ancient DNA analysis of MOB in sedimentary records is another method that can be used to improve the assessment of the evolution of C-CH_4 transfers in food webs when coupled with $\delta^{13}C$ analysis of consumer remains (Belle et al., 2015; Belle, Verneaux, et al., 2016). Finally, some studies have highlighted particular polyunsaturated fatty acids in sediments as biomarkers of MOB and methanogenic Archaea (Bowman et al., 1991; Elvert et al., 2016; Naeher et al., 2014). Polyunsaturated fatty acid analyses of sedimentary records may provide additional information on CH_4 production and transfer dynamics. Analysis of these lipid biomarkers directly on consumer remains, such as *Daphnia* ephippia, may constitute a future challenge in studies of C-CH_4 transfers in the pelagic compartments of lakes.

5 | CONCLUSION

Daphnia δ^{13}C showed strong seasonal variations in lakes Remoray, Bonlieu, Retournemer, and Longemer. Throughout the summer stratification period, $\delta^{13}C_{Daphnia}$ was close to $\delta^{13}C_{seston}$ in the four lakes studied. After the water column mixing occurred, the differences between $\delta^{13}C_{Daphnia}$ and $\delta^{13}C_{seston}$ increased, with more highly ^{13}C-depleted *Daphnia* signatures. Particularly low $\delta^{13}C_{Daphnia}$ values in lakes Remoray, Bonlieu, and Retournemer reflected the potential transfer of C-CO_2 from respiration in the summer stratification period and the transfer of a non-negligible part of C-CH_4 in pelagic food webs in winter due to seasonal stratification mechanisms. Less negative values were obtained during the sampling of Lake Longemer. This result reflects a smaller proportion of C-CO_2 from respiration transferred during summer stratification and does not allow us to confidently conclude that methanogenic carbon contributed to the pelagic food web during the winter period. This study does not allow us to identify the factors controlling the intensity of these C pathways in pelagic food webs, and therefore the differences observed between lakes. The importance of these different carbon pathways in food webs can be potentially influenced by several factors, such as temperature or origin and quantity of organic matter sedimented. Indeed, laboratory studies have highlighted that these factors can influence the intensity of methanogenic processes (Duc et al., 2010). Moreover, as deep oxygen conditions and summer hypolimnetic methane storage mechanisms depend largely on thermal stratification (Vachon et al., 2019), the strength of thermal stratification could be an important controlling factor of the intensity of these C pathways in pelagic food webs. Future large-scale studies involving larger numbers of lakes and variables would be necessary to identify these control factors.

Ephippia seem to be produced either in the summer stratification period or after the autumnal turnover depending on the lake studied. As CH_4 availability in the water column seems seasonal in these types of lakes, the information provided by ephippia δ^{13}C signatures depends on the production periods of the ephippia. Therefore, using $\delta^{13}C_{ephippia}$ to study past transfers of specific carbon sources into pelagic compartments is problematic in stratified lakes if used alone and requires a multi-proxy paleolimnological approach to provide a better understanding of their past evolution. This study also showed that $\delta^{13}C_{ephippia}$ appears to be homogeneously distributed in the deep area of a medium-sized lake with a single basin. A single core retrieved from the deepest part of the lake thus should provide a representative sample of the ephippia produced in the lake.

AUTHOR CONTRIBUTIONS

All authors devised the study. V.E. compiled the data. Statistical analyses were carried out by V.E. V.E. wrote the original draft, and all authors commented on and contributed to revising the draft.

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DATA AVAILABILITY STATEMENT

Data are available from the authors upon reasonable request.

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