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PRIMARY RESEARCH PAPER

Discrepancies between the stable isotope compositions of water, macrophyte carbonates and organics, and mollusc shells in the littoral zone of a charophyte-dominated lake (Lake Lednica, Poland)

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Abstract The aim of this study was to better understand the relations between carbon and oxygen stable isotope values of ambient water, mollusc shells, macrophytes and their carbonate encrustations, commonly used in palaeolimnological studies. Water, molluscs and macrophytes were sampled from the littoral zone in Lake Lednica, NW Poland. The influence of carbon species assimilated during photosynthesis and the net intensity of photosynthesis resulting from the size of charophyte species and the density of their stands were postulated to be the most important factors causing the species-specific $\delta^{13}C$ values of charophyte thalli and encrustations. It was suggested that photosynthetic activity of charophytes affected not only the $\delta^{13}C$ values of charophyte encrustations but also mollusc shells by changing $\delta^{13}C$ values of DIC within charophyte stands. In addition, incorporation of metabolic carbon into the shell was proposed as the main cause of both the ^{13}C depletion of mollusc shells relative to $\delta^{13}C$ values of DIC and the species-specific $\delta^{13}C$ values of shells. Mollusc shells were precipitated at the isotope equilibrium or close to the equilibrium with $\delta^{18}O$ values of lake water. Charophyte encrustations were found to be ^{18}O depleted due to the kinetic isotope effects during intense photosynthesis and thus fast precipitation of the calcite.

Keywords Stable C and O isotopes · Lake water · Mollusc shells · Macrophytes · Encrustations · Characeae

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Introduction

Numerous studies discussed the factors controlling carbon ($\delta^{13}C$) and oxygen ($\delta^{18}O$) stable isotope composition of freshwater carbonates, including mollusc shells or carbonates precipitated as a by-product of photosynthetic activity of autotrophs. For over a decade now, there has been an increasing number of isotope studies of charophytes (macroscopic algae from the *Characeae* family), in particular encrustations precipitated on the stems of those macroscopic algae (Coletta et al., 2001; Andrews et al., 2004; Pentecost et al., 2006; Pełechaty et al., 2010). Investigations of the isotope composition of freshwater mollusc shells have over 50-year history (e.g., Fritz & Poplawski, 1974) with the most recent studies focused on the detailed sclerochronological isotope record of

mussel growth increments (e.g., Kaandorp et al., 2003; Schöll-Barna et al., 2012; Yoshimura et al., 2010). Attention was also paid to the $\delta^{13}C$ values of macrophyte organic parts (Keeley & Sandquist, 1992; Leng et al., 2005). Determination of factors controlling the stable isotope composition of modern carbonates and organic matter makes the application of their $\delta^{13}C$ and $\delta^{18}O$ values possible in the studies of both recent lakes and lacustrine sediments.

Oxygen stable isotope composition of mollusc shells and charophyte encrustations is primarily controlled by $\delta^{18}O$ values of water and water temperature (Coletta et al., 2001; Kaandorp et al., 2003; Andrews et al., 2004; Shanahan et al., 2005). Many studies have demonstrated that mollusc shells tend to precipitate in isotopic equilibrium with water (Fritz & Poplawski, 1974; Dettman et al., 1999; Kaandorp et al., 2003; Anadón et al., 2010; Versteegh et al., 2010). In contrast, it was shown (McConnaughey, 1989; Andrews et al., 2004; Pentecost et al., 2006) that due to rapid photosynthesis observed in charophytes, precipitation of their encrustations is exposed to kinetic isotope effects. Kinetic isotope effects occur because the reaction rates of light isotopes are faster compared to the heavy isotopes. Discrimination against the heavy isotopes of carbon and oxygen during the hydration and hydroxylation of CO_2 (McConnaughey, 1989) leads to decreased carbon and oxygen stable isotope values of carbonates, compared to $\delta^{13}C$ values of DIC (dissolved inorganic carbon) and $\delta^{18}O$ values of water. In contrast, kinetic isotope effects in mollusc shells are regarded as small or absent (McConnaughey & Gillikin, 2008). Comparison of $\delta^{18}O$ values of molluscs and encrustations must consider 0.6‰ enrichment in aragonite shells compared to calcite encrustations (Tarutani et al., 1969), both precipitated in isotopic equilibrium with water.

The primary factor responsible for the differences in the $\delta^{13}C$ values observed in macrophytes is the source of carbon available for photosynthesis. In most freshwater lakes in temperate climate, the most frequent form of carbon occurring in water is HCO_3^- as it dominates at pH values between 7 and 9.5. At decreased water pH, HCO_3^- is accompanied and finally replaced by CO_2, e.g., at pH 5.5, 80% of carbon occurs as CO_2 (Stumm & Morgan, 2012). The difference between $\delta^{13}C$ values of HCO_3^- and aquatic CO_2 is dependent on the water temperature and ranges between 7 and 12‰, with HCO_3^- being ^{13}C enriched (Mook et al., 1974; Romanek et al., 1992; Zhang et al., 1995). Because numerous aquatic macrophytes have the ability to use both carbon species, the $\delta^{13}C$ value of tissue or thalli differs to a large extent. $\delta^{13}C$ values reported for different aquatic plants range between −50 and −11‰ (Leng et al., 2005).

Carbon stable isotope composition of charophyte thalli and encrustations is also controlled by photosynthetically driven metabolic effects (Andrews et al., 2004, and references therein). During photosynthesis, a higher proportion of $^{12}CO_2$ is incorporated preferentially by charophytes to form ^{13}C-depleted thalli. In consequence, the remaining DIC in the direct surrounding of the stems, i.e., in microhabitat, becomes ^{13}C enriched. $CaCO_3$ precipitated from such DIC is ^{13}C enriched compared to $\delta^{13}C$ values of DIC more distant from the stems (cf. Pentecost & Spiro, 1990; Andrews et al., 1997).

In molluscs, both inorganic carbon derived from the ambient DIC and organic carbon originating from the mollusc food can be bound to form the shell. Because the $\delta^{13}C$ value of the average organic matter in freshwaters is approximately 20‰ lower compared to the carbon stable isotope composition of DIC (Leng et al., 2005), the different proportion of the two carbon sources in the shell is subsequently reflected in its $\delta^{13}C$ value. The amount of carbon derived from mollusc food was estimated at 6–10% of the total C built into the shell (McConnaughey et al., 1997); however, the exact percentage can differ between the species. Also, feeding habits of molluscs are important because $\delta^{13}C$ values differ among macrophytes and phytoplankton, which are the primary sources of food to freshwater molluscs. $\delta^{13}C$ values of the aquatic macrophyte organic matter were shown to vary between −50 and −11‰, and $\delta^{13}C$ in lacustrine phytoplankton can range between −47 and −12‰ (Leng et al., 2005 and references therein). Thus, the food preferences of molluscs will result in the differences between the shell $\delta^{13}C$ values.

Another explanation of the differences between $\delta^{13}C$ values of freshwater mollusc shells was proposed by McConnaughey et al. (1997) who noted a clear distinction between the $\delta^{13}C$ values measured in aquatic gill-breathing snails and aquatic lung-breathing snails, with the former to have higher $\delta^{13}C$ values and closer to $\delta^{13}C_{DIC}$. McConnaughey et al. (1997)

suggested that the low $\delta^{13}C$ values in pulmonate snails result from their ability to breath ^{13}C-depleted atmospheric CO_2.

Interpretation of the $\delta^{13}C$ values of shells and encrustations must consider the different aragonite-HCO_3^- and calcite-HCO_3^- fractionation factors. The experimental laboratory study of Romanek et al. (1992) showed the temperature-independent 2.7 and 1‰ enrichment of aragonite and calcite, respectively, relative to HCO_3^-.

Temporal and spatial conditions of charophyte and mollusc growth must also be considered when interpreting $\delta^{13}C$ and $\delta^{18}O$ values. The length and starting point of macrophyte and mollusc growth and their habitat may all result in different isotope composition of thalli, tissues, encrustations, and shells.

In most studies investigating the factors controlling $\delta^{13}C$ and $\delta^{18}O$ values of freshwater carbonates and organic matter, authors focused on the comparison between the stable isotope composition of encrustations, shells, macrophyte tissues or thalli and $\delta^{13}C$ values of DIC and $\delta^{18}O$ values of water (e.g., Coletta et al., 2001; Andrews et al., 2004; Pentecost et al., 2006; McConnaughey & Gillikin, 2008; Bucci et al., 2009; Pełechaty et al., 2010). However, equally important is understanding the relationship between the isotope composition of different components of the recent environment, as it helps choose the best material to be analysed isotopically in palaeolacustrine studies. Also, simultaneous isotope analyses of shells, encrustations and organic matter allow a more comprehensive reconstruction of the past environment because of the temporal and spatial differences in precipitation or growth of those materials.

In the present study, we compared carbon and oxygen stable isotope values of ambient water, mollusc shells, macrophyte organic matter and carbonate encrustations of macrophytes (primarily charophytes), on which molluscs occurred. All the studied materials were sampled from the littoral zone of Lake Lednica, NW Poland (Fig. 1), a calcium-rich water body known for abundant and diverse charophyte vegetation (Pełechaty, 2005 and references therein; Pełechaty et al., 2015). An approach to include different components of the environment within the lake littoral into a comparative isotope study allows the determination of shifts in $\delta^{13}C$ and $\delta^{18}O$ values between the components and makes the interpretation of those shifts easier and more complete. Since it has been known that charophytes can strongly influence $\delta^{13}C$ value of DIC and $\delta^{18}O$ values of water in their direct surrounding (Coletta et al., 2001; Andrews et al., 2004; Pentecost et al., 2006; Pełechaty et al., 2010), the question was asked how this affects the stable isotope composition of mollusc shells, commonly attached to those macrophytes.

Location

Lake Lednica is situated in the southern part of the Gniezno Lake District, approximately 35 km east of the city of Poznań in west-central Poland (52°33′N, 17°23′E; Fig. 1). Lake Lednica is an elongated water body with an area of 3.4 km^2 filling the southern part of a tunnel valley extending between Janowiec and Lednogóra. The lake is 7.3 km long and has a maximum width of 0.83 km; its maximum depth is 15.1 m. In addition to direct precipitation, limited surface run-off and groundwater input, the lake is fed by several small, temporary streams, which mainly flow during early spring and after the events of intense precipitation. One permanent surface outflow is located in the south-eastern part of the lake (Kolendowicz, 1992). The lake catchment is relatively small, with an area of approximately 38 km^2. Due to the insignificant inflow in relation to the lake volume, $\sim 2.4 \times 10^7 m^3$, the modern water residence time of the lake is relatively long and has been estimated at 6 years (Jańczak, 1991). This is consistent with an estimated annual water exchange of 18% (Tybiszewska & Szulczyńska, 2003). Physical and chemical properties of Lake Lednica waters, based on the summer values, indicate Secchi disc visibility on average more than 2.0 m, high oxygen saturation, solute content and high concentrations of Ca^{2+} above 115 mgl^{-1} (Pełechaty et al., 2015). The calcium-rich and hard meso-eutrophic waters of Lake Lednica feature a high diversity of charophytes (Characeae), dominant in the vegetation of the lake. Charophyte meadows, extensively developed in the littoral zone of the lake, offer a variety of habitats for the mollusc fauna and significantly contribute to the sedimentation of lake marl. Calcium carbonate represents up to 80% of the modern sediments deposited in the littoral of Lake Lednica.

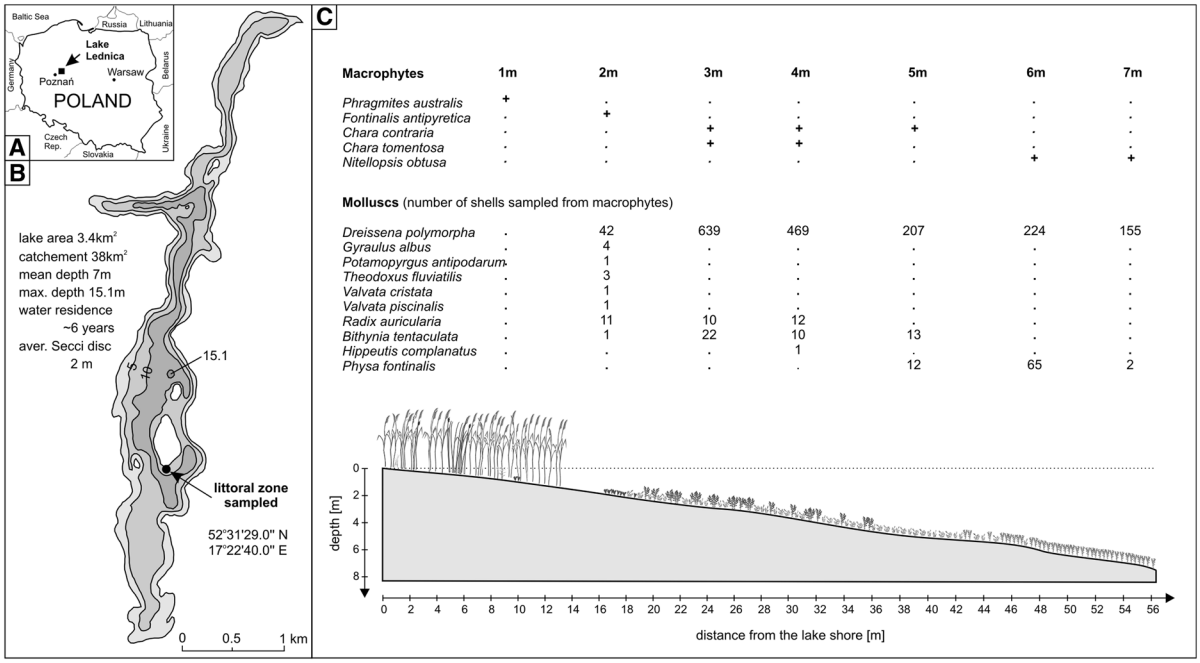

Fig. 1 Location of the study area. Littoral zone sampled is indicated with a *black dot* and an *arrow*. Longitude and latitude provided indicate location of the shallowest sampling site (i.e., 2 m, the starting point of the transect) within a transect along which water, macrophyte and mollusc samples were taken. The transect along the littoral zone investigated is provided with information on macrophyte and mollusc species occurring at each depth sampled. For molluscs, the number of individual shells occurring on macrophytes is given

The study area is influenced by both Atlantic and continental air masses, the former commonly prevailing. The present climate is characterized by mean annual precipitation of 500 mm and mean annual temperature of 7.9°C (mean July 17.9°C; mean January −2.4°C). A seasonal precipitation minimum causes an effective moisture deficit in the summer months (Kondracki, 2000).

Materials and methods

Fieldwork

Macrophytes with live molluscs attached were sampled within the littoral zone of Lake Lednica (Fig. 1) at a water depth between 2 and 7 m, at 1-m intervals. The sampling sites were set along a transect running perpendicular to the isobaths of the lake. Macrophytes were sampled with an anchor and placed in tight-sealed plastic bags.

Along with macrophytes, water samples for carbon and oxygen stable isotope analyses of DIC (dissolved inorganic carbon) and water, respectively, were collected at a depth of 0.5 m and from the above-bottom water layer (directly above macrophyte stands) using a bathometer (5-l Uwitec Plexiglas Watersampler). Water samples were placed in 10 ml glass septa test tubes and preserved with mercury chloride ($HgCl_2$). Separate vials were used for the collection of water for analysis of $\delta^{13}C$ of DIC and $\delta^{18}O$ of water.

Basic physical and chemical analyses of water above the studied charophyte patches, including water temperature, oxygen concentration, conductivity and pH, were performed using a portable field measurement equipment (Elmetron CX-401).

Laboratory work

All the live molluscs present on macrophytes were handpicked in the laboratory and identified to a species level under a low-power binocular microscope (Zeiss

Stemi 2000-C). Molluscs were treated with 50% ethyl alcohol for preservation until further treatment. Soft parts were handpicked with tweezers. All the empty shells were treated with 10% H_2O_2 for 48 h to eliminate organics that might influence the isotope results. Subsequently the shells were cleaned with a nylon brush under tap water and dried at room temperature overnight. Samples composed of 10 shells each (Table 1) were homogenized in an agate mortar and placed in Eppendorf vials. Selection of 10 shells per each sample results from within population variability in C and O isotope values observed in freshwater mollusc shells (Apolinarska, 2013; Apolinarska & Pełechaty, unpublished). In our studies, we have shown that mean isotope values of at least several, preferably 10 shells, are representative of $\delta^{13}C$ values of DIC and $\delta^{18}O$ values of water; however, species-specific offsets from equilibrium were observed. To avoid differences in the isotope composition of shells resulting from different age of particular individuals and hence differences in $\delta^{13}C$ values of DIC and $\delta^{18}O$ values of water during the shell growth, shells of uniform size within one species were selected for stable isotope analyses (Table 1). Also, all individuals with growth ceases visible on shells, indicative of winter cessation of the shell growth were excluded from the $\delta^{13}C$ and $\delta^{18}O$ analyses.

Subsequent to the collection of the mollusc shells, the macrophytes were air dried and homogenized in an agate mortar. Each sample of macrophytes prepared for stable isotope analyses was composed of 10 individual specimens. Each homogenized macrophyte sample was divided in half. One half for stable isotope composition of carbonates was placed in Eppendorf vials. The second half of each sample was used for carbon isotope determinations in organic matter. The samples were acid-washed with 20% HCl for 2 h to remove carbonates and rinsed with distilled water. The procedure was repeated three times. Finally, the samples were oven dried at 50°C and ground to a homogeneous powder using an agate mortar.

Isotope analyses

Fourteen water samples were analysed for $\delta^{13}C_{DIC}$ by an automated equilibration unit, Gas Bench II, coupled in continuous flow mode to a Delta V Advantage isotope ratio mass spectrometer at GeoZentrum

Table 1 Carbon and oxygen stable isotope composition of multi-shell homogenized samples

Depth (m)	n_{tot}	*Dreissena polymorpha*				n_{tot}	*Radix auricularia*				n_{tot}	*Bithynia tentaculata*				n_{tot}	*Physa fontinalis*			
		n	Shell length (mm)	$\delta^{13}C$ (‰)	$\delta^{18}O$ (‰)		*n*	shell height (mm)	$\delta^{13}C$ (‰)	$\delta^{18}O$ (‰)		*n*	Shell height (mm)	$\delta^{13}C$ (‰)	$\delta^{18}O$ (‰)		*n*	Shell height (mm)	$\delta^{13}C$ (‰)	$\delta^{18}O$ (‰)
2	42	10	7–8	−7.22	−4.37	13	10	3–6	−10.63	−4.24	1									
3	639	10	7–8	−6.63	−3.91	12	10	4–6	−9.91	−4.16	22	10	2–3	−8.32	−4.35					
4	469	10	7–8	−7.10	−4.11	10	10	4–6	−10.06	−4.17	10	10	2–3	−8.41	−4.50					
5	207	10	7–8	−6.48	−3.32						13	10	2–4	−8.89	−4.17	12	10	3	−11.59	−4.00
6	224	10	7–8	−6.38	−3.03											65	10	3	−13.01	−3.85
7	155	10	7–8	−6.19	−2.84											2				

Total abundance of species studied at each depth interval (n_{tot}), number of the shells analysed (*n*) and shell length or height are provided

Nordbayern, Erlangen, Germany. A sample volume of ~ 0.7 ml was injected into 12 ml Labco Exetainer™ vials which were prepared with phosphoric acid and pre-flushed with helium (purity 99.999%). Samples were analysed in duplicates and the reported value is the mean value. All values are reported in the standard δ-notation in per mil (‰) versus Vienna Pee Dee Belemnite (V-PDB) (Coplen, 2011). The datasets were corrected for the instrumental drift and normalized to the V-PDB scale by assigning a value of +1.95 and −46.6‰ to NBS 19 and LSVEC, respectively (Brand et al., 2014). External reproducibility was based on repeated analyses of a control sample prepared from sodium bicarbonate and DIC-free, ultrapure water. The precision of the control sample was better than 0.1‰ (1 sigma) for $\delta^{13}C_{DIC}$.

$\delta^{18}O$ values of water were measured using an isotope ratio infrared spectroscopy (IRIS) analyzer, based on wavelength-scanned cavity ring-down spectroscopy, L 1102-*i* WS-CRDS, at GeoZentrum Nordbayern, Erlangen, Germany. All values are reported in the standard δ-notation in per mil (‰) versus Vienna Standard Mean Ocean Water (V-SMOW). Four sequential injections of each sample were measured, and raw data were corrected for sample-to-sample memory. The reported value is the mean value. The datasets were corrected for instrumental drift during the run and normalized to the V-SMOW/SLAP scale by assigning a value of 0‰ and −55.5‰ to V-SMOW2 and SLAP2, respectively (Brand et al., 2014). For normalization, two laboratory reference materials, which were calibrated directly against V-SMOW2, were measured in each run. External reproducibility, based on repeated analyses of a control sample, was better than 0.1‰. For a detailed description of the analysis procedure, refer to van Geldern & Barth (2012).

The $\delta^{13}C$ and $\delta^{18}O$ values of 14 samples of molluscs, each consisting of 10 shells, and the same number of macrophyte carbonates were measured using a Gas Bench II hooked up to a Finnigan MAT 253 gas source mass spectrometer (both Thermo Fisher Scientific, Bremen, Germany) at the Institute of Geoscience at J.W. Goethe University in Frankfurt am Main, Germany. Details concerning the analytical setup are given in Spötl & Vennemann (2003). For a single analysis, 50–120 mg of carbonate was loaded into Labco Exetainer™ vials. Carrara marble was analysed with the samples, and its isotopic composition was calibrated against NBS 19 (Fiebig et al., 2005). The results are expressed as per mil (‰) deviations from the PDB carbonate standard and have an analytical precision of ± 0.06‰ for carbon and ±0.08‰ for oxygen.

Organic carbon isotope ($\delta^{13}C_{org}$) determinations of macrophytes (14 samples, each consisting of 10 complete individuals) were measured using a Flash Elemental Analyzer 1112 connected to the continuous flow inlet system of a MAT 253 gas source mass spectrometer at the Institute of Geosciences, Goethe University Frankfurt, Germany. All values are reported in the standard δ-notation in per mil (‰). USGS 24 standard was analysed along with the samples in order to prove the accuracy and precision. Both samples and standards reproduced within ±0.2‰.

Results

Isotope composition of DIC and water

Oxygen stable isotope composition of surface and above-bottom water (mean values −3.89 and −3.97‰, respectively) and carbon stable isotope values of surface DIC (mean −4.48‰) were uniform within the sampling sites in the littoral zone of Lake Lednica (Table 2; Fig. 2). The variability in isotope values was almost within the analytical error. A shift was observed only in the $\delta^{13}C$ values measured in the above-bottom DIC. The $\delta^{13}C_{DIC}$ values decreased from −4.38‰ at a water depth of 2 m to −8.86‰ at a depth of 7 m (Fig. 2).

Table 2 Carbon and oxygen stable isotope composition of DIC and water, respectively, measured in surface and bottom waters

Depth (m)	$\delta^{13}C_{DIC}$ (‰)		$\delta^{18}O_{water}$ (‰)	
	Surface	Bottom	Surface	Bottom
2	−4.46	−4.38	−3.91	−3.89
3	−4.36	−4.41	−3.88	−4.07
4	−4.42	−5.22	−3.91	−3.76
5	−4.58	−5.64	−3.87	−4.02
6	−4.56	−5.32	−3.89	−3.94
7	−4.48	−8.86	−3.90	−4.14

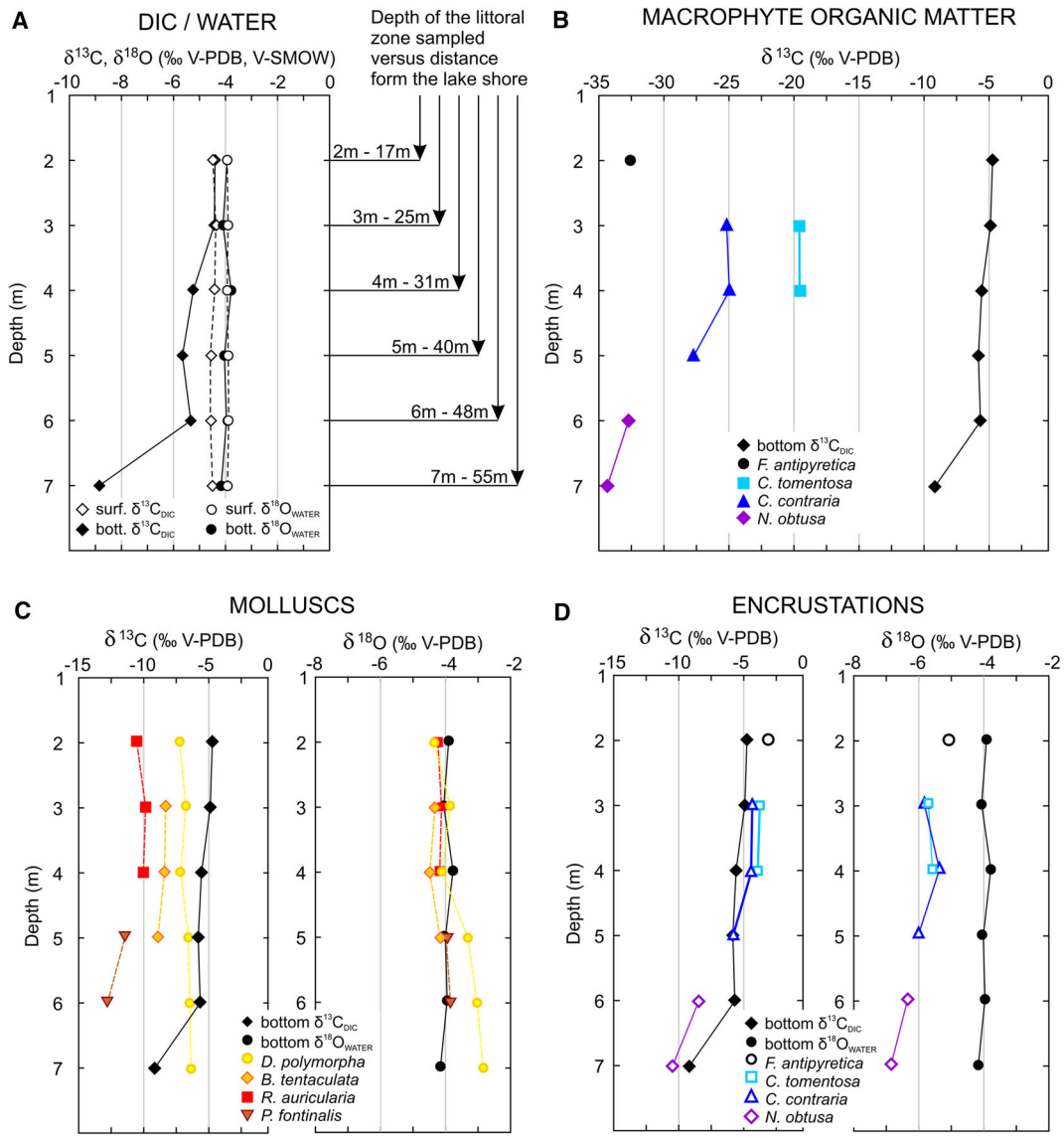

Fig. 2 Changes in the stable isotope composition of DIC, water, macrophyte organic matter and encrustations and mollusc shells observed with increasing water depth in the Lake Lednica littoral zone. **A** carbon and oxygen stable isotope composition of DIC and water, respectively, *Surf.* surface water and DIC. *Bott.* bottom water and DIC; **B** $\delta^{13}C$ values of macrophyte organic matter; **C** carbon and oxygen stable isotope composition of mollusc shells; **D** $\delta^{13}C$ and $\delta^{18}O$ values of encrustations

Isotope composition of macrophytes

Along the study transect, the littoral zone vegetation was dominated by charophytes. *Chara tomentosa* L. and *Chara contraria* Kütz. were present at a depth between 3 and 5 m. In deeper stands, *Nitellopsis obtusa* (Desv.) J. Groves was the dominant species, while the moss species, *Fontinalis antipyretica* L., was present at a depth of 2 m (Table 3).

Both $\delta^{13}C$ and $\delta^{18}O$ values of encrustations occurring on macrophytes revealed a decreasing tendency along with an increasing water depth (Fig. 3; Table 3).

Table 3 Carbon and oxygen stable isotope values in macrophytes and their encrustations

Depth (m)	*Fontinalis antipyretica*			*Chara tomentosa*				*Chara contraria*				*Nitellopsis obtusa*			
	$\delta^{13}C_{ORG}$ (‰)	$\delta^{13}C_{CARB}$	$\delta^{18}O_{CARB}$	*n*	$\delta^{13}C_{ORG}$ (‰)	$\delta^{13}C_{CARB}$	$\delta^{18}O_{CARB}$	*n*	$\delta^{13}C_{ORG}$ (‰)	$\delta^{13}C_{CARB}$	$\delta^{18}O_{CARB}$	*n*	$\delta^{13}C_{ORG}$ (‰)	$\delta^{13}C_{CARB}$	$\delta^{18}O_{CARB}$
2	−32.5	−2.77	−5.05												
3				10	−19.4	−3.46	−5.68	10	−25.1	−3.93	−5.76				
4				10	−19.4	−3.51	−5.56	10	−24.9	−4.06	−5.29				
5								10	−27.6	−5.45	−5.96				
6												10	−32.7	−8.15	−6.32
7												10	−34.3	−10.23	−6.83

Incomplete record of macrophytes along the increasing depth in the littoral zone reflects changes in the spatial distribution of species

$\delta^{13}C$ values ranged between −2.77 and −10.23‰ and $\delta^{18}O$ values decreased from −5.05 to −6.83‰.

Carbon stable isotope values of the macrophyte organics revealed a trend similar to the $\delta^{13}C$ values in carbonate encrustations of those macrophytes but were strongly ^{13}C depleted (Table 3; Fig. 3). The highest values were measured in *C. tomentosa* (−19.4‰), while the lowest in *N. obtusa* (−34.3‰). Low $\delta^{13}C$ values were also observed in *F. antipyretica* (−32.5‰).

$\delta^{13}C$ and $\delta^{18}O$ values of mollusc shells

Populations of molluscs occurring on macrophytes within the littoral zone of Lake Lednica were dominated by *Dreissena polymorpha* (Pallas) with *Bithynia tentaculata* (Linnaeus), *Radix auricularia* (Linnaeus), *Physa fontinalis* (Linnaeus). Other gastropod species were also present; however, only a few shells were collected. Species composition and mollusc abundance changed with the depth (Fig. 1; Table 2). The molluscs, including *D. polymorpha*, *B. tentaculata* and *R. auricularia*, were most numerous at 3 and 4 m of depth and occurred on both charophyte species growing at this depth, *C. contraria* and *C. tomentosa*. *P. fontinalis* was collected from charophytes with delicate stems, i.e., *C. contraria* and *N. obtusa*, at greater depths. This snail was most abundant at 6 m. In contrast, the most diverse mollusc assemblage was found on *F. antipyretica* at 2 m of depth.

The shells of *D. polymorpha*, the only mollusc species present throughout the studied depth gradient, became ^{13}C and ^{18}O enriched with increasing water depth (Table 1; Fig. 3). Although $\delta^{18}O$ values measured in snail shells varied between the analysed mollusc species, they followed the ^{18}O enrichment recorded by *D. polymorpha*. (Table 1; Fig. 3). The difference in the $\delta^{18}O$ values between the species was usually below 0.5‰. The increased difference in $\delta^{18}O$ values was noted between the shells of *D. polymorpha* and *P. fontinalis* (mean 0.74‰). The differences in $\delta^{13}C$ values between the species were higher, up to 6.6‰. Moreover, the species could be arranged from the least to the most depleted of ^{13}C: *D. polymorpha*, *B. tentaculata*, *R. auricularia* and *P. fontinalis* (Fig. 3). Offsets in $\delta^{13}C$ values between the species were similar at all depths sampled, except for the $\delta^{13}C$ values in the shells of *D. polymorpha* at a depth between 5 and 6 m (Table 1; Fig. 3).

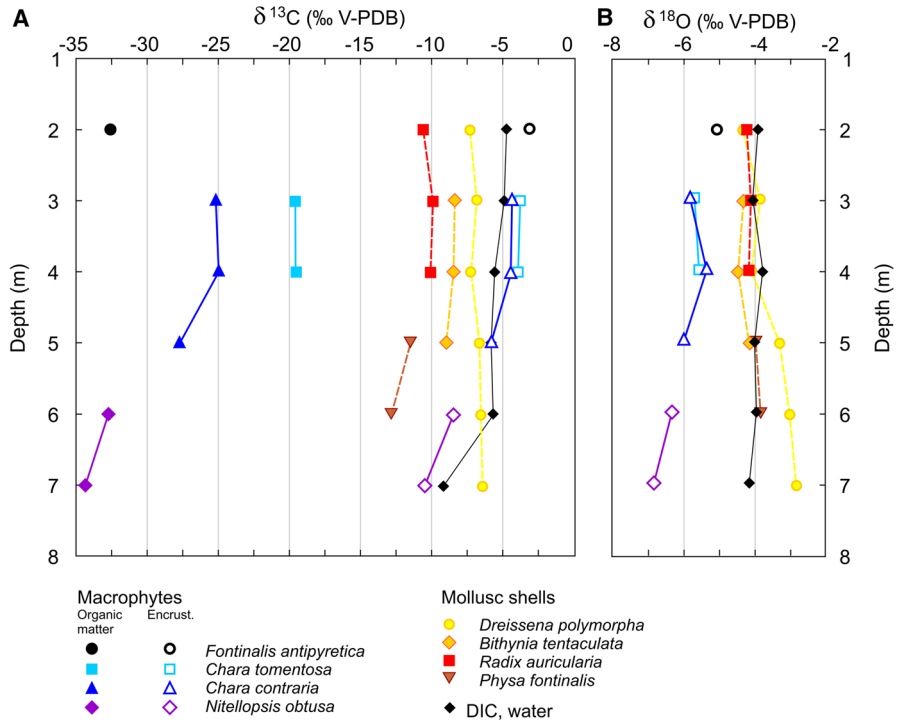

Fig. 3 Carbon (**A**) and oxygen (**B**) stable isotope values of DIC, water, mollusc shells, macrophytes encrustations and organic matter. Differences in isotope values are noted not only between macrophyte organic matter, encrustations and mollusc shells but also between species of each of the groups of organisms studied. Those differences are seen best in $\delta^{13}C$ record

Discussion

Stable isotope composition of DIC and water in the littoral zone of Lake Lednica

Very uniform $\delta^{18}O$ and $\delta^{13}C$ values of surface and above-bottom water and surface DIC, respectively (Table 2; Fig. 2), indicate spatial homogeneity of the isotope composition of the lake waters. This homogeneity indicates an active mixing of the water column within the littoral zone in Lake Lednica. However, isotope homogeneity was not observed for $\delta^{13}C$ values of the above-bottom DIC. While at a depth between 2 and 6 m, the difference between the surface and above-bottom $\delta^{13}C_{DIC}$ was still relatively small, i.e., within 1‰, it increased strongly at the deepest site investigated (Fig. 2). This difference, increasing with depth, can be explained by an increasing amount of isotopically light carbon (^{12}C) from decomposition of organic matter on the lake bottom. This observation indicates that the degree of water mixing by the wave action was limited at a depth of 7 m and below. This is confirmed by the findings of Pełechaty (2005) who has shown abrupt changes in the physical and chemical properties of the water in Lake Lednica starting at a depth of 7 m. Significant deforestation of the catchment area is conducive to good mixing of waters in the lake (Pełechaty, 2005). The still small difference between $\delta^{18}O$ values of the surface and the above-bottom water at 7 m is in contradiction with the decreased $\delta^{13}C$ values of DIC. The limited water mixing at this depth would increase the difference between $\delta^{18}O$ values of the surface and the above-bottom water due to the preferential evaporative loss of $H_2^{16}O$ from the surface waters. However, it is suggested that depending on the strength of the factors affecting $\delta^{13}C_{DIC}$ and $\delta^{18}O_{water}$, the isotope values change more or less instantly and to a varying extent. Since the release of ^{12}C-depleted CO_2 from the decomposition of the organic matter on the lake bottom can be intense under dense macrophyte patches, as in the present study, the change in $\delta^{13}C_{DIC}$ values is significant (Fig. 2).

The stable isotope composition of the waters in Lake Lednica described above is confirmed by the earlier studies performed in this lake (Apolinarska, 2013; Apolinarska & Pełechaty, unpublished). Carbon stable isotope values of DIC and oxygen stable isotope values of water remained nearly stable in the surface waters and $\delta^{18}O$ values were also nearly constant in the above-bottom waters (depth of 0.5–7 m) in late

spring (June) and mid-summer (August). $\delta^{13}C$ values of the above-bottom DIC were still uniform in late spring; however, similarly to the present study, the decrease in $\delta^{13}C_{DIC}$ with increasing water depth was observed in summer.

Relations between $\delta^{13}C$ and $\delta^{18}O$ values

Carbon stable isotope composition of macrophytes and encrustations

The ^{13}C-depleted values of the above-bottom DIC were followed by a decrease in $\delta^{13}C$ values of both the macrophyte organic matter (in this study in *F. antipyretica* tissues and charophyte thalli) and encrustations occurring on the macrophytes (Fig. 3). Whereas both the charophyte thalli and encrustations became ^{13}C depleted with depth, their isotope relation to the ambient DIC was opposite. The organic parts were ^{13}C depleted relative to $\delta^{13}C_{DIC,}$ whereas ^{13}C enrichment in relation to $\delta^{13}C_{DIC}$ was noted in most of the encrustations Fig. 3). Both are related to the preferential $^{12}CO_2$ incorporation by macrophytes during photosynthesis and formation of ^{13}C-depleted thalli. This leaves the remaining DIC in the direct surrounding of the stems, available during $CaCO_3$ precipitation, ^{13}C enriched (Pentecost & Spiro, 1990; Andrews et al., 1997; Andrews et al., 2004, and references therein). Carbon stable isotope values of charophyte encrustations were commonly found higher compared to $\delta^{13}C$ values of DIC (Coletta et al., 2001; Pentecost et al., 2006; Pełechaty et al., 2010). The extent of the ^{13}C enrichment of the encrustations relative to $\delta^{13}C_{DIC}$ values must be corrected by 1‰ resulting from the calcite-HCO_3^- fractionation factor (Romanek et al., 1992). In the present study, this brings most of the encrustations closer to $\delta^{13}C_{DIC}$.

As evidenced in the present study, the difference between $\delta^{13}C$ values of charophyte encrustations and organic parts appears to be species-specific, i.e., about 24‰ in *N. obtusa* followed by about 21‰ in *C. contraria* and about 16‰ in *C. tomentosa* (Fig. 3). Those species-specific offsets in $\delta^{13}C$ values between organic matter and encrustations indicate strong control of the macrophyte over the carbon stable isotope composition of its thalli and encrustations. It is suggested that the extent of the above-observed differences is related to the size of the charophyte species and the density of the stands it forms. Among the three species studied, *C. tomentosa* is the largest species, with the thickest stems. In conditions of intensive photosynthesis in dense charophyte stands, such as those formed by *C. tomentosa*, the local shortage of DIC available to these macroalgae may occur. In such a situation, the algae are less selective in carbon isotopes and more ^{13}C is built into charophyte stems. Still, this intensive photosynthesis in dense charophyte stands leads to strong ^{13}C enrichment of the local, i.e., within a stand, DIC. In consequence, high $\delta^{13}C$ values of encrustations are observed. Less intensive photosynthesis in *C. contratia*, a smaller and less heavily encrusted species, is reflected in lower carbon stable isotope values in both encrustations and organic matter. *C. contraria* derived from the same depths as *C. tomentosa* had 5.5‰ lighter thalli and 0.5‰ lighter encrustations. In the present study, the greatest difference between the thalli and encrustations was found for *N. obtusa*. Furthermore, while encrustations of *C. tomentosa* and *C. contraria* were ^{13}C enriched relative to $\delta^{13}C_{DIC}$, encrustations of *N. obtusa* were ^{13}C depleted relative to DIC (Fig. 3). *N. obtusa* is a smaller and ecorticate species producing less carbonates than *Chara* species (Pukacz et al., 2014). Because its growth is slower, the preferential uptake of $H^{12}CO_3^-$ for photosynthetic purposes was not modified by the shortage of the carbon source suggested above for *C. tomentosa*. Also, *N. obtusa* was found at greater depths within the littoral zone of Lake Lednica, where, as discussed earlier, release of ^{12}C-enriched carbon from decomposing organic matter results in ^{13}C-depleted DIC. The lower the $\delta^{13}C$ values of the DIC available to the macrophytes, the more ^{13}C depleted the tissues and the thalli are. Low $\delta^{13}C$ values will also be measured in encrustations. Similar correlation between the size of the charophyte species and $\delta^{13}C$ values of its thalli was also made in charophytes sampled from five lakes in western Poland (Pełechaty et al., 2010; Pronin et al., unpublished). In these studies, encrustations of *Chara rudis* and *C. tomentosa* (both tall charophyte species with thick stems) were ^{13}C enriched compared to DIC, whereas *Chara globularis* (small species with thin stems) encrustations were ^{13}C depleted (Pelechaty et al., 2013a, b; Pronin et al., unpublished).

The difference between $\delta^{13}C$ values of encrustations and organic parts was even greater in the analysed moss species, *F. antipyretica,* compared to

the analogue difference in charophytes (Fig. 3). Tissues of the moss were also far more ^{13}C depleted relative to $\delta^{13}C$ values of DIC (Fig. 3). We suggest that the low $\delta^{13}C$ values measured in *F. antipyretica* (Table 3) are indicative of CO_2 as the carbon source to this moss species, whereas ^{13}C-enriched thalli of charophytes indicate HCO_3^- as the main source of carbon assimilated by those macroalgae during photosynthesis. Depending on the water temperature CO_2 is between 7 and 12‰ ^{13}C depleted relative to HCO_3^- (Mook et al., 1974; Romanek et al., 1992; Zhang et al., 1995). Assimilation of CO_2 in addition to HCO_3^- is also suggested for *N. obtusa*. At deeper sites where this species occurred (Fig. 3), the proportion between CO_2 and HCO_3^- was different compared to the shallower sites, due to lower pH, and more CO_2 is available to the macrophytes.

Because of the discontinuous presence of the macrophyte species along the investigated transect, *F. antipyretica*, in particular (Fig. 3), interpretation of the differences between $\delta^{13}C$ values of macrophyte organic parts, encrustations they precipitated and DIC of their surrounding environment was limited in the present study. The above listed differences were studied in detail on two charophyte species, *C. tomentosa* and *C. globularis*, sampled from several lakes in western Poland (Pronin et al., unpublished). To verify whether the offsets are constant irrespective of the environmental characteristics, lakes with different physical and chemical properties, different in size and depth, were selected for the study. Similarly to the results of the present study, the difference between $\delta^{13}C$ values of organic parts, carbonates and DIC were observed by the above-cited authors to be species-specific. $\delta^{13}C$ values of *C. tomentosa* encrustations were by 16‰ ^{13}C enriched relative to thalli, on average. The same difference was observed in this study (Fig. 3). Although Pronin et al. (unpublished) did not investigate *C. contraria*, they studied a similar in size charophyte species, *C. globularis*. Interestingly, encrustations of both species were 21‰ ^{13}C enriched compared to organic parts, on average. The above presented comparison of data indicates that the differences in $\delta^{13}C$ values discussed are species specific and relatively constant irrespective of the lake. This is further discussed by Pronin et al. (submitted). The extent of the offset between $\delta^{13}C$ values of encrustations and organic parts is dependent on the charophyte size.

Carbon stable isotope composition of mollusc shells

Because mollusc was collected from the macrophytes, the common pool of DIC was available during precipitation of both the shells and the carbonates occurring on the macrophytes. The assumed common pool of DIC is also based on the similar time of growth of charophytes and molluscs studied. The majority of aquatic macrophytes, occurring in lakes in temperate climate, including many charophyte species, are annual (discussed in more detail below). Growth starts in spring and continues till autumn, when they gradually decay. However, among charophyte species studied here, *C. tomentosa* and *N. obtusa* are perennial species and, although with significant limitation, continue their development during winter. *C. contraria* is claimed to be an annual charophyte but can overwinter under favourable conditions. The common water moss (*F. antipyretica*) is also expected to perform a perennial growth pattern. Despite the above, however, our earlier studies in Lake Lednica evidenced significant reduction in vegetation cover during winter with the most pronounced increase in growth between May and July, particularly in case of charophytes (this being also observed in other lakes and by other authors, e.g., Pelechaty et al. 2013a and references therein). Most of the freshwater gastropods inhabiting temperate climate have a 1 to 2-year life span (Frömming 1956; Taft et al., 2012). *D. polymorpha* was observed to live up to 7 years (Stańczykowska & Lewandowski, 1993). After hatching, which is usually observed in May and June, molluscs live attached to macrophytes until autumn when macrophytes decay. In winter, molluscs live on the lake bottom, usually among plant detritus. To avoid differences in mollusc life span, shells of uniform size within one species, with no growth ceases visible on shells, indicative of winter cessation of the shell growth, were selected for stable isotope analyses in this study. Also, because of the small sizes of the species studied, migration of the gastropods, if present, is regarded to be restricted.

However, despite the assumed common pool of DIC, $\delta^{13}C$ values of the mollusc shells were ^{13}C depleted relative to $\delta^{13}C$ values of encrustations (Fig. 3) which is consistent with the isotope records of past environments (von Grafenstein et al., 2000; Yu, 2000; Hammarlund et al., 2003; Apolinarska & Hammarlund, 2009; Andersson et al., 2010). This

^{13}C depletion of shells indicates either a different carbon pool used during precipitation, existence of vital effects or other isotope fractionation mechanisms influencing the stable isotope composition of shells. Interpretation of the $\delta^{13}C$ values of shells and encrustations must consider different aragonite-HCO_3^- and calcite-HCO_3^- fractionation factors (Romanek et al., 1992). Correction of the $\delta^{13}C$ values of the aragonitic mollusc shells and calcite encrustations by 2.7 and 1‰, respectively, brings most of the encrustations closer to $\delta^{13}C_{DIC}$ values, whereas it results in even a larger difference between $\delta^{13}C$ values of mollusc shells and both encrustations and DIC (Fig. 3).

Despite this general ^{13}C depletion of the shells, carbon stable isotope values of molluscs were species specific (Fig. 3). The order of the species from isotopically heaviest to isotopically lightest, i.e., *D. polymorpha, B. tentaculata, R. auricularia and P. fontinalis*, confirmed the results of our previous study where stable isotope composition of the shells of several snails from the littoral zone of Lake Lednica was compared (Apolinarska & Pełechaty, unpublished). The species-specific $\delta^{13}C$ values in freshwater mollusc shells were also observed by Fritz & Poplawski (1974), Aucour et al., (2003), Shanahan et al. (2005), De Francesco & Hassan (2013).

The most plausible explanation of both ^{13}C depletion of mollusc shells relative to $\delta^{13}C$ values of DIC and the species-specific $\delta^{13}C$ values in shells is linked with the proportion between ambient and metabolic carbon incorporated into the shell and $\delta^{13}C$ values of the mollusc food. Among the studied molluscs, the most ^{13}C-enriched shells were found in *D. polymorpha*, the only species using filter feeding as an exclusive feeding mode. Filter feeding is also among the possible feeding alternatives of *B. tentaculata*, the snail with the $\delta^{13}C$ values intermediate between *D. polymorpha* and the two prosobranchs studied, i.e., *R. auricularia* and *P. fontinalis*. Although the $\delta^{13}C$ values of particulate organic matter (POM) in Lake Lednica were not measured, it is suggested that the POM filtered by *D. polymorpha* and *B. tentaculata* could have been ^{13}C enriched. At high pH values, as those measured in the surface waters of Lake Lednica (> 8, Pełechaty et al., 2015), HCO_3^- is the main carbon species in the waters and thus the primary source of carbon for phytoplankton. Because the $\delta^{13}C$ values of HCO_3^- are about 9–10‰ higher compared to the $\delta^{13}C$ values of CO_2 in Lake Lednica, also higher $\delta^{13}C$ values will be observed in phytoplankton (Talbot & Johannessen, 1992), which is regarded as one of the main constituents of the POM in epilimnion of lakes.

Carbon stable isotope values of most of the studied molluscs follow the changes in $\delta^{13}C$ values of encrustations, macrophyte organic matter and DIC, and become ^{13}C depleted with water depth (Fig. 3). *D. polymorpha* is the only exception, with $\delta^{13}C$ values of the shells increasing along the depth gradient (Fig. 3). The approximately 1‰ increase in $\delta^{13}C$ values of *D. polymorpha* shells is considered as an additional proof of the influence of the metabolic carbon on $\delta^{13}C$ values of its shell. The composition of the POM available for filter feeding organisms changes within the water column in a lake. At shallower depths, the POM is composed mainly of primary producers, i.e., phytoplankton, algae, whereas at increased water depths, the percentage of organisms from higher trophic levels, i.e., zooplankton, increases. Approximately 1‰ increase in the $\delta^{13}C$ values is observed between each trophic level (DeNiro & Epstein, 1978; Vander Zanden, 2001). Because *D. polymorpha* is a filter feeder, the type of food ingested by this species changes with depth, which is reflected in $\delta^{13}C$ values of its shell.

In contrast to *D. polymorpha*, *P. fontinalis*, a small snail with thin, delicate shells, was the most ^{13}C depleted among the studied molluscs (Fig. 3). In addition to diatoms, other algae, including charophytes, are the primary source of food for this snail. In the littoral zone of Lake Lednica, *P. fontinalis* was found exclusively on *C. contraria* and *N. obtusa*, smaller, the less heavily encrusted charophytes with lower $\delta^{13}C$ values of their thalli, compared to *C. tomentosa*. Other mollusc species did not show such preferences. We suggest that feeding on *C. contraria* and *N. obtusa* was a reason of the most ^{13}C-depleted shells of *P. fontinalis* among the species studied.

The clear distinction between the $\delta^{13}C$ values measured in aquatic prosobranch snails and aquatic pulmonate snails, as proposed by McConnaughey et al. (1997), was observed in the present study. Shells of the gill-breathing molluscs, *B. tentaculata* and *D. polymorpha*, were ^{13}C enriched compared to the lung-breathing snails, *R. auricularia* and *P. fontinalis* (Fig. 3). Despite the above conformity, we are sceptical to apply this explanation here. *P. fontinalis* sampled from Lake Lednica was a fully aquatic species, occurring at the depths of 5 and 6 m, and thus,

the possibility of active migration to the water surface was excluded. A similar conclusion was drawn for *R. auricularia*. Although this species was found at shallower depths compared to *P. fontinalis* at all the sites sampled, i.e., between 2 and 4 m, macrophytes to which the snail was attached were fully submerged.

Oxygen stable isotope composition of shells and encrustations

The life cycle of charophytes is depending on the species, annual or perennial and is mainly controlled by the local climatic conditions (Martin et al., 2003). During our previous field studies in Lake Lednica, most charophytes were absent in early spring, and thus, they were regarded to have an annual life cycle in this lake. Mollusc shells studied here were also regarded to have precipitated in the year of collection, as already explained above. Despite the similar growth period of charophytes and molluscs, and thus precipitation under similar water $\delta^{18}O$ values and water temperature, $\delta^{18}O$ values of encrustations and shells were different (Fig. 3). The increased oxygen stable isotope values of mollusc shells observed along the increasing water depth were accompanied by the decreased charophyte $\delta^{18}O$ values (Fig. 3). Due to those different trends, the offset between the two carbonates was not systematic and ranged between 1 and 4‰. ^{18}O-enriched values of mollusc shells compared to charophyte $CaCO_3$ (Fig. 3) are consistent with the stable isotope compositions described in previous studies (von Grafenstein et al., 2000; Yu, 2000; Hammarlund et al., 2003; Hodell et al., 2005; Apolinarska & Hammarlund, 2009; Andersson et al., 2010).

To verify whether shells and encrustations were precipitated in the isotope equilibrium with water, we used equations of Grossman & Ku (1986) and Craig (1965), respectively. The equation of Grossman & Ku (1986) presents the relationship between temperature, oxygen stable isotope composition of water and biogenic aragonite:

$$T\,^{\circ}\mathrm{C} = 20.6 - 4.34\left(\delta^{18}\mathrm{O}_{\mathrm{ARAGONITE}} - \delta^{18}\mathrm{O}_{\mathrm{WATER}}\right), \quad (1)$$

where $\delta^{18}O_{ARAGONITE}$ (V-PDB) is the oxygen stable isotope composition of the aragonite mollusc shell and $\delta^{18}O$ of water (V-SMOW) is subtracted by 0.2‰ to relate to the V-PDB standard (Wurster & Patterson, 2001).

The precipitation temperature of encrustations was estimated using the equation of Craig (1965):

$$T\,^{\circ}\mathrm{C} = 16.9 - 4.2\left(\delta^{18}\mathrm{O}_{\mathrm{CALCITE}} - \delta^{18}\mathrm{O}_{\mathrm{WATER}}\right) + 0.13\left(\delta^{18}\mathrm{O}_{\mathrm{CALCITE}} - \delta^{18}\mathrm{O}_{\mathrm{WATER}}\right)^2, \quad (2)$$

where $\delta^{18}O_{CALCITE}$ is the oxygen stable isotope value of calcite encrustation and $\delta^{18}O_{WATER}$ is the oxygen stable isotope composition of water.

Because the $\delta^{18}O$ value of water was measured in the present study only once (water was sampled in July along with macrophytes and mollusc), $\delta^{18}O_{WATER}$ used in Eqs. 1 and 2 was derived from the previous, detailed study of the physical, chemical and isotopic properties of waters in Lake Lednica, with the samples collected at monthly intervals (Pełechaty & Apolinarska, unpublished). According to the aforementioned previous study, $\delta^{18}O$ values of water in Lake Lednica changed from approximately −4.7 to −4.0‰ between May and July (approximate growth time of investigated shells and encrustations), with the mean value of −4.3‰, which was applied in calculations. We decided to use those previously measured values because $\delta^{18}O$ values of water sampled in July were consistent in both studies.

Mean temperature of precipitation of the snail shells calculated using the equation of Grossman & Ku (1986) ranged between 20.6 and 17.8°C and decreased with the lake depth (Fig. 4). Such temperatures are consistent with the mean water temperatures measured in the littoral zone of Lake Lednica between May and July (Pańczakowa, 1991; Fiszer & Michałkiewicz, 1998; Pełechaty, 2005; Pełechaty & Apolinarska, unpublished). Hence, the snail shells precipitated at the isotope equilibrium or close to the equilibrium with water, which is consistent with conclusions of the previous isotope studies of mollusc shells (Fritz & Poplawski, 1974; Leng et al., 1999; Dettman et al., 1999; Wurster & Patterson, 2001; Wu et al., 2007).

Lower temperatures of shell precipitation calculated for *D. polymorpha* (Fig. 4) indicate an earlier start of the growth of this species in the spring. This refers particularly to the *D. polymorpha* shells sampled at 5 m and below where the calculated precipitation temperature (14.2–15.5°C) is lower by approximately 3–3.5°C compared to the calculated precipitation temperature of *P. fontinalis* shells (Fig. 4). At the earlier start of *D. polymorpha* growth, the $\delta^{18}O$ values of shells would have also been

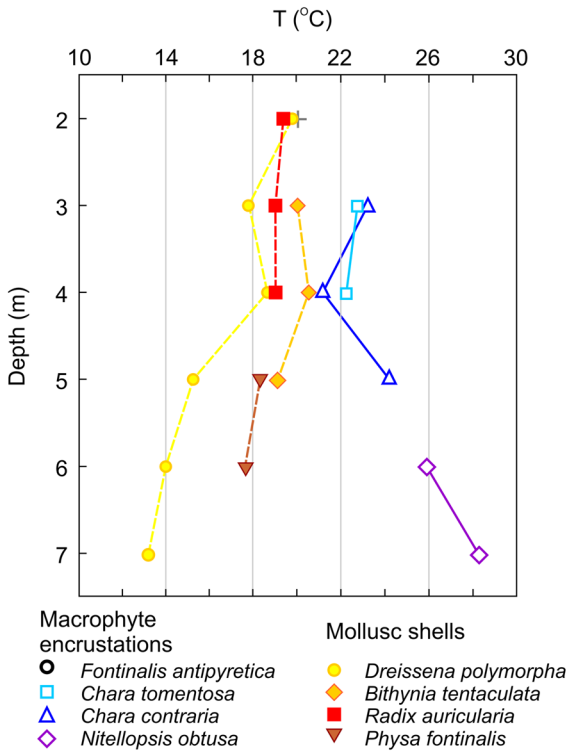

Fig. 4 Mean temperature of precipitation of macrophyte encrustations and mollusc shells calculated using equation of Craig (1965) and Grossman & Ku (1986), respectively. Despite the expected similar time of precipitation of mollusc shells and macrophyte encrustations and thus similar $\delta^{18}O$ values of water and water temperature during precipitation, the temperatures calculated differ in a wide range. The range of the temperatures calculated increases with depth

influenced by lower $\delta^{18}O$ values of water. In consequence, the actual difference in the temperature of shell precipitation by the snails and the bivalve should be even higher.

We suggest that small differences in the time of the species growth, or other vital effects not defined here resulted in the differences in $\delta^{18}O$ values between the species.

In all cases, the estimated precipitation temperatures of encrustations are higher compared to the temperatures of mollusc shell precipitation (Fig. 4). We interpret this difference as a result of the kinetic isotope effects during fast precipitation of biogenic carbonates, leading to decreased oxygen stable isotope values of carbonates compared to $\delta^{18}O$ values of water (McConnaughey, 1989). Kinetic effects are well recognized in charophyte encrustations (McConnaughey, 1989; Andrews et al., 2004; Pentecost et al., 2006) but they are regarded to be small or absent in molluscs (McConnaughey & Gillikin, 2008). The decreased $\delta^{18}O$ values in encrustations result in higher temperatures calculated (compare Eq. 2). Among the studied macrophytes, the estimated precipitation temperatures of encrustations sampled from *F. antipyretica* (the moss species) were consistent with the temperatures calculated for mollusc shells precipitation, and those carbonates are regarded to precipitate in isotopic equilibrium with water. However, this conclusion needs to be confirmed by further isotope study because it is based on the results of a single isotope measurement of *F. antipyretica* encrustations (Fig. 4).

The strongly increased estimated precipitation temperatures of *C. contraria* and *N. obtusa* encrustation, at a depth below 5 m (water temperatures as high as 28°C were calculated; Fig. 4), are unrealistic in the littoral zone of Lake Lednica. Also, those temperatures are opposite to the expected decrease in the water temperature with depth, recorded in mollusc shells (Fig. 4). ^{18}O depletion in the encrustations (Fig. 3), resulting in high temperatures calculated (Fig. 4), cannot be linked with kinetic isotope effects, because those effects are expected to be stronger at shallower stands where photosynthesis is more intensive due to higher light availability. Also, it is unlikely that kinetic isotope effects would have been stronger in *N. obtusa* compared to *C. tomentosa*. As suggested earlier, the latter species is a taller, thicker charophyte, and subsequently more intensive photosynthesis and stronger kinetic effects are expected in *C. tomentosa*. In the second, more likely explanation, lower than expected $\delta^{18}O$ values of encrustations are linked to the earlier start of the growth of the charophytes in conditions with lower $\delta^{18}O$ values of water. We suggest that the charophytes from deeper stands survived winter and thus recorded lower $\delta^{18}O$ values of water. This interpretation is confirmed by ^{13}C-depleted encrustations of *N. obtusa*.

Conclusions

In the present study, we have shown the discrepancies between the carbon and oxygen stable isotope composition of water, DIC, macrophyte carbonates and organics, and mollusc shells sampled from the littoral zone of charophyte-dominated Lake Lednica, Poland.

Isotope values were found to be species specific in macrophytes, both carbonate encrustations and thalli or tissues, and in the mollusc shells. Discussion of the factors controlling the stable isotope composition of the materials studied allowed the determination of factors responsible for the differences in the observed isotope values. The species-specific differences in $\delta^{13}C$ values of macrophyte thalli or tissues and co-occurring encrustations were controlled by the species of carbon assimilated during photosynthesis, i.e., the proportion between CO_2 and HCO_3^- assimilated, and the intensity of photosynthesis. The latter modifies the local $\delta^{13}C$ values of DIC and influences $\delta^{13}C$ values of both encrustations and shell of molluscs attached to macrophytes. Discrepancies between the $\delta^{13}C$ values of mollusc shells were regarded to be controlled by preferences of the species for the food source and the amount of organic carbon built into the shell. Oxygen stable isotope values of mollusc shells were found to be precipitated in equilibrium with $\delta^{18}O$ values of water, or close to the equilibrium. $\delta^{18}O$ values of encrustations were modified by kinetic effects resulting from fast photosynthesis in charophytes, whereas the $\delta^{18}O$ values of carbonates precipitated on *F. antipyretica* (the moss species) were consistent with the values measured in mollusc shells and were regarded to precipitate in isotopic equilibrium with water. Based on the low $\delta^{18}O$ values recorded in charophyte encrustations sampled from the water depth of 5 m and below, it was suggested that charophytes may survive winter at the deeper stands, i.e., may be perennial.

The complexity of the factors controlling $\delta^{13}C$ and $\delta^{18}O$ values of macrophyte carbonates and organics, and mollusc shells sampled from the littoral zone of the lake and the differences in the observed isotope values stress the need for careful selection of the material for palaeolimnological investigations where $\delta^{13}C$ and $\delta^{18}O$ values in carbonates and organic matter are commonly measured.

Because interpretations in the present study are based on dataset of one transect from one lake only, there is a need for further investigation and verification of the results obtained, which is planned for Lake Lednica and other Chara-lakes in the region.

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