DE GRUYTER
OPEN

Inter- and intra-specific variability in δ^{13}C and δ^{18}O values of freshwater gastropod shells from Lake Lednica, western Poland

KARINA APOLINARSKA[1] and MARIUSZ PEŁECHATY[2]

[1] *Institute of Geology, Faculty of Geographical and Geological Sciences, Adam Mickiewicz University, Krygowskiego Str. 12, 61-680 Poznań, Poland.*
E-mail: karinaap@amu.edu.pl
[2] *Department of Hydrobiology, Faculty of Biology, Adam Mickiewicz University, Umultowska Str. 89, 61-614 Poznań, Poland.*
E-mail: marpelhydro@onet.poczta.pl

ABSTRACT:

Apolinarska, K. and Pełechaty, M. 2017. Inter- and intra-specific variability in δ^{13}C and δ^{18}O values of freshwater gastropod shells from Lake Lednica, western Poland. *Acta Geologica Polonica*, **67** (3), 441–458. Warszawa.

This study focuses on the inter- and intra-specific variability in δ^{13}C and δ^{18}O values of shells and opercula of gastropods sampled live from the littoral zone of Lake Lednica, western Poland. The δ^{13}C and δ^{18}O values were measured in individual opercula of *Bithynia tentaculata* and in shells of *Bithynia tentaculata*, *Gyraulus albus*, *Gyraulus crista*, *Lymnaea* sp., *Physa fontinalis*, *Radix auricularia*, *Theodoxus fluviatilis* and *Valvata cristata*. The gastropods selected for the study are among the species most commonly found in European Quaternary lacustrine sediments. The carbon isotope composition of the gastropod shells was species-specific and the same order of species from the most to the least ^{13}C-depleted was observed at all sites sampled. Differences in shell δ^{13}C values between species were similar at all sampling sites, thus the factors influencing shell isotopic composition were interpreted as species-specific. The δ^{18}O values of shells were similar in all the species investigated. Significant intra-specific variability in shell δ^{13}C and δ^{18}O values was observed not only within the populations of Lake Lednica, which can be explained by heterogeneity of δ^{13}C DIC, δ^{18}O water and water temperature between the sites where macrophytes with snails attached were sampled, but also between individuals sampled from restricted areas of the lake's bottom. The latter points to the importance of factors related to the ontogeny of individual gastropods.

Key words: C and O stable isotopes; Recent gastropod shells; Freshwater; Inter-specific differences; Intra-specific variability; Lake Lednica.

INTRODUCTION

Gastropod shells are a common constituent of lacustrine sediments deposited within the littoral zone of lakes (Alexandrowicz 2013). Qualitative and quantitative changes in gastropod assemblages observed along sediment sequences have been interpreted in terms of past ecological conditions, e.g. lake depth, macrophyte cover and water movement (Alexandrowicz 1999; Yang *et al.* 2001; Wasylikowa *et al.* 2006; Szymanek 2013, 2014). Such malacological analysis reliably describes the past habitat of gastropods because most species found in Quaternary sediments are present in recent mollusc faunas, and

their ecological preferences are well known. In addition to this ecological approach, the application of gastropod shells in palaeolimnological studies has also used their stable carbon ($\delta^{13}C$) and oxygen ($\delta^{18}O$) isotopic composition (Böttger *et al.* 1998; Leng *et al.* 1999; Yu 2000; Hammarlund *et al.* 2003; Anadón *et al.* 2006; Baroni *et al.* 2006; Hassan *et al.* 2012; Szymanek *et al.* 2016).

Reliable palaeoclimatic reconstructions based on the stable isotope composition of gastropod shells may only be performed if the shells are precipitated in isotopic equilibrium with the water or the shift from equilibrium is known. The carbon and oxygen isotopic composition of recent gastropod shells and its relation to $\delta^{13}C$ values of DIC and $\delta^{18}O$ values of water has been studied relatively often (White *et al.* 1999; Wu *et al.* 2007; Anadón *et al.* 2010; Taft *et al.* 2012). From the available data, the expectation is that freshwater gastropod shells are precipitated in oxygen isotope equilibrium with the ambient water or in values close to equilibrium (Fritz and Poplawski 1974; Leng *et al.* 1999; Wu *et al.* 2007; Anadón *et al.* 2010). Thus, it is expected that species from a common habitat should record similar $\delta^{18}O$ values. In contrast, the stable carbon isotopic composition of gastropod shells is regarded as being out of equilibrium with ambient DIC (Aucour *et al.* 2003; Wu *et al.* 2007). The $\delta^{13}C$ values reported are shifted to be more negative than would be expected in the isotopic equilibrium with DIC by between 1 and 9‰ (McConnaughey *et al.* 1997; Aucour *et al.* 2003; Shanahan *et al.* 2005).

The results of the studies discussing $\delta^{13}C$ and $\delta^{18}O$ values of recent shells are confirmed by the data on the stable isotope composition of gastropod shells from lacustrine sediments (Böttger *et al.* 1998; Apolinarska and Hammarlund 2009; Álvarez *et al.* 2015). The differences in $\delta^{18}O$ values between species are usually small and below 1‰. In contrast, the range of $\delta^{13}C$ values of gastropod species is significant, i.e. between 2 and 7‰ (Baroni *et al.* 2006; Apolinarska 2009; De Francesco and Hassan 2013). The differences in isotope values between species result from a number of factors including shell mineralogy, gastropod physiology, habitat-related factors or the source of carbon in the shell (Shanahan *et al.* 2005 and references therein), all of which should be considered when interpreting $\delta^{13}C$ and $\delta^{18}O$ values of gastropod shells.

In response to changes in ecological conditions within the lake littoral zone, the species composition of gastropods changes throughout the history of a lake. As a consequence, it is not possible to perform isotope analysis based on a single species throughout the full length of the sediment sequence. When the difference in isotope values between species is known one species can be replaced with another. Because most of the studies investigating isotopic composition of recent gastropod shells have concentrated on single species, differences in isotope values between species are poorly known, which is the starting point for this study. An exception are the studies of Aucour *et al.* (2003) presenting the results of stable isotope analyses of four gastropod species [*Bithynia tentaculata* (Linnaeus 1758), *Theodoxus fluviatilis* (Linnaeus 1758), *Viviparus viviparus* (Linnaeus 1758) and *Radix auricularia* (Linnaeus 1758)] from the rivers Rhône and Saône in France and Shanahan *et al.* (2005) who studied $\delta^{13}C$ and $\delta^{18}O$ values in five gastropod species [*Helisoma duryi* (Wetherby 1879), *Melanoides tuberculate* (O.F. Müller 1774), *Physa virgata* (Gould 1855), *Pyrgulopsis* sp. and *Tyronia* sp.] from springs in southern Nevada.

Information on the differences in isotope values between species of recent gastropods can be supplemented with the results of isotope studies of subfossil shells where $\delta^{13}C$ and $\delta^{18}O$ values of different species from the same sediment samples have been analysed (Böttger *et al.* 1998; Jones *et al.* 2002; Apolinarska and Hammarlund 2009). However, stable isotope records of subfossil shells are often highly variable, with $\delta^{13}C$ and $\delta^{18}O$ values changing by more than 1‰ between subsequent samples (Anadón *et al.* 2006; Baroni *et al.* 2006; Apolinarska 2009). As a consequence, determination of species-specific offsets in shell isotope values is difficult because the differences in shell $\delta^{13}C$ and $\delta^{18}O$ values between gastropod species are far from being constant. Such variability in isotope composition of shells is observed mainly in studies where few gastropod shells were analysed per sediment layer (Anadón *et al.* 2006; Baroni *et al.* 2006; Apolinarska 2009). It has been shown that the differences in $\delta^{13}C$ and $\delta^{18}O$ values between individual shells sampled from 1-cm thick sediment samples can be as high as several per mill (Jones *et al.* 2002; Escobar *et al.* 2010; Apolinarska *et al.* 2015a), and thus individual shells are not representative of isotopic values of ambient DIC and water during sediment deposition. This variability in isotopic composition between shells was linked to changes in climatic and environmental conditions during deposition of the sediment sample which the shells were taken from. The optimum number of shells analysed per sediment sample was discussed by Jones *et al.* (2002), Escobar *et al.* (2010) and Apolinarska *et al.* (2015a). However, it was also shown (Shanahan *et al.* 2005) that > 2‰

variability in isotope values exists within the population, which indicates that even if recent gastropods are considered, isotope values of single shells may fail to be representative of ambient isotope conditions. This further emphasizes the need to analyse at least several shells per sediment layer in palaeolimnological studies.

In the present study, we aimed to determine inter- and intra-specific variability in $\delta^{13}C$ and $\delta^{18}O$ values of gastropod shells sampled live at several sites within the littoral zone of Lake Lednica, western Poland. The gastropods species selected for the present study, including *Bithynia tentaculata* (Linnaeus 1758), *Gyraulus albus* (O.F. Müller 1775), *Gyraulus crista* (Linnaeus 1758), *Lymnaea* sp., *Physa fontinalis* (Linnaeus 1758), *Radix auricularia* (Linnaeus 1758), *Theodoxus fluviatilis* (Linnaeus 1758) and *Valvata cristata* O.F. Müller 1775, are among the species most commonly found in Quaternary lacustrine sediments. To the best of our knowledge, no other study has compared the isotope composition of as many as eight gastropod species commonly co-occurring in European Quaternary lacustrine sediments. Such an approach allows the better understanding of the inter-specific relations in $\delta^{13}C$ and $\delta^{18}O$ values recorded in shells derived from one ecosystem, which is of value for palaeolimnological studies. If possible, inter- and intra-specific variability in $\delta^{13}C$ and $\delta^{18}O$ values of gastropod shells sampled from Lake Lednica are compared with the results of other studies to see whether the differences in values are species-specific and constant irrespective of the sampling site.

Site description

Lake Lednica is situated in the southern part of the Gniezno Lake District, 35 km east of the city of Poznań in western Poland (52°33′N, 17°23′E; Text-fig. 1). Lake Lednica is an elongated body of water with an area of 3.4 km² filling the southern part of a tunnel valley extending between Janowiec and Lednogóra. The lake is 7300 m long and has a maximum width of 825 m; 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 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 km². The physicochemical properties of Lake Lednica waters based on samples collected monthly in the summer between June and
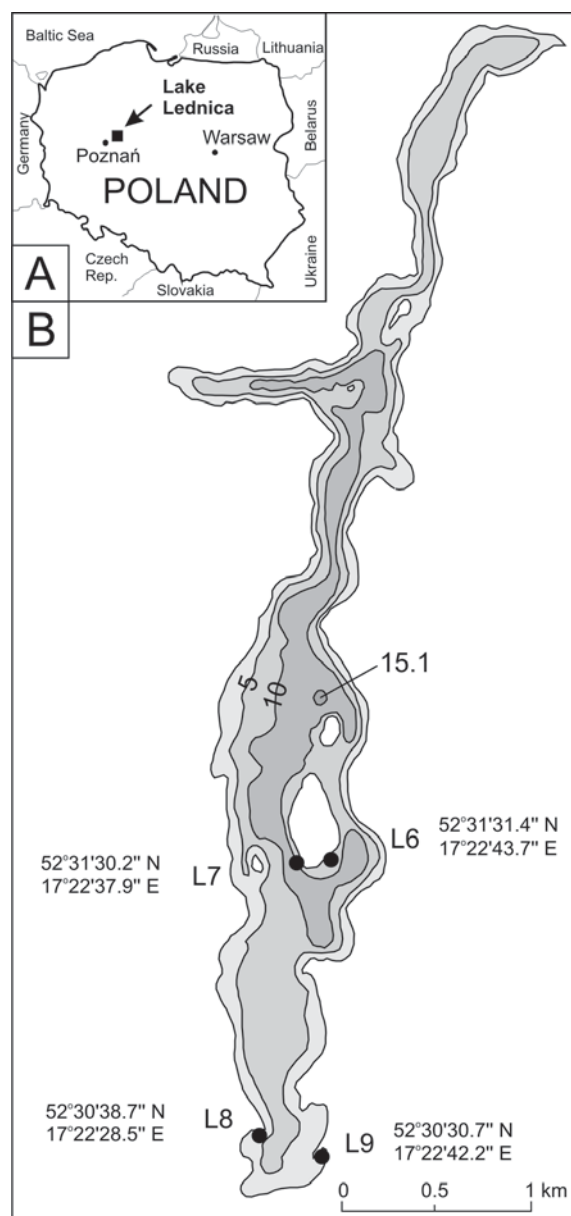
Text-fig. 1. A – Location of Lake Lednica in Poland. B – Lake Lednica with sampling sites (L6, L7, L8, L9) indicated with black dots (after Apolinarska 2013)

August at three pelagic sites include visibility by Secchi disc of 2.3 ± 0.6 m (mean ± SD), high oxygen saturation, conductivity and Ca^{2+} content (10.8 ± 1.0 mg L^{-1}, 794 ± 22 µS cm^{-1}, 110.7 ± 4.0 mg L^{-1}, respectively), (Pełechaty *et al*. 2015). The calcium rich, meso-eutrophic waters of Lake Lednica support a high diversity of charophytes (Characeae), dominant in the lake's vegetation. Charophyte meadows, extensively developed in the lake's littoral zone, offer a variety of habitats for mollusc faunas and

significantly contribute to the sedimentation of lake marl. Calcium carbonate constitutes up to 80% of the modern sediments deposited in the littoral zone of Lake Lednica.

The study area is influenced by both Atlantic and continental air masses, the former commonly prevailing. The present climate is characterised by a mean annual precipitation of 500 mm (minimum in February, 30 mm; maximum in June, 90 mm) and a mean annual temperature of 7.9°C (mean July 17.9°C; mean January -2.4°C) (Kondracki 2000). The mean oxygen isotope values of precipitation during 25 years of systematic measurements in Cracow, southern Poland (data for Poznań are not available) range between -13.5‰ in February and -7‰ in June (Duliński *et al.* 2001).

MATERIALS AND METHODS

Fieldwork

Live gastropods occurring in the Lake Lednica littoral zone were collected in June and August 2011. An Ekman's bottom grab sampler (AMS 445.11; dimensions 0.15×0.15×0.15 m) allowed for the collection of both macrophytes and bottom sediments simultaneously, and thus, ensured collection of both epiphytic and benthic gastropod species. Sampling sites were selected on the basis of the observations made during earlier reconnaissance, which allowed us to recognize mollusc distribution within the littoral zone. In June, the samples were taken from a number of sites located in four areas within the lake's littoral zone (L6, L7, L8, L9; Text-fig. 1) between one and five meters of depth. In August sampling was restricted to the sites with the most abundant molluscs observed in June. At selected sites sampling was extended to six and seven meters of depth. Samples were put into plastic bags and transported to the Institute of Geology, Poznań.

Concurrently with the macrophytes and lake bottom sediments, water samples for stable carbon and oxygen isotope analyses were collected at a depth of 0.5 m and directly above the macrophytes using a bathometer (5-l Uwitec Plexiglas Watersampler), further referred to as surface water and above-bottom water. All water samples were placed in 10-mL glass septa test tubes and preserved with $HgCl_2$. Separate vials were used for the collection of water for $\delta^{13}C$ and $\delta^{18}O$ analyses. At the same sites and depths, water temperatures were measured using an Elmetron CX-401 portable meter.

Laboratory work and isotope analyses

In the laboratory, all the live molluscs present on macrophytes were handpicked. Sediments were passed through a 1-mm sieve under running water; however, no live benthic molluscs were found. Live molluscs were treated with 50% ethyl alcohol for conservation until further treatment. Soft parts were handpicked with tweezers. Empty shells were placed in 10% H_2O_2 for 48 h to eliminate organics that might interfere with the isotope results. All shells were identified to a species level and measured (height and width) under a low-power binocular microscope (Zeiss Stemi 2000-C). Prior to stable isotope analyses the mineralogy of selected shells and opercula was verified by X-ray diffraction analysis performed at the Institute of Geology, University of Poznań. Later, individual shells and opercula of *B. tentaculata* were homogenised in an agate mortar and placed in Eppendorf vials. Ten individual shells and opercula per site of each of the gastropod species present were analysed whenever possible, and as many shells and opercula as possible were analysed otherwise (Appendix: Supplementary material – Table 1). Within each sampling site, intra-specific isotope analyses were performed on the shells and opercula of similar height and width (Appendix: Supplementary material – Table 1).

The stable isotope compositions ($\delta^{13}C$ and $\delta^{18}O$) of the gastropod shells and opercula and water samples were analysed at the Isotope Dating and Environment Research Laboratory in Warsaw, Poland. Carbonates were dissolved using 100% phosphoric acid (density 1.9) at 75°C using a Kiel IV online carbonate preparation line connected to a ThermoFinnigan Delta+ mass spectrometer. All values are reported as δ values, where $\delta = (R_{sample}/R_{standard} - 1) \times 1000$, in per mil relative to V-PDB by assigning a $\delta^{13}C$ value of 1.95‰ and a $\delta^{18}O$ value of -2.20‰ to NBS19. Reproducibility was checked on the basis of the long-term repeatability of NBS19 analysis and was better than ± 0.07 and 0.12‰, for $\delta^{13}C$ and $\delta^{18}O$, respectively.

Isotope analyses of both dissolved inorganic carbon ($\delta^{13}C_{DIC}$) and water ($\delta^{18}O_{water}$) were conducted using a GasBench-II headspace autosampler online with a Finnigan MAT 253 isotope ratio mass spectrometer (IRMS). To ensure the precision of the $\delta^{13}C_{DIC}$ results, four international carbonate standards were measured in each series of samples: NBS 18, NBS 19, LSVEC and IAEA-CO-9. The precision of the oxygen isotope results was verified by measuring three international standards (V-SMOW, GISP, SLAP) and one internal standard in each series of samples. $\delta^{18}O_{water}$ values are reported in per mil relative to V-SMOW.

Statistical analyses

Descriptive statistics, group comparisons and correlation analysis were applied with the use of Statistica 10 software (StatSoft Inc., Tulsa, OK, USA). To determine the differences between two groups of data the Mann-Whitney *U*-test was applied (surface water vs. above-bottom water, June vs. August samples) and ANOVA via the Kruskal-Wallis *H*-test was performed for more than two groups (inter-specific differentiation). The choice of tests was conditioned by different numbers of shells found during the field study. A correlation coefficient was calculated in order to determine if there were relationships between the isotope compositions of gastropod shells and the depth of their occurrence and the length of their shells. Intra-specific variability was described with the use of the coefficient of variation. For all the listed statistical tests and correlation, $P < 0.05$ was applied as being statistically significant. The study results are presented graphically as scatter biplots and box and whisker diagrams.

RESULTS

Snail species collected

The following species were collected from macrophytes sampled in Lake Lednica: *Bithynia tentaculata*, *Gyraulus albus*, *Gyraulus crista*, *Lymnaea* sp., *Physa fontinalis*, *Radix auricularia*, *Theodoxus fluviatilis* and *Valvata cristata*. Out of eight gastropod species identified during the study, only two species, *B. tentaculata* and *T. fluviatilis*, occurred in June and August. Other species were noted either in June or in August (Appendix: Supplementary material – Table 1). The juvenile shells grouped into *Lymnaea* sp. were characterized by very similar shell shape and dimensions to minimize the possible differences in isotope values resulting from species-specific preferences, including food and habitat. The latter was common for all the species studied as the snails were sampled from macrophytes. The preservation of original molluscan shell aragonite, and calcite in opercula, of selected individuals was confirmed by X-ray diffraction analysis.

Water temperature

The temperature of the surface waters in June ranged between 21.6 and 22.4°C whereas in August water temperatures were 0.5°C lower, on average, and ranged between 21.1 and 22°C (Appendix: Supplementary material – Table 1). The highest temperature of the surface waters, i.e. 23°C, was measured in an isolated bay in the southern part of the lake (Text-fig. 1). The difference in water temperature between the surface and bottom samples increased with depth. Up to 5 m of depth, the difference did not exceed 0.7°C; in most samples, however, it ranged between 0.3 and 0.5°C. At 6 and 7 m the difference increased to 1.3 and 3.1°C, respectively, as the above-bottom waters became colder with depth (Appendix: Supplementary material – Table 1).

Isotope composition of DIC and water

At the time of the field studies the stable C and O isotope compositions of DIC and water, respectively, were spatially homogenous. Strong depletion in ^{13}C was observed only at one, seven meters deep, sampling site. Neither in June nor in August were the differences between the surface waters and those sampled from above the macrophytes from which the snail shells were collected (referred to as above-bottom waters in Text-fig. 2) statistically significant (Mann-Whitney *U*-test, $P > 0.05$). In contrast, δ^{13}C-$_{DIC}$ and δ^{18}O$_{water}$ values differed significantly between the June and August samples (Mann-Whitney *U*-test, for δ^{13}C$_{DIC}$ $P = 0.000$ and for δ^{18}O$_{water}$ $P = 0.0000$). Water sampled in August was ^{13}C and ^{18}O enriched as compared to June, with visibly narrower variability of δ^{18}O values (Text-fig. 2).
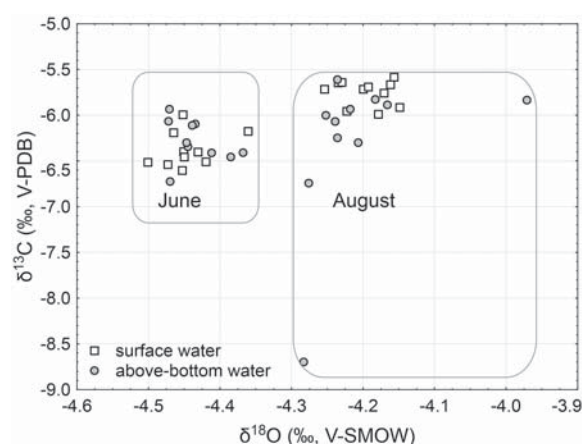
Text-fig. 2. Stable C and O isotope composition of DIC and water, respectively, at all the sites sampled (after Apolinarska 2013, modified). Surface (open squares) and above-bottom waters (filled circles) are distinguished. Note the ^{13}C and ^{18}O enrichment in DIC and water, respectively, in August
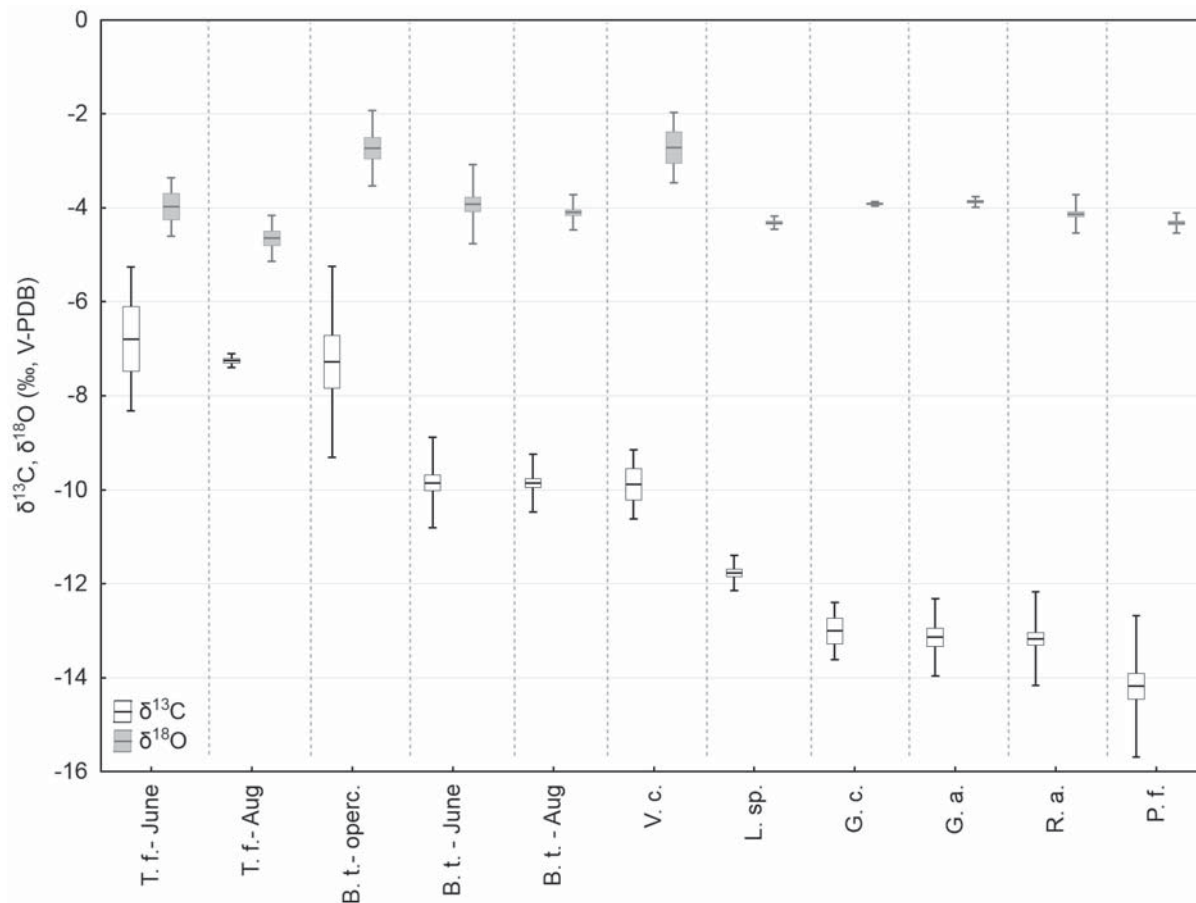
Text-fig. 3. Mean δ^{13}C and δ^{18}O values, standard errors and standard deviations in shells and opercula of the species investigated from Lake Lednica. Note that the sequence of δ^{13}C mean values measured in the shells of particular species was not followed by δ^{18}O values. Abbreviations used: G.c. – *Gyraulus crista,* B.t. – *Bithynia tentaculata,* B.t.-operc. – *Bithynia tentaculata* operculum, G.a. – *Gyraulus albus,* R.a. – *Radix auricularia,* *L*.sp. – *Lymnaea* sp., P.f. – *Physa fontinalis,* V.c. – *Valvata cristata,* T.f. – *Theodoxus fluviatilis*

Inter-specific differences in δ^{18}O and δ^{13}C values of shells

Despite the spatial homogeneity of the isotopic composition of water, a statistically significant differentiation in δ^{13}C and δ^{18}O values of shells appeared among the species collected during the study reported (Kruskal-Wallis *H*-test, for δ^{13}C P = 0.000 and for δ^{18}O P = 0.0000). δ^{13}C mean values of shells ranged from -14.18‰ in *P. fontinalis* to -6.79‰ in *T. fluviatilis* (Table 1). In contrast to δ^{13}C values, the δ^{18}O mean values were within a much smaller range, i.e., between -4.65‰ in *T. fluviatilis* collected in August and -2.71‰ in *V. cristata*. For species collected both in June and August the differences in isotopic compositions between the two months were insignificant regarding the δ^{13}C mean values while the δ^{18}O mean values revealed depletion in [18]O towards August (Table 1, Text-fig. 3). A sorting of species, from the least to the most de-

pleted in ^{13}C, was not followed by the δ^{18}O values of shells (Text-fig. 3). Along with different mean values, particular snail species also exhibited different ranges of δ^{13}C and δ^{18}O values, as presented in Table 1.

Intra-specific variation in δ^{18}O and δ^{13}C values of mollusc shells

In addition to species-to-species differentiation, the study also provides evidence of intra-species variability of isotope signatures between shells. This internal variation in isotope compositions of shells within each species is presented twofold, i.e. at individual sampling sites (Text-fig. 4) and within the whole population in the lake (Table 1), irrespective of the site-specific characteristics.

The ranges between the minimum and maximum carbon stable isotope values, observed for a given species at individual sampling sites, are between 0.15
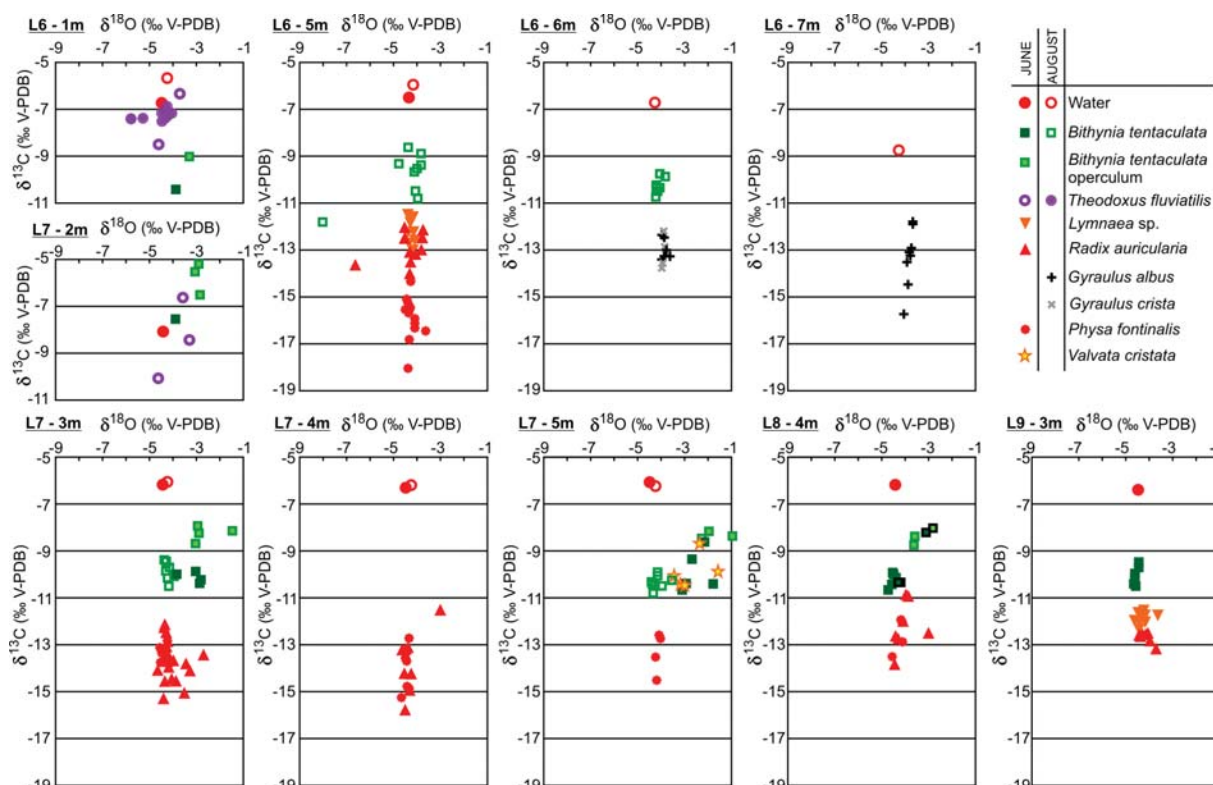
Text-fig. 4. Intra-specific variability in shell δ¹³C and δ¹⁸O values observed at the sites studied. Each symbol represents carbon and oxygen isotope composition of individual shell

and 3.92‰, with the mean value of 1.57‰ (Text-fig. 4). The ranges of δ¹⁸O values at individual sampling sites are much smaller, i.e. between 0.12 and 1.86‰, with the mean value of 0.78‰ (Text-fig. 4; Appendix: Supplementary material – Table 1). The ranges of δ¹³C and δ¹⁸O values observed within all sites for a given species studied in Lake Lednica are visibly wider and reach between 0.53 and 6.01‰, mean value 3.33‰, and 0.12 and 2.94‰, with mean value 1.53‰, respectively (Table 1).

Species	δ¹³C (‰)						δ¹⁸O (‰)					
	N	Mean	Median	Minimum	Maximum	St. dev.	CV	Mean	Median	Minimum	Maximum	St. dev.	CV
Theodoxus fluviatilis June	5	-6.79	-6.44	-8.49	-4.67	1.53	22.57	-3.98	-3.71	-4.66	-3.31	0.63	15.76
Theodoxus fluviatilis August	10	-7.26	-7.27	-7.46	-6.93	0.15	2.01	-4.65	-4.43	-5.74	-4.26	0.49	10.46
Bithynia tentaculata opercula	10	-8.33	-8.29	-8.96	-7.88	0.34	4.09	-2.66	-2.91	-3.63	-0.95	0.91	34.38
Bithynia tentaculata August	38	-9.85	-9.92	-10.75	-8.50	0.61	6.19	-4.10	-4.16	-4.75	-2.30	0.37	9.06
Valvata cristata	5	-9.88	-10.05	-10.45	-8.67	0.73	7.35	-2.71	-3.01	-3.44	-1.58	0.75	27.57
Bithynia tentaculata June	30	-9.99	-10.08	-10.64	-8.17	0.54	5.43	-3.92	-4.41	-4.72	-1.78	0.86	21.85
Lymnaea sp.	20	-11.77	-11.72	-12.62	-11.14	0.37	3.16	-4.32	-4.35	-4.59	-4.09	0.14	3.14
Gyraulus crista	5	-13.01	-13.07	-13.65	-12.10	0.61	4.70	-3.91	-3.92	-3.97	-3.85	0.04	1.14
Gyraulus albus	18	-13.14	-13.18	-15.49	-11.57	0.83	6.28	-3.87	-3.88	-4.07	-3.63	0.12	3.01
Radix auricularia	55	-13.17	-13.01	-15.67	-10.85	1.00	7.56	-4.13	-4.26	-4.67	-2.71	0.41	9.92
Physa fontinalis	30	-14.18	-13.65	-17.93	-11.92	1.51	10.65	-4.32	-4.36	-4.67	-3.65	0.21	4.87
Lake water	38	-6.34	-6.38	-6.73	-5.62	0.24	3.75	-4.43	-4.45	-4.50	-4.33	0.04	0.99

Table 1. Compilation of the δ¹³C and δ¹⁸O values of the snail species, DIC and water from Lake Lednica including mean, median, minimum and maximum isotope values. Number of shells analyzed (N), standard deviation (st. dev.) and coefficient of variation (CV, showing internal variability of isotope values in particular species) are also provided
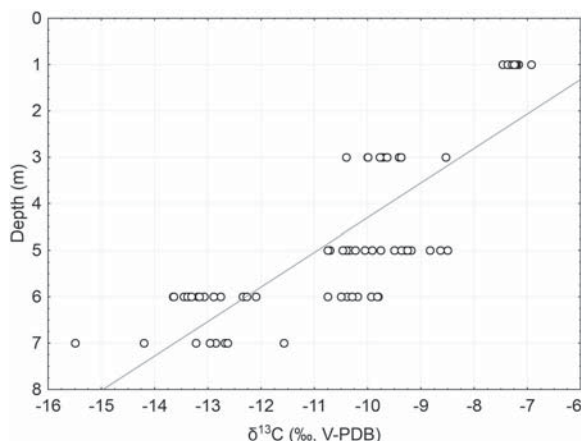
Text-fig. 5. The relationship between the depth of the occurrence of snails and the stable C isotope composition of their shell. Decrease in $\delta^{13}C_{shell}$ with depth (r = -0.8) is interpreted as resulting from increasing with depth proportion between release of ^{12}C from decomposition of organic matter and photosynthetic activity of macrophytes
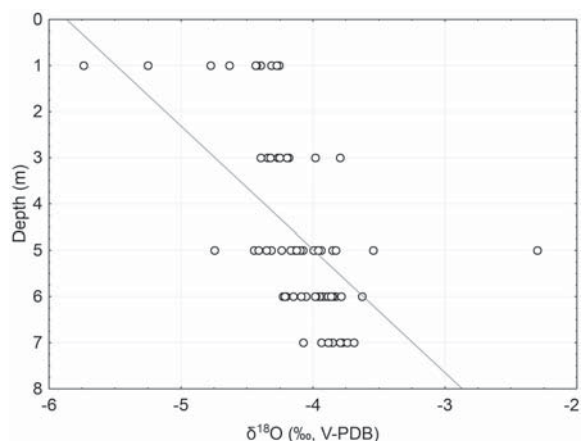
Text-fig. 6. The relationship between the depth of snails' occurrence and the stable O isotope composition of their shell. Increase in $\delta^{18}O_{shell}$ values with depth (r = 0.6) is related to the water temperature gradient

The internal variability of the isotope values in particular species is reflected in the values of the coefficient of variation (CV, Table 1; Appendix: Supplementary material – Fig. 1). The opercula of *B. tentaculata* revealed the highest CV values for $\delta^{18}O$, indicating the wide range of values represented by particular individuals. In case of shells, a June sample of *T. fluviatilis* reached the highest CV values of $\delta^{13}C$, exceeding 20% and followed by high CV values of $\delta^{18}O$. *V. cristata* and *B. tentaculata* (June sample) had the second and third highest CV values of $\delta^{18}O$ after the opercula of *B. tentaculata* (Table 1; Appendix: Supplementary material – Fig. 1). In contrast to the above species, *G. crista* and *Lymnaea sp.*

revealed minor internal variability of stable C and O isotope signatures.

Relationships between $\delta^{13}C$ and $\delta^{18}O$ values of shells and depth

Since the gastropod shells were collected from a variety of depths (Appendix: Supplementary material – Table 1), the relationships between the depth of the occurrence of the snails and the stable C and O isotope composition of their shells was tested. Although statistically significant (p = 0.00000 for $\delta^{13}C$ and P = 0.00005 for $\delta^{18}O$ values), the relationships were not very strong. $\delta^{13}C$ values decreased with the depth (r = -0.4) while the values of $\delta^{18}O$ revealed a reverse tendency (r = 0.3). The relationships appeared to be much stronger when only material collected in August was tested (r = -0.8, p = 0.0000 for $\delta^{13}C$, and r = 0.6, p = 0.00000 for $\delta^{18}O$ values; Text-figs 5, 6). These tendencies were also observed in samples collected in June, but the correlations were weaker and comparable to overall correlations mentioned above.

DISCUSSION

Below, the inter- and intra-specific variability in the isotopic composition of gastropod shells sampled from Lake Lednica is discussed in terms of the factors influencing shell $\delta^{13}C$ and $\delta^{18}O$ values. If possible, the results obtained are compared with data from other studies in order to verify whether the differences in shell isotope values observed between species from Lake Lednica are species-specific and constant irrespective of the sampling site or shell age.

Inter-specific differences in carbon stable isotope composition of shells

Although discussion of shell $\delta^{13}C$ values in terms of isotopic equilibrium or an offset from the equilibrium with $\delta^{13}C$ values of DIC is impossible because of lack of data on seasonal changes in $\delta^{13}C$ values of DIC, in our opinion the actual extent of ^{13}C-depletion of shells relative to DIC is not far from being presented in Text-fig. 7. We base this assumption on the fact that the gastropod shell main growth period is concentrated on the spring and summer seasons which are represented in the present study by $\delta^{13}C$ values of DIC measured in June and August.

The same order of species from the most to the least depleted in ^{13}C observed at different sampling sites (Text-fig. 7), i.e. *P. fontinalis*, *R. auricularia*, *G.*
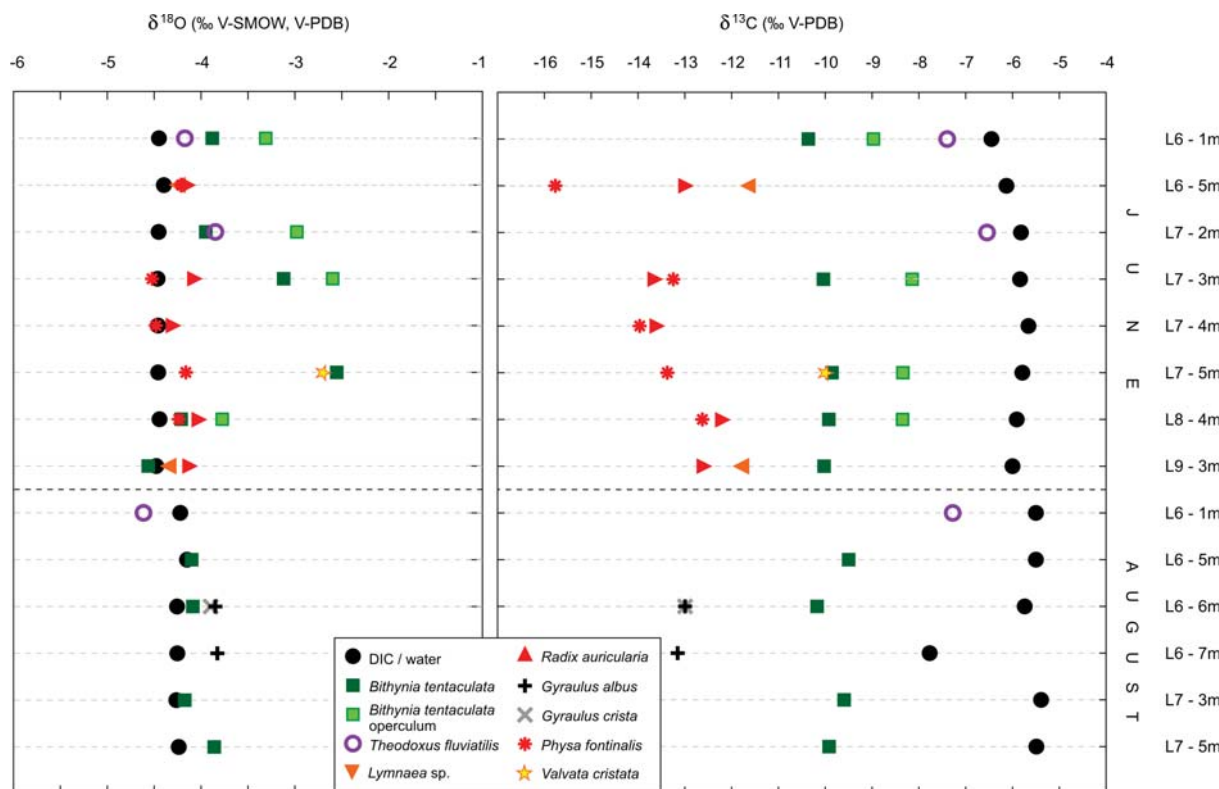
Text-fig. 7. Mean shell $\delta^{13}C$ and $\delta^{18}O$ values and above-bottom DIC $\delta^{13}C$ and water $\delta^{18}O$ values, respectively, presented separately for each sampling site from Lake Lednica

crista, *G. albus*, *Lymnaea* sp., *V. cristata*, *B. tentaculata*, *T. fluviatilis*, and similar offsets in $\delta^{13}C$ values between species allow us to conclude that the factors responsible for the differences observed are constant and species-specific.

Because of the different fractionation factors between calcite and water and aragonite and water, comparison of shell $\delta^{13}C$ values must consider the differences in shell mineralogy between species. Among the gastropod species studied all shells are composed of aragonite, whereas the opercula of *B. tentaculata* are known to be calcitic (Piechocki 1979). In some studies calcite was found in *B. tentaculata* subfossil shells; however, its presence was regarded as resulting from diagenetic and post-depositional changes in shell mineralogy (Anadón *et al.* 2010 and references therein). The average 1.6‰ ^{13}C-enrichment of the opercula relative to the shells of *B. tentaculata* (Text-fig. 7) disagrees with the expected ~1.7‰ depletion of calcitic opercula relative to aragonitic shells, both precipitated in equilibrium with DIC (Romanek *et al.* 1992). A similar difference between mean $\delta^{13}C$ values of opercula and shells, i.e. 1.2‰, was observed in specimens sampled from a Lake Geneva sediment se-

quence (Anadón *et al.* 2010), whereas, opercula from Lake Rosnowskie were on average 2.4‰ enriched in ^{13}C compared to shells derived from the same sediments (Apolinarska *et al.* 2015b). The formation of shell and operculum in *B. tentaculata* is a continuous, prolonged process, and thus the differences observed do not result from precipitation during different seasons. We suggest that the differences observed can be linked with mechanisms of shell and operculum formation, the former precipitated from extrapallial fluid occurring between shell and mantle and the latter secreted on the snails foot (Marin and Luquet 2004). However, the above suggestion requires detailed investigation of shell and operculum formation in the context of their isotope composition. This is of particular value for palaeolimnological studies because shells and opercula of *B. tentaculata* are among the most common gastropod remains in lacustrine sediments and their stable C and O isotopic compositions have been frequently used in palaeoclimatic studies (Hammarlund *et al.* 1999; Hammarlund *et al.* 2003; Anadón *et al.* 2006; Apolinarska 2009). However, little is still known about the relation between isotope composition of shells and opercula.

Strong differences in shell $\delta^{13}C$ values between species are not likely to be explained by habitat preferences of snails and thus habitat-related differences in $\delta^{13}C$ values of DIC. All species were collected from macrophytes and no preferences as for the location of the species within the macrophyte beds were observed.

Based on the data on snail reproduction time, growth rate and relatively short life span, usually below one year, it is assumed that most shells investigated in the present study were precipitated in the year of the research. *B. tentaculata* and *T. fluviatilis* with a life expectancy of between one and three years (Wächtler 2001) and two to three years (Piechocki 1979), respectively, may be the only exceptions. Larger shells of both of the species may reflect the integrated isotope record of up to three years. In order to limit the shells investigated to specimens precipitated in the year of sampling all shells with growth ceases visible, i.e. marks indicative of winter shell growth cessation, were excluded from isotope studies. Although the shells are regarded as precipitated in the same year, the exact time of shell formation can differ between species and results from the asynchronous spawning of snails. Due to the different threshold temperature required to start breeding, spawning is extended in time and occurs between March and May, usually peaking in April (Piechocki 1979). In consequence, juvenile snails occur during a few weeks. Because of active ^{12}C assimilation by primary producers during the spring phytoplankton pick, $\delta^{13}C$ values of DIC change significantly in terms of weeks and thus mean $\delta^{13}C$ values of DIC are not equal for all snails.

$\delta^{13}C$ values of prosobranch snails (*B. tentaculata, V. cristata*) were clearly distinct from pulmonate species (*G. crista, G. albus, R. auricularia, P. fontinalis*), with the latter group ^{13}C-depleted by more than 3‰ on average, compared to the former group (Text-figs 3, 7). This difference can be explained by incorporation into the shell structure of carbon from inhaling atmospheric CO_2 by lung breathing snails (McConnaughey *et al*. 1997). This mechanism, together with the influence of metabolic carbon on shell $\delta^{13}C$ values, was considered to be responsible for the strong ^{13}C depletion in *R. auricularia* shells compared with $\delta^{13}C$ values of DIC in the rivers Rhône and Saône (Aucour *et al*. 2003). Incorporation of carbon from atmospheric CO_2 was also proposed to explain the ^{13}C-depletion of *Physa virgata* shells (Shanahan *et al*. 2005). In the latter work, gastropods were observed to migrate on the reeds close to the water table of the lake. Similarly, Aucour *et al*. (2003) suggested migration of *R. auricularia* to the water surface and

thus into contact with atmospheric CO_2. In contrast, all pulmonate species sampled from Lake Lednica occurred on fully submerged macrophytes at water depths of between 3 and 7 meters and migration to the water surface to inhale atmospheric air is unlikely. Instead, feeding habits may be important here.

Incorporation of metabolic carbon into shell structure is regarded as the most plausible reason of ^{13}C-depletion of shells relative to DIC (McConnaughey and Gillikin 2008). It is proposed that the extent of the depletion is proportional, both to the dietary preferences of gastropods and to the amount of metabolic carbon bound to the shell. As a result, species-specific differences between $\delta^{13}C$ values of gastropods and DIC are noted. The dietary preferences of the species studied are similar, as most of the gastropods feed on macrophytes and epiphytic phytoplankton. Filter-feeding is among the feeding alternatives of *B. tentaculata* and *V. cristata*. Because of the possible differences in $\delta^{13}C$ values of primary producers, food preferences of the gastropods may result in different $\delta^{13}C$ values of shells. Isotope study of macrophytes in Lake Lednica (Apolinarska *et al*. 2015c) has shown strong differences in $\delta^{13}C$ values between species. Carbon isotope composition of macrophyte organics varied between -19.4‰ in *Chara tomentosa* (Linnaeus 1758) to -34.3‰ in *Nitellopsis obtusa* [(Desvaux) J. Groves 1919]. It is possible that feeding strategy, not gill or lung breathing, results in the $\delta^{13}C$ difference between prosobranch (filter-feeders) and pulmonate gastropods observed in Lake Lednica. We base this suggestion on the results of our above-cited study (Apolinarska *et al*. 2015c), where shells of bivalve species *Dreissena polymorpha* (Pallas 1769), an active filter-feeder, restricted to this feeding strategy, were at all sites ^{13}C-enriched relative to shells of both prosobranch and pulmonate gastropods. Exact conclusions on the influence of metabolic carbon on shell $\delta^{13}C$ values require detailed monitoring of the $\delta^{13}C$ values of ambient DIC and the possible food sources performed in the gastropods' natural habitat and also under controlled conditions in a laboratory.

Despite the differences in $\delta^{13}C$ values between the species analysed, and the small variability of DIC $\delta^{13}C$ values in the well-mixed waters of the Lake Lednica littoral zone, the relationship between the depth of snail habitat and the carbon stable isotope composition of shells was statistically significant, but not strong (Text-fig. 5). Decrease in $\delta^{13}C_{shell}$ with depth (r = -0.4; Text-fig. 5) is interpreted as resulting from the proportion of the release of ^{12}C from the decomposition of organic matter and that from photosynthetic

activity of macrophytes increasing with depth. This relationship shows that although δ^{13}C values of shells are species-specific and presumably shifted from isotope equilibrium with DIC they record relative changes in δ^{13}C values of the ambient DIC.

Consistent with observations in the present study, ^{13}C-depletion of shells relative to DIC and species-specific δ^{13}C values have been observed in other isotope studies of both recent and subfossil gastropods. Similar to that noted in Lake Lednica, gastropods of the genus *Physa* and species in the family Lymnaeidae have commonly been found among the most ^{13}C depleted (Fritz and Poplawski 1974; Böttger *et al*. 1998; Aucour *et al*. 2003; Apolinarska 2009; De Francesco and Hassan 2013). Shell δ^{13}C values of *Physa* have been between 2.0 and 5.2‰ lower compared to other gastropod species (Shanahan *et al*. 2005; Baroni *et al*. 2006; Apolinarska *et al*. 2015c). In the present study ^{13}C-depletion in *Physa fontinalis* shells relative to other species analysed ranges between 0.5 and 4‰, with the smallest difference observed between *P. fontinalis*, *Lymnaea* sp. and *Radix auricularia* (Text-figs 3, 7). If compared to isotope values of the species most commonly analysed in the studies of both recent and fossil shells, i.e. *B. tentaculata*, depletion of *P. fontinalis* is not constant but changes between 2.7‰ in the study of Apolinarska *et al*. (2015c), 3‰ in the present study, to 3.3‰ measured by Baroni *et al*. (2006). This variable shift in δ^{13}C values between the two species may result from variable values of one or, which seems more likely, both of them. Based on the above data, ^{13}C-depletion in *P. fontinalis* shells is suggested to be species-specific, however, it is not possible to provide an exact shift in δ^{13}C values relative to other gastropods.

In contrast to *Physa*, the difference in carbon isotope values between species of the family Lymnaeidae and *B. tentaculata* is highly variable (Fritz and Poplawski 1974; Böttger *et al*. 1998; Baroni *et al*. 2006; De Francesco and Hassan 2013; Apolinarska *et al*. 2015c). Comparison of shell δ^{13}C values of *Radix auricularia* and *Bithynia tentaculata* sampled live from Lake Lednica (Apolinarska *et al*. 2015c), the present study and rivers Rhône and Saône (Aucour *et al*. 2003) revealed high variability in the extent of ^{13}C-depletion, with shells of *Radix auricularia* depleted in ^{13}C by 1.7, 3.0 and 4.2‰, respectively. The extent of depletion is even more variable, from close to 0 to 5.2‰, when shell δ^{13}C values of different species of the family Lymnaeidae are compared with the carbon isotope composition of other gastropod species (Fritz and Poplawski 1974; Böttger *et al*. 1998; Baroni *et al*. 2006; De Francesco and Hassan 2013;

Apolinarska *et al*. 2015c). However, in most studies this depletion ranges between 2 and 3‰. Interesting data on δ^{13}C values of *Lymnaea stagnalis* shells were presented by Fritz and Poplawski (1974). *L. stagnalis* sampled from several small lakes in southwestern Ontario was on average 4.5‰ ^{13}C-depleted relative to δ^{13}C values of three species of *Helisoma* genus, whereas the carbon isotope composition of *L. stagnalis* grown in the laboratory under controlled conditions was within δ^{13}C values recorded for other species. The authors suggested that the artificial environment in the tanks and uniform food prevented the recording of the natural variability in the gastropods ecosystem. Thus, site-specific conditions are very important factors controlling shell isotope values. We regard them as a reason why offsets in the isotope composition of species are not constant, despite the fact that species-specific isotope values can be observed.

In contrast, to the above-discussed species, shells of the gastropod *Theodoxus fluviatilis* were most enriched in ^{13}C, with δ^{13}C values between 1.5 and 7.2‰ higher compared to those measured in the other gastropod species studied and closest to δ^{13}C values of DIC (Text-figs 3, 7). Those data are consistent with the results of Aucour *et al*. (2003) who found *T. fluviatilis* shells between 0.4 and 5.5‰ ^{13}C-enriched compared to other mollusc species sampled at six different sites in the rivers Rhône and Saône (Aucour *et al*. 2003). Shells of *T. fluviatilis* were by 1.5 (Aucour *et al*. 2003) and 2‰ (present study) ^{13}C-enriched relative to associated *B. tentaculata*. Again, the differences in isotope values are species-specific but not constant between studies. However, because few shells of *T. fluviatilis* were sampled, and these from the shallowest sites within Lake Lednica littoral zone exclusively, our results should be confirmed in further study.

Oxygen stable isotope composition of gastropod shells

Although the waters within the Lake Lednica littoral zone are well mixed by wave action and show insignificant spatial changes in the isotope composition of water and water temperature, a statistically significant but not strong relationship between the depth of occurrence and δ^{18}O values (r = 0.6; Text-fig. 6) of bulk shell material was noted. The 0.9‰ increase in mean δ^{18}O values of bulk shell material with depth (Text-fig. 6) agrees with > 3°C difference in water temperature measured at the shallowest and deepest sampling sites (Appendix: Supplementary material –Table 1). This calculation was based on 0.24‰ increase in shell δ^{18}O values per 1°C decrease

in water temperature (Craig 1965; Kim and O'Neil 1997). Thus, the expected sensitivity of shell $\delta^{18}O$ values to change in water temperature was recorded by the gastropod species studied. As already discussed above, the precise calculations used to show whether the shells were precipitated in isotope equilibrium with water are not possible because of lack of data on seasonal changes in water $\delta^{18}O$ values and water temperature. Precipitation of gastropod shells in isotopic equilibrium or close to equilibrium with water $\delta^{18}O$ values has been shown independently for several species, including the taxa studied here, i.e. *Lymnaea peregra* (White *et al.* 1999; Taft *et al.* 2012), *Physa virgata* (Shanahan *et al.* 2005), *Gyraulus sibirica* (Wu *et al.* 2007) and *Bithynia tentaculata* (Anadón *et al.* 2010). These findings are indirectly confirmed here by the small inter-specific variability in mean $\delta^{18}O$ values (Text-fig. 3) indicating that shell $\delta^{18}O$ values of all the species analysed in the present study can be reliably used in palaeolimnological studies.

Although $\delta^{18}O$ values were found to be similar in the species studied, they were not the same (Text-figs 3, 7). Strong ^{18}O-enrichment in some of the larger shells of *B. tentaculata* (Text-fig. 7) and increased CV values for this species (Table 1; Appendix: Supplementary material – Fig. 1), show that some of the shells may have grown in the year preceding, despite the fact that shells with growth ceases were excluded from the study. It is proposed that *B. tentaculata* recorded an integrated isotope signal from the previous year that included periods of increased stagnancy of the lakes' water, resulting in $\delta^{13}C$ and $\delta^{18}O$ changes in the shells with depth.

Similarly to those observed in $\delta^{13}C$ values, strong differences in $\delta^{18}O$ values were noted between the shells and opercula of *Bithynia tentaculata* (Text-fig. 7). According to the different fractionation factors between calcite and water and aragonite and water, $\delta^{18}O$ values of opercula should be 0.6‰ ^{18}O-depleted compared to shells (Tarutani *et al.* 1969). In contrast to the values expected, the opercula were 0.62‰ ^{18}O-enriched on average, however, the difference was usually between 0.5 and 0.55‰. Similar to our results, a 0.47‰ difference between opercula and shells from Lake Geneva sediments was observed by Anadón *et al.* (2006), whereas in subfossil material from Lake Rosnowskie (Apolinarska *et al.* 2015b) opercula were 0.61‰ ^{18}O-enriched compared to shells of *B. tentaculata*. Thus, the difference in ^{18}O values between shells and opercula is very constant, irrespective of the sampling site and shell age. As already suggested discussing the $\delta^{13}C$ values of opercula, we propose that a different mechanism

of precipitation as responsible for the significant shift between shell and opercula.

Similar to the findings of the present study, a small inter-specific difference in $\delta^{18}O$ values, usually < 1‰, between species of recent gastropods, was measured by Fritz and Poplawski (1974) and De Francesco and Hassan (2013). Observations of the oxygen stable isotope composition of recent molluscs are in agreement with the results of the studies of subfossil shells by Fritz and Poplawski (1974), Böttger *et al.* (1998), Apolinarska (2009), Apolinarska and Hammarlund (2009) and De Francesco and Hassan (2013).

Although, precipitation of shell in equilibrium with $\delta^{18}O$ water was suggested for a number of species, as pointed out above, the intra-specific variability in shell $\delta^{18}O$ values, discussed in detail in the next section, must also be considered.

Intra-specific differences in the stable isotope composition of shells

Investigation of $\delta^{13}C$ and $\delta^{18}O$ values of individual gastropod shells from Lake Lednica was carried out to show the extent of intra-specific variability in the isotopic composition of shells sampled within the lake littoral zone. We intended to test whether the 2‰ variability in shell isotope values of gastropods from springs in Nevada (Shanahan *et al.* 2005) was specific to that site or if it is of broader meaning.

The mean differences in $\delta^{13}C$ and $\delta^{18}O$ values within populations of all the species sampled from Lake Lednica were 3.3‰ and 1.6‰, respectively (Table 1). Although the waters in the lake's littoral zone are regarded as well mixed by wave action and exhibit minor changes in their physicochemical (Pełechaty 2005) and isotopic composition, a number of factors can result in heterogeneity of $\delta^{13}C$ DIC, $\delta^{18}O$ water and water temperature between sites where macrophytes with snails attached were sampled. Those factors include differences in depth, density of macrophyte patches or variable exposure to wave action and thus differences in the degree of water mixing. Also, one population includes snails at different ontogenic stages. In most freshwater snails spawning occurs twice a year, in early spring and in summer, additionally, most species have a one to two-year life span (Frömming 1956; Piechocki 1979). In consequence, at least two generations can co-occur in the lake. Also, as discussed above, spawning is not synchronous and within one generation individual snails may have experienced slightly different environmental conditions during growth.

To minimize the influence of within-lake hetero-

geneity of DIC δ^{13}C values, water δ^{18}O values and water temperature on δ^{13}C and δ^{18}O values of shells, variability in isotope composition among mono-specific shells occurring in a restricted area in the lake littoral zone, i.e. at a single sampling site, was investigated. Further, to eliminate the influence of different ontogenic stages, and hence shell precipitation under variable stable isotope composition of water and water temperature, within one species shells of uniform sizes were chosen for isotope analyses (Appendix: Supplementary material – Table 1). Despite these restrictions the mean intra-specific variability in δ^{13}C and δ^{18}O values of the shells collected from a macrophyte patch covering the bottom surface of ~0.02 m^2, was 1.5‰ and 0.8‰, respectively (Text-fig. 4). This intra-specific variability in the carbon and oxygen isotope values of shells is very likely to have resulted from factors internal to the snail. Physiologic processes influencing shell isotope values can include the variable proportion of metabolic and environmental carbon built into the shell, different mollusc growth rates, infection by parasites, and other biologically controlled processes. Some of the variation in δ^{13}C and δ^{18}O values can result from the location of snails within the macrophyte patches.

The intra-specific differences in shell isotope values observed by Shanahan *et al.* (2005) and those described in the present paper are not unique to gastropods. The within-species variation in δ^{13}C values of marine and freshwater bivalves studied by Keith *et al.* (1964) was 2.45‰ and 2.21‰, respectively. The range of δ^{18}O values observed in the same molluscs was less than one per mill with the average value of 0.59‰. Lorrain *et al.* (2004) observed 1 to 2.5‰ variability in δ^{13}C values between scallops derived from a dredge haul that sampled the bottom over a distance of less than 500 m. δ^{13}C and δ^{18}O values in *Dreissena polymorpha* shells sampled from Lake Lednica varied by 2 and 1.5‰, on average (Apolinarska 2013).

CONCLUSIONS

Isotope investigation of eight recent freshwater gastropod species from Lake Lednica, western Poland, provided insight into the inter-specific differences in δ^{13}C and δ^{18}O values in shells between *Bithynia tentaculata*, *Gyraulus albus*, *Gyraulus crista*, *Lymnaea* sp., *Physa fontinalis*, *Radix auricularia*, *Theodoxus fluviatilis* and *Valvata cristata*. The mollusc species investigated are widely distributed in Europe both contemporarily and in Quaternary lake deposits.

Among the gastropods studied the same order of species from the most to the least ^{13}C-depleted was observed at all sites sampled, i.e. *P. fontinalis*, *R. auricularia*, *G. crista*, *G. albus*, *Lymnaea* sp., *V. cristata*, *B. tentaculata* and *T. fluviatilis*. Offsets in δ^{13}C values between gastropods were found to be very similar at all sites sampled and it was concluded that factors responsible for the differences observed are constant and species-specific. A clear difference was observed between δ^{13}C values of prosobranch snails, i.e., *B. tentaculata* and *V. cristata* and pulmonate species, i.e. *G. crista*, *G. albus*, *R. auricularia* and *P. fontinalis*, with the latter group ^{13}C-depleted by more than 3‰ on average, compared to the former group. Although δ^{13}C values of shells are species-specific and presumably shifted from isotope equilibrium with DIC, they record relative changes in δ^{13}C values of the ambient DIC.

Inter-specific variability in shell δ^{18}O values was much lower compared to δ^{13}C records. Mean δ^{18}O values were very similar in all the species analysed. Opercula of *Bithynia tentaculata* were found as an exception, however, the difference in δ^{18}O values between shells and opercula of *B. tentaculata* was found to be very similar at all sampling sites.

Intra-specific variability in shell δ^{13}C and δ^{18}O values was noted in all the species investigated even when shells were sampled from a very restricted area within a lake, with homogenous environmental characteristics. Differences in isotope values between individual shells showed that the stable isotope composition of one shell cannot be assumed as representative of a population.

Because of the inter- and intra-specific variability in δ^{13}C and δ^{18}O values of gastropod shells derived from Lake Lednica, in studies interpreting the isotope composition of shells in terms of the characterization of the recent environment or the reconstruction of past environmental conditions it is advisable to prepare mono-specific samples, each composed of at least several shells. The number of shells analysed depends on the isotope variability within the sediment studied. This approach refers primarily to the δ^{13}C values, and is due to the significant species-specific differences observed. Despite those inter-specific differences, the statistically significant relationship between the depth of snail habitat and the carbon stable isotope composition of bulk shell material indicates that shell δ^{13}C values followed the DIC δ^{13}C values in Lake Lednica. Thus, changes in shell δ^{13}C values can be used to reconstruct relative changes in ancient DIC δ^{13}C values. Whenever two or more species are used within a sediment sequence, their records need to overlap to verify the differences in δ^{13}C values.

REFERENCES

Alexandrowicz, S.W. 1999. *Bithynia tentaculata* (Linnaeus, 1758) as an indicator of age and deposition environment of quaternary sediments. *Folia Malacologica*, **7**, 79–88.

Alexandrowicz, W.P. 2013. Evolution of the malacological assemblages in North Poland during the Late Glacial and Early Holocene. *Folia Quaternaria*, **70**, 39–69.

Álvarez, D., Fagel, N., Araneda, A., Jana-Pinninghoff, P., Keppens, E. and Urrutia, R. 2015. Late Holocene climate variability on the eastern flank of the Patagonian Andes (Chile): A δ^{18}O record from mollusks in Lago Cisnes (47°S). *The Holocene,* **25**, 1220–1230.

Anadón, P., Martín-Rubio, M., Robles, F., Rodriguez-Lázaro, J., Utrilla, R. and Vázquez, A. 2010. Variation in Sr uptake in the shell of the freshwater gastropod *Bithynia tentaculata* from Lake Arreo (northern Spain) and culture experiments. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **288**, 24–34.

Anadón, P., Moscariello, A., Rodríguez-Lázaro, J. and Filippi, M.L. 2006. Holocene environmental changes of Lake Geneva (Lac Léman) from stable isotopes (δ^{13}C, δ^{18}O) and trace element records of ostracod and gastropod carbonates. *Journal of Paleolimnology*, **36**, 117–117.

Apolinarska, K. 2009. Reconstructions of the early and middle Holocene climate and environment based on δ^{13}C and δ^{18}O records in biogenic carbonates; Lake Niepruszewskie, western Poland. *Acta Geologica Polonica*, **59**, 359–370.

Apolinarska, K. 2013. Stable isotope compositions of recent *Dreissena polymorpha* (Pallas) shells: paleoenvironmental implications. *Journal of Paleolimnology*, **50**, 353–364.

Apolinarska, K. and Hammarlund, D. 2009. Multi-component stable isotope records from Late Weichselian and early Holocene lake sediments at Imiolki, Poland: palaeoclimatic and methodological implications. *Journal of Quaternary Science*, **24**, 948–959.

Apolinarska, K., Pełechaty, M. and Kossler, A. 2015. Within-sample variability of δ^{13}C and δ^{18}O values of freshwater gastropod shells and the optimum number of shells to measure per sediment layer in the Paddenluch palaeolacustrine sequence, Germany. *Journal of Paleolimnology*, **54**, 305–323.

Apolinarska, K., Pełechaty, M. and Noskowiak, D. 2015. Differences in stable isotope compositions of freshwater snails from surface sediments of two Polish shallow lakes. *Limnologica – Ecology and Management of Inland Waters*, **53**, 95–105.

Apolinarska, K., Pełechaty, M. and Pronin, E. 2015. 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). *Hydrobiologia*, **768**, 1–17.

Aucour, A., Sheppard, S. and Savoye, R. 2003. δ^{13}C of fluvial mollusk shells (Rhone River): a proxy for dissolved inorganic carbon? *Limnology and Oceanography*, **48**, 2186–2193.

Baroni, C., Zanchetta, G., Fallick, A.E. and Longinelli, A. 2006. Mollusca stable isotope record of a core from Lake Frassino, northern Italy: hydrological and climatic changes during the last 14 ka. *The Holocene*, **16**, 827–837.

Böttger, T., Hiller, A., Junge, F.W., Litt, T., Mania, D. and Scheele, N. 1998. Late Glacial stable isotope record, radiocarbon stratigraphy, pollen and mollusc analyses from the Geisental area, Central Germany. *Boreas*, **27**, 88–100.

Craig, H. 1965. The measurement of oxygen isotope temperatures. In: Tongiorgi, E. (Ed.), Stable isotopes in Oceanographic Studies and Paleotemperatures, Spoleto, Italy, pp. 161–182.

Duliński, M., Florkowski, T., Grabczak, J. and Różański, K. 2001. 25 lat systematycznych pomiarów składu izotopowego opadów na terenie Polski. *Przegląd Geologiczny*, **49**, 250–256.

De Francesco, C.G. and Hassan, G.S. 2013. Stable isotope composition of freshwater mollusk shells from central-western Argentina. *Revista Brasileira de Paleontologia*, **16**, 213–224.

Escobar, J., Curtis, J., Brenner, M., Hodell, D. and Holmes, J. 2010. Isotope measurements of single ostracod valves and gastropod shells for climate reconstruction: evaluation of within-sample variability and determination of optimum sample size. *Journal of Paleolimnology*, **43**, 921–938.

Fritz, P. and Poplawski, S. 1974. [18]O and ^{13}C in the shells of freshwater molluscs and their environments. *Earth and Planetary Science Letters*, **24**, 91–98.

Frömming, E. 1956. Biologie der mitteleuropäischen Süßwasserschnecken. 313 pp. Duncker & Humblot, Berlin.

Hammarlund, D., Björck, S., Buchardt, B., Israelson, C. and Thomsen, C.T. 2003. Rapid hydrological changes during the Holocene revealed by stable isotope records of lacustrine carbonates from Lake Igelsjön, southern Sweden. *Quaternary Science Reviews*, **22**, 353–370.

Hammarlund, D., Edwards, T.W.D., Bjorck, S., Buchardt, B. and Wohlfarth, B. 1999. Climate and environment during the Younger Dryas (GS-1) as reflected by composite stable isotope records of lacustrine carbonates at Torreberga, southern Sweden. *Journal of Quaternary Science*, **14**, 17–28.

Hassan, F.A., Hamdan, M.A., Flower, R.J. and Keatings, K. 2012. The oxygen and carbon isotopic records in Holocene freshwater mollusc shells from the faiyum paleolakes, Egypt: Their paloenvironmental and paleoclimatic implications. *Quaternary International*, **266**, 175–187.

Jones, M.D., Leng, M.J., Eastwood, W.J., Keen, D.H. and Turney, C.S.M. 2002. Interpreting stable-isotope records from freshwater snail-shell carbonate: a Holocene case study from Lake Gölhisar, Turkey. *The Holocene*, **12**, 629–634.

Keith M.L., A.G.M., Eichler R. 1964. Carbon and oxygen isotopic composition of mollusk shells from marine and freshwater environments. *Geochimica et Cosmochimica Acta*, **28**, 1757–1786.

Kim, S.-T. and O'Neil, J.R. 1997. Equilibrium and nonequilibrium oxygen isotope effects in synthetic carbonates. *Geochimica et Cosmochimica Acta*, **61**, 3461–3475.

Kolendowicz, L. 1992. Wahania poziomu wód Jeziora Lednickiego w świetle badań osadów terasowych. *Badania Fizjograficzne nad Polską Zachodnią*, **43A**, 47–53.

Kondracki, J. 2000. Geografia regionalna Polski. 440 pp. Państwowe Wydawnictwo Naukowe, Warsaw.

Leng, M.J., Lamb, A.L., Lamb, H.F. and Telford, R.J. 1999. Palaeoclimatic implications of isotopic data from modern and early Holocene shells of the freshwater snail *Melanoides tuberculata*, from lakes in the Ethiopian Rift Valley. *Journal of Paleolimnology*, **21**, 97–106.

Lorrain, A., Paulet, Y.-M., Chauvaud, L., Dunbar, R., Mucciarone, D. and Fontugne, M. 2004. δ¹³C variation in scallop shells: Increasing metabolic carbon contribution with body size? *Geochimica et Cosmochimica Acta*, **68**, 3509–3519.

Marin, F. and Luquet, G. 2004. Molluscan shell proteins. *Comptes Rendus Palevol*, **3**, 469–492.

McConnaughey, T. and Gillikin, D. 2008. Carbon isotopes in mollusk shell carbonates. *Geo-Marine Letters*, **28**, 287–299.

McConnaughey, T.A., Burdett, J., Whelan, J.F. and Paull, C.K. 1997. Carbon isotopes in biological carbonates: Respiration and photosynthesis. *Geochimica et Cosmochimica Acta*, **61**, 611–622.

Pełechaty, M. 2005. Does spatially varied phytolittoral vegetation with significant contribution of charophytes cause spatial and temporal heterogeneity of physical-chemical properties of the pelagic waters of a tachymictic lake? *Polish Journal of Environmental Studies*, **14**, 63–73.

Pełechaty, M., Ossowska, J., Pukacz, A., Apolinarska, K. and Siepak, M. 2015. Site-dependent species composition, structure and environmental conditions of *Chara tomentosa* L. meadows, western Poland. *Aquatic Botany*, **120**, **Part A**, 92–100.

Piechocki, A. 1979. Mięczaki (Mollusca). Ślimaki (Gastropoda) 187 pp. Państwowe Wydawnictwo Naukowe, Warszawa-Poznań.

Romanek, C.S., Grossman, E.L. and Morse, J.W. 1992. Carbon isotope fractionation in synthetic aragonite and calcite: Effects of temperature and precipitation rate. *Geochimica et Cosmochimica Acta*, **56**, 419–430.

Shanahan, T.M., Pigati, J.S., Dettman, D.L. and Quade, J. 2005. Isotopic variability in the aragonite shells of freshwater gastropods living in springs with nearly constant temperature and isotopic composition. *Geochimica et Cosmochimica Acta*, **69**, 3949–3966.

Szymanek, M. 2013. Palaeoecology of the Holsteinian lake in vicinity of Wilczyn (eastern Poland) based on molluscan studies. *Geological Quarterly*, **57**, 637–648.

Szymanek, M. 2014. Environmental changes of the Mazovian (Holsteinian/~MIS 11) palaeolake near Szymanowo (eastern Poland) in the light of malacological analysis. *Acta Geologica Polonica*, **64**, 249–260.

Szymanek, M., Bińka, K. and Nitychoruk, J. 2016. Stable ¹⁸O and ¹³C isotope records of *Viviparus diluvianus* (Kunth, 1865) shells from Holsteinian (MIS 11) lakes of eastern Poland as palaeoenvironmental and palaeoclimatic proxies. *Boreas*, **45**, 109–121.

Taft, L., Wiechert, U., Riedel, F., Weynell, M. and Zhang, H. 2012. Sub-seasonal oxygen and carbon isotope variations in shells of modern Radix sp. (Gastropoda) from the Tibetan Plateau: potential of a new archive for palaeoclimatic studies. *Quaternary Science Reviews*, **34**, 44–56.

Tarutani, T., Clayton, R.N. and Mayeda, T.K. 1969. The effect of polymorphism and magnesium substitution on oxygen isotope fractionation between calcium carbonate and water. *Geochimica et Cosmochimica Acta*, **33**, 987–996.

Wächtler, D.K. 2001. Reproductive Biology and Life History Strategy of *Bithynia tentaculata* (Linnaeus, 1758) and *Bithynia leachii* (Sheppard, 1823). 175 pp. Vom Fachbereich Biologie der Universität Hannover.

Wasylikowa, K., Witkowski, A., Walanus, A., Hutorowicz, A., Alexandrowicz, S.W. and Langer, J.J. 2006. Palaeolimnology of Lake Zeribar, Iran, and its climatic implications. *Quaternary Research*, **66**, 477–493.

White, R.M.P., Dennis, P.F. and Atkinson, T.C. 1999. Experimental calibration and field investigation of the oxygen isotopic fractionation between biogenic aragonite and water. *Rapid Commun Mass Spectrom*, **13**, 1242–1247.

Wu, J., Schleser, G.H., Lücke, A. and Li, S. 2007. A stable isotope record from freshwater lake shells of the eastern Tibetan Plateau, China, during the past two centuries. *Boreas*, **36**, 38–46.

Yang, J., Karrow, P.F. and Mackie, G.L. 2001. Paleoecological analysis of molluscan assemblages in two marl deposits in Waterloo Region, southwestern Ontario, Canada. *Journal of Paleolimnology*, **25**, 313–328.

Yu, Z. 2000. Ecosystem response to Lateglacial and early Holocene climate oscillations in the Great Lakes region of North America. *Quaternary Science Reviews*, **19**, 1723–1747.

APPENDIX: Supplementary material

	August								June										
Location	L7	L7	L7	L6	L6	L6	L6	L6	L9	L8	L8	L8	L7	L7	L7	L6	L6	L6	L6
Depth	5m	5m	5m	3m	7m	6m	5m	1m	3m	4m	4m	5m	4m	4m	3m	3m	2m	5m	1m
Water temperature – surface	21.9	21.9	21.9	22.0	21.7	21.4	21.5	21.1	23.0	22.4	22.4	21.8	21.7	21.7	21.9	21.9	22.1	22.2	21.6
Water temperature – above bottom	20.8	20.8	20.8	21.2	18.6	20.1	20.8	21.3	22.4	21.9	21.9	21.4	21.3	21.3	21.3	21.3	21.5	21.3	21.6
Gyraulus crista – N						5						5				4			
Gyraulus crista – height						2						7–9				10–11			
Gyraulus crista – $\delta^{13}C$ mean						-12.99						-9.85				-10.04			
Gyraulus crista – $\delta^{13}C$ min.						-13.65						-10.55				-10.25			
Gyraulus crista – $\delta^{13}C$ max.						-12.1						-8.55				-9.81			
Gyraulus crista – $\delta^{18}O$ mean						-3.91						-2.54				-3.13			
Gyraulus crista – $\delta^{18}O$ min.						-3.97						-3.11				-3.86			
Gyraulus crista – $\delta^{18}O$ max.						-3.85						-1.78				-2.8			
Bithynia tentaculata – N	2	3	5	10		8	10		10	5	5								1
Bithynia tentaculata – height	8–10	4–5	1.5–3	4		2–3	2–4		3–4	2.5–3.5	10–12								10
Bithynia tentaculata – $\delta^{13}C$ mean	-9.48	-10.18	-10.37	-9.65		-10.21	-9.51		-10.01	-10.15	-9.59								-10.4
Bithynia tentaculata – $\delta^{13}C$ min.	-10.47	-10.4	-10.75	-10.4		-10.75	-10.71		-10.47	-10.64	-10.32								-10.4
Bithynia tentaculata – $\delta^{13}C$ max.	-8.5	-9.91	-10.05	-8.53		-9.8	-8.63		-9.48	-9.89	-8.17								-10.4
Bithynia tentaculata – $\delta^{18}O$ mean	-3.13	-4.01	-4.29	-4.2		-4.11	-4.13		-4.54	-4.53	-3.87								-3.87
Bithynia tentaculata – $\delta^{18}O$ min.	-3.96	-4.35	-4.41	-4.4		-4.23	-4.75		-4.65	-4.72	-4.32								-3.87
Bithynia tentaculata – $\delta^{18}O$ max.	-2.3	-3.54	-4.17	-3.8		-3.83	-3.83		-4.42	-4.37	-3.14								-3.87
Bithynia tentaculata operculum – N					8						1	2				4			1
Bithynia tentaculata operculum – height					2–3						5	5				4			1
Bithynia tentaculata operculum – $\delta^{13}C$ mean					-13.2						-7.99	-8.29				-8.17			-8.96
Bithynia tentaculata operculum – $\delta^{13}C$ min.					-15.49						-7.99	-8.37				-8.59			-8.96
Bithynia tentaculata operculum – $\delta^{13}C$ max.					-11.57						-7.99	-8.22				-7.88			-8.96
Bithynia tentaculata operculum – $\delta^{18}O$ mean					-3.84						-3.37	-1.44				-2.58			-3.32
Bithynia tentaculata operculum – $\delta^{18}O$ min.					-4.07						-3.37	-1.93				-3.02			-3.32
Bithynia tentaculata operculum – $\delta^{18}O$ max.					-3.69						-3.37	-0.95				-1.45			-3.32
Gyraulus albus – N						9													
Gyraulus albus – height						2–3													
Gyraulus albus – $\delta^{13}C$ mean						-13.05													
Gyraulus albus – $\delta^{13}C$ min.						-13.64													
Gyraulus albus – $\delta^{13}C$ max.						-12.27													
Gyraulus albus – $\delta^{18}O$ mean						-3.89													
Gyraulus albus – $\delta^{18}O$ min.						-3.98													
Gyraulus albus – $\delta^{18}O$ max.						-3.63													

Species	N	height	$\delta^{13}C$ mean	$\delta^{13}C$ min.	$\delta^{13}C$ max.	$\delta^{18}O$ mean	$\delta^{18}O$ min.	$\delta^{18}O$ max.
Radix auricularia	10	4–5	-13.13	-13.95	-12.17	-4.2	-4.5	-3.81
	10	3–4	-13.01	-14.04	-12.2	-4.35	-4.67	-4.22
	10	6–8	-14.25	-15.29	-13.36	-3.79	-4.41	-2.71
	4	3–4	-12.61	-13.1	-11.3	-4.15	-4.67	-3.01
	4	4–5	-14.64	-15.61	-14.09	-4.42	-4.55	-4.27
	7	3–4	-12.17	-13.76	-10.85	-4.04	-4.46	-3.02
	10	4	-12.64	-13.15	-12.35	-4.13	-4.41	-3.69
Lymnaea sp.	10	3–4	-11.77	-12.62	-11.14	-4.28	-4.41	-4.09
	10	4	-11.77	-12.22	-11.52	-4.34	-4.59	-4.14
Physa fontinalis	10	2–3	-15.86	-17.93	-14.23	-4.26	-4.52	-3.65
	6	3–4	-13.19	-13.68	-12.75	-4.46	-4.54	-4.26
	7	3–5	-13.94	-15.17	-12.67	-4.46	-4.67	-4.33
	4	2.5–3	-13.33	-14.51	-12.61	-4.13	-4.23	-4
	4	3–4	-12.56	-13.44	-11.92	-4.26	-4.56	-4.13
Theodoxus fluviatilis	2	4	-7.4	-8.49	-6.3	-4.17	-4.64	-3.71
	3	4	-6.39	-8.06	-4.67	-3.85	-4.66	-3.31
	10	2–3	-7.26	-7.46	-6.93	-4.64	-5.74	-4.26
Valvata cristata	5	5	-9.88	-10.45	-8.67	-2.71	-3.44	-1.58

Table 1. Compilation of the $\delta^{13}C$ and $\delta^{18}O$ values of the snail species, DIC and water from Lake Lednica including mean, median, minimum and maximum isotope values. Number of shells analyzed (N), standard deviation (st. dev.) and coefficient of variation (CV, showing internal variability of isotope values in particular species) are also provided
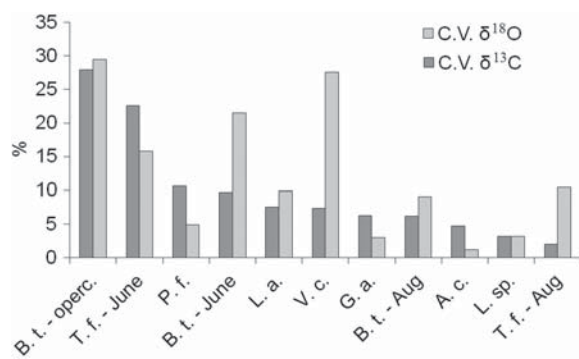
Fig. 1. Internal variability of isotope values in particular species reflected in values of coefficient of variation (CV). Abbreviations used: G.c. – *Gyraulus crista,* B.t. – *Bithynia tentaculata,* B.t.-operc. – *Bithynia tentaculata* operculum, G.a. – *Gyraulus albus,* R.a. – *Radix auricularia,* L.sp. – *Lymnaea* sp., P.f. – *Physa fontinalis,* V.c. – *Valvata cristata,* T.f. – *Theodoxus fluviatilis*