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

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ABSTRACT:


This study focuses on the inter- and intra-specific variability in $\delta^{13}$C and $\delta^{18}$O values of shells and opercula of gastropods sampled live from the littoral zone of Lake Lednica, western Poland. The $\delta^{13}$C and $\delta^{18}$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 $\delta^{13}$C values between species were similar at all sampling sites, thus the factors influencing shell isotopic composition were interpreted as species-specific. The $\delta^{18}$O values of shells were similar in all the species investigated. Significant intra-specific variability in shell $\delta^{13}$C and $\delta^{18}$O values was observed not only within the populations of Lake Lednica, which can be explained by heterogeneity of $\delta^{13}$C DIC, $\delta^{18}$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 palaeoclimate 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 tuberculata (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 isotopic 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 $> 2\%_o$
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 δ¹³C and δ¹⁸O 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 δ¹³C and δ¹⁸O values recorded in shells derived from one ecosystem, which is of value for palaeolimnological studies. If possible, inter- and intra-specific variability in δ¹³C and δ¹⁸O 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 August at three pelagic sites include visibility by Secchi disc of 2.3 ± 0.6 m (mean ± SD), high oxygen saturation, conductivity and Ca²⁺ 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 (Dulfiński 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 mollusks 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 HgCl₂. Separate vials were used for the collection of water for δ¹³C and δ¹⁸O 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% H₂O₂ 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 (δ¹³C and δ¹⁸O) 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 δ = (Rsample/Rstandard – 1) × 1000, in per mil relative to V-PDB by assigning a δ¹³C value of 1.95‰ and a δ¹⁸O value of -2.2‰ 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 δ¹³C and δ¹⁸O, respectively.

Isotope analyses of both dissolved inorganic carbon (δ¹³Cdic) and water (δ¹⁸Owater) 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 δ¹³Cdic 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. δ¹⁸Owater 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, $\delta^{13}$C-DIC and $\delta^{18}$O-water values differed significantly between the June and August samples (Mann-Whitney $U$-test, for $\delta^{13}$C-DIC $P = 0.000$ and for $\delta^{18}$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 $\delta^{18}$O values (Text-fig. 2).
Inter-specific differences in δ¹³C and δ¹⁸O values of shells

Despite the spatial homogeneity of the isotopic composition of water, a statistically significant differentiation in δ¹³C and δ¹⁸O values of shells appeared among the species collected during the study reported (Kruskal-Wallis H-test, for δ¹³C $P = 0.000$ and for δ¹⁸O $P = 0.0000$). δ¹³C mean values of shells ranged from -14.18‰ in P. fontinalis to -6.79‰ in T. fluviatilis (Table 1). In contrast to δ¹³C values, the δ¹⁸O 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 δ¹³C mean values while the δ¹⁸O mean values revealed depletion in ¹⁸O towards August (Table 1, Text-fig. 3). A sorting of species, from the least to the most depleted in ¹³C, was not followed by the δ¹⁸O values of shells (Text-fig. 3). Along with different mean values, particular snail species also exhibited different ranges of δ¹³C and δ¹⁸O values, as presented in Table 1.

Intra-specific variation in δ¹⁸O and δ¹³C 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
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).

### 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.
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 δ¹⁸O, 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 δ¹³C, exceeding 20% and followed by high CV values of δ¹⁸O. *V. cristata* and *B. tentaculata* (June sample) had the second and third highest CV values of δ¹⁸O 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 δ¹³C and δ¹⁸O 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 δ¹³C and P = 0.00005 for δ¹⁸O values), the relationships were not very strong. δ¹³C values decreased with the depth (r = -0.4) while the values of δ¹⁸O 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 = 0.00000 for δ¹³C, and r = 0.6, p = 0.00000 for δ¹⁸O 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 δ¹³C and δ¹⁸O 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 δ¹³C values in terms of isotopic equilibrium or an offset from the equilibrium with δ¹³C values of DIC is impossible because of lack of data on seasonal changes in δ¹³C values of DIC, in our opinion the actual extent of ¹³C-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 δ¹³C values of DIC measured in June and August.

The same order of species from the most to the least depleted in ¹³C observed at different sampling sites (Text-fig. 7), i.e. *P. fontinalis, R. auricularia, G.*
crista, G. albus, Lymnaea sp., V. cristata, B. tentaculata, T. fluviatilis, and similar offsets in δ¹³C 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 δ¹³C 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‰ ¹³C-enrichment of the opercula relative to the shells of 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 (Wächter 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 cessation visible, 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 Nitelopsis 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 $\delta^{13}C_{\text{shell}}$ with depth ($r = -0.4$; Text-fig. 5) is interpreted as resulting from the proportion of the release of $^{13}C$ from the decomposition of organic matter and that from photosynthetic
activity of macrophytes increasing with depth. This relationship shows that although δ13C values of shells are species-specific and presumably shifted from isotope equilibrium with DIC they record relative changes in δ13C values of the ambient DIC.

Consistent with observations in the present study, 13C-depletion of shells relative to DIC and species-specific δ13C 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 13C depleted (Fritz and Poplawski 1974; Böttger et al. 1998; Aucour et al. 2003; Apolinarska 2009; De Francesco and Hassan 2013). Shell δ13C 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 13C-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 δ13C 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, 13C-depletion in P. fontinalis shells is suggested to be species-specific, however, it is not possible to provide an exact shift in δ13C 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). However, in most studies this depletion ranges between 2 and 3‰. Interesting data on δ13C 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‰ 13C-depleted relative to δ13C values of three species of Helisoma genus, whereas the carbon isotope composition of L. stagnalis grown in the laboratory under controlled conditions was within δ13C 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 13C, with δ13C values between 1.5 and 7.2‰ higher compared to those measured in the other gastropod species studied and closest to δ13C 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‰ 13C-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) 13C-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 δ18O values (r = 0.6; Text-fig. 6) of bulk shell material was noted. The 0.9‰ increase in mean δ18O 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 δ18O values per 1°C decrease
in water temperature (Craig 1965; Kim and O’Neil 1997). Thus, the expected sensitivity of shell δ18O 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 δ18O values and water temperature. Precipitation of gastropod shells in isotopic equilibrium or close to equilibrium with water δ18O 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 by the small inter-specific variability in mean δ18O values (Text-ﬁg. 3) indicating that shell δ18O values of all the species analysed in the present study can be reliably used in palaeoecological studies.

Although δ18O values were found to be similar in the species studied, they were not the same (Text-figs 3, 7). Strong 18O-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 δ13C and δ18O changes in the shells with depth.

Similarly to those observed in δ13C values, strong differences in δ18O 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, δ18O values of opercula should be 0.6‰ 18O-depleted compared to shells (Tarutani et al. 1969). In contrast to the values expected, the opercula were 0.62‰ 18O-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‰ 18O-enriched compared to shells of B. tentaculata. Thus, the difference in δ18O values between shells and opercula is very constant, irrespective of the sampling site and shell age. As already suggested discussing the δ13C 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 δ18O 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 δ18O water was suggested for a number of species, as pointed out above, the intra-specific variability in shell δ18O values, discussed in detail in the next section, must also be considered.

**Intra-specific differences in the stable isotope composition of shells**

Investigation of δ13C and δ18O 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 δ13C and δ18O 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 (Pelechaty 2005) and isotopic composition, a number of factors can result in heterogeneity of δ13C DIC, δ18O 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-
genity of DIC $\delta^{13}$C values, water $\delta^{18}$O values and water temperature on $\delta^{13}$C and $\delta^{18}$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 $\delta^{13}$C and $\delta^{18}$O values of the shells collected from a macrophyte patch covering the bottom surface of ~0.02 m$^2$, was 1.5\%o and 0.8\%o, 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 $\delta^{13}$C and $\delta^{18}$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 $\delta^{13}$C values of marine and freshwater bivalves studied by Keith et al. (1964) was 2.45\%o and 2.21\%o, respectively. The range of $\delta^{18}$O values observed in the same molluscs was less than one per mill with the average value of 0.59\%o. Lorrain et al. (2004) observed 1 to 2.5\% variability in $\delta^{13}$C values between scallops derived from a dredge haul that sampled the bottom over a distance of less than 500 m. $\delta^{13}$C and $\delta^{18}$O values in Dreissena polymorpha shells sampled from Lake Lednica varied by 2 and 1.5\%o, 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 $\delta^{13}$C and $\delta^{18}$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 $\delta^{13}$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 $\delta^{13}$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\%o on average, compared to the former group. Although $\delta^{13}$C values of shells are species-specific and presumably shifted from isotope equilibrium with DIC, they record relative changes in $\delta^{13}$C values of the ambient DIC.

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

Intra-specific variability in shell $\delta^{13}$C and $\delta^{18}$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 $\delta^{13}$C and $\delta^{18}$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 $\delta^{13}$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 $\delta^{13}$C values followed the DIC $\delta^{13}$C values in Lake Lednica. Thus, changes in shell $\delta^{13}$C values can be used to reconstruct relative changes in ancient DIC $\delta^{13}$C values. Whenever two or more species are used within a sediment sequence, their records need to overlap to verify the differences in $\delta^{13}$C values.
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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*