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$\delta^{13}C$ of fluvial mollusk shells (Rhône River): A proxy for dissolved inorganic carbon?

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Abstract

The relationship between the $\delta^{13}C$ of dissolved inorganic carbon (DIC) and modern mollusk aragonite from rivers was calibrated for the purpose of reconstructing DIC paleochemistry from the shell record. The $\delta^{13}C$ values of aragonitic bivalves (*Dreissena polymorpha, Corbicula fluminea*), prosobranch gastropods (*Bithynia tentaculata, Theodoxus fluviatilis, Viviparus viviparus*), and an air-breathing pulmonate gastropod (*Limnea auricularia*) were analyzed from several locations on the Rhône River (−13.7‰ to −6.0‰) and its major tributary, the Saône River (−11.4‰ to −10.2‰). The $\delta^{13}C_{DIC}$ varied from −11.5‰ to −7.5‰, and the $\delta^{13}C$ of particulate inorganic matter (POM) varied from −31.7‰ to −25.4‰. At a given site, the $\delta^{13}C$ of all species except the pulmonate were within 1‰ of each other. Whole-shell $\delta^{13}C$ correlated positively with $\delta^{13}C_{DIC}$, with a slope close to unity. Bioaragonite–DIC fractionations were 0–1.5‰ for bivalves and 0–2.7‰ for gastropods (excluding the pulmonates). Applying these fractionations, bivalves that live in open water are a reliable proxy, monitoring the average $\delta^{13}C_{DIC}$ value to within its natural ~2‰ temporal variation within the growth period. For the suspension feeders (bivalves) using POM as a food source, the $\delta^{13}C$ of whole shells and bulk POM indicated that the incorporation of carbon derived from respiratory sources lay in the range 10–30%. Fine-scale analyses of growth increments of *C. fluminea* could not be related simply to $\delta^{13}C$ DIC because metabolic and seasonal variations in $\delta^{13}C_{DIC}$ produced similar isotopic fluctuations (≤2.5‰).

Dissolved inorganic carbon (DIC) in rivers, the dominant dissolved riverine carbon reservoir, presents a wide range of $\delta^{13}C$ values from −28‰ to −1‰ (Mook 1970; Hitchon and Krouse 1972; Longinelli and Edmond 1983; Cameron et al. 1995; Yang et al. 1996; Barth and Veizer 1999; Telmer and Veizer 1999), in contrast to the restricted range of 0–2‰ for most seawaters (Kroopnick 1980). It is determined by geological processes on the watershed (i.e., alteration–dissolution that involved, as carbon-bearing reactants, the solid carbonate phases, atmospheric CO_2, and soil biogenic CO_2; e.g., Salomons and Mook 1986). It is also sensitive to riverine processes such as CO_2 escape or exchange with the atmosphere and the balance between primary production and respiration (Mook 1970). Anthropogenic additions of nitrate and phosphate, input from waste organic carbon, and extensive damming alter photosynthetic fixation, organic matter oxidation, and CO_2 exchange with the atmosphere and thus the $\delta^{13}C$ of DIC ($\delta^{13}C_{DIC}$) (Bühl et al. 1991; Flintrop et al. 1996). In contrast to the instantaneous and direct samples of DIC, proxies that can monitor the evolution of DIC back through time are necessary so that the changes of riverine DIC chemistry in the past and the effect of common industrial modifications in and along rivers can be assessed. Mollusk shells potentially record both recent and past variations of the $\delta^{13}C$ value of DIC, but few studies of mollusks are from freshwater lakes and rivers, and conclusions have been contradictory (Fritz and Poplawski 1974; Bühl et al. 1991; Dettman et al. 1999). The isotopic relationship between shell carbonate and DIC is not necessarily simply governed by the equilibrium fractionation factor between carbonate and DIC and its sensitivity to variations in temperature (Grossman and Ku 1986) and speciation (a function of pH). For example, respiratory carbon derived from food can be incorporated into shell carbonate (e.g., Tanaka et al. 1986; McConnaughey et al. 1997).

To improve our understanding of the $\delta^{13}C$ of mollusks and its potential application as a proxy for riverine $\delta^{13}C_{DIC}$, we have determined (1) interspecific isotope fractionations as a possible result of the vital effects arising from physiological differences in six different species, (2) fractionation between shell aragonite and DIC, and (3) intrashell isotopic variations for individual bivalves. The present work is part of a larger study on the carbon isotope cycle in a major European river, the Rhône River, and its important tributary the Saône River (Aucour et al. 1999). The range of $\delta^{13}C_{DIC}$ is between −13‰ and −4‰ in the Rhône River system and between −11.5‰ and −7.5‰ for the section studied here (Aucour et al. 1999). For the calibration, we used the bivalve *Dreissena polymorpha*, which has been present in French rivers for the last one and a half centuries and is now very common. For the purpose of reconstructing paleoriver isotopic compositions, the calibration was also extended to other genera of bivalves and gastropods that are present in West European Quaternary deposits.

Acknowledgments

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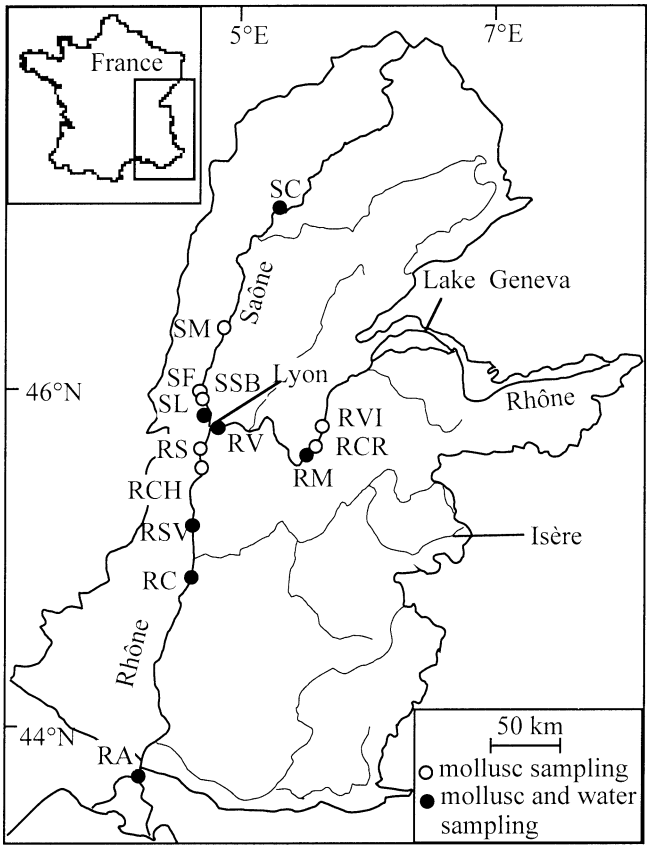

Fig. 1. Map of the watershed of the Rhône River, showing the sampling stations on the Rhône and Saône Rivers. Note that the confluence of the Saône is 8 km downstream from sampling Sta. RV.

Material and methods

River water was collected at seven sites in the Rhône and the Saône (Fig. 1) in March, July, and September 1996. The Rhône and Saône at Lyon were resampled for DIC at the end of February 1999 and in March and May 2000. For the determination of $\delta^{13}C_{DIC}$, river water was filtered in the field by pressure filtration on cellulose nitrate 47-mm filters (porosity 0.45 μm) to completely fill a 1-liter bottle. DIC was precipitated as $BaCO_3$ at pH $>$ 10 (Aucour et al. 1999). The $\delta^{18}O$ value of water was determined by equilibration of water with CO_2 (Socki et al. 1992). The $\delta^{13}C$ value of particulate organic matter (POM), was determined after filtration of 1–2.5 liters of water on precombusted glass microfiber 47-mm filters (GF/F Whatman) within 24 h after sampling, decarbonation by 10% HCl, and combustion of the filter with cupric oxide. Mollusks were sampled at 14 sites in the Rhône and Saône (Fig. 1).

The selected species included bivalves (*D. polymorpha, Corbicula fluminea*), prosobranch gastropods (*Bithynia tentaculata, Theodoxus fluviatilis, Viviparus viviparus*), and a pulmonate gastropod (*Limnea auricularia*). One to several shells were collected live from either the topside of rocks, which are exposed to the flow, or their underside. Whole shells were cleaned of organic matter by the action of H_2O_2 and then gently crushed. A shell represents up to 2 yr of growth for the sampled gastropods (Mouthon 1982) and up to several years for the bivalves (McMahon 1983; Mouthon 2001). Periods of growth are marked by conspicuous concentric growth ridges on the outer surface of the valve of the bivalve *C. fluminea*. One valve was sectioned along the axis of maximum growth. In the outer shell layer (Fig. 2), the cross section showed visible internal growth increments, which were oblique to the outer surface. The inner and outer layers differed by a structure that was, respectively, finely cross-lamellar and complexly cross-lamellar. Growth increments were sampled with a diamond-studded dental drill on two specimens collected in the Saône (Sta. SL, Fig. 1) in April 2000. Carbonate powder (about 0.5 mg) was obtained without sectioning the shell. A shallow groove was drilled along the growth ridge, targeting the shallower growth increments (Fig. 2). The first 10 mm from the hinge were not sampled because the outer layer is very thin. The drilled powders represent a minute fraction in weight of the shell. After such subsampling, the ''whole'' shell was crushed before analysis. Carbonates were reacted with 100% orthophosphoric acid at 30°C for several hours. All isotope ratios were determined on CO_2 using a VG PRISM mass spectrometer. The $\delta^{18}O$ and $\delta^{13}C$ values of the carbonate samples and the $\delta^{13}C$ values of particulate organic carbon are reported versus PDB (Pee Dee Belemnite). The $\delta^{18}O$ values of the waters are given relative to SMOW (Standard Mean Ocean Water). The fractionation between substances A and B, $\varepsilon_{A\text{-}B}$, is the deviation of the fractionation factor, $\alpha_{A\text{-}B}$, from unity (per mil) and is related to δ by Eq. 1.

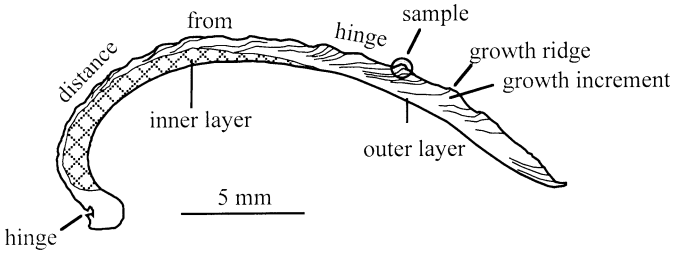

Fig. 2. Cross section of shell of the bivalve *Corbicula fluminea*, illustrating how sample powder was selected by drilling (grey circle). Internal growth increments are visible in the outer layer. Note that there are several growth increments per year.

$$\varepsilon_{A\text{-}B} = (\alpha_{A\text{-}B} - 1)10^3 = \frac{(\delta^{13}C_A - \delta^{13}C_B)}{(1 + 10^{-3}\delta^{13}C_B)} \quad (1)$$

Results

C isotope compositions of the six species of mollusks are presented in Table 1. They range from −13.7‰ to −6.0‰ (PDB), or from −11.4‰ to −6.0‰ if *Limnea* is excluded. However, from any given station, all species except the pulmonate gastropod (*L. auricularia)* generally have $\delta^{13}C$ within 1‰ of each other. Table 2 presents the measured DIC, POM, and water analyses. The $\delta^{13}C_{DIC}$ values as a whole (1996–2000) range from −12.3‰ to −7.6‰. On the basis of the March, July, and September samples from the same year, the temporal variations range from 1.0‰ to 2.7‰ (av-

Table 1. Stable isotope composition of mollusks collected in the Rhône River and in the Saône River.

Location*	Mollusk species	$\delta^{13}C$ (‰) PDB	$\delta^{18}O$ (‰) PDB
Rhône River			
Vions (RVI)	*Dreissena polymorpha*	−7.2	−10.8
	Limnea auricularia	−11.3	−9.4
	Theodoxus fluviatilis	−6.4	−10.6
Cressin (RCR)	*D. polymorpha*	−7.5	−9.7
	T. fluviatilis	−6.2	−9.5
Murs (RM; 6)	*D. polymorpha*	−8.1	−9.3
	T. fluviatilis	−6.0	−9.9
Villeurbanne-Lyon (RV; 8)	*Bithynia tentaculata*	−7.5	−11.5
	Corbicula fluminea	−8.4	−11.1
	D. polymorpha	−8.3	−9.9
	T. fluviatilis	−7.8	−10.2
	Viviparus viviparus	−8.6	−11.0
Solaise (RS)	*D. polymorpha*	−9.1	−9.6
left riverside	*L. auricularia*	−13.7	−9.7
	T. fluviatilis	−8.2	−10.5
right riverside	*L. auricularia*	−12.9	−9.8
Chasse (RCH)	*B. tentaculata*	−8.6	−10.8
	L. auricularia	−12.8	−9.5
St Vallier (RSV; 13)	*B. tentaculata*	−9.6	−9.6
	C. fluminea	−9.4	−9.8
	D. polymorpha	−9.4	−9.1
	D. polymorpha	−9.4	−9.0
Charmes (RC; 15)	*D. polymorpha*	−9.2	−10.0
Arles (RA; 17)	*B. tentaculata*	−9.6	−10.4
	C. fluminea	−9.5	−10.3
	D. polymorpha	−8.5	−11.5
	T. fluviatilis	−8.1	−10.0
Saône River			
Charrey (SC; 10)	*C. fluminea*	−11.0	−7.2
	D. polymorpha	−10.8	−7.2
	T. fluviatilis	−10.7	−7.3
Macon (SM)	*C. fluminea*	−11.0	−7.3
Furieux (SF)	*C. fluminea*	−10.7	−8.5
	V. viviparus	−11.1	−7.5
Saint-Bernard (SSB)	*B. tentaculata*	−11.1	−7.3
	D. polymorpha	−10.5	−6.6
	C. fluminea	−11.0	−7.2
Lyon (SL; 11)	*B. tentaculata*	−11.1	−6.8
	B. tentaculata	−10.9	−8.4
	C. fluminea	−11.2	−6.4
	C. fluminea (C-1)†	−11.1	−8.2
	C. fluminea (C-2)†	−11.3	−7.9
	D. polymorpha	−10.8	−6.7
	D. polymorpha	−10.2	−7.8
	V. viviparus	−11.4	−7.3

* Letters in parentheses indicate abbreviations used in Fig. 1; numbers refer to stations in Aucour et al. (1999).
† Samples subject to growth band subsampling.

erage 1.6‰) for the Rhône and 0.4‰ to 1.0‰ (average 0.7‰) for the Saône, with the July value usually most depleted in ^{13}C. On average, the Saône is systematically more negative than the Rhône above the confluence at Lyon. For the Rhône and Saône Stas. RV and SL sampled in 1996, 1999, and 2000, the ranges are 2.5‰ and 1.2‰, respectively, although differences among average annual values or values for the same season are <1‰. It is noteworthy that the Rhône–Saône system was in spate during the July 1996 sampling because of unusually heavy rainfall for the season; therefore, the July series represents an uncommon hydrological event (Aucour et al. 1999). Taking the average of the spring, summer, and fall values of the Rhône–Saône as the best estimate of DIC during the main growing season, the range is −11.3‰ to −8.7‰. This range of values is thus more restricted than that for the mollusks, even excluding

Table 2. Carbon isotope composition of DIC and POM and oxygen isotope composition of water in the Rhône and Saône Rivers at the locations of water sampling.

Location*	Sampling period	DIC $\delta^{13}C$ (‰) PDB	POM $\delta^{13}C$ (‰) PDB	Water $\delta^{18}O$ (‰) SMOW
RM	Mar 96	−8.2	−27.4	−11.8
	Jul 96	−10.3	−25.4	−11.4
	Sep 96	−7.6	−25.8	−11.7
RV†	Mar 96	−9.6	−28.4	−10.7
	Jul 96	−11.1	−27.1	−10.5
	Sep 96	−9.5	−27.4	−10.9
RSV	Mar 96	−10.3	−30.0	−9.9
	Jul 96	−11.3	−27.7	−9.8
	Sep 96	−10.4	−27.3	−9.8
RC	Mar 96	−10.3	−29.7	−10.0
	Jul 96	−11.0	−27.0	−9.9
	Sep 96	−9.6	−26.7	−11.0
RA	Mar 96	−10.2	−28.3	−9.8
	Jul 96	−9.9	−26.0	−10.3
	Sep 96	−9.0	−26.4	−10.4
SC	Mar 96	−10.4	−33.0	−7.9
	Jul 96	−11.4	−26.9	−7.5
	Sep 96	−10.9	−28.5	−7.3
SL†	Mar 96	−11.5	−31.7	−8.5
	Jul 96	−11.3	−27.3	−8.3
	Sep 96	−11.1	−28.4	−7.8

* Letters indicate abbreviations used in Table 1.

† Additional measurements of $\delta^{13}C$ DIC for station RV: −11.7‰ in Feb 1999, −9.2‰ in Mar 2000, and −10.1‰ in May 2000 and for station SL: −12.3 in Feb 1999, −11.9‰ in Mar 2000, and −12.3‰ in May 2000.

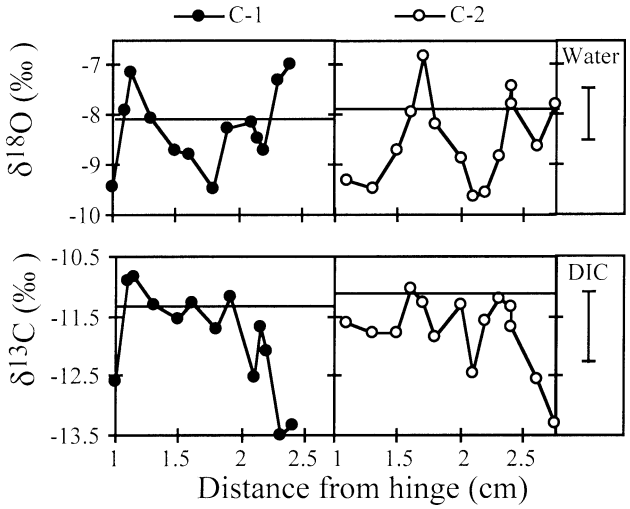

Fig. 3. $\delta^{18}O$ and $\delta^{13}C$ values of growth bands for two shells of *Corbicula fluminea* plotted against distance from the hinge. The two shells (C-1 and C-2) were sampled in the Saône River at Lyon (Sta. SL). Horizontal solid lines represent the δ values of the whole shell. Also shown are vertical bars for the measured range of $\delta^{18}O$ water and $\delta^{13}C_{DIC}$ at this station.

Limnea. All the DIC δ values are within the mollusk range. The bivalve–DIC fractionation is typically 0–1.5‰, whereas the gastropod–DIC fractionation (excluding the pulmonate) is 0–2.7‰.

The $\delta^{13}C$ values of the POM used as a food source by the suspension feeders (bivalves) are presented in Table 2. They range from −30.0‰ to −25.4‰ (average −27.4‰) in the Rhône and from −33.0‰ to −26.9‰ (average −29.3‰) in the Saône. The latter is thus systematically ~2‰ more negative than the Rhône. The less negative $\delta^{13}C$ values of POM around −26‰ fall within the typical range for C3 terrestrial plants. Values between −33‰ and −26‰ are consistent with mixtures of terrestrial and phytoplanktonic organic matter. The sample (Sta. SC, March 1996) that presents the most negative $\delta^{13}C$ value of −33‰ indicates a dominant phytoplanktonic origin. It gives a fractionation $\varepsilon_{b\text{-}p}$ of 22.8‰ between bicarbonate (the main form of DIC) and phytoplankton during photosynthesis. We used this $\varepsilon_{b\text{-}p}$ value when we estimated the isotopic composition of the phytoplankton ingested by the bivalves.

Temperatures of shell secretion T (degrees Kelvin) were estimated from the experimental aragonite–water fractionation equation (Eq. 2).

$$1{,}000 \ln(\alpha_{ar\text{-}H2O}) = 2.559(10^6 T^{-2}) + 0.715 \quad (2)$$

This equation applies to many aragonitic mollusks (Grossman and Ku 1986; Dettman et al. 1999). The O isotope composition of the shell (Table 1) and the average O isotope composition of the local river water for March, July, and September (Table 2) were used in the calculation. The $\delta^{18}O$ values of river water range from −11.8‰ to −9.8‰ in the Rhône (average of the March, July, and September values ranged from −11.6‰ to −9.8‰), and from −8.5‰ to −7.3‰ in the Saône (averages from −7.6‰ to −8.2‰). The most negative $\delta^{18}O$ of Rhône waters is upstream from Lyon and reflects the influx from glacial meltwaters through Lake Geneva, whose surface waters are at −12.5‰ (Fontes and Gonfiantini 1970). The higher $\delta^{18}O$ of Saône waters reflect its lowland character. The calculated temperatures generally fall between 15°C and 25°C, with a few values falling between 10°C and 15°C. We found no significant correlation between the carbon isotope fractionation between shell and DIC and the temperature of secretion, which was estimated as above for bivalves and gastropods.

Growth ring series were analyzed in two specimens (C-1 and C-2) of *C. fluminea* collected in the Saône at Lyon (Sta. SL, Fig. 3). The most recent growth increment in each specimen was near the outer shell margin and thus furthest from the hinge. The two shells presented similar $\delta^{18}O$ fluctuations, whose amplitudes are larger than the measured temporal variation of $\delta^{18}O$ of the river water (Table 2). The $\delta^{18}O$ fluctuations can be interpreted as annual temperature cycles, with more positive $\delta^{18}O$ values indicating the cold season. Shell lengths of a population of *Corbicula* had been measured by Mouthon (2001) at the same site between 1996 and 1999. Comparing his results on shell length and the $\delta^{18}O$ profile in the shells, we assigned the $\delta^{18}O$ peaks at 21 mm (C-1) and 24 mm (C-2) to the cold season 1998–1999 and those at 11 mm (C-1) and 17 mm (C-2) to the cold season 1997–1998. The individuals C-1 and C-2 should be about 3.5 yr old, and the sampled growth band series should represent the last 2.5 yr of growth. Mouthon (2001) reported that sexual maturity is reached when the animal is about 10 mm in size (i.e.,

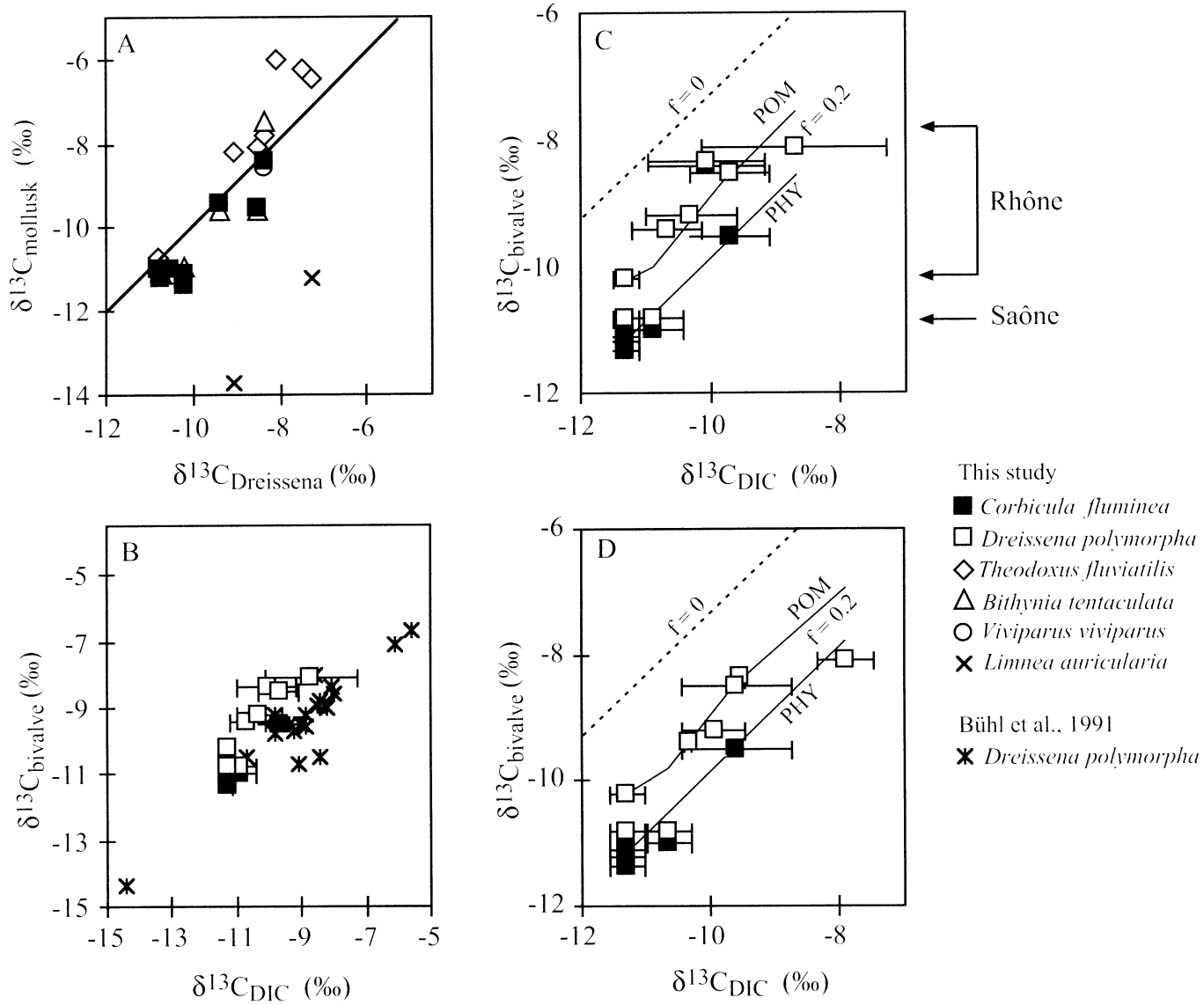

Fig. 4. (A) $\delta^{13}C$ of mollusk versus $\delta^{13}C$ of associated *Dreissena polymorpha.* The solid line is the 1 : 1 line. (B) Summary plot showing $\delta^{13}C$ data in bivalves from the Rhône–Saône Rivers and from the Rhine River versus DIC. (C) $\delta^{13}C_{DIC}$ versus $\delta^{13}C$ of bivalves. The $\delta^{13}C_{DIC}$ is the average of the March, July, and September 1996 values. The vertical error bar represents the standard deviation for the three measurements on DIC. The equation of the regression line is $\delta^{13}C_{DIC} = 0.6\delta^{13}C_{bivalve} - 5.1$ ($n = 16$, $r^2 = 0.74$, $P < 0.001$). The dashed line is the isocurve for $f = 0$. The thin lines are isocurves for $f = 0.2$, either for the food source POM (POM) or phytoplankton (PHY). (D) Same plot as in panel C, but with average $\delta^{13}C_{DIC}$ for March and September only. The equation of the regression line is $\delta^{13}C_{DIC} = 0.7\delta^{13}C_{bivalve} - 3.2$ ($n = 16$, $r^2 = 0.76$, $P < 0.001$).

about 1 yr old) and that the mollusk generally dies in its fourth year. In both shells, the $\delta^{13}C$ of growth rings are either similar to or deviate negatively from the value of the whole shell, particularly in the last increments secreted before collection. The carbon isotopic compositions of ''whole'' shells of C-1 and C-2 are similar to each other and to those of other shells collected at the same station.

Discussion

To visualize the interspecific variations in the shell, the $\delta^{13}C$ of the various species were plotted versus the $\delta^{13}C$ values of the associated *D. polymorpha* (Fig. 4A). There is good correlation between the $\delta^{13}C$ of *C. fluminea, B. tentaculata, T. fluviatilis,* and *V. viviparus* and that of *D. polymorpha* ($r^2 = 0.85$, $n = 22$, $P < 0.001$). These species are well distributed over the whole $\delta^{13}C$ range, with the data aligned close to the bisectrix, indicating that the observed trend is systematic. At a given site, however, the gastropod *T. fluviatilis* is enriched in ^{13}C by 1–2‰. The two pulmonates (*L. auricularia*) are both depleted in ^{13}C by ~4.5‰ relative to *D. polymorpha. L. auricularia* is the only species we studied that can breathe air; it is discussed further below.

The $\delta^{13}C_{DIC}$ value of the Saône around −11‰ (Fig. 4C)

indicates that DIC is essentially controlled by the reaction of soil biogenic CO_2 and carbonate minerals on the carbonate-dominated lowland watersheds (Aucour et al. 1999). In the Rhône, the greater variation in $\delta^{13}C_{DIC}$ and less negative values reflect the mixing of lowland tributaries with alpine tributaries and waters from Lake Geneva. Alpine tributaries and Lake Geneva present less negative $\delta^{13}C_{DIC}$ because of carbonate dissolution by atmospheric CO_2, H_2SO_4, or both, and partial equilibration of the waters with the atmosphere (Aucour et al. 1999).

The relationship between average $\delta^{13}C_{DIC}$ and $\delta^{13}C$ for bivalves is shown including (Fig. 4C) and excluding (Fig. 4D) the July DIC sample, which represented an uncommon, short hydrological event. Significant positive correlations are found, with the slope of the regression line ~0.7. This demonstrates that the $\delta^{13}C$ of these bivalve whole shells closely records the variation in $\delta^{13}C_{DIC}$ values along the river and that the $\delta^{13}C$ of a bivalve can be used to estimate the DIC isotope composition of the associated waters. It should be recalled that a shell represents a time-integrated river water composition during the period of its growth rather than a discharge-integrated signal. Sources of error in the estimation of average $\delta^{13}C_{DIC}$ during shell growth are linked to the temporal variation of $\delta^{13}C_{DIC}$, the discrete (rather than time-integrated) sampling method of DIC, and the variable timing of growth among individuals and species. The temporal fluctuations in $\delta^{13}C_{DIC}$ (Table 2) and the relative uncertainty in the estimation of average $\delta^{13}C_{DIC}$ during shell growth (Fig. 4C) are smaller in the Saône than in the Rhône. Because of the high concentration of DIC in the Saône waters, processes that vary with season, such as gas exchange with the atmosphere and redox reactions (respiration, photosynthesis), do not appear to have a significant effect on the budget of carbon isotopes (Aucour et al. 1999). In the Rhône upstream of Lyon, the relatively large fluctuations of $\delta^{13}C_{DIC}$ of 2.5‰ reflect the contribution of waters from Lake Geneva, alpine watersheds, and low- to medium-altitude terrains.

The C isotope fractionation between aragonite and bicarbonate that was determined experimentally on inorganic precipitates ($\varepsilon_{ar\text{-}HCO3} = 2.7 \pm 0.6$‰; Romanek et al. 1992) differs from that given by shell aragonite. The bioaragonite–bicarbonate fractionation is smaller. This observation applies to bivalves as well as to gastropods. The use of DIC instead of the bicarbonate $\delta^{13}C$ value does not change the interpretations of the results because the bicarbonate ion is >95% of the DIC at the pH of Rhône waters (~8). Previous data obtained on shells of freshwater mollusks by Fritz and Poplawski (1974) and Bühl et al. (1991) are consistent with our results. All these data indicate that the experimentally determined fractionation between inorganic aragonite and bicarbonate (Romanek et al. 1992) cannot be directly applied to reconstruct the variations in $\delta^{13}C_{DIC}$ in freshwaters.

A current explanation for the depletion in mollusk shell of ^{13}C relative to equilibrium precipitation with DIC is the incorporation of metabolic carbon from respiratory sources into the shell (Tanaka et al. 1986; McConnaughey et al. 1997). Isotopic and chemical equilibrium among the DIC species is assumed to occur in the precipitation medium, which is the extrapallial fluid (Wilbur and Saleuddin 1983). Following the assumptions and equations developed in Tanaka et al. (1986), CO_2 is exchanged between the precipitation medium and the ambient water by diffusion through cell membranes. The carbon isotopic composition of the shell carbonate is related to the isotopic composition of bicarbonate in ambient water and to the isotopic composition of metabolic CO_2 in the mass balance derived Eq. 3 for the fraction of metabolic carbon in the shell, *f*.

$$f = \frac{(\delta^{13}C_s - \varepsilon_{ar\text{-}b} - \delta^{13}C_b)}{(\delta^{13}C_{meta} + \varepsilon_{b\text{-}CO_2} - \delta^{13}C_b)} \quad (3)$$

$\delta^{13}C_s$, $\delta^{13}C_b$, and $\delta^{13}C_{meta}$ are the $\delta^{13}C$ values of the shell, bicarbonate, and metabolic CO_2, respectively. $\varepsilon_{ar\text{-}b}$ is the fractionation between the carbonate and bicarbonate ion (Eq. 1), and $\varepsilon_{b\text{-}CO2}$ is the fractionation between bicarbonate and aqueous CO_2. In our calculations, we used $\varepsilon_{ar\text{-}b} = 2.7$‰ (Romanek et al. 1992). The value of $\varepsilon_{b\text{-}CO2}$ depends on temperature (Salomons and Mook 1986) and ranges from 9.0‰ to 10.1‰ for our estimated isotopic temperatures of shell secretion (15–25°C). Equation 3 for *f* differs from that used by McConnaughey et al. (1997). They took a mixing model between respiratory CO_2 and ambient DIC to fix the isotopic composition of the precipitating fluids. Their model yields an estimated *f* value that is about half of that given by Eq. 3.

The incorporation of metabolic carbon by the bivalves is discussed in terms of the sources of food, POM (Table 2), and phytoplankton. Phytoplankton is a priori the most labile and easily metabolized fraction. At a temperature of 20°C, $\varepsilon_{b\text{-}CO2}$ is taken to be 9.6‰. The sensitivity of *f* to the input value $\delta^{13}C_{meta}$ is visualized in Fig. 4C,D. The bivalves fall within a relatively narrow band that corresponds to *f* values of 0.1–0.3 if POM is taken as the food source. Isocurves, which were calculated for a constant incorporation of metabolic carbon, are shown in Fig. 4C,D for $f = 0$ (dashed line) and $f = 0.2$, with POM or phytoplankton as the food source (thin solid lines). A change in food source alone (POM/phytoplankton) introduces a variation of up to 1‰ in shell $\delta^{13}C$ or, inversely, a significant uncertainty in the determination of the amount of metabolic carbon incorporated.

The growth ring series of *C. fluminea* show an overall variation between the latest and youngest increments that exceeds that of the temporal variations of river DIC at this station (Table 2; Fig. 3). The latest increment, which grew in the third year, presents a drop in $\delta^{13}C$ that can be related only in part to a decrease in $\delta^{13}C_{DIC}$. This implies that the increments record an ontogenetic increase in the incorporation of metabolic carbon. There is no visible effect of the temperature of secretion and the amount of metabolic carbon incorporated, as shown by the lack of correlation between the $\delta^{13}C$ and $\delta^{18}O$ values of the incremental samples. The present data base indicates that it is not possible to use the growth increments of *C. fluminea* to reconstruct fine-scale seasonal or interannual fluctuations of the river composition. Note that the mean isotopic composition of the incremental samples is slightly depleted in ^{13}C relative to the whole shell. This mismatch suggests that the inner layer or the first increments of the outer layer (not subsampled here) or both should incorporate less metabolic carbon than the shell as a whole.

Previous studies of $\delta^{13}C_{DIC}$ and $\delta^{13}C$ bioaragonite relation-

ships from rivers are essentially limited to the Rhine River (Bühl et al. 1991), a major world river, and two minor shallow (<0.5 m) rivers in Michigan (Dettman et al. 1999). A comparable positive relationship is observed for *D. polymorpha* from the Rhine with a slope close to one and bioaragonite–DIC fractionations of about 0 ± 1‰ (Fig. 4B). The δ range for the Rhine (Bühl et al. 1991) is larger than ours, but the DIC was only measured once in the fall. Pluriannual time series of $\delta^{13}C_{DIC}$ for the lower Rhine (Mook 1970) indicate that the $\delta^{13}C_{DIC}$ fluctuations from spring to fall are small (1‰ to 2‰) and similar to our measurements. The Michigan rivers, each sampled at a single site for DIC, but bimonthly over 21 months, gave for growth increments of unionids a bioaragonite–DIC fractionation between +1.4‰ and −6.3‰. The mostly negative, highly variable fractionations were interpreted in terms of a significant and variable incorporation of metabolic carbon in the bioaragonite. Although it seems that fine-scale, seasonal or pluriannual reconstructions of the fluctuation of $\delta^{13}C_{DIC}$ cannot be derived from growth increments of either unionids or *Corbicula* sp., at least the $\delta^{13}C$ value of *Corbicula* (and *Dreissena*) can be used to estimate $\delta^{13}C_{DIC}$ to within 1‰ or so. Bioaragonite–DIC fractionations down to −6.3‰ in the unionids suggest a higher and more variable respiratory carbon input than in our bivalve species, possibly related to their semi-infaunal habitat. These observations suggest that bivalves that live in contact with open river waters can be applied as a proxy for riverine $\delta^{13}C_{DIC}$.

The Limneaid *L. auricularia,* with a lung in which air is renewed by periodically returning to the water surface and opening the pneumostome (McMahon 1983), has a more complex carbon history. They have more depleted $\delta^{13}C$ values than associated bivalves and prosobranchs, consistent with results for lake environments reported by Fritz and Poplawski (1974). The isotopic compositions of *L. auricularia* at about −13‰ from Stas. RS and RCH below the Rhône–Saône confluence (Table 1) approach the value calculated for aragonite precipitated in isotopic equilibrium with respiratory CO_2 (−15.5‰). The latter value is obtained by assuming that $f = 1$ in Eq. 3. Note that it can vary widely depending on food source (decreasing it by up to −4.6‰ for a phytoplanktonic food source) or whether CO_2 diffusion between the lung, which is taken as the gas reservoir, and the surrounding atmosphere is taken into account (increasing it by up to 4.4‰) (O'Leary 1984). The $\delta^{13}C$ of *L. auricularia* (Fig. 4A) falls in the lower range of $\delta^{13}C$ values reported for land snails in areas with pure C3 plant vegetation (−13‰ to −5‰) (Lécolle 1983; Léone et al. 2000). This can be explained by the depletion in ^{13}C of the phytoplanktonic food source relative to terrestrial C3 plants ($\delta^{13}C = -26$‰), by a higher contribution of atmospheric CO_2 ($\delta^{13}C = -8$‰) in the land snails, or both.

The greater variation in $\delta^{13}C$ values of shell aragonite and DIC from the Rhône reflects the greater diversity of origins and histories of Rhône waters (alpine, lowland, and lake) relative to the lowland-dominated Saône. The proxy developed for modern bivalves should be applicable to fossil systems, so long as the shell has preserved its aragonitic mineralogy and the species does not live in a semi-infaunal habitat (i.e., it lives in open water). Also, the observed variations of $\delta^{13}C$ among bivalves and prosobranchs strongly suggest that associated fossil species should be analyzed until the bioaragonite–DIC fractionations are understood in more detail.

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