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Carbon, nitrogen, oxygen, and hydrogen-stable isotope ratios in *Artemia* from different geographical origin

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Abstract

Stable isotope ratios $^{13}C/^{12}C$, $^{15}N/^{14}N$, $^{18}O/^{16}O$, and D/H have been determined in *Artemia* biomass and its chitin-derived substrates of various wild and laboratory-cultured strains of *Artemia monica* and *A. franciscana.*

The comparison of the isotopic data from a given *Artemia* population of unknown origin with the isotopic fingerprint of well-characterized *Artemia* populations from known origin, allows identification of the environment of origin and might make strain identification possible. The confidence of identification can be increased by utilizing four independent isotopic parameters of chitin-derived compounds rather than using only two parameters of the entire biomass as such.

To ensure positive identification with this new technique, further isotopic measurements are to be performed to evaluate seasonal and other factors contributing to the isotopic variability in brine shrimp from various environments.

Introduction

Stable isotopes of carbon, nitrogen, and hydrogen in different types of animals have been used in many ecological, environmental, physiological, and climatic studies (see reviews by Fritz and Fontes, 1980 ; Fry and Sherr, 1984). The most extensive compilation of stable isotopic data from 75 arthropod species, including *Artemia*, collected in 59 localities and grown in the laboratory, mainly utilized the poly-amino-sugar chitin from arthropod exoskeletons. This amino-sugar is well-defined chemically and allows for higher accuracy in interspecies comparisons than with ill-defined whole biomass of selected tissues (Schimmelmann, 1985). Briefly, it was shown that the $^{13}C/^{12}C$ ratios in marine arthropod chitins are different from those of terrestrial chitins, whereas chitin levels from brackishwater environments are intermediate. The $^{18}O/^{16}O$ ratios largely relate to the ratios of the waters available to primary biomass producers, which, in case of terrestrial meteoric waters, can be correlated with the climate. The D/H ratios of carbon-bound hydrogen reflect dietary influences that, for a given genus like *Artemia*, can also be climatically interpreted in the light of the D/H ratios of meteoric and ambient waters available to the local

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biota. Finally, $^{15}N/^{14}N$ ratios are relatively species-independent and therefore characteristics for a given environment.

Natural populations of *Artemia* inhabit aquatic environments which due to high rates of evaporation and/or closed-basin character, display extreme salinities, chemical compositions of dissolved salts, pH, etc., that are likely to cause large variabilities of the environmentally and climatically sensitive stable isotope ratios in *Artemia.*

The stable isotope ratios of laboratory cultured brine shrimp populations are largely influenced by the respective isotopic compositions of their diets and ambient waters (DeNiro and Epstein, 1978, 1981 ; Schimmelmann 1985).

The objective of this study was to evaluate the potential of stable isotope ratios in chitin-derived compounds and whole biomass from *Artemia* to serve as an analytical tool to identify brine shrimp from certain saline ecosystems.

Materials and methods

Two natural *Artemia* populations of different species were examined. *Artemia monica* was collected at Mono Lake, USA in June 1983, and *Artemia franciscana* from the San Francisco Bay area was supplied as commercial product by the San Francisco Bay Brand Co. (purchased in 1983).

Furthermore, cultures of brine shrimp (*A. franciscana*) from San Francisco Bay (one culture) and Great Salt Lake (three cultures) were raised in a closed flow-through culture system as described in Lavens *et al.* (1985). Temperature and salinity were kept at 25 ± 1 °C and 35 ± 1 ‰ respectively. The population density was 10 individuals/l. With algal growth being excluded, the composition of the diet (50 % corn bran, 50 % rice bran ; overall $\delta^{13}C = -19.6$ ‰, $\delta^{15}N = +4.8$ ‰ ; δ values are defined below) was kept constant for all populations raised.

We used the chromatographically purified D-glucosamine ("deacetylated chitin-monomer") in its hydrochloride form (GlcN · HCl) and dehydrated chitose instead of macromolecular chitin isolates, which still may be contaminated by covalently bounded proteinaceous compounds or may show compositional and isotopic heterogeneities due to deacetylation and adsorption effects (Muzzarelli, 1977 ; Schimmelmann, 1985). The methods for processing whole biomass, chitin, and chitin-derived compounds for stable isotope analyses have been described in detail elsewhere (Northfelt *et al.*, 1981 ; Schimmelmann, 1985 ; Schimmelmann and DeNiro, 1986). The results are expressed in δ-notation, *i.e.* in per mil enrichment [δ value > zero] or depletion [δ value < zero] of the heavy isotope in the sample relative to a standard :

$$\delta\,{}^{x}A = \frac{({}^{x}A/{}^{z}A)\ \text{sample} - ({}^{x}A/{}^{z}A)\ \text{standard}}{({}^{x}A/{}^{z}A)\ \text{standard}} \cdot 1\,000\ ‰\ ;$$

and ${}^{x}A = {}^{18}O$, ^{15}N, ^{13}C, or D, and ${}^{z}A = {}^{16}O$, ^{14}N, ^{12}C, or H.

The standards are "Vienna Standard Mean Ocean Water" (V-SMOW) for $\delta\,{}^{18}O$ values and δD values, the Peedee Belemnite (PDB) carbonate for $\delta\,{}^{13}C$ values and atmospheric nitrogen (AIR) for $\delta\,{}^{15}C$ values. The precisions of the measurements, given as standard deviations of the means calculated for 20 replicate analyses, are ± 0.2 ‰ for $\delta\,{}^{18}O$ values, ± 0.1 ‰ for $\delta\,{}^{15}N$ and $\delta\,{}^{13}C$ values, and ± 3 ‰ for δD values.

Results and discussion

The use of GlcN · HCl (for $\delta\ ^{13}C$, $\delta\ ^{15}N$, and $\delta\ ^{18}O$ values) and of dehydrated chitose (for δ D values) from chitin permits the determination of four independent stable isotope ratios. Whole biomass allows only for the determination of $\delta\ ^{13}C$ and $\delta\ ^{15}N$ values, because the presence of sulfur in complex organic matter interferes with the determination of the $\delta\ ^{18}O$ value, and the exchangeability of non-carbon-bound hydrogen (*e.g.* OH-and NH_2- hydrogen) with ambient water hydrogen during processing of the sample renders D/H ratios in whole biomass and in macromolecular chitin isolates unreliable.

Fig. 1 and 2 show *Artemia* chitin-derived δ values. Dashed lines compare ranges of δ values of natural fully marine arthropods with δ values of not fully marine chitins. Data from marine arthropods cluster within relatively small ranges reflecting the largely thermally and isotopically homogeneous environmental and climatic conditions in the marine environment. In contrast, *Artemia* data, and those from non-marine arthropods in general (Schimmelmann, 1985), fall within larger ranges due to the extreme variance of their habitats.

Taking the precisions of the measurements into account, we observed no overlapping between the data among natural *Artemia* populations and those among *Artemia franciscana* grown in the laboratory. The two strains of *A. franciscana* that were raised under controlled conditions showed only minor isotopic variances that can be related to small variances in overall dietary and water isotopic compositions during growth, but do not give sufficient evidence for biochemical chitin-metabolic differences among strains of *Artemia.*

We noted that the four independent isotopic parameters measured for a given chitin represent a four-dimensional vector characterizing a given *Artemia* population. Two-dimensional graphic display used in Fig. 1 and 2 can be completed by similar graphs $\delta\ ^{13}C$ *versus* δD, $\delta\ ^{13}C$ *versus* $\delta\ ^{18}O$, $\delta\ ^{15}N$ *versus* D, and $\delta\ ^{15}N$ *versus* $\delta\ ^{18}O$. Measurements on whole biomass, on the contrary, yield only two independent stable isotope ratios, $\delta\ ^{13}C$ and $\delta\ ^{15}N$, with only one graph possible.

Table I presents δ values of whole biomass and GlcN · HCl from cultured strains of *Artemia franciscana* that can be compared with the δ values of the diet given above. The metabolic isotope fractionation between diet and GlcN · HCl for carbon of 2.1 ± 0.3 ‰ (n = 4) and for nitrogen

TABLE I

Carbon and nitrogen stable isotopic compositions of whole biomass and of D-glucosamine hydrochloride from cultured *Artemia*

Whole biomass		D-glucosamine hydrochloride	
$\delta\ ^{13}C$ (‰)	$\delta\ ^{15}N$ (‰)	$\delta\ ^{13}C$ (‰)	$\delta\ ^{15}N$ (‰)
	Artemia franciscana		
	San Francisco Bay strain		
- 19.2	+ 5.3	- 21.8	- 6.4
	Artemia franciscana		
	Great Salt Lake strain, three populations measured		
- 19.2	+ 4.4	- 21.1	- 4.8
- 19.3	+ 4.8	- 21.8	- 4.5
- 19.0	+ 4.6	- 21.9	- 5.6

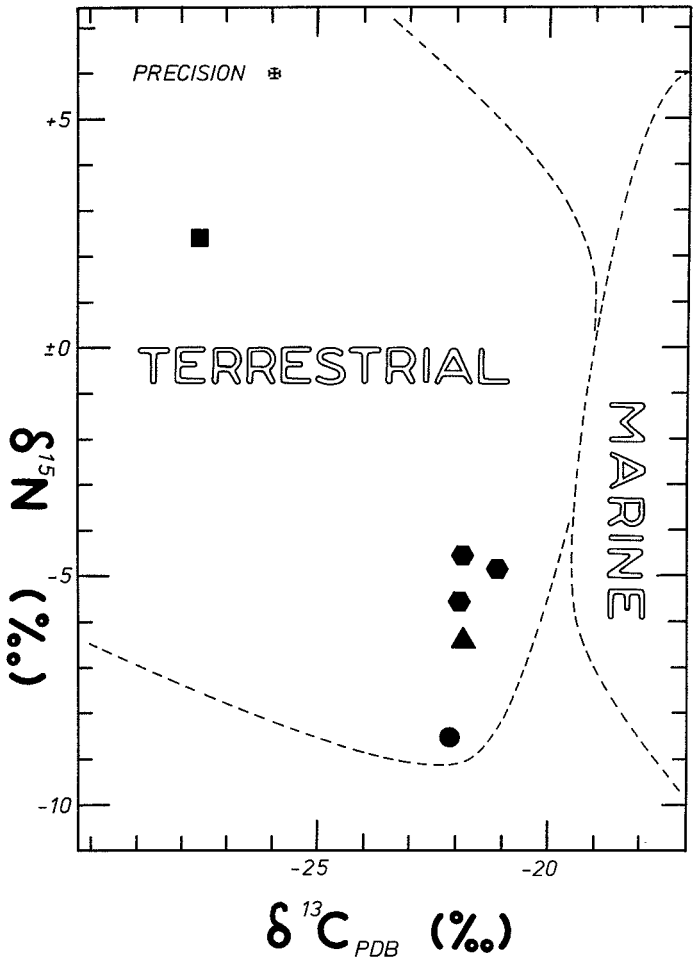

FIG. 1. Carbon and nitrogen stable isotope ratios of D-glucosamine hydrochloride from chitin. The ranges of marine and terrestrial arthropod chitins are indicated (after Schimmelmann, 1985).
(■) *Artemia monica* from Mono Lake ; (●) *Artemia franciscana* from San Francisco Bay area ; (▲) *Artemia franciscana*, San Francisco Bay strain, grown in the laboratory ; (■) *Artemia franciscana*, Great Salt Lake strain, grown in the laboratory, three populations measured.

of 10.1 ± 0.7 ‰ (n = 4), and the isotopic differences between whole biomass and diet averaging for carbon 0.4 ± 0.1 ‰ (n = 4) and for nitrogen 0.0 ± 0.3 ‰ (n = 4) are quantitatively similar to results from other arthropods (Schimmelmann and DeNiro, 1985) and emphasize systematic isotopic differences between amino-sugar (chitin, GlcN · HCl) and whole biomass (largely proteinaceous). When free from other organic debris, total biomass of *Artemia* appears to be a reliable substrate for infra-genus comparisons, but its limitations to $\delta\ ^{13}C$ and $\delta\ ^{15}N$ make it an inferior substrate when compared to chitin-derived compounds. In any case, due to the isotopic differences between the two types of organic substrates, comparisons are valid only among δ values of whole biomass, or among chitin-derived δ values.

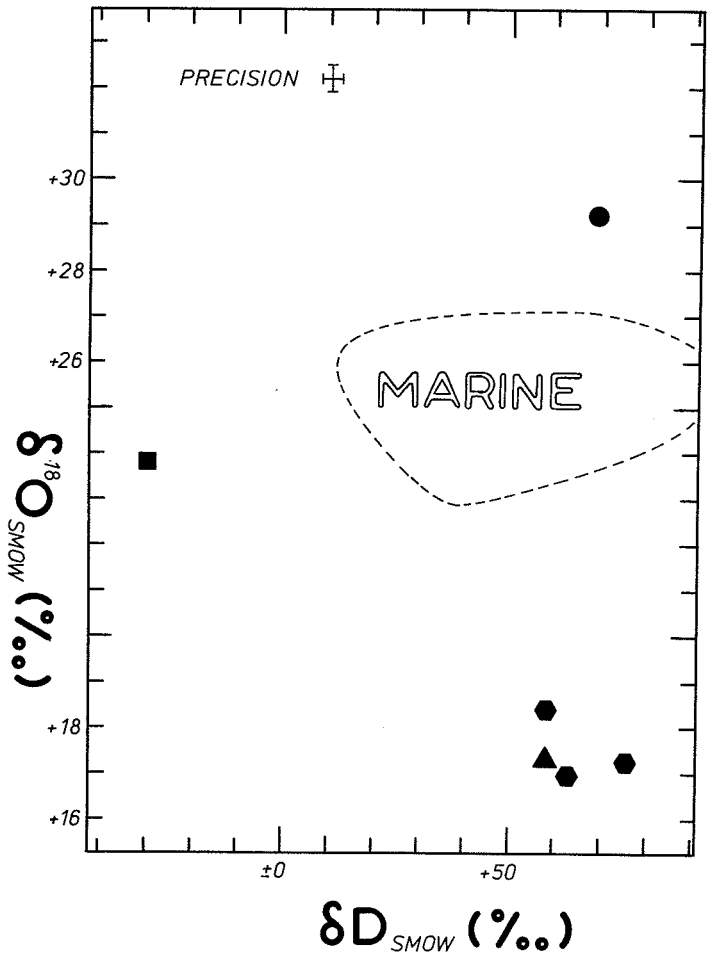

FIG. 2. Hydrogen and oxygen stable isotope ratios in D-glucosamine hydrochloride and dehydrated chitose. The range of marine arthropod chitins is indicated (after Schimmelmann, 1985). For definition of symbols, see Fig. 1.

Conclusions

Stable isotope ratios in whole biomass and in chitin-derived substrates from *Artemia* are highly sensitive environmental indicators. The comparison of isotopic data from a given *Artemia* population of unknown origin with the isotopic signatures of well-characterized *Artemia* populations from known source areas, allows analytical identification, regardless of the strain involved. The confidence of identification can be increased by utilizing four independent isotopic parameters in chitin-derived compounds rather than using only two parameters in whole biomass. Positive identification should be preceded by isotopic measurements of *Artemia* populations from

potential source areas to evaluate seasonal and other factors contributing to isotopic variability in brine shrimp within the various environments.

Acknowledgements

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