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Acid washing effect on elemental and isotopic composition of whole beach arthropods: Implications for food web studies using stable isotopes

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ABSTRACT

Inorganic carbon removal through acidification is a common practice prior to isotopic analysis of macroinvertebrate samples. We have experimentally tested the effect of acidification on the elemental and isotopic composition of a range of beach arthropod species. Acidification resulted in a significant depletion of 7.7% and 1.2% on average for carbon and nitrogen, respectively, suggesting that acid washing affects body carbon compounds other than carbonates. With a few exceptions, $\delta^{13}C$ and $\delta^{15}N$ showed no changes following 1 N HCl attack. Based on those exceptions, our results show that only those samples with a high $CaCO_3$ content result in impoverished ^{13}C as a consequence of acidification. Those suspected to be carbonate-free are not significantly affected. Concerning $\delta^{15}N$ values, only high carbonate species were affected when treated with HCl. As a standard protocol, it is recommended to acidify only carbonate-rich samples prior to $\delta^{13}C$ analyses. When possible, muscle tissue samples should be used instead of the entire organism.

1. Introduction

Carbon and nitrogen isotopic ratios are very useful tools for the study of food web structures in aquatic ecosystems (e.g., Jennings et al., 1997; Pinnegar and Polunin, 2000; Vizzini and Mazzola, 2003; Fry, 2006). This approach helps to elucidate the origin of the ingested organic matter (Fry and Sherr, 1984; Owens, 1987; Preston, 1992) and to characterize flows of mass and energy through ecosystems (Fry, 1988; Owens, 1988; Hobson and Welch, 1992; Rau et al., 1992; Hesslein et al., 1993).

Most arthropod species contain carbonates which contribute to the global animal isotopic signature. It is common that samples are acidified before analysis in order to avoid the alteration of the $\delta^{13}C$ values by the high ^{13}C content of this non-dietary carbon fraction (e.g., Nieuwenhuize et al., 1994; Yokohama et al., 2005). The chemical reaction that follows the acidification treatment is:

$$CaCO_3 + 2HCl \rightarrow CO_2^{\uparrow} + CaCl_2 + H_2O \quad (1)$$

However, the need for and the convenience of using HCl as part of the sample preparation for duel isotopic analysis is still a matter of controversy that has only recently begun to be directly addressed (e.g., Soreide et al., 2006; Mateo et al. (in press)). A few observations have reported that acidification

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may also alter $\delta^{15}N$ values (Pinnegar and Polunin, 1999), supposedly as a consequence of partial loss of compounds containing nitrogen (Bunn et al., 1995; Jacob et al., 2005) such as chitin, proteins, and glycoprotein (Goering et al., 1990; Shafer et al., 1994). Based on this, some authors prefer to divide the sample into two aliquots, one acidified for the $\delta^{13}C$ analysis and another one not acidified for the $\delta^{15}N$ analysis (Polunin et al., 2001; Bouillon et al., 2002; Nyssen et al., 2002). Other studies have reported no significant effect by HCl attack on the isotopic signatures of marine animals, concluding that acidification of the samples was unnecessary (e.g., Chanton and Lewis, 1999; Grey et al., 2001; Nyssen et al., 2005). Some authors have tried to limit the extent of the eventual effect of acidification by acidifying samples before drying and grinding (Bunn et al., 1995) or using more gentle carbonate removal methods such as wetting samples in weak acid (0.1 N HCl; e.g., Hobson et al., 2002). However, only a few studies have presented data on the elemental composition (C and N) and discussed the effects of acidification on the elemental composition (Bunn et al., 1995). To contribute to the assessment of these methodological effects in beach macroinvertebrates, we evaluated the effects of acidification on C and N elemental composition and on the $\delta^{13}C$ and $\delta^{15}N$ values of some representative beach arthropods. The effect of acid washing on the carbon and nitrogen composition of these species has never been reported. To this end, the isotopic and elemental compositions of acidified and non-acidified aliquots have been compared.

Recently, Jacob et al. (2005) reported a positive linear relation between the effect of acidification on invertebrate $\delta^{13}C$ values and an estimate of its carbonate content. Since then, however, the sample's $CaCO_3$ content related to acid washing effect on isotopic values has received little attention. An exception is a study by Ng et al. (2007), where they calculated a carbonate proxy (as in Jacob et al., 2005) and recommended acid rinsing only for carbonate-rich algae. We hypothesize that acidification of invertebrate samples prior to analytical procedures involves changes both in the elemental and in the isotopic composition that can seriously confound food web analysis based on isotopic trophic scenarios. More specifically, we hypothesize that the most common acidification procedure (i.e., 1 N HCl acid washing until bubbling cessation) results in the removal of dietary (i.e., not from carbonates) carbon and nitrogen. We finally provide strong evidence supporting that the carbonate content of the invertebrate sample can be one of the most useful criteria for deciding on the need for acidifying the samples prior to isotopic analysis. To assess this problem, we have selected various beach and semi-terrestrial macroinvertebrate species and compared the elemental and isotopic compositions of acid-washed to raw aliquots and their relation with a carbonate proxy. In the light of the results, simple criteria for sample pre-treatment are proposed for standardization.

2. Materials and methods

Sample collection was carried out in May 2005 on the beach of Burano (N 42°23′51″; E 11°22′40″, Grosseto, Italy) using pitfall cross traps that intercepted macroinvertebrates. Six pitfall cross traps were deployed along the beach at 3-m intervals from the shoreline to the base of the dune. The traps were kept active for 24 h. The individuals captured were stored in thermally-sealed plastic bags and frozen at the site. Several individuals of the macroinvertebrate species *Arctosa cinerea* (Araneae, Lycosidae), *Geophilus* sp. (Geophilomorpha, Geophilidae), *Parallelomorphus laevigatus* (Coleoptera, Carabidae), *Phaleria bimaculata* (Coleoptera, Tenebrionidae), *Phaleria provincialis* (Coleoptera, Tenebrionidae), *Pimelia bipunctata* (Coleoptera, Tenebrionidae), *Scarites buparius* (Coleoptera, Carabidae), *Talitrus saltator* (Amphipoda, Talitridae) and *Tylos europaeus* (Isopoda, Tylidae) were selected. All individuals were taxonomically determined, age classified, cleaned with distilled water and oven-dried at 60 °C for 48 h. Finally, they were kept in a dry, dark place until processing. Four replicates of each species were used for elemental and isotopic analysis. Replicates consisted of 5–8 whole individuals of the same species. All replicates were re-dried (placed in the oven overnight at 60 °C) and milled to a fine homogeneous powder using an agate mortar and pestle. The powder was stored in a dry environment (relative humidity under 20%). About half of each of the replicates was acidified by adding 1 N HCl drop-by-drop until cessation of bubbling (Nieuwenhuize et al., 1994). Samples were then left in excess of acid for 3 h. This procedure was selected because it is considered to be the most widely used standard. The other half was kept untreated. The acidified sub-samples were re-dried at 60 °C for 24 h, and milled again to a fine homogenous powder. Samples were not rinsed with distilled water after acidification to avoid the alteration of the isotope values by the lixiviation of some organic carbon and nitrogen compounds (e.g., Cloern et al., 2002).

Finally, an aliquot of 0.7 mg dry weight (±0.05 mg) of each sample was weighed and placed in a tin capsule for solid samples. The encapsulated samples were kept under constant laboratory temperature (20 °C) and humidity conditions until analysis. Elemental and isotopic composition was determined for the gasses evolved from a single combustion using a Finnigan Delta S isotope ratio mass spectrometer (Conflo II interface) at the Scientific-Technical Services of the University of Barcelona. Isotopic values are reported in the δ_{VPDB} notation relative to the standards Vienna Pee Dee Belemnite and atmospheric nitrogen for carbon and nitrogen, respectively ($\delta_{sample} = 1000\ [(R_{sample}/R_{standard}) - 1]$, $R = {}^{13}C/{}^{12}C$, or $R = {}^{15}N/{}^{14}N$). Analytical precision based on the standard deviation of internal standards (atropine, IAEA CH3, CH6, CH7, and USGS40 – analytical grade L-glutamic acid, for carbon, and atropine, IAEA N1, NO3, N2, and USGS40, for nitrogen) ranged from 0.11 to 0.06‰ (mean = 0.09‰) for carbon, and from 0.06 to 0.28‰ (mean = 0.16‰) for nitrogen.

All data were checked for normality (Kolmogorov–Smirnov test) and for variance homogeneity (Levene's test). One-way ANOVAs were used to test the null hypothesis of no overall difference in the elemental composition and isotopic values between acidified and untreated samples. Post-hoc comparisons were then used to test specific differences between pairs (single species, raw vs. acidified samples). A proxy was used to estimate the content of $CaCO_3$ and to assess its role on the effect of acid washing on the stable isotope ratios (Jacob et al., 2005):