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The effects of acidification on the stable isotope signatures of marine algae and molluscs

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Abstract

Carbon (^{13}C) and nitrogen (^{15}N) stable isotope analysis has become increasingly important in the study of energy flow and tropho-dynamics in many ecosystems. Prior to analysis, samples are often pre-treated with acids to remove inorganic carbonates which may bias the results. The effects of pre-analysis acidification on isotopic values are, however, still poorly understood for marine producers (e.g. algae and cyanobacteria), and consumers (e.g. molluscs), which may confound the comparability of different studies. In this study, such effects (untreated vs. decalcified samples) were examined at two different sampling periods (summer and winter). Acidification did not seem to affect the isotopic composition of consumers, but reduced both $\delta^{13}C$ and $\delta^{15}N$ of producers. This effect was consistent for the two sampling periods, although both producers and consumers had more enriched $\delta^{13}C$ and $\delta^{15}N$ values in summer. Acidification had the most distinct, negative effect on the isotopic values of samples which had low carbonate contents. It is, therefore, important to be aware of temporal variations in sample isotopic values and especially the effects of sample treatment when attempting to compare different studies. As an attempt to standardize protocols, it is recommended that only acid-washing carbonate-rich samples is adopted as the most appropriate pre-analysis treatment.

Keywords: Sample treatment; Inorganic carbon; Carbonates; Temporal variation

1. Introduction

The predictable, stepwise enrichment of carbon (^{13}C) and nitrogen (^{15}N) stable isotopes across trophic levels is useful for studying organic matter flow, dietary source and trophic pathways within food webs in both aquatic and terrestrial ecosystems (Peterson and Fry, 1987; Vander Zanden and Rasmussen, 2001). In aquatic ecosystems, trophic fractionations of ^{13}C and ^{15}N are, on average, +1‰ and +3.4‰, respectively, which enable the use of ^{13}C as a carbon source tracer, and ^{15}N to reveal trophic relationships (Peterson and Fry, 1987; Michener and Schell, 1994).

The simultaneous measurement of $\delta^{13}C$ and $\delta^{15}N$, however, has some major methodological uncertainties. Tissue fat content, for example, is known to affect $\delta^{13}C$ but not $\delta^{15}N$ (Fry, 1988; Rau et al., 1992), whilst the effects of preservation by formalin and/or ethanol can vary greatly between species and studies (Bosley and Wainright, 1999; Kaehler and Pakhomov, 2001). Tissue inorganic carbon content, i.e. calcium carbonate ($CaCO_3$), is another source of variation which may bias isotopic values (Fry, 1988; Cloern et al., 2002; Jacob et al., 2005).

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Table 1
$\delta^{13}C$ and $\delta^{15}N$ (mean ± S.D.) of producer and consumer species with different sample treatments in the two sampling periods

Sample	Taxonomic group	Species	$\delta^{13}C$				$\delta^{15}N$			
			September		February		September		February	
			Untreated	Decalcified	Untreated	Decalcified	Untreated	Decalcified	Untreated	Decalcified
Producers	Biofilm	A matrix of mucus, bacteria and microalgae	−11.66 ± 0.05	−12.73 ± 0.03	−12.73 ± 0.40	−16.61 ± 0.84	7.74 ± 0.24	5.60 ± 0.14	2.21 ± 0.96	1.02 ± 2.06
	Cyanobacteria	Black cyanobacterial mat*	–	–	−6.97 ± 0.18	−7.80 ± 0.11	–	–	3.47 ± 0.24	2.47 ± 0.14
		Kyrtuthrix maculans	−1.87 ± 0.08	−2.44 ± 0.13	−3.50 ± 0.06	−4.62 ± 0.03	0.50 ± 0.16	−0.79 ± 0.17	0.73 ± 0.10	0.24 ± 0.66
		Lyngbya sp.*	–	–	−10.64 ± 0.12	−11.83 ± 0.20	–	–	6.55 ± 0.13	4.48 ± 0.93
	Chlorophyta	*Ulva* spp.*	–	–	−15.41 ± 0.54	−16.94 ± 0.36	–	–	6.92 ± 0.41	5.84 ± 0.29
	Phaeophyta	*Endarachne binghamiae**	–	–	−16.80 ± 0.19	−17.28 ± 0.13	–	–	7.66 ± 0.26	4.99 ± 0.53
		Hapalospongidion gelatinosum	−6.75 ± 0.17	−6.76 ± 0.23	−7.88 ± 0.06	−8.39 ± 0.10	9.17 ± 0.26	7.21 ± 0.07	7.39 ± 0.20	6.08 ± 0.21
		*Hincksia mitchelliae**	–	–	−16.18 ± 0.17	−16.99 ± 0.03	–	–	7.32 ± 0.10	4.85 ± 0.40
		*Sargassum hemiphyllum**	–	–	−16.48 ± 0.57	−16.57 ± 0.43	–	–	5.69 ± 0.33	4.50 ± 0.44
	Rhodophyta	Encrusting corallines	−6.47 ± 0.14	−10.92 ± 0.10	−8.80 ± 0.40	−16.89 ± 0.05	11.46 ± 0.33	8.24 ± 0.42	7.44 ± 0.93	5.97 ± 0.63
		*Gelidium pusillum**	–	–	−16.62 ± 0.19	−16.29 ± 0.37	–	–	5.36 ± 0.34	6.44 ± 0.74
		Hildenbrandia spp.	−7.08 ± 0.12	−6.46 ± 0.12	−8.70 ± 0.28	−9.28 ± 0.35	9.15 ± 0.32	7.09 ± 0.09	7.36 ± 0.16	7.24 ± 0.07
		*Porphyra suborbiculata**	–	–	−15.22 ± 0.83	−14.96 ± 1.12	–	–	6.39 ± 0.26	5.35 ± 0.62
Consumers	Polyplacophora	*Acanthopleura japonica*	−12.09 ± 1.00	−11.86 ± 0.81	−12.75 ± 1.33	−13.04 ± 1.54	12.35 ± 0.30	12.17 ± 0.40	10.89 ± 0.54	11.63 ± 0.68
	Gastropoda	*Cellana toreuma*	−10.66 ± 0.94	−11.16 ± 0.22	−12.14 ± 0.98	−12.19 ± 1.50	9.50 ± 0.97	10.22 ± 0.36	9.06 ± 0.69	9.66 ± 0.86
		Chlorostoma argyrostoma	−11.25 ± 0.59	−11.06 ± 0.42	−12.28 ± 0.39	−12.09 ± 0.51	10.66 ± 0.46	11.13 ± 0.27	10.26 ± 0.55	10.27 ± 0.41
		Lunella coronata	−11.24 ± 0.47	−11.46 ± 0.14	−11.76 ± 0.54	−11.17 ± 0.12	10.39 ± 0.36	10.66 ± 0.06	9.97 ± 0.29	10.33 ± 0.62
		Monodonta labio	−11.65 ± 0.95	−11.19 ± 0.37	−12.36 ± 1.16	−11.92 ± 1.32	9.41 ± 0.57	9.89 ± 0.41	9.54 ± 0.56	9.76 ± 0.36
		Nerita albicilla	−10.61 ± 0.49	−11.08 ± 0.24	−13.03 ± 0.67	−13.03 ± 0.65	11.40 ± 0.36	11.56 ± 0.13	11.33 ± 0.52	11.66 ± 0.41

Species with an asterisk (*) are strongly seasonal and only present in winter.