

Sample acidification effects on carbon and nitrogen stable isotope ratios of macrofauna from a *Zostera noltii* bed

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Abstract. Acidification treatment has been used in most stable isotope studies to eliminate inorganic non-dietary carbon, allowing $\delta^{13}\text{C}$ measurement of organic carbon needed for trophic studies. Because only limited information about the acidification effects on isotopic ratios exists in the literature, we provide an assessment of such effects for a wide range of benthic macroinvertebrates from estuarine intertidal sediments with sparse *Zostera noltii* Hornem. vegetation. Our results revealed only few significant $\delta^{13}\text{C}$ shifts for macrobenthos; however, $\delta^{13}\text{C}$ decrease after acidification was substantial for several species. We conclude that acidification is not necessary for removing calcium carbonate in a majority of macrobenthos, but we also suggest preliminary testing before deciding it can be omitted. The magnitude of the $\delta^{15}\text{N}$ shifts was substantial for some species and variability in $\delta^{15}\text{N}$ values of replicate acidified samples exceeded that of non-acidified samples. Therefore, sample acidification should be avoided for $\delta^{15}\text{N}$ determination. Moreover, acidification effects vary considerably among species belonging to a single class, rendering generalisations from single-species information potentially spurious.

Additional keywords: decarbonation, inorganic carbon, macrobenthos, sample treatment, seagrass.

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Introduction

Stable isotopes have been used in many studies dealing with food-web analysis and trophic interactions (see review by [Boecklen *et al.* 2011](#)). Among others, stable carbon and nitrogen isotope analyses are widely used in aquatic food-web studies, giving information about both the utilisation of the food resources and the trophic level of the consumers ([Peterson and Fry 1987](#)).

Many studies have already investigated the effects of sample-preparation procedures on stable isotope ratios, mainly focusing on preservation methods (e.g. [Bosley and Wainright 1999](#); [Ryba and Burgess 2002](#); [Sarakinis *et al.* 2002](#); [Barrow *et al.* 2008](#)) and the acidification of samples (e.g. [Bunn *et al.* 1995](#); [Jacob *et al.* 2005](#); [Søreide *et al.* 2006](#); [Ng *et al.* 2007](#); [Jaschinski *et al.* 2008](#); [Kolasinski *et al.* 2008](#); [Mateo *et al.* 2008](#); [Serrano *et al.* 2008](#)). The latter is crucial for a correct analysis of tissue carbon isotopic composition because it removes inorganic, non-dietary carbon present in, for instance, shells. Calcium carbonate (CaCO_3) is the main source of inorganic carbon in most organisms and is highly enriched in ^{13}C compared with organic carbon, because of differences between organic matter synthesis and calcium carbonate precipitation ([Craig 1953](#); [DeNiro and Epstein 1978](#); [Kennedy *et al.* 2005](#)). Acidification of the

samples with dilute HCl before isotope analysis has been widely used to eliminate CaCO_3 , so as to increase the accuracy of diet analysis. The use of HCl also leads to the formation of CaCl_2 , a hygroscopic compound which rapidly integrates water, potentially hampering accurate weighing and evaluation of %C and %N of the samples; however, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ measurement does not necessarily require determination of sample mass and %C and %N.

There is evidence that sample acidification may also affect nitrogen isotopic composition. Because carbonates do not contain nitrogen, a loss of organic ^{15}N is assumed to be a result of acidification, attributed to small losses of acid-soluble proteins and amino acids via the volatilisation of oxidised nitrogenous organic compounds ([Goering *et al.* 1990](#); [Bunn *et al.* 1995](#)). Nevertheless, different studies have produced highly equivocal results on the direction and magnitude of the acidification effect on $\delta^{15}\text{N}$ signatures. For instance, $\delta^{15}\text{N}$ increase as a result of sample acidification has been reported for penaeid shrimps ([Bunn *et al.* 1995](#)), fish ([Bunn *et al.* 1995](#); [Pinnegar and Polunin 1999](#)), bivalves ([Yokoyama *et al.* 2005](#)), octopus ([Carabel *et al.* 2006](#)), Arctic crustaceans ([Søreide *et al.* 2006](#)) and tropical brachyurans ([Kolasinski *et al.* 2008](#)). In contrast, decreases in $\delta^{15}\text{N}$ were observed for blue crabs ([Fantle *et al.* 1999](#)), ghost