

## NOTE

## $\delta^{15}\text{N}$ evidence for nitrogen fixation associated with macroalgae from a seagrass-mangrove-coral reef system

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**ABSTRACT:** Early studies using acetylene reduction have suggested that macroalgae may contribute to nitrogen enrichment of waters near coral reefs via nitrogen fixation by their epiphytic cyanophytes. Our objectives were to investigate the potential of stable nitrogen isotope analysis for detecting nitrogen fixation in near-reef macroalgae, and to compare these findings with those for different fixing and non-fixing autotrophs from other systems. We made collections of near-reef algae in Puerto Rico, seagrasses and macroalgae from the northeast coast of the U.S., and freshwater algae from streams in Quebec, Canada. The mean  $\pm$  SD  $\delta^{15}\text{N}$  for our near-reef algae was  $0.3 \pm 1.0\text{‰}$ , values which were significantly lower than those from our other sampling areas and also well below published values for other marine benthic nitrogen fixers (ca 2‰) and non-fixers (ca 6‰). Our results provide a useful test of the value of stable nitrogen analysis in detecting nitrogen fixation of near-reef algae, thereby supporting previous non-isotope work in suggesting that this macroalgal-cyanophyte complex may provide an important source of fixed nitrogen to reef systems.

**KEY WORDS:** Benthic macroalgae ·  $\delta^{15}\text{N}$  · Inferred nitrogen fixation

Because nitrogen concentrations in tropical oceanic waters near coral reefs are so very low (Webb et al. 1975, D'Elia & Wiebe 1990), nitrogen often becomes limiting to algal growth (Williams & Carpenter 1988, Berner 1990). As a result, nitrogen fixation is recognized as being an important feature of many coral reef algae (Burriss 1976, Potts & Whitton 1977, Wilkinson & Fay 1979, Shashar et al. 1994, Williams & Carpenter 1997). This enhanced nitrogen fixation often leads to a positive balance and net export of nitrogen from reefs to surrounding waters (D'Elia & Wiebe 1990).

Macroalgae are dominant features of shallow tropical waters (Baravetzka & Larkum 1986, Hillis-Colin-

vauz 1986) and are frequently covered by epiphytic blue-green algae such as *Calothrix* sp. (Hillis-Colinvaux 1980) that are capable of nitrogen fixation as indicated by acetylene reduction (Capone 1977, Capone et al. 1977, Goldner 1980). Macroalgae may therefore contribute to the nitrogen enrichment of waters near coral reefs (Baravetzka & Larkum 1986, Berner 1990). Similarly, the presence of epiphytic cyanophytes on tropical seagrasses is also associated with high rates of nitrogen fixation (Goering & Parker 1972, McRoy et al. 1973, Goldner 1980).

The first objective of the present study was to investigate the potential of stable nitrogen isotope analysis for detecting nitrogen fixation associated with macroalgae situated in seagrass meadows or mangrove stands located near coral reefs. An ancillary objective was to compare these findings with similar  $\delta^{15}\text{N}$  values obtained for different autotrophs, both non-fixing and presumably fixing forms, from other systems.

Notwithstanding inter-site variability in physiological isotopic fractionation in relation to differences in  $\delta^{15}\text{N}$  of source DIN (dissolved inorganic nitrogen), an overall gradient exists in  $^{15}\text{N}$  enrichment of autotrophs from terrestrial ( $\delta^{15}\text{N}$  range from  $-9$  to  $8\text{‰}$  centered about a mode of  $-1\text{‰}$ ) to freshwater ( $\delta^{15}\text{N}$  range from  $-3$  to  $10\text{‰}$  centered about a mode of  $3\text{‰}$ ) to marine ( $\delta^{15}\text{N}$  range from  $-3$  to  $18\text{‰}$  centered about a mode of  $6\text{‰}$ ) environments (France 1995). Irregularities in this pattern with  $\delta^{15}\text{N}$  values approaching the atmospheric signature of  $0\text{‰}$  are suggestive of nitrogen fixation (e.g. Virginia & Delwiche 1982, Minagawa & Wada 1986, Gu & Alexander 1993).

With the exception of a few estuarine studies that have suggested or identified benthic microalgae as being nitrogen fixers (Pulich & Scalan 1987, Currin et

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al. 1995, France 1998), there is scant  $\delta^{15}\text{N}$  evidence that any other marine benthic autotrophs are capable of this process. Seagrass-epiphyte complexes have been suggested to be able to fix nitrogen based on the atypically low  $\delta^{15}\text{N}$  values ( $3.5 \pm 1.4$  SD‰; Fry et al. 1986) of these plants relative to  $\delta^{15}\text{N}$  values for other marine autotrophs (ca 6‰; France 1995). The few  $\delta^{15}\text{N}$  values that exist for coral reef algae ( $2.5 \pm 2.0$ ‰; Yamamuro et al. 1995) are comparable to those for estuarine benthic microalgae ( $2.2 \pm 2.1$ ‰), implying the presence of nitrogen fixation.

Calcareous green algae (*Halimeda incrassata*, *Penicillus capitatus* and *Avrainvillea longicaulis*) were collected from within or near seagrass (*Thalassia testudinum*) beds, and the red alga *Acanthophora spicifera* was collected from mangrove (*Rhizophora mangle*) prop roots. All 21 samples were obtained from locations situated inshore and near fringing coral reefs along the southwestern coast of Puerto Rico, near the Isla Magueyes Laboratories, University of Puerto Rico (France & Holmquist 1997).

We sampled other autotrophs for comparison. Benthic algae not thought to be capable of nitrogen fixation were sampled from freshwater streams in the Laurentian mountains of southern Quebec (Canada) and from seagrass beds in Boston Harbor (MA, USA). Freshwater benthic algae ( $n = 18$ ) representing filamentous greens (*Cladophora*, *Spyrogira*, *Microspora*) and diatom (*Tabellaria*, *Cymbella*, *Achnanthes*) epilithon were obtained from streams of ranging hydrodynamic and chemical characteristics (France & Cattaneo 1998). Temperate marine benthic algae ( $n = 6$ ) representing macroalgae (*Ectocarpus* sp., *Ceramium* sp., *Desmarestia* sp., *Enteroporia bantrophora*, *Chondrus crispus*) were sampled from within or near seagrass beds at several stations in Boston Harbor. We also sampled detritus composed predominantly of seagrass (*Zostera marina*) remains from outer Boston Harbor ( $n = 4$ ) to obtain plant-epiphyte complexes similar to those previously suggested by  $\delta^{15}\text{N}$  analysis to be capable of fixing nitrogen. All samples were acid washed, gently rinsed and hand-cleaned to remove inorganic and obvious organic contaminants and were analyzed for nitrogen isotopes on a VG Micromass 903E triple-collector (Dept of Earth Sciences, Univ. of Waterloo) and a Europa Tracermass (Dept of Biology, McGill Univ.) mass spectrometer with an average SD of  $\pm 0.20$ ‰ for paired standards following weight-related bias (beam value) correction.

The significant ( $t$ -test,  $p < 0.05$ ) enrichment in  $^{15}\text{N}$  observed for non-nitrogen-fixing algae from Boston Harbor ( $6.5 \pm 0.7$  SD‰) compared to those from Quebec streams ( $3.8 \pm 2.4$ ‰) agrees with literature data for these different types of algae (Fig. 1) as well as for vascular and algal autotrophs in general (France 1995).

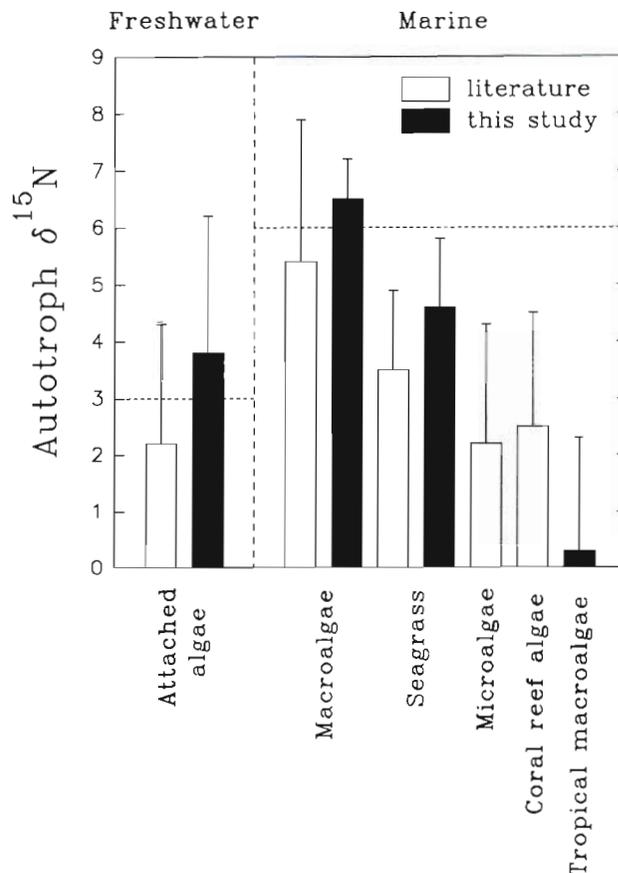


Fig. 1. Stable nitrogen isotope ratios ( $\delta^{15}\text{N}$ ) +SD for macroalgal-cyanophyte complexes from a seagrass-mangrove-coral reef system in Puerto Rico ('tropical macroalgae') compared to data for other autotrophs sampled in this study [seagrass-epiphyte detritus and macroalgae from Boston Harbor ('seagrass' and 'macroalgae' respectively); filamentous-epilithic algae from Quebec streams ('attached algae')] as well as to global data summarized from the literature [white histograms, data sources are papers cited in the text or referenced in France (1995)]. Sample sizes from left to right for literature data are 65, 21, 20, 12 and 4, and for data from this study are 18, 6, 4 and 21. Horizontal dotted lines denote average  $\delta^{15}\text{N}$  values for freshwater and marine autotrophs compiled by France (1995)

Compared to the global marine average for all autotrophs,  $\delta^{15}\text{N}$  values of the seagrass-epiphyte detritus from Boston Harbor are low ( $4.6 \pm 1.2$ ‰) supporting previous stable isotope results (Fry et al. 1986) which indicate that the standing seagrass-epiphyte complex is capable of a limited nitrogen fixation (Fig. 1). The average  $\delta^{15}\text{N}$  value found for macroalgae from the seagrass-mangrove system of Puerto Rico ( $0.3 \pm 1.0$ ‰) is significantly below (ANOVA, Duncan's multiple range test,  $p < 0.05$ ) averages recorded for the other identified marine nitrogen fixers of coral reef algae, seagrass and microalgae (Fig. 1). Tropical

macroalgae-cyanophyte complexes displayed inter-specific variability in their  $\delta^{15}\text{N}$  values: *Avrainvillea longicaulis*  $2.0 \pm 0.1\text{‰}$ , *Acanthophora spicifera*  $1.4 \pm 1.4\text{‰}$ , *Penicillus capitatus*  $0.9 \pm 0.9\text{‰}$ , and *Halimeda incrassata*  $-1.7 \pm 1.3\text{‰}$ , corroborating via  $\delta^{15}\text{N}$  analysis the magnitude and variability in nitrogen fixation associated with these species previously estimated through acetylene reduction (Goldner 1980).

Measurements of  $\delta^{15}\text{N}$  in coral tissues ( $2.5 \pm 1.6\text{‰}$ ,  $n = 70$ ) have been interpreted as demonstrating nitrogen fixation by endolithic cyanophytes (Yamamuro et al. 1992, 1995, Muscatine & Kaplan 1994). The especially low  $\delta^{15}\text{N}$  values presumably originating from cyanophytes associated with macroalgae found in this study ( $0.3 \pm 1.0\text{‰}$ ) suggest that this particular epiphyte-substrate complex may represent a major source of fixed nitrogen to oligotrophic reef systems.

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