Vol. 167: 297–299, 1998 | MARINE ECOLOGY PROGRESS SERIES Mar Ecol Prog Ser | Published June 18

NOTE

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

R. France[1,*], J. Holmquist[2], M. Chandler[3], A. Cattaneo[4]

[1]Harvard University, Graduate School of Design, 48 Quincy St, Cambridge, Massachusetts 02138, USA
[2]Dept Marine Sciences, University of Puerto Rico, Mayaguez Campus, Isla Magueyes Laboratories, PO Box 908 Lajas, Puerto Rico 00667, USA
[3]New England Aquarium, Central Wharf, Boston, Massachusetts 02110, USA
[4]Dept Sciences Biologiques, Universite de Montreal, CP 6128, Succ. Centre Ville, Montreal, Quebec H3C 3J7, Canada

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 ± SD $\delta^{15}N$ for our near-reef algae was 0.3 ± 1.0‰, 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 · $\delta^{15}N$ · 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 (Burris 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-Colinvaux 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 $\delta^{15}N$ 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 $\delta^{15}N$ of source DIN (dissolved inorganic nitrogen), an overall gradient exists in ^{15}N enrichment of autotrophs from terrestrial ($\delta^{15}N$ range from −9 to 8‰ centered about a mode of −1‰) to freshwater ($\delta^{15}N$ range from −3 to 10‰ centered about a mode of 3‰) to marine ($\delta^{15}N$ range from −3 to 18‰ centered about a mode of 6‰) environments (France 1995). Irregularities in this pattern with $\delta^{15}N$ values approaching the atmospheric signature of 0‰ 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

*E-mail: rfrance@gsd.harvard.edu

al. 1995, France 1998), there is scant $\delta^{15}N$ 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 $\delta^{15}N$ values (3.5 ± 1.4 SD‰; Fry et al. 1986) of these plants relative to $\delta^{15}N$ values for other marine autotrophs (ca 6‰; France 1995). The few $\delta^{15}N$ values that exist for coral reef algae (2.5 ± 2.0‰; Yamamuro et al. 1995) are comparable to those for estuarine benthic microalgae (2.2 ± 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 testudium*) 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., *Entropuria 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 $\delta^{15}N$ 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 ±0.20‰ for paired standards following weight-related bias (beam value) correction.

The significant (t-test, $p < 0.05$) enrichment in ^{15}N observed for non-nitrogen-fixing algae from Boston Harbor (6.5 ± 0.7 SD‰) compared to those from Quebec streams (3.8 ± 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).

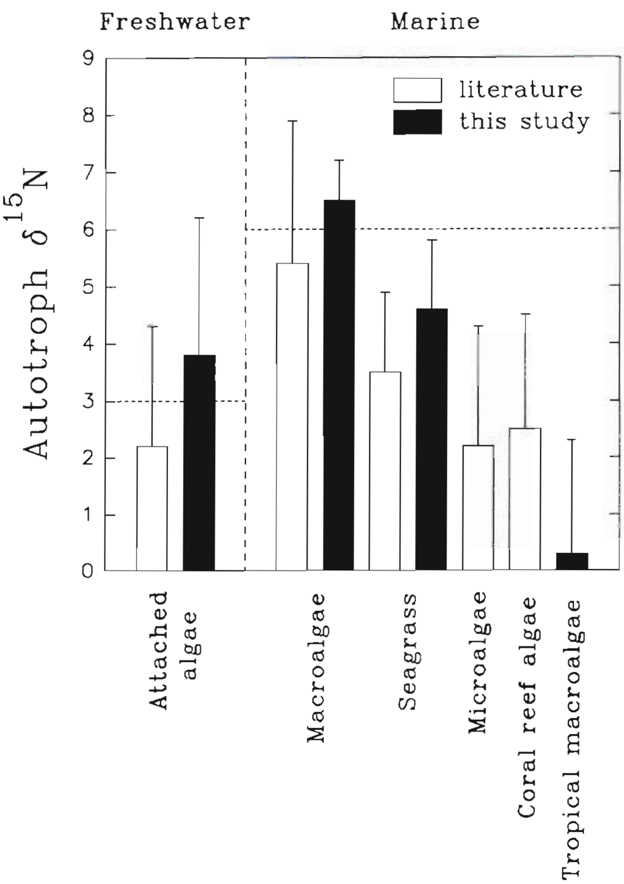

Fig. 1. Stable nitrogen isotope ratios ($\delta^{15}N$) +SD for macroalgal-cyanophyte complexes from a seagrass-mangrove-coral reef system in Puerto Rico ('tropical macroalgae') compared to data for other autotrophs sampled in this study [seagrass-epiphyte detritus and macroalgae from Boston Harbor ('seagrass' 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 $\delta^{15}N$ values for freshwater and marine autotrophs compiled by France (1995)

Compared to the global marine average for all autotrophs, $\delta^{15}N$ values of the seagrass-epiphyte detritus from Boston Harbor are low (4.6 ± 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 $\delta^{15}N$ value found for macroalgae from the seagrass-mangrove system of Puerto Rico (0.3 ± 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 interspecific variability in their $\delta^{15}N$ values: *Avrainvillea longicaulis* 2.0 ± 0.1‰, *Acanthophora spicifera* 1.4 ± 1.4‰, *Penicillus capitatus* 0.9 ± 0.9‰, and *Halimeda incrassata* –1.7 ± 1.3‰, corroborating via $\delta^{15}N$ analysis the magnitude and variability in nitrogen fixation associated with these species previously estimated through acetylene reduction (Goldner 1980).

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

Acknowledgements. This work was funded by a Canadian NSERC Strategic Grant to R. Peters and R.F., a US Army Research Office Grant to J.H., the New England Aquarium, and an NSERC operating grant to A.C. Travel support for R.F. to Puerto Rico was supplied by Circo Caribe through D. Soles. R. Drimmie, S. Mazumder, G. Cheong, M. Patton, V. Angus, P. Bethiaume and D. Ballantine are thanked for logistic assistance.

LITERATURE CITED

Baravetzka MA, Larkum AWD (1986) Reef algae. Oceanus 29: 49–54

Berner T (1990) Coral-reef algae. In: Dubinsky Z (ed) Coral reefs. Ecosytems of the World 25. Elsevier, New York, p 253–264

Burris RH (1976) Nitrogen fixation by blue-green algae of the Lizard Island area of the Great Barrier Reef. Aust J Plant Physiol 3:41–51

Capone DG (1977) $N_2(C_2H_2)$ fixation by macroalgal epiphytes. Proc 3rd Int Coral Reef Symp. Univ Guam Press, Mangilao, p 337–342

Capone DG, Taylor DL, Taylor BF (1977) Nitrogen fixation (acetylene reduction) associated with macroalgae in a coral-reef community in the Bahamas. Mar Biol 40:29–32

Currin CA, Newell SY, Paerl HW (1995) The role of standing dead *Spartina alterniflora* and benthic microalgae in salt marsh food webs: considerations based on multiple stable isotope analysis. Mar Ecol Prog Ser 121:99–116

D'Elia CF, Wiebe WJ (1990) Biogeochemical nutrient cycles in coral-reef ecosystems. In: Dubinsky Z (ed) Coral reefs. Ecosystems of the world 25. Elsevier, New York, p 49–74

France RL (1995) Source variability in $\delta^{15}N$ of autotrophs as a potential aid in measuring allochthony in freshwaters. Ecography 18:318–320

France RL (1998) Estimating the assimilation of mangrove detritus by fiddler crabs in Laguna Joyuda, Puerto Rico, using dual stable isotopes. J Trop Ecol (in press)

France RL, Cattaneo A (1998) $\delta^{13}C$ variability of benthic algae: effects of water colour via modulation by stream current. Freshwat Biol (in press)

France RL, Holmquist JG (1997) $\delta^{13}C$ variability of macroalgae: effects of water motion via baffling by seagrasses and mangroves. Mar Ecol Prog Ser 147:305–308

Fry B, Macko SA, Zieman JC (1986) Review of stable isotopic investigations of food webs in seagrass meadows. Florida Mar Res Publ 42:189–209

Goering JJ, Parker PL (1972) Nitrogen fixation by epiphytes on sea grasses. Limnol Oceanogr 17:320–323

Goldner LL (1980) Nitrogen fixation (acetylene reduction) in shallow water Bahamian environments. Bull Mar Sci 30: 444–453

Gu B, Alexander V (1993) Estimation of N_2 fixation based on differences in the natural abundances of ^{15}N among freshwater N_2-fixing and non-N_2-fixing algae. Oecologia 96: 43–48

Hillis-Colinvaux L (1980) Ecology and taxonomy of *Halimeda* primary producer of coral reefs. Adv Mar Biol 17:1–327

Hillis-Colinvaux L (1986) Have reefs been misnamed? Oceanus 29:43–48

McRoy CP, Goering JJ, Chaney B (1973) Nitrogen fixation associated with seagrasses. Limnol Oceanogr 18:998–1002

Minagawa M, Wada E (1986) Nitrogen isotope ratios of red tide organisms in the East China Sea: a characterization of biological nitrogen fixation. Mar Chem 19:245–259

Muscatine L, Kaplan IR (1994) Resource partioning by reef corals as detemined from stable isotope composition. 2. $\delta^{15}N$ of zooxanthellae and animal tissue vs depth. Mar Biol 100:185–193

Potts M, Whitton BA (1977) Nitrogen fixation by blue-green algal communities in the intertidal zone of the lagoon of Aldabra Atoll. Oecologia 27:275–283

Pulich WM, Scalan RS (1987) Organic carbon and nitrogen flow from marine cyanobacteria to semiaquatic food webs. Contrib Mar Sci 30:27–37

Shashar N, Cohen Y, Loya Y, Sar N (1994) Nitrogen fixation (acetylene reduction) in stony corals: evidence for coral-bacteria interactions. Mar Ecol Prog Ser 111:259–264

Virginia RA, Delwiche CC (1982) Natural ^{15}N abundance of presumed N_2-fixing and non N_2-fixing plants from selected ecosystems. Oecologia 54:273–325

Webb KL, DuPaul WD, Wiebe W, Sottile W, Johannes RE (1975) Enewetak (Eniwetok) Atoll: aspects of the nitrogen cycle on a coral reef. Limnol Oceanogr 20:198–210

Wilkinson CR, Fay P (1979) Nitrogen fixation in coral reef sponges with symbiotic cyanobacteria. Nature 279: 527–529

Williams SL, Carpenter RC (1988) Nitrogen-limited primary productivity of coral reef algal turfs: potential contribution of ammonium excreted by *Diadema antillarum*. Mar Ecol Prog Ser 47:145–152

Williams SL, Carpenter RC (1997) Grazing effects on nitrogen fixation in coral reef algal turfs. Mar Biol 130:223–231

Yamamuro M, Kayanne H, Minagawa M (1995) Carbon and nitrogen stable isotopes of primary producers in coral reef ecosystems. Limnol Oceanogr 40:617–621

Yamamuro M, Minagawa M, Kayanne H (1992) Preliminary observation on food webs in Shiraho coral reef as determined from carbon and nitrogen stable isotopes. Proc 7th Int Coral Reef Symp, Vol 1 Univ Miami Press, Miami, p 352–355

Editorial responsibility: Otto Kinne (Editor), Oldendorf/Luhe, Germany

Submitted: January 26, 1998; Accepted: April 21, 1998
Proofs received from author(s):