

NOTE

$\delta^{13}\text{C}$ variability of macroalgae: effects of water motion via baffling by seagrasses and mangroves

Robert L. France^{1,*}, Jeff G. Holmquist²¹WDNRG Limnetics, 417 Haney St, Winnipeg, Manitoba, Canada R3R 0Y5²Department of Marine Sciences, University of Puerto Rico, Mayaguez Campus, Isla Magueyes Laboratories, PO Box 908 Lajas, Puerto Rico 00667, USA

ABSTRACT: It has been argued that the ^{13}C -enrichment of benthic compared to pelagic consumers is a function of differential diffusion resistance in the boundary layer that surrounds algae in these 2 environments. We pursued a field test and confirmed the hypothesis that differences in water motion may ultimately translate into differing $\delta^{13}\text{C}$ values by sampling benthic algae both inside and outside the flow-shielded habitats of seagrass beds and mangrove forests. Macroalgae collected within seagrass beds were ^{13}C -enriched relative to macroalgae at the edge or outside the grass canopy. However, the inverse held in the mangrove system where presumably photoassimilation by sheltered macroalgae of respiratory CO_2 from the mineralization of mangrove detritus more than compensated for the effect of reduced water motion. Our findings suggest the application of stable isotope analysis for the fine-scale study of foodweb structure on the basis of local differences in water motion, but also caution against the potential for such processes to confound conclusions drawn from data collected on a larger scale.

KEY WORDS: Water motion · Benthic macroalgae · ^{13}C -enrichment

Stable carbon isotope analyses are important for elucidating material flow in foodwebs (Fry & Sherr 1984). As an important marine example, offshore pelagic consumers are often depleted in ^{13}C compared to benthic consumers (McConnaughey & McRoy 1979, Fry et al. 1983, Fry & Sherr 1984, Rodelli et al. 1984, Hobson et al. 1994, France 1995a, Jarman et al. 1996). The basis for this relationship, however, is not completely clear. France (1995a), using previous physiological arguments (Smith & Walker 1980, Wheeler 1980, Keeley & Sandquist 1992), suggested that the ^{13}C -enrichment of

benthic compared to pelagic consumers was a consequence of physical phenomena at low trophic levels, i.e. low diffusion resistance in the boundary layer surrounding planktonic algae exposed to turbulent conditions versus higher diffusion resistance for benthic algae exposed to less water motion. Hecky & Hesslein (1995) elaborated on this hypothesis and, expanding the findings of Farquhar (1983), developed a theoretical model to predict carbon isotopic fractionation in relation to boundary layer thickness. Hecky & Hesslein's (1995) model is supported by France's (1995a, b) compilations of worldwide $\delta^{13}\text{C}$ data on benthic and planktonic algae. However, field studies demonstrating an effect of water motion on intraspecific $\delta^{13}\text{C}$ of aquatic autotrophs are few and often conducted for reasons other than those of direct concern here (Osmond et al. 1981, Raven et al. 1982, Cooper & McRoy 1988, Simenstad et al. 1993). Here we report on the first explicit test of the influence of water motion on $\delta^{13}\text{C}$ variability of benthic algae.

Because water flow is greatly reduced by seagrass canopies (e.g. Fonseca et al. 1982, Harlin et al. 1982, Bulthuis et al. 1984, Fonseca & Fisher 1986, Fonseca 1989, Ackerman & Okubo 1993) and mangrove prop roots (e.g. Scoffin 1970, Hoffmeister 1974, Odum et al. 1982), resulting in a decreased thickness of diffusion boundary layers (Koch 1993), we determined $\delta^{13}\text{C}$ values of various macroalgae as a function of location relative to stands of these comparatively large vascular plants. We collected 3 species of calcareous green algae (*Halimeda incrassata*, *Penicillus capitatus*, *Avrainvillea longicaulis*) at least 10 m within seagrass (*Thalassia testudinum*) beds, as well as at the sand-seagrass ecotone and at least 5 m outside beds. We collected the red alga (*Acanthophora spicifera*) from prop roots of mangroves (*Rhizophora mangle*) located in

*Present address: Department of Biology, McGill University, 1205 Ave. Dr Penfield, Montreal, Quebec, Canada H3A 1B1. E-mail: rfrance@bio1.lan.mcgill.ca

areas of low water motion surrounded by dense stands, as well as from open channels between such stands where water motion was expected to be greater. We sampled during September and December 1994 on the southwestern coast of Puerto Rico (near Isla Magueyes Laboratories, University of Puerto Rico). Replicate sampling sites were established along a 15 km section of coastline. All samples were collected from water depths between 1 and 2 m to avoid potential artifacts due to differing light levels (Wefer & Killingley 1986) and atmospheric exposure (Cooper & McRoy 1988).

To remove carbonates, all macroalgae samples were ground and acid washed until evolution of CO_2 ceased (Wefer & Killingley 1986). Samples were stored frozen until they could be analyzed for carbon isotopes on a Europa Tracermass mass spectrometer interfaced with a Robo-prep-CN analyzer which produced an average SD of $\pm 0.3\text{‰}$ for duplicate measurements (France 1996a). The data demonstrated marked departures from normality (probit plots) and were marginal in terms of homogeneity of variance (Cochran's test). As these problems were not rectified by various transformations, we used sign tests for our analyses. All per-contrast error rates less than 0.05 were also significant at 0.048 after compensation for multiple comparisons via the sequential Bonferroni adjustment (to family-wise error rate; Holm 1979, Rice 1989).

We examined water motion at our seagrass stations using clod cards (plaster of paris blocks attached to plastic cards; Doty 1971, Thompson & Glenn 1994). Sixteen pairs of cards (one of each pair in seagrass, the other in nearby sand) were deployed at our stations. This method uses plaster (calcium sulfate hemihydrate) dissolution rate as an estimator of multi-directional water motion. The approach was particularly useful for measurements in our grassbeds, because the dense canopy of seagrass would have precluded use of larger apparatuses. We prepared the cards following the methods of Thompson & Glenn (1994), except that we used contact cement rather than silicone cement for attachment of the blocks to the cards. The cards were anchored to the substrate with 15 cm galvanized nails and were left in place for 4 d before recovery. Each pair was deployed at intervals of several days apart so as to include temporal as well as spatial variance in water motion.

There were no significant differences in $\delta^{13}\text{C}$ for samples collected from outside grassbeds compared to the periphery of beds for each of the 3 species of benthic macroalgae ($p > 0.05$). Differences did exist between these combined samples and those collected from deep inside the seagrass beds (Fig. 1; *Halimeda incrassata*, $p < 0.001$; *Penicillus capitatus*, $p = 0.016$; family-wise error rate = 0.016). Limited sampling of the third species of calcareous green alga (*Avrainvillea*

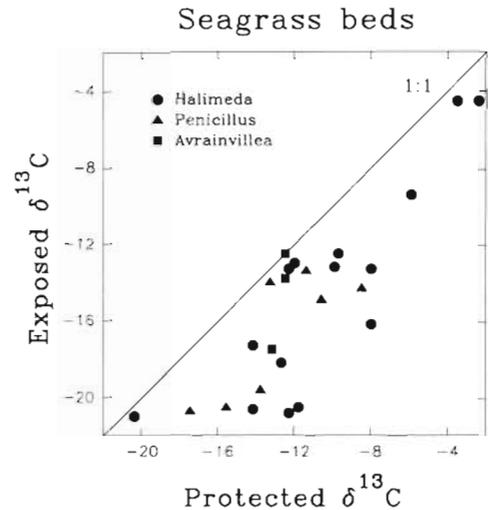


Fig. 1. *Halimeda incrassata*, *Penicillus capitatus* and *Avrainvillea longicaulis*. Site-specific comparisons of stable carbon isotope ratios ($\delta^{13}\text{C}$) for 3 species of benthic macroalgae collected from protected locations of low water motion inside seagrass beds and from exposed locations of greater water motion at the periphery or outside such beds

longicaulis) showed the same trend as *H. incrassata* and *P. capitatus* (Fig. 1). One-tailed sign tests indicated greater water motion in sand habitats than in seagrass beds at our stations ($p = 0.0037$). Therefore, macroalgae of all 3 species were found to be enriched in ^{13}C by 0 to 9‰ inside the seagrass beds, where they were shielded from the higher water motion existing at the periphery and outside the grassbeds. These findings, which are similar to those determined with respect to water current for vascular macrophytes (Osmond et al. 1981), offer support for the contentions of France (1995a) and the model of Hecky & Hesslein (1995), i.e. increasing boundary layer thickness through reducing water motion decreases the isotopic discrimination against ^{13}C , resulting in higher $\delta^{13}\text{C}$ values.

Epiphytic macroalgae from mangroves displayed the opposite trend. *Acanthophora spicifera* collected from open channels were enriched in ^{13}C compared to samples from protected areas ($p = 0.0039$; Fig. 2). In this case, as for seagrasses and particulate organic matter (Fleming et al. 1982, Zieman et al. 1984, Lin et al. 1991, Hemminga et al. 1994), it appears that the photoassimilation of respiratory CO_2 originating from the mineralization of mangrove detritus (usually having $\delta^{13}\text{C}$ values of about -26 to -30‰) by macroalgae located within protected areas superseded any possible effect of reduced water motion on isotopic fractionation. As a result, this process resulted in lower, not higher, $\delta^{13}\text{C}$ values. Our results for epiphytic macroalgal $\delta^{13}\text{C}$ from mangroves therefore support Smith & Kroopnick's (1981) suggestion, based on their work with coral reef

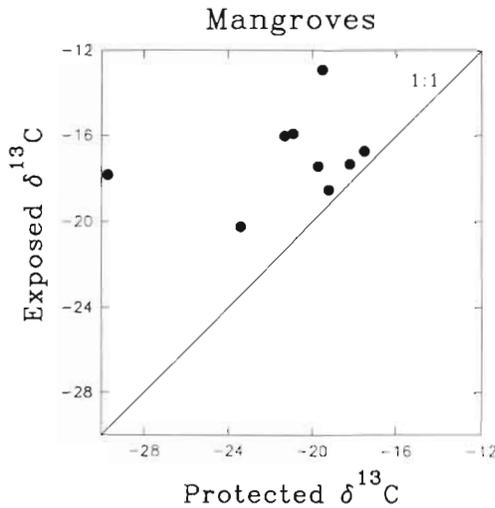


Fig. 2. *Acanthophora spicifera*. Site-specific comparisons of stable carbon isotope ratios for epiphytic algae collected from protected locations surrounded by mangrove islets and from exposed locations in tidal channels between such islets

organisms, that the magnitude of carbon isotopic fractionation can be used as a convenient measure of aquatic metabolism, particularly the balance between the production of new compared to the recycling of old organic matter (cf. Rosenfeld & Mackay 1991, del Giorgio & France 1996, France et al. 1997). It is unlikely, however, that a similar process can be invoked to describe the isotopic differences in macroalgal $\delta^{13}\text{C}$ inside and outside the seagrass beds; i.e. if assimilation of decomposing seagrass detritus (usually having $\delta^{13}\text{C}$ values of about -15 to -8‰) were a major factor, one might expect epiphytic algal and seagrass values to be similar, which is seldom the case (France 1996b).

The present work indicates that, in addition to such environmental factors as temperature and light, water motion may play a major role in regulating intraspecific variability in the stable carbon isotopic composition of marine macroalgae. Because carbon undergoes little (DeNiro & Epstein 1978, France & Peters 1997) or no (France 1995c, 1996c) further fractionation with food assimilation, variability in benthic algal $\delta^{13}\text{C}$ will consequently be reflected by variability in consumer $\delta^{13}\text{C}$. As a result, isotopic differences between benthic and planktonic foodwebs may be reduced in situations where benthic algae are ^{13}C -depleted through their exposure to conditions of either high water motion or decomposing mangrove detritus. For example, France (1995a) found greater overlap in the frequency distributions of worldwide $\delta^{13}\text{C}$ data between offshore consumers and those from rocky shores and mangrove swamps than between offshore consumers and those from seagrass meadows. Conversely, our results suggest applications of stable carbon isotope analysis for

fine-scale elucidation of material flow in foodwebs on the basis of local differences in water motion.

Acknowledgements. This work was supported by a Canadian NSERC Strategic Grant to R. Peters and R.L.F. and a US Army Research Office grant (DAAH04-93-2-0020) to J.G.H. Travel support for R.L.F. was supplied by Circo Canbe through D. Soles. S. Mazumder, M. Schaepler, G. Cheong and G. Cabana aided in sample preparation and analysis, as did D. Ballantine with algal taxonomy and J. Schmidt-Gengenbach with clod card preparation and deployment.

LITERATURE CITED

- Ackerman JD, Okubo A (1993) Reduced mixing in a marine macrophyte canopy. *Funct Ecol* 7:912–919
- Bulthuis DA, Brand GW, Mobley MC (1984) Suspended sediments and nutrients in water ebbing from seagrass-covered and denuded tidal mudflats in a southern Australian embayment. *Aquat Bot* 20:257–266
- Cooper IW, McRoy CP (1988) Stable carbon isotope ratio variations in marine macrophytes along intertidal gradients. *Oecologia* 77:238–241
- del Giorgio PA, France RL (1996) Ecosystem-specific patterns in the relationship between zooplankton and POM or microplankton $\delta^{13}\text{C}$. *Limnol Oceanogr* 41:359–365
- DeNiro MJ, Epstein S (1978) Influence of diet on the distribution of carbon isotopes in animals. *Geochim Cosmochim Acta* 42:495–506
- Doty MS (1971) Measurement of water movement in reference to benthic algal growth. *Bot Mar* 14:32–35
- Farquhar GD (1983) On the nature of carbon isotope discrimination in C_4 species. *Aust J Plant Physiol* 10:205–226
- Fleming M, Lin G, Da Silva L (1982) Influence of mangrove detritus in an estuarine ecosystem. *Bull Mar Sci* 47:663–669
- Fonseca MS (1989) Sediment stabilization by *Halophila decipiens* in comparison to other seagrasses. *Estuar Coast Shelf Sci* 29:501–507
- Fonseca MS, Fisher JS (1986) A comparison of canopy friction and sediment movement between four species of seagrass with reference to their ecology and restoration. *Mar Ecol Prog Ser* 29:15–22
- Fonseca MS, Fisher JS, Zieman JC, Thayer GW (1982) Influence of the seagrass, *Zostera marina* L., on current flow. *Estuar Coast Shelf Sci* 15:351–364
- France RL (1995a) Carbon-13 enrichment in benthic compared to planktonic algae: foodweb implications. *Mar Ecol Prog Ser* 124:307–312
- France RL (1995b) Differentiation between littoral and pelagic food webs in lakes using stable carbon isotopes. *Limnol Oceanogr* 40:1310–1313
- France RL (1995c) Carbon isotopic variability in the composite pelagic foodweb of four oligotrophic lakes: feeding diversity or metabolic fractionations? *J Plankton Res* 17:1993–1997
- France RL (1996a) Carbon isotope ratios in logged and unlogged boreal forests: examination of the potential for determining wildlife habitat use. *Environ Manage* 20:249–255
- France RL (1996b) Stable isotopic survey of the role of macrophytes in the carbon flow of aquatic foodwebs. *Vegetatio* 124:67–72
- France RL (1996c) Absence or masking of metabolic fractionations of ^{13}C in a freshwater benthic food web. *Freshwat Biol* 36:1–6

- France R, del Giorgio P, Westcott K (1997) Productivity and heterotrophy influences on zooplankton $\delta^{13}\text{C}$ in northern temperate lakes. *Aquat Microb Ecol* 12:85–93
- France RL, Peters RH (1997) Ecosystem differences in the trophic enrichment of ^{13}C in aquatic foodwebs. *Can J Fish Aquat Sci* (in press)
- Fry B, Scalani RS, Parker PL (1983) $^{13}\text{C}/^{12}\text{C}$ ratios in marine food webs of the Torres Strait, Queensland. *Aust J Mar Freshwat Res* 54:707–716
- Fry B, Sherr EB (1984) $\delta^{13}\text{C}$ measurements as indicators of carbon flow in marine food webs. *Contrib Mar Sci* 27:15–47
- Harlin MM, Thorne-Miller B, Boothroyd JC (1982) Seagrass-sediment dynamics of a flood-tidal delta in Rhode Island (USA). *Aquat Bot* 14:127–138
- Hecky RE, Hesslein RH (1995) Contributions of benthic algae to lake food webs as revealed by stable isotope analysis. *J North Am Benthol Soc* 14:631–653
- Hemminga MA, Slim FJ, Kazunga J, Ganssen GM, Nieuwenhuize J, Kruyt NM (1994) Carbon outwelling from a mangrove forest with adjacent seagrass beds and coral reefs (Gazi Bay, Kenya). *Mar Ecol Prog Ser* 106:291–301
- Hobson KA, Piatt JF, Pitocchelli J (1994) Using stable isotopes to determine seabird trophic relationships. *J Anim Ecol* 63:786–798
- Hoffmeister JE (1974) Land from the sea: the geologic study of south Florida. Univ of Miami Press, Coral Gables, FL
- Holm S (1979) A simple sequentially rejective multiple test procedure. *Scand J Stat* 6:65–70
- Jarman WM, Hobson KA, Sydeman WJ, Bacon CE, McLaren EB (1996) Influence of trophic position and feeding location on contaminant levels in the Gulf of the Farallones food web revealed by stable isotope analysis. *Environ Sci Technol* 30:654–660
- Keeley JE, Sanquist DR (1992) Carbon: freshwater plants. *Plant Cell Environ* 15:1021–1035
- Koch EW (1993) Hydrodynamics, diffusion-boundary layers and photosynthesis of the seagrasses *Thalassia testudinum* and *Cymodocea nodosa*. *Mar Biol* 116:767–776
- Lin G, Banks T, Sternberg L (1991) Variation in $\delta^{13}\text{C}$ values for the seagrass *Thalassia testudinum* and its relations to mangrove carbon. *Aquat Bot* 40:333–341
- McConnaughey T, McRoy CP (1979) Food-web structure and the fractionation of carbon isotopes in the Bering Sea. *Mar Biol* 53:257–262
- Odum WE, McIvor CC, Smith TJ (1982) The ecology of the mangroves of South Florida; a community profile. *US Fish Wildl Serv FWS/OBS-81/24*
- Osmond CB, Valaane N, Haslam SM, Votila P, Roksandic Z (1981) Comparisons of $\delta^{13}\text{C}$ values in leaves of aquatic macrophytes from different habitats in Britain and Finland: some implications for photosynthesis processes in aquatic plants. *Oecologia* 50:117–124
- Raven JA, Beardall J, Griffiths H (1982) Inorganic C-sources for *Lemanea*, *Cladophora* and *Ranunculus* in a fast-flowing stream: measurements of gas exchange and of carbon isotope ratio and their ecological implications. *Oecologia* 53:68–78
- Rice WR (1989) Analyzing tables of statistical tests. *Evolution* 43:223–225
- Rodelli MR, Gearing JN, Gearing PJ, Marshall N, Saseumar A (1984) Stable isotope ratio as a tracer of mangrove carbon in Malaysian ecosystems. *Oecologia* 61:326–333
- Rosenfeld JS, Mackay RJ (1991) Assessing the food base of stream ecosystems: alternatives to the P/R ratio. *Oikos* 50:141–147
- Scoffin TP (1970) The trapping and binding of subtidal carbonate sediments by marine vegetation in Bimini Lagoon, Bahamas. *J Sediment Petrol* 40:249–273
- Simenstad CA, Duggins DO, Quay PD (1993) High turnover of inorganic carbon in kelp habitats as a source of $\delta^{13}\text{C}$ variability in marine food webs. *Mar Biol* 116:147–160
- Smith FR, Walker NA (1980) Photosynthesis by aquatic plants: effects of unstirred layers in relation to assimilation of CO_2 and HCO_3^- and to carbon isotopic discrimination. *New Phytol* 86:245–259
- Smith SV, Kroopnick P (1981) Carbon-13 isotopic fractionation as a measure of aquatic metabolism. *Nature* 294:252–253
- Thompson TL, Glenn EP (1994) Plaster standards to measure water motion. *Limnol Oceanogr* 39:1768–1779
- Wefer G, Killingley JS (1986) Carbon isotopes in organic matter from a benthic alga *Halimeda incrassata* (Bermuda): effects of light intensity. *Chem Geol* 59:321–326
- Wheeler WN (1980) Effect of boundary layer transport on the fixation of carbon by the giant kelp *Macrocystis pyrifera*. *Mar Biol* 56:103–110
- Zieman JC, Macko SA, Mills AL (1984) Role of seagrasses and mangroves in estuarine food webs: temporal and spatial changes in stable isotope composition and amino acid content during decomposition. *Bull Mar Sci* 35:380–392

This note was submitted to the editor

Manuscript first received: August 15, 1996
Revised version accepted: February 24, 1997