Estuarine, Coastal and Shelf Science (1988) **26**, 429–436

Changes in Carbon and Hydrogen Stable Isotope Ratios of Macroalgae and Seagrass During Decomposition

Gwen E. Fenton and David A. Ritz

Zoology Department, University of Tasmania, Box 252C G.P.O. Hobart, 7001, Tasmania, Australia

Received 11 August 1987 and in revised form 20 October 1987

Keywords: isotope ratios; decomposition; macroalgae; sea grasses; Tasmania

Stable isotope ratios of carbon and hydrogen were determined for six algae and one seagrass species during a 60-day decomposition experiment. Changes in the carbon ratios were in the order of 1‰. However, significant isotopic changes were observed for hydrogen in all species and in most cases these changes were substantial. These changes probably reflect differential leaching of isotopically distinct compounds.

Introduction

Stable isotope analysis is now widely used as a means of tracing material flow through food chains with the isotopes of carbon being the most commonly used (see Fry & Sherr, 1984, for a review). The method is based on the fact that, while the isotopes of, for example, carbon and hydrogen are fractionated during photosynthesis and chemosynthesis, there is little further fractionation within higher trophic levels. Animal isotopic ratios are largely diet dependent. Thus the stable isotope ratio of an animal reflects that of its diet, effectively constituting a natural tag (DeNiro & Epstein, 1978; Thayer *et al.*, 1978). Numerous studies document the value of the method in identifying carbon sources for animals living in seagrass environments (Fry & Parker, 1979), mangroves (Rodelli *et al.*, 1984), salt marshes (Haines, 1977), and in the plankton (Checkley & Entzeroth, 1985). There are less data for hydrogen isotopic ratios but these too have been found to be useful in food chain studies (Estep & Dabrowski, 1980; Macko *et al.*, 1983). As the stable isotope method aims to identify and track the major dietary sources, a knowledge of the isotopic variability of these sources is necessary. Gearing *et al.* (1984) noted that several assumptions have frequently been made in the literature;

(1) each plant produces a relatively constant isotopic signature;
(2) this signature does not change during decomposition to detritus; and
(3) the isotopic ratio of a consumer is virtually identical to that of its diet.

The second of these assumptions has been examined here and the first assumption in Fenton and Ritz (in prep.).

0272–7714/88/040429+08 $03.00/0

© 1988 Academic Press Limited

TABLE 1. List of species used in the decomposition experiment and their site of collection

	Species	Site
Phaeophyta	*Ecklonia radiata*	One Tree Point
	Acrocarpia paniculata	One Tree Point
	Hormosira banksii	Killora Bay
Rhodophyta	*Gigartina* sp.	One Tree Point
Chlorophyta	*Ulva taeniata*	One Tree Point
	Ulva spathulata	Killora Bay
Marine angiosperm	*Heterozostera tasmanica*	Killora Bay

It is probable that a low percentage of macroalgal and seagrass biomass in the sea is eaten while the plants are still living (Mann, 1972; Fenchel, 1977). Most macrophyte production passes through a detrital phase before it is consumed by animals. Hence it is of great interest to know whether any fractionation of stable isotopes accompanies the decomposition of macrophyte tissue. Previous work on decomposition both *in situ* and in the laboratory suggests there is very little fractionation in the case of carbon (e.g. Stephenson *et al.*, 1986). However, a significant fractionation of hydrogen isotopes during decomposition of *Ulva* sp. was reported by Macko *et al.* (1983).

In the present study, we investigated the changes in stable isotope ratios of carbon and hydrogen during decomposition of a representative range of common macroalgae in Southern Tasmania, including members of the Chlorophyta, Phaeophyta and Rhodophyta, and a seagrass.

Materials and Methods

Six algae and one seagrass species were collected in October 1985 from one of two sites on Bruny Island, Southern Tasmania, namely One Tree Point and Killora Bay. The species of algae are listed in Table 1. A sample of seawater (surface) was collected from both sites and analysed for δD.

The algae and seagrass were dried for 48 h at 60 °C, crumbled and sieved to a particle size of 125–250 μm to give a homogeneous sample. Triplicate sub-samples of each species weighing 1–3 g were placed in 1-l beakers which were then filled with filtered (3 μm) seawater and covered. The beakers were maintained at 13 °C in a 12/12 light/dark regime for 60 days. The water was changed every alternate day by decanting off 750 ml and refilling with filtered seawater. The seawater used throughout had an isotopic ratio of $\delta D = +0{\cdot}4‰$.

Samples were collected from each beaker periodically throughout the experiment on pre-combusted (4 h at 450 °C) GF/C filters (1-μm pore size), wrapped in pre-combusted aluminium foil (4 h at 450 °C) and dried for 48 h at 60 °C. Each sample was then crushed using a mortar and pestle and stored in glass vials (both pre-cleaned in chromic acid and rinsed in distilled water) in a desiccator until analysed.

In order to examine more closely the isotopic ratios of mucilage produced by algae early in the decomposition process, the first two days of the above experiment were repeated for *Ecklonia radiata* and *Hormosira banksii*. These two species were selected for further study because, in the early stages of the initial decomposition when considerable mucilage was

released, the δD of one species became more negative while that of the other became more positive. Care was taken to collect the densest concentration of mucilage from the base of the beaker by decanting off as much seawater as possible. Samples of seawater and of seawater-containing mucilage were treated as seawater samples for isotopic analysis.

Stable isotope analysis

Organic combustion

Organic material (10–13 mg) was mixed with 1·5 g of pre-fired CuO (4 h at 800 °C in a muffle furnace), placed in a 10 cm quartz tube containing two 9 cm lengths of Cu wire mesh and sealed with quartz wool, all of which had previously been pre-combusted under vacuum for 20 min at 900 °C and cooled for a further 30 min under high vacuum (10^{-6} T). The sample tube was then loaded into the quartz combustion tube (pre-combusted as for sample tube) and attached to the high-vacuum line and evacuated. The sample was then combusted at 900 °C for 20 min and the resultant CO_2 and H_2O separated and collected using liquid nitrogen and dry-ice/acetone. The water was frozen directly onto HNO_3-cleaned zinc (8–30 mesh, BDH Analar) and converted into hydrogen by heating at 450 °C for 45 min according to the method of Coleman *et al.* (1982).

Water samples

A 5 μl water sample was pipetted into a prepared zinc tube under a positive pressure of high purity dry nitrogen gas. The tube was attached to a vacuum line and the water frozen with liquid nitrogen prior to evacuating the tube. Once evacuated the tap was closed and the sample thawed, refrozen and then re-evacuated to ensure all nitrogen was removed. The sample was then converted to hydrogen as above.

Isotopic analysis

The CO_2 and H_2 were subsequently analysed on a VG Micromass 602D stable isotope mass spectrometer. The results are reported in $\delta^{13}C$ and δD notation with respect to the international standards PDB and SMOW, respectively. A minimum of three replicates of each sample were analysed with a reproducibility of $\pm 0{\cdot}2$‰ for $\delta^{13}C_{PDB}$ and ±3‰ for δD. Reference reproducibility was less than 0·09‰ for $\delta^{13}C$ and $\delta^{18}O$ and less than 2‰ for δD.

Results

Carbon

The carbon results are given in Figure 1. Significant changes in $\delta^{13}C$ values occurred in all species except *Acrocarpia paniculata* which maintained an almost constant isotopic ratio throughout the experiment. The variations over time were, however, small with final values between 0·55 and 1·22‰ from their initial values. The largest isotopic change was observed during the first day for all algae except for *Ulva taeniata* and *U. spathulata* in which the largest change occurred between days 13 and 31. This initial change was in a positive direction for all species except *Hormosira banksii* and *Ecklonia radiata*. However, *E. radiata* was the only species which at the finish of the experiment was more negative than it started, with $\Delta = -0{\cdot}92$‰. In contrast to the algae, the seagrass *Heterozostera tasmanica* was slower to show isotopic change with the maximum change occurring at the end of the experiment between days 31 and 59. Despite this slower rate of change the final value of *H. tasmanica* was 1·22‰ more positive than its initial value, which was the largest change in carbon observed in this experiment.

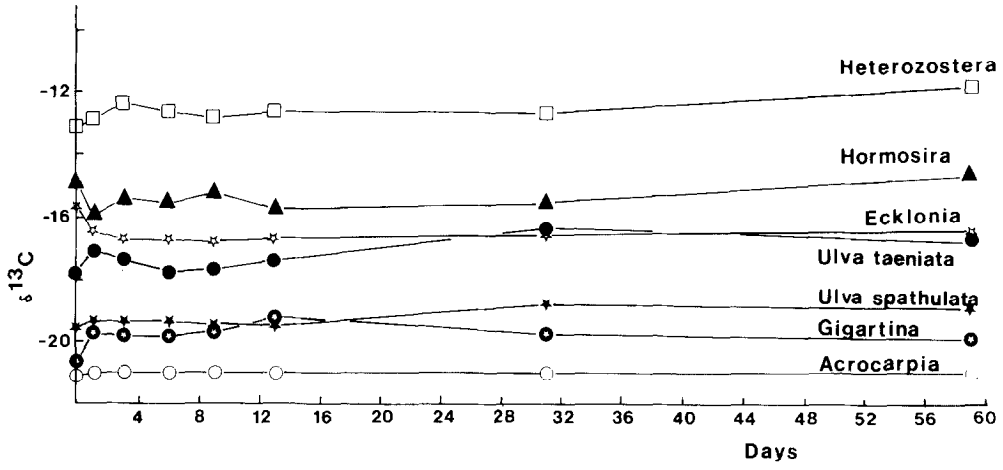

Figure 1. $\delta^{13}C_{PDB}$ values of the algae and seagrass species during the 60-day decomposition experiment. Each value represents the mean of at least triplicate analyses. The standard deviation was always less than ±0·2‰ and, therefore, too small to be drawn on the graph.

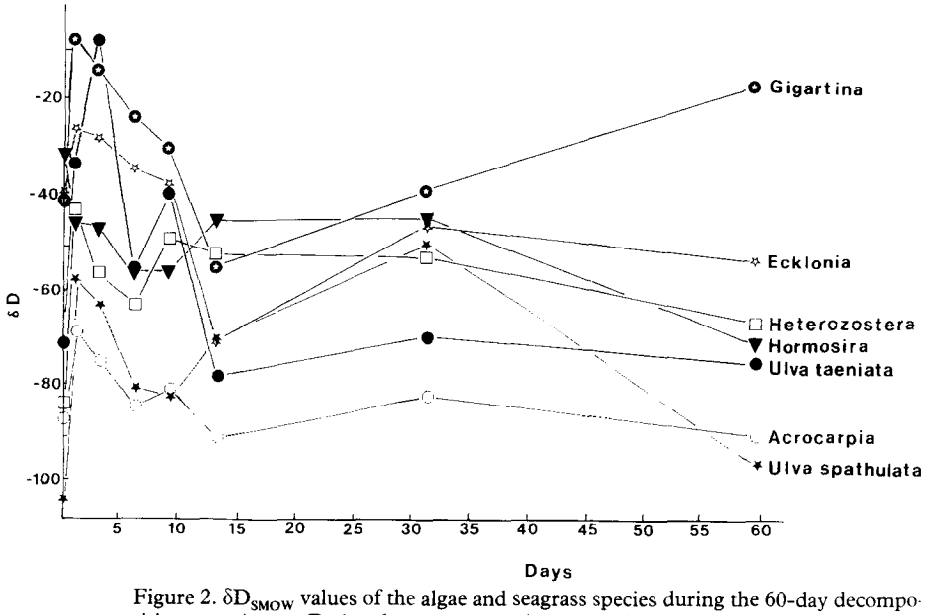

Figure 2. δD_{SMOW} values of the algae and seagrass species during the 60-day decomposition experiment. Each value represents the mean of at least triplicate analyses. The standard deviation was always less than ±3‰ and, therefore, too small to be drawn on the graph.

Hydrogen

The hydrogen results are given in Figure 2. Significant changes in δD over time occurred in all species and in most cases were substantial. However, by examining initial and final values alone the extent of these changes would not have been apparent, for example,

TABLE 2. Deuterium results from the mucilage experiment: each value represents a minimum of triplicate analyses with an error of less than 3‰

Sample	δD_{SMOW}‰
Seawater	+12·03
E. radiata mucilage Day 1	+5·10
E. radiata mucilage Day 2	+4·24
H. banksii mucilage Day 1	+3·37
H. banksii mucilage Day 2	+3·88

Acrocarpia paniculata $\Delta D = 4{\cdot}8‰$, *Ulva taeniata* $\Delta D = 6‰$, and *Ulva spathulata* $\Delta D = 9{\cdot}7‰$.

A. paniculata showed the least isotopic change for hydrogen, as was the case for carbon, although the changes were significant. The largest change occurred during the first day, but by day 6 the δD had virtually resumed its initial value from which it did not substantially deviate thereafter. *Heterozostera tasmanica* followed a similar pattern of isotopic decomposition to *A. paniculata* although the extent of the changes was substantially greater for the seagrass.

The pattern of substantial change in the first day was observed in all species and was in a positive direction (becoming isotopically heavier) for all species except *Hormosira banksii* which became isotopically lighter. After the initial positive change all species, except *U. taeniata*, had become more negative by day 3. *U. taeniata* did not show this trend until day 6 by which time all species were becoming more negative.

Ulva spathulata became progressively more negative until day 9; subsequently it became slightly more positive upto day 31, after which it again became more negative until at day 59 it had almost returned to its initial isotopic ratio, with a final value of $\delta D = -97{\cdot}07‰$ compared to an initial value of $\delta D = -106{\cdot}76‰$. *U. taeniata* did not follow the same pattern as *U. spathulata*. *U. taeniata* reached a second positive peak by day 13 after which it hardly changed and at the end of the experiment was similar to its initial value.

Gigartina sp. and *Ecklonia radiata* were most negative on day 13 and thereafter became more positive. In both these species the final isotopic ratios were significantly different from their initial values.

The δD value for surface water from One Tree Point was $\delta D = +5{\cdot}8‰$, which was not significantly different from that at Killora Bay with $\delta D = +3{\cdot}1‰$.

The hydrogen isotopic ratios of the mucilage produced by *Ecklonia radiata* and *Hormosira banksii* during the first two days of decomposition are given in Table 2. No species difference was observed between the mucilage values, but the mucilage of both species was more negative than the surrounding seawater.

Discussion

In general these results show that, during the processes of decay and disintegration, the $\delta^{13}C$ of algae and the seagrass *Heterozostera tasmanica* change little over 60 days. These results are consistent with the findings reported for decomposing marsh grasses (Haines, 1977), phytoplankton (Gearing *et al.*, 1984), seagrasses and mangroves (Zieman *et al.*, 1984), and for algae (Stephenson *et al.*, 1986). Despite the fact that mucilage released by

decomposing plant material promotes the culture of micro-organisms (Linley *et al.*, 1981), the microbial decomposition does not appear to result in isotopic fractionation (Haines, 1977). However, a recent study by Macko & Estep (1984) found isotopic fractionation of ^{13}C (up to 11‰) and ^{15}N (up to 22‰) associated with heterotrophic microbiological activity in single-species cultures grown on individual pure compounds. In a natural environment with a diversity of of substrates and species, such large effects may be cancelled out and in fact go undetected. However, if the organic source is dominated by a single compound (e.g. cellulose), or if one microbial population is dominant, the isotopic composition of source material could be altered significantly (Macko & Estep, 1984) and therefore would be worth investigating for macroalgal decomposition.

In contrast to the results for carbon, the δD changed quite markedly in all plants. A change in hydrogen isotopic ratio during decay has been reported by Macko *et al.* (1983) for *Ulva* sp. who found that δD of the alga became progressively more positive over the course of 56 days. The magnitude of this variation, ranging from −158·5‰ at the start to −112‰ at the end of the experiment, contrasts with the present results in which the δD of the two species of *Ulva* did not conform to this pattern and varied markedly from each other. Macko *et al.* (1983), when trying to rationalize their results for the decomposition of *Ulva*, suggested that the observed changes could be the result of either exchange with the isotopically heavier surrounding water or that during decomposition the organically bound hydrogen may preferentially lose the light isotope. Estep and Hoering (1980, 1981) have shown experimentally that organic hydrogen in living or in sonicated algal cells does not exchange to any great extent with the surrounding water. Only when the sonicated algal cells were denatured (by heating at 56 °C for 24 h) and resuspended in isotopically heavy water, was hydrogen exchanged. The drying procedure used to prepare the plants in the present study was not such a severe treatment, but may have been sufficient to denature cells and promote exchange of organically bound hydrogen with the water (which was isotopically similar to that found at the study sites at the time of collection). Even if such an exchange did occur this can hardly explain the fact that, in some cases, the δD rapidly became isotopically heavier, while in *Hormosira banksii* it became lighter.

The changes observed are most likely the result of differential rates of leaching. For example, some species released large quantities of mucilage within the first day of the dried material being placed in water (e.g. *Ecklonia radiata*) while others (e.g. *H. banksii*) were much more resistant to breakdown. *H. banksii* differed from all other plants in that it showed a fairly consistent trend towards becoming isotopically lighter with time. However, the results of the mucilage experiment did not show any difference between *E. radiata* and *H. banksii*. The release of an isotopically negative substance (i.e. mucilage) will render the plant more positive; therefore, because *E. radiata* produced substantially greater quantities of mucilage it would be expected to show a greater positive shift relative to *H. banksii*. The results obtained here showed this to be true for *E. radiata* but do not explain why *H. banksii* became more negative during the first day. Clearly mucilage production alone cannot explain the changes observed in these algae. Further investigation into the isotopic composition of leachates throughout the decomposition process is needed to explain the patterns observed here.

Species differences in the patterns of isotopic decomposition may be partially due to their differing abilities to resist desiccation in the environment. Sieburth (1969) found considerable organic exudation from algae as a result of desiccation (caused by low tide) and subsequent re-immersion in seawater. The desiccation, although artificially produced in the present study, resulted in extensive exudation from the species examined in this

experiment, but the extent of this exudation may reflect their habitat exposure to such desiccation. For example, *H. banksii* occurs in a different habitat to the other plants, being essentially a littoral species (Edgar, 1984). *Gigartina* sp. and both *Ulva* species also extend into the littoral but their distribution is subtidal. *H. banksii* might, therefore, be expected to be more resistant to desiccation and its tough epidermis lends support to this idea.

In conclusion, the assumption examined here that a plant's isotopic signature does not change during decomposition appears to be basically true for carbon but not for hydrogen. In terms of food-web analysis, although carbon isotopes are useful in characterizing both living and dead macrophytes, they cannot be used to determine which state the plant was in when consumed. The complexity of the changes observed in hydrogen isotopic ratios during breakdown suggests that they, too, cannot be used to discriminate between live and detrital plant matter in the diets of animals.

Acknowledgements

We thank Miss S. Adrichem and Mr M. Power, Central Science Laboratory for technical assistance and Mr R. B. Mawbey and Mr R. Holmes for assistance in the collection of the algae. Algal identifications were kindly made by Dr D. P. Thomas and Mr C. Sanders. This work was supported by a Marine Science and Technology Grant No. 84/1858.

References

Checkley Jr, D. M. & Entzeroth, L. C. 1985 Elemental and isotopic fractionation of carbon and nitrogen by marine planktonic copepods and implications to the marine nitrogen cycle. *Journal of Plankton Research* **7**, 553–568.

Coleman, M. L., Shepherd, T. J., Durham, J. J., Rouse, J. E. & Moore, G. R. 1982 Reduction of water with zinc for hydrogen isotope analysis. *Analytical Chemistry* **54**, 993–995.

DeNiro, M. & Epstein, S. 1978 Influence of diet on the distribution of carbon isotopes in animals. *Geochimica et Cosmochimica Acta* **42**, 495–506.

Edgar, G. J. 1984 General features of the ecology and biogeography of Tasmanian subtidal rocky shore communities. *Papers and Proceedings of the Royal Society of Tasmania* **118**, 173–186.

Estep, M. F. & Dabrowski, H. 1980 Tracing food webs with stable hydrogen isotopes. *Science* **209**, 1537–1538.

Estep, M. F. & Hoering, T. C. 1980 Biogeochemistry of the stable hydrogen isotopes. *Geochimica et Cosmochimica Acta* **44**, 1197–1206

Estep, M. F. & Hoering, T. C. 1981 Stable hydrogen isotope fractionations during autotrophic and mixotrophic growth of microalgae. *Plant Physiology* **67**, 474–477.

Fenchel, T. 1977 Aspects of the decomposition of seagrasses. In *Seagrass Ecosystems: A Scientific Perspective* (McRoy, C. P. & Helfferich, C., eds). M. Dekker, New York. p. 123–145.

Fry, B. & Parker, P. L. 1979 Animal diet in Texas seagrass meadows: ^{13}C evidence for the importance of benthic plants. *Estuarine and Coastal Marine Science* **8**, 499–509.

Fry, B. & Sherr, E. B. 1984 $\delta^{13}C$ measurements as indicators of carbon flow in marine and freshwater ecosystems. *Contributions to Marine Science* **27**, 13–47.

Gearing, J. N., Gearing, P. J., Rudnick, D. T., Requejo, A. G. & Hutchins, M. J. 1984 Isotopic variability of organic carbon in a phytoplankton-based, temperate estuary. *Geochimica et Cosmochimica Acta* **48**, 1089–1098.

Haines, E. B. 1977 The origins of detritus in Georgia salt marsh estuaries. *Oikos* **29**, 254–260.

Linley, E. A. S., Newell, R. C. & Bosman, S. A. 1981 Heterotrophic utilisation of mucilage released during fragmentation of kelp (*Ecklonia maxima* and *Laminaria pallida*). I. Development of microbial communities associated with the degradation of kelp mucilage. *Marine Ecology Progress Series* **4**, 31–41.

Macko, S. A. & Estep, M. L. F. 1984 Microbial alteration of stable nitrogen and carbon isotopic compositions of organic matter. *Organic Geochemistry* **6**, 787–790.

Macko, S. A., Estep, M. L. F. & Lee, W. Y. 1983 Stable hydrogen isotope analysis of foodwebs on laboratory and field populations of marine amphipods. *Journal of Experimental Marine Biology and Ecology* **72**, 243–249.

Mann, K. H. 1972 Macrophyte production and detritus food chains in coastal waters. *Memorie Istituto Italiano di Idrobiologia dott* **29 Suppl,** 353–383.

Rodelli, M. R., Gearing, J. N., Gearing, P. J., Marshall, N. & Sasekumar, A. 1984 Stable isotope ratio as a tracer of mangrove carbon in Malaysian ecosystems. *Oecologia (Berlin)* **61,** 326–333.

Sieburth, J. McN. 1969 Studies on algal substances in the sea. III. The production of extracellular organic matter by littoral marine algae. *Journal of Experimental Biology an Ecology* **3,** 290–309.

Stephenson, R. L., Tan, F. C. & Mann, K. H. 1986 Use of stable carbon isotope ratios to compare plant material and potential consumers in a seagrass bed and a kelp bed in Nova Scotia, Canada. *Marine Ecology Progress Series* **30,** 1–7.

Thayer, G. W., Parker, P. L., LaCroix, M. W. & Fry, B. 1978 The stable isotope carbon ratio of an eelgrass *Zostera marina* bed. *Oecologia (Berlin)* **35,** 1–12.

Zieman, J. C., Macko, S. A. & Mills, A. L. 1984 Role of seagrasses and mangroves in estuarine food webs: temporal and spatial changes in stable isotope composition and amino acid content during decomposition. *Bulletin of Marine Science* **35,** 380–392.