Variability in the fractionation of stable isotopes during degradation of two intertidal red algae

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A B S T R A C T

Macroalgae contribute to intertidal food webs primarily as detritus, with unclear implications for food web studies using stable isotope analysis. We examined differences in the thallus parts of two South African rhodophytes (*Gelidium pristoides* and *Hypnea spicifera*) and changes in overall $\delta^{13}C$, $\delta^{15}N$ signatures and C:N ratios during degradation in both the field and laboratory. We hypothesized that both degrading macroalgal tissue and macroalgal-derived suspended particulate material (SPM) would show negligible changes in $\delta^{13}C$, but enriched $\delta^{15}N$ signatures and lower C:N ratios relative to healthy plants. Only C:N laboratory ratios conformed to predictions, with both species of macroalgae showing decomposition related changes in $\delta^{13}C$ and significant depletions in $\delta^{15}N$ in both the field and laboratory. In the laboratory, algal tissue and SPM from each species behaved similarly (though some effects were non-significant) but with differing strengths. *Gelidium pristoides* $\delta^{13}C$ increased and C:N ratios decreased over time in tissue and SPM; $\delta^{15}N$ became depleted only in SPM. *Hypnea spicifera*, $\delta^{13}C$, $\delta^{15}N$ and C:N ratios all decreased during degradation in both SPM and algae.

Over 60 days in the field, $\delta^{13}C$ was depleted in both species (1–2‰) and in naturally senescent *Gelidium pristoides* fronds. $\delta^{15}N$ was depleted in *Hypnea spicifera* (approx. 1‰), while C:N ratios of both species were unaffected. The two species differed in $\delta^{13}C$, $\delta^{15}N$ and C:N after degradation, but only in C:N beforehand. We suggest isotope changes in the laboratory mainly reflect microbial effects, while in the field these are combined with leaching due to constant water replenishment and agitation. Differences between these two species in the isotope responses to degradation highlight the difficulty of linking the signature of SPM to its multiple sources.

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1. Introduction

At temperate and high latitudes, extensive beds of kelps have productivities comparable to the most productive terrestrial ecosystems (Barnes and Hughes, 1982). Despite this, there are very few grazing species that utilize this material directly and consequently the primary production of kelps vastly exceeds herbivorous consumption (Newell and Lucas, 1981) and most enters the detrital food chain (e.g. Newell and Lucas, 1981; Mann, 1988; Duggins et al., 1989; Kaehler et al., 2000, 2006; Fredriksen, 2003). On the west coast of South Africa for example, the kelps *Ecklonia maxima* and *Laminaria pallida* account for upwards of 65% of the detritus found in intertidal suspended particulate matter or SPM (Bustamante and Branch, 1996). In the same fashion, primary production by salt marsh plants (e.g. *Spartina alterniflora*) can be very high, with only a small percent of the above-ground vegetation being consumed by grazers (e.g. Hackney and De La Cruz, 1980; Valiela et al., 1985; Benner et al., 1987; Buth, 1987; Currin et al., 1995). The rest degrades and enters the detrital food web and many studies have underlined the importance of detrital material to salt marsh and estuarine ecosystems (e.g. Haines, 1977; Haines and Montague, 1979; Hughes and Sherr, 1983; Paterson and Whitfield, 1997).

Studies of algal–grazer interactions in the intertidal zone indicate that the biomass and distribution of some macroalgae can be limited by grazers (Williams, 1993). However, many benthic grazers in both marine and freshwater systems also feed on the periphyton of macrophytes, or on epilithic microalgae, including algal sporelings which show much higher turnover rates and contain less refractory material than macrophytes (McQuaid, 1996; Hillebrand et al., 2002). Consequently, as with salt marshes and kelp beds, the

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bulk of other macroalgal production is likely to enter a detrital food chain (McQuaid and Branch, 1985), so that understanding the process of macroalgal decomposition is central to elucidating food webs in these communities.

In recent years, food web resolution in a number of estuarine and coastal communities has been achieved through stable isotope analysis (e.g. Bustamante and Branch, 1996; Froneman, 2001; Fredriksen, 2003; Carmichael et al., 2004; Hill et al., 2006). However, few of these studies have considered isotopic changes in organic carbon sources (i.e. phytoplankton, macroalgae) due to decomposition. If isotope fractionation occurs during degradation, the isotopic signatures of many detritivores may bear little resemblance to the signatures of living algae. This will complicate the interpretation of trophic relationships where the bulk of primary production is consumed as detritus. The majority of macrophyte detritus is fibrous and nitrogen poor, but microbial colonization during decomposition results in reduced fiber content and increased available nitrogen (Stuart et al., 1981), making the previously refractory detritus digestible for detritivores and filter feeders (Mann, 1988; Levinton et al., 2002). Because of microbial colonization, the $\delta^{13}C$ and $\delta^{15}N$ values of macroalgal detritus will not necessarily reflect those of the living alga, but rather a combination of signatures from the decomposing alga, plus the various microbial communities colonizing it (Newell and Lucas, 1981; Newell et al., 1982). In addition the alga's signature may be altered by leaching during decomposition (Benner et al., 1991; Asada et al., 2005)

Although a number of studies have addressed the isotopic effects of degradation in aquatic vegetation, the focus has been on angiosperms and the results have often been conflicting. There appears to be little or no change in $\delta^{13}C$ or $\delta^{15}N$ during decay in the seagrasses Zostera noltii (Machás et al., 2006), Thalassia testudinum and Zostera marina (Zieman et al., 1984; Stephenson et al., 1986; Fenton and Ritz, 1988) or the mangrove Rhizophora mangle (Wooller, 2003). In contrast, Spartina alterniflora showed distinct $\delta^{13}C$ depletion during diagenesis in salt marsh sediments (Benner et al., 1987, 1991; Alberts et al., 1988; Fogel et al., 1989) and $\delta^{15}N$ depletion has been described in degrading mangroves (Zieman et al., 1984) and in seven species of tropical angiosperms (2–6‰), with no significant accompanying changes in $\delta^{13}C$ (Currin et al., 1995; Fellerhoff et al., 2003).

In comparison to angiosperms, little work has been done on shifts in the isotopic ratios of decomposing macroalgae. Fenton and Ritz (1988) reported both small enrichments and depletions in carbon during decomposition and Stephenson et al. (1986) described a lack of change in carbon isotope composition associated with the degradation of kelp and the formation of detritus. As a consequence, the isotopic differences between living macroalgae and their detritus are poorly understood. The south coast of South Africa is too warm to support extensive kelp beds, but maintains a high biomass of intertidal and subtidal algae that appear to contribute large amounts of organic carbon to intertidal food webs (Hill et al., 2006). Although there are beds of Zostera capensis that occur in nearby estuaries, these estuaries are few and small, so that the contribution of angiosperm production to the intertidal zone is thought to be minimal (Taylor and Allanson, 1995). A substantial percentage of intertidal SPM is thus likely to be macroalgal detritus. The aims of this study were, firstly, to conduct a preliminary investigation of isotopic variation of different tissue types within a single plant and secondly to elucidate changes in C:N ratios, $\delta^{13}C$ and $\delta^{15}N$ signatures during decomposition of two abundant intertidal rhodophytes: Gelidium pristoides and Hypnea spicifera. We hypothesized that (1) in agreement with the work done by Stephenson et al. (1986), $\delta^{13}C$ signatures macroalgae would demonstrate very little change during both degradation and the formation

of detritus; (2) $\delta^{15}N$ signatures would become enriched due to colonizing bacteria preferentially retaining organic nitrogen during biochemical processing (e.g. see Macko and Estep, 1984); and (3) C:N ratios of degrading plants would decrease relative to healthy plants as a result of microbial mineralization during decomposition (Kirstensen, 1994).

2. Methods

2.1. Study site

The macroalgae Gelidium pristoides and Hypnea spicifera were chosen for decomposition studies as they are important contributors to macroalgal biomass and primary production on the South African coast and are likely to contribute large amounts of SPM to the intertidal zone. Both G. pristoides and H. spicifera have a poly-siphonia-type life history (Carter, 1985; Reis and Yoneshigue-Valentin, 2000), and possess erect fronds borne on creeping rhizomes (De Clerck et al., 2005). Algal samples were collected from Port Alfred (33°27′21 S, 26°52′29 E), an exposed rocky shore on the south coast of South Africa.

2.2. Sample design

2.2.1. Basic structural differences
Ten samples of each species were collected from the lower intertidal zone and rinsed in distilled water (dH₂0) to remove external salts and epiphytes. Each sample was separately spun in a salad spinner for 60 s to remove excess water and then weighed. Samples were subsequently dried to a constant weight at 60 °C, and wet weight:dry weight ratios were calculated.

2.2.2. Differences between thallus parts
Ten fresh samples each of healthy fronds, senescent fronds and rhizomes were dissected from ten specimens of each species as a precursor to the lab and field studies in order to investigate isotopic variability within a single plant. Samples of the midsection of the frond, between frond tip and rhizome, were also collected from Hypnea spicifera only.

2.2.3. Field study
Twenty holes were drilled into the rocks in the sub-littoral fringe of the intertidal zone at Port Alfred and eye-hooks were secured in the holes using plastic anchors. Twenty mesh bags (150 μm mesh; 20 × 20 cm) were each filled with 100 g of freshly collected macroalgae (frond tips only; either Gelidium pristoides ($n = 10$) or Hypnea spicifera ($n = 10$)) which were subsequently cut up into small pieces (1 cm bit) to induce decomposition. The mesh bags were then attached to eye-hooks using zip-ties. Bags were left on the rocks for 60 days and the degrading macroalgae were sampled at time zero, day 30 and day 60.

2.2.4. Laboratory study
Since most physical changes in algal structure (bleaching and loss of rigidity) were seen within the first two weeks of the field study, a shorter-term laboratory experiment was undertaken. 100 g of fresh frond tips from Gelidium pristoides ($n = 10$) or Hypnea spicifera ($n = 10$), cut up into 1 cm pieces, were added to each of twenty 10 L buckets filled with seawater, strongly aerated (to maintain agitation) and left to decompose for 30 days. Algae were sampled at time zero and day 30. SPM samples at time zero were obtained from 5-L surface seawater samples (i.e. same as water in buckets) and later SPM samples were obtained from each bucket on day 30. A microbial film developed on the walls of all the buckets as the experiment progressed and this material (biofilm) was sampled

from each bucket on day 30 only. Water temperature throughout the experiment was ambient, ranging between 18 and 20 °C.

2.3. Sample preparation

All macroalgae were rinsed in dH_2O, visible epiphytes were removed and algae were oven dried (60 °C, 48 h). Water samples were filtered through pre-combusted (500 °C, 6 h) GF/F Whatman® filters (0.45 μm pore size), using a vacuum pump (≤4 cm Hg) and then oven dried at 60 °C for 24 h. Zooplankton and other large particles were manually removed under a dissecting microscope at 16× magnification from GF/F filters before drying. Biofilm was rinsed with dH_2O and also oven dried (60 °C, 48 h).

2.4. Isotopic analysis

C:N ratios, $\delta^{13}C$ and $\delta^{15}N$ signatures of all samples were determined using a continuous flow Isotope Ratio Mass Spectrometer (IRMS), after sample combustion in an on-line Carlo–Erba preparation unit at the University of Cape Town, South Africa. Sucrose, valine and Merck gelatin were used as standards, calibrated against International Atomic Energy reference materials. Results are expressed in standard delta notation, $\delta X = ([R_{sample}/R_{standard}] - 1) \times 1000$, where X is the element in question and R is the ratio of the heavy over the light isotope. Precision of replicate determinations for both carbon and nitrogen was ±0.05‰.

2.5. Data analysis

2.5.1. Basic structural differences
A t-test (independent, by groups) was performed on wet/dry ratios of Gelidium pristoides and Hypnea spicifera using Stastistica v7 (StatSoft Inc, 2004).

2.5.2. Differences between thallus parts
Separate one-way ANOVAs were performed for Gelidium pristoides and Hypnea spicifera to assess the effect of thallus part on $\delta^{13}C$, $\delta^{15}N$ and C:N.

2.5.3. Field study
Two-way ANOVAs were performed separately to assess species-specific differences in $\delta^{13}C$, $\delta^{15}N$ and C:N ratio through time, using species and time as fixed factors.

2.5.4. Laboratory study
Separate two-way ANOVAs were performed for each species to assess the effects of time and material (alga or SPM) on $\delta^{13}C$, $\delta^{15}N$ and C:N ratios. A further two-way ANOVA was performed for day 30 only, using species and material as fixed factors to assess species-specific differences in $\delta^{13}C$, $\delta^{15}N$ and C:N ratios between SPM, algae and biofilm.

All ANOVA were Model I and performed using Statistica v7 (StatSoft Inc. 2004). In the event of significant effects, Newman–Keuls post hoc comparisons were used to determine homogenous groups. The prerequisites for parametric analyses were tested using Kolmogorov–Smirnov's and Lilliefor's tests for normality and Levene's test for homogenous variances. Data transformation was not required for any analysis.

3. Results

3.1. Basic structural differences

No significant differences were found between wet/dry ratios of Gelidium pristoides and Hypnea spicifera ($T_{0.5(18)} = -0.20$, $p = 0.84$).

3.2. Differences between thallus parts

Patterns of isotopic signatures between different parts of the alga differed between species, but clearly indicated significant differences in $\delta^{13}C$, $\delta^{15}N$ and C:N ratios among thallus parts within a single plant. The holdfast of Gelidium pristoides was significantly more depleted in $\delta^{13}C$ than both the healthy and senescent fronds ($F_{2, 14} = 16.17$, $p < 0.0001$). The $\delta^{15}N$ signatures of thallus parts decreased in the sequence holdfast > healthy fronds > senescent fronds ($F_{2, 14} = 32.06$, $p < 0.0001$). No significant differences were seen in C:N ratios. The $\delta^{15}N$ signatures of Hypnea spicifera showed healthy and senescent fronds > mid-frond > holdfast ($F_{3, 19} = 19.63$, $p < 0.0001$) with C:N ratios of healthy fronds being significantly lower than all other thallus parts ($F_{3, 19} = 6.01$, $p < 0.0001$). No significant differences were seen in $\delta^{13}C$ (Fig. 1).

3.3. Field study

Two-way ANOVA indicated significant time × species interactions for both $\delta^{13}C$ ($F_{2, 44} = 4.95$, $p = 0.01$) and $\delta^{15}N$ ($F_{2, 44} = 5.05$, $p = 0.01$), while C:N ratios showed only a species effect ($F_{2, 44} = 374.71$, $p < 0.01$).

For both species, macroalgal $\delta^{13}C$ was significantly depleted (1–2‰) at days 30 and 60, relative to day zero, however, the effect was stronger for Hypnea spicifera (Fig. 2A). $\delta^{15}N$ was similarly significantly depleted (approx. 1‰) after 30 and 60 days in H. spicifera, but not in Gelidium pristoides. Both $\delta^{13}C$ and $\delta^{15}N$ differed significantly between species after, but not before degradation. In all cases values were 1–2.5‰ ($\delta^{13}C$) or 1–1.5‰ ($\delta^{15}N$) higher in G. pristoides.

Neither species showed any effect of time on their C:N ratios, which were consistently, significantly higher in Gelidium pristoides (mean 13.3 ± 1.12 SD) than in Hypnea spicifera (mean 8.4 ± 0.40 SD).

3.4. Laboratory study

The results differed between species. For Gelidium pristoides both $\delta^{15}N$ and C:N ratios, all effects were significant, including the time × material interaction ($F_{1, 36} = 6.53$, $p < 0.02$; $F_{1, 36} = 17.36$, $p < 0.01$ for $\delta^{15}N$ and C:N ratios respectively). The $\delta^{13}C$ ratios enriched over time in both algal material and SPM, although the effect was not significant for the algal tissue (Fig. 2B) or for the time × material interaction in $\delta^{13}C$ ($p = 0.22$). $\delta^{13}C$ and C:N ratios in Hypnea spicifera were significantly affected by time ($F_{1, 36} = 9.47$, $p < 0.01$; $F_{1, 36} = 43.01$, $p < 0.01$ respectively) and material ($F_{1, 36} = 18.04$, $p < 0.01$; $F_{1, 36 =} 27.72$, $p < 0.01$ respectively), with both SPM and algal material decreasing over time, although the decrease was less for SPM than algae tissue. The $\delta^{15}N$ ratios showed no significant effects (Fig. 2B).

3.4.1. Effect of time
After thirty days of decomposition, $\delta^{13}C$ signatures of Hypnea spicifera thalli were on average 1.5‰ more depleted than fresh samples, and the C:N ratios of both Gelidium pristoides and H. spicifera decreased significantly from day 0 to day 30. No other significant changes were seen in either species. SPM collected from the G. pristoides buckets showed signatures at day 30 to be enriched in $\delta^{13}C$ but depleted in $\delta^{15}N$ relative to day 0, while C:N ratios showed no significant change. SPM from the H. spicifera buckets showed the reverse: a decrease in C:N ratio from day 0 to day 30, but no change in $\delta^{13}C$ or $\delta^{15}N$ (Fig. 2B).

For both species $\delta^{13}C$ did not differ between algal material and SPM at day zero, but was significantly higher for SPM on day 30. For $\delta^{15}N$ ratios, SPM and algal tissue differed only for Gelidium pristoides on day 30, when SPM was depleted relative to the
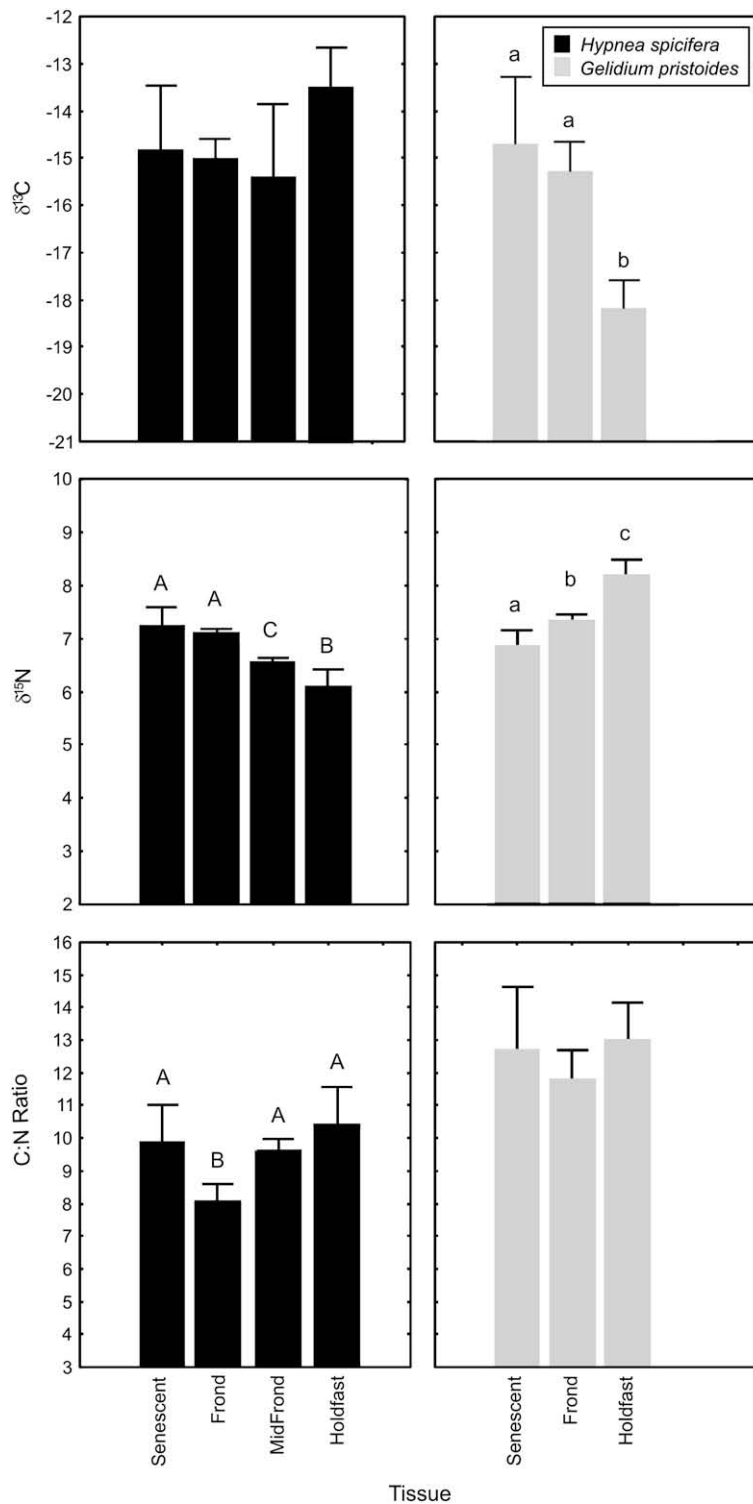
Fig. 1. Isotopic differences in thallus parts of *Hypnea spicifera* and *Gelidium pristoides* for δ^{13}C, δ^{15}N and C:N ratios (senescent = senescent frond material). Values are means + SD. Uppercase letters indicate homogenous groups within *H. spicifera* and lowercase letters indicate homogenous groups within *G. pristoides* (Newman–Keuls, $p < 0.05$).

thallus parts. The results for C:N ratios were more complex. Thallus parts and SPM differed only on day 30 for *Hypnea spicifera* and on day zero and 30 for *G. pristoides*. In both cases SPM had lower C:N ratios.

The two-way ANOVA for day 30 allowed the inclusion of biofilm as a material (see Fig. 3). At day 30 *Gelidium pristoides* showed significant differences among all three material types, with δ^{13}C

signatures of SPM > algal tissue > biofilm ($F_{2, 29} = 12.77$, $p < 0.01$) and δ^{15}N signatures of algal tissue and biofilm > SPM ($F_{2, 29} = 17.76$, $p < 0.01$). Significant differences were also seen between material types in *Hypnea spicifera* at day 30, with SPM and biofilm δ^{13}C signatures > algal tissue ($F_{2, 29} = 12.25$, $p < 0.01$). In both *G. pristoides* and *H. spicifera* the algal C:N ratio > SPM and biofilm ($F_{2, 29} = 18.22$; $F_{2, 29} = 15.11$ respectively, $p < 0.01$; Fig. 3).
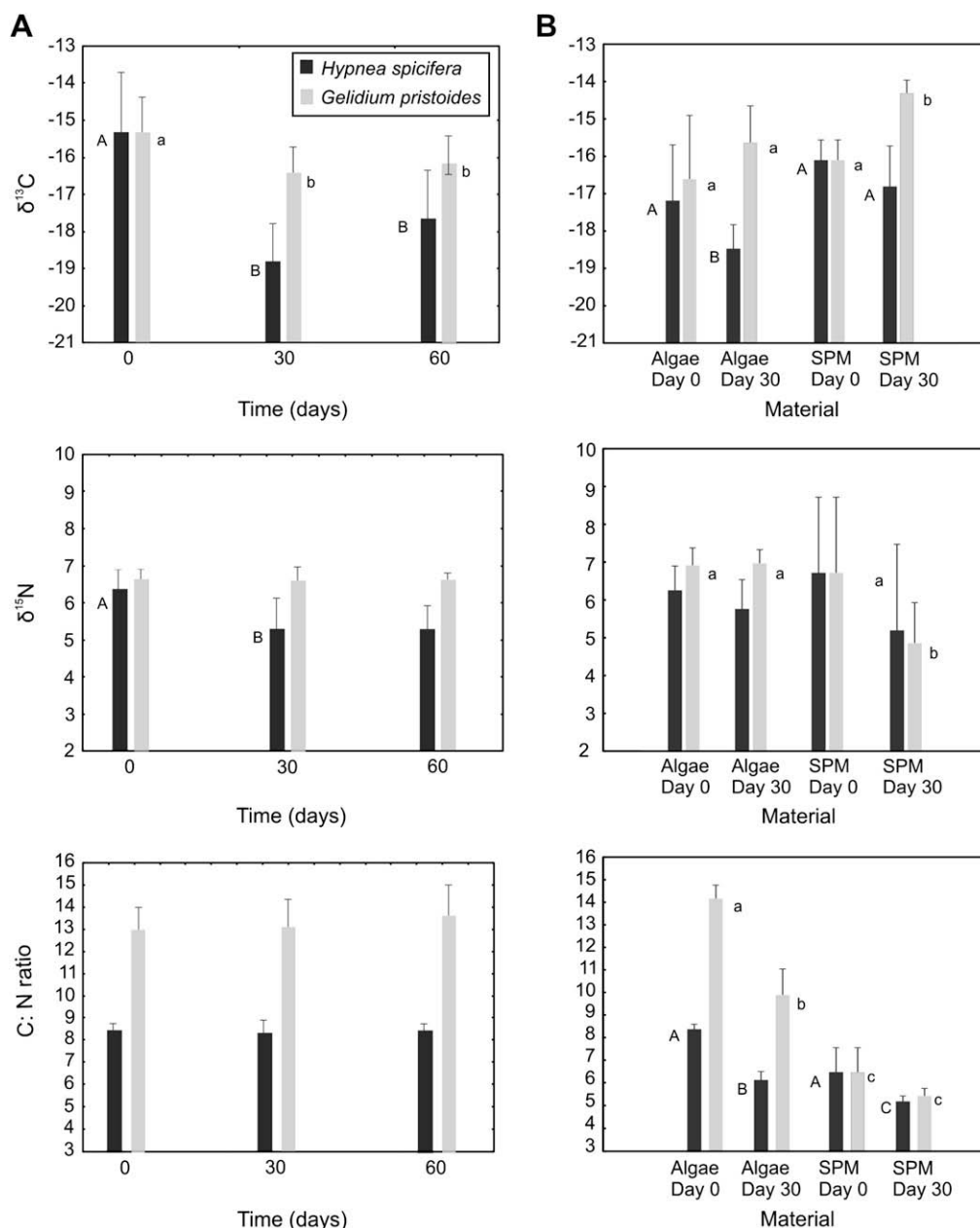
Fig. 2. Effects of decomposition in *Hypnea spicifera* and *Gelidium pristoides* on $\delta^{13}C$, $\delta^{15}N$ and C:N ratios in the field (left) and laboratory (right). Values are means + SD. Uppercase letters indicate homogenous groups within *H. spicifera* and lowercase letters indicate homogenous groups within *G. pristoides* (Newman–Keuls, $p < 0.05$).

4. Discussion

Only the C:N ratios in the laboratory experiments conformed to our hypotheses, while $\delta^{15}N$ signatures of both macroalgae (field and lab) and SPM (lab only), with one exception, depleted during degradation and all carbon signatures showed isotope changes presumably related to decomposition (Table 1).

4.1. Isotopic shifts in carbon

Contrary to our predictions, both algal species showed decomposition related changes in $\delta^{13}C$ signatures of thallus parts, with *Hypnea spicifera* (lab and field) and *Gelidium pristoides* (field only) showing up to 2.0‰ depletion after thirty and sixty days of decomposition, while only the laboratory $\delta^{13}C$ signatures of *G. pristoides* were not significantly altered. In contrast to thallus parts,

signatures for *G. pristoides*-derived SPM (lab) enriched in $\delta^{13}C$ over time. Fenton and Ritz (1988) also observed changes in $\delta^{13}C$ ratios over a 60-day decomposition experiment but these were in the order of less than 1.0‰, and of the six macroalgae they examined, only the $\delta^{13}C$ ratio of the kelp *Ecklonia radiata* remained depleted by day 60. Combined with our findings, this indicates that carbon changes during decomposition are largely species specific. Shifts in carbon similar to those observed here were seen during early diagenesis of the angiosperm *Spartina alterniflora* by Benner et al. (1987, 1991) with large depletions (up to 4.0‰) that were attributed to polysaccharide removal through leaching, resulting in decomposed material having lower ^{13}C due to enrichment in lignin-derived carbon (Benner et al., 1991). The preferential loss of carbohydrates and proteins, which are particularly susceptible to microbial degradation (Hedges et al., 1988; Harvey et al., 1995), would lead to a decrease in the $\delta^{13}C$ ratios of the remaining organic
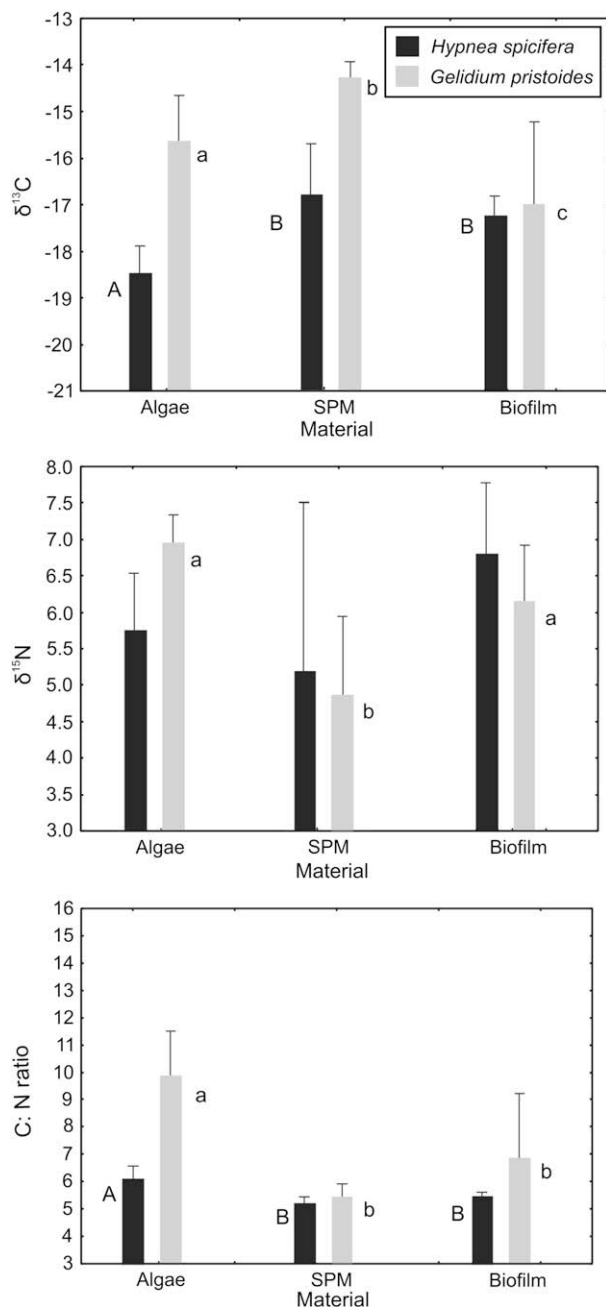
Fig. 3. Species-specific differences in *Gelidium pristoides* and *Hypnea spicifera* for $\delta^{13}C$, $\delta^{15}N$ and C:N ratios in the laboratory at day 30. Values are means + SD. Uppercase letters indicate homogenous groups within *H. spicifera* and lowercase letters indicate homogenous groups within *G. pristoides* (Newman–Keuls, $p < 0.05$).

Table 1
Predicted and observed changes in isotope composition of algal tissue and tissue-derived SPM. – indicates no clear trend, * denotes a significant change.

	Hypotheses	Field		Laboratory			
	Both algae	*G. pristoides*	*H. spicifera*	*G. pristoides*		*H. spicifera*	
				Algae	SPM	Algae	SPM
$\delta^{13}C$	–	▼*	▼*	▲	▲*	▼*	▼
$\delta^{15}N$	▲	–	▼*	▼	▼*	▼*	▼
C:N ratio	▼	–	–	▼*	▼	▼*	▼*

matter. Although other studies have shown that decomposition does not affect $\delta^{13}C$ ratios in vascular plants, angiosperms (Currin et al., 1995; Schweizer et al., 1999; Fellerhoff et al., 2003; Machás et al., 2006) or kelp (Stephenson et al., 1986), we suggest that other macroalgae (e.g. *Gelidium* and *Hypnea* sp.) undergo leaching during decomposition. Assuming different rates of leaching for carbohydrates with different isotopic signatures, this would result in variable algal signatures during degradation.

Rhodophyta synthesize organic halogen-containing compounds such as halo-ketones, brominated phenols and terpenes (Fenical, 1975; Hay and Fenical, 1988; Hughes et al., 1991) that are thought to be defensive chemicals used to deter herbivory (Hay and Fenical, 1988). Leaching of such chemicals during degradation may alter the carbon isotopic composition of residual plant detritus. In our case, the leaching process would be greater under field conditions due to constant water replenishment as well as greater agitation, which may explain the consistent $\delta^{13}C$ depletion for both algae in the field. Thus, while changes in the laboratory would be due to the microbial effects with weak leaching, results from the field would include microbial effects plus strong leaching.

4.2. Isotopic shifts in nitrogen

Changes in isotopic nitrogen composition were species and/or material specific and the observed trends were opposite to the predicted results, albeit some of these trends were non-significant. Under virtually all conditions, $\delta^{15}N$ ratios were depleted during degradation (by a maximum of 1.0‰) in the algae tissue and SPM derived from both species. The exceptions to this were the absence of marked changes in $\delta^{15}N$ signatures of the thallus parts of *Gelidium pristoides* in either the field or laboratory. The depletions observed in this study may be explained by either the gain of a nitrogen fraction depleted in ^{15}N or a loss of one enriched in ^{15}N. A number of studies have reported decreases in $\delta^{15}N$. Fourqurean and Schrlau (2003), for example, interpreted the 2‰ decrease in $\delta^{15}N$ seen during the decomposition of seagrasses and mangroves as a response to the immobilization of environmental N sources by microbes during decomposition. Lehmann et al. (2002) observed isotopic depletions in decomposing organic matter and suggested their results were likely to be due to the addition of a ^{15}N depleted fraction from bacterial growth using soluble nitrogenous compounds. Similarly, we suggest that our changes in the isotopic composition of decomposing macroalgae can be ascribed to bacterial biosynthesis.

The predicted changes hypothesized for nitrogen isotope signatures were based on work by Caraco et al. (1998) who found a 15‰ increase in $\delta^{15}N$ leaf material in litter bag experiments and Macko and Estep (1984) who reported microbial alteration of the $\delta^{15}N$ composition of organic substrates by the marine bacterium *Vibrio harveyi*, which showed bacterial fractionation between 0 and 22‰ depending on the amino acids or sugars available in the substrate. They suggested that microbial communities assimilate ^{15}N enriched dissolved organic or inorganic nitrogen and could preferentially retain organic nitrogen during biochemical processing. However, work done by Fogel and Tuross (1999) and Fellerhoff et al. (2003) indicates that changes in $\delta^{15}N$ during decomposition/diagenesis of organic plant material are not always unidirectional and vary greatly between species. In fact both Caraco et al. (1998) and Lehmann et al. (2002) discuss changes in $\delta^{15}N$ in relation to the assimilation of dissolved inorganic nitrogen (DIN) by bacteria during decomposition and hypothesize that the magnitude and direction of isotopic shifts during decomposition are dependant on the nature of the microbial communities as well as the isotopic composition of the DIN. The effects of bacterial colonization could therefore play a large role in isotopic shifts in nitrogen during

macroalgal decomposition. In natural environments, it is likely that species and substrate diversity will influence bacterial populations and Macko and Estep (1984) and Fellerhoff et al. (2003) suggest that large isotope fractionations may then cancel each other out, resulting in a more consistent $\delta^{15}N$ signature. However, in cases where a homogenous microbial community dominates the organic substrate (e.g. high sulphur, anaerobic marine sediments), isotopic signatures will be significantly altered. This is of particular importance to macroalgae decomposition as there is indirect evidence that algal species differ in the bacterial communities they support (Šyvokeine et al., 1988). In addition, Harvey et al. (1995) reported that during peak microbial growth in the decay of their phytoplankton incubations, bacteria account for more than 20% of total biomass. So it is unsurprising that the interactions between the decomposition of organic matter and bacterial growth/decay complicate the understanding of nitrogen isotope evolution during macroalgal degradation.

4.3. Changes in C:N ratios

If the C:N ratio of an animal is constant, then limitations of available nitrogen may restrict C utilization (Hessen, 1992), resulting in compensation for low food quality through increased feeding rates (Cruz-Rivera and Hay, 2000; Norderhaug et al., 2003). However, there may be an upper limit to the C:N ratio above which an alga will be unable to support consumer growth (Hessen and Bjerkeng, 1997). Russel-Hunter (1970) estimated that consumers require a diet with a C:N ratio of 17 or less. Since some macroalgae (e.g. kelp) often have C:N ratios above this limit (Sjøtun et al., 1996; Elser et al., 2000; Norderhaug et al., 2003), the effects of decomposition on C:N ratios become important. Both *Gelidium pristoides* and *Hypnea spicifera* had C:N ratios well below 17, but the ratio of *H. spicifera* was lower than that of *G. pristoides*, suggesting that it is the better food. Indeed, there is little evidence of direct grazing on *G. pristoides*, while *H. spicifera* often shows signs of fish cropping (pers obs). Also algal fronds, which are the parts consumed, have better C:N ratios than holdfasts. In situ bacterial growth is likely to result in lower C:N ratios as the C:N of bacterial biomass generally ranges from 4 to 5 (Müller, 1977) and although we saw no significant changes in C:N ratios under field conditions, bacterial colonization of the thallus parts of both macroalgae and macroalgal-derived SPM was clearly evident from our lab experiments, where C:N ratios decreased significantly after 30 days of decomposition. Alternatively, loss of particulate organic carbon and nitrogen (POC and PON) from the plant detritus through leaching may have caused the decrease, as suggested by Kirstensen (1994), who reported that after a 3–5 day initial leaching phase, the temporal pattern of POC and PON loss from algal detritus was greatly increased with higher rates for the former, resulting in a 5–28% reduced C:N ratio. The mechanisms underlying changes in C:N ratios are likely to be tied to those responsible for isotopic changes. Potential explanations include: bacterial colonization and subsequent nitrogen fixation during macrophyte decay (Thornton and McManus, 1994; Fellerhoff et al., 2003), increased carbon loss through microbial respiration or mineralization (Fellerhoff et al., 2003), or the removal of carbohydrates (containing only C, H and O) during the initial stages (first 100 days) of decomposition (Fogel and Tuross, 1999). Pagiro and Thomaz (1999) ascribed their decreases in C:N ratio during degradation to immobilization of nitrogen by microorganisms and a decrease in carbon content through respiration.

4.4. Differences between species

Although fresh samples of *Gelidium pristoides* and *Hypnea spicifera* had comparable isotopic values, there were clear differences

between them after 30 and 60 days of decomposition. *Gelidium pristoides* had consistently more enriched carbon and nitrogen isotope ratios throughout the experiments, and demonstrated higher C:N ratios. This may simply reflect the biochemical composition of each species and their subsequent ease of decomposition, with *H. spicifera* experiencing more rapid leaching and microbial colonization than *G. pristoides*. As the basic life history of both macroalgae is similar, it is possible that structural differences between the two are responsible for different rates of leaching, however, no significant differences in wet/dry ratios between *G. pristoides* and *H. spicifera* were found, indicating that structural elements do not differ drastically between the two. A more complex explanation would be the species-specific support of colonies of unique microflora (Macko and Estep, 1984; Šyvokeine et al., 1988; Hamilton and Lewis, 1992). Carbon and nitrogen signatures of biofilm samples were very similar between species, which suggests that the signatures of developing biofilm may have more to do with microbial colonies than the algae themselves and the higher C:N ratio of *G. pristoides* would accordingly indicate either higher microbial mineralization in *G. pristoides* or higher colonization by nitrogen fixing bacteria in *H. spicifera*. Interpreting differences between species can be further complicated by the potential for macroalgae to exhibit tissue-specific growth rates, resulting in a variety of distinct isotopic signatures within a single plant as shown in this study and a number of others (Fry and Sherr, 1984; Stephenson et al., 1984; Gearing, 1991; Michener and Schell, 1994).

The two species showed tissue-specific trends that were largely opposite. *Hypnea spicifera* showed no significant differences in $\delta^{13}C$, while these were marked in *Gelidium pristoides*, holdfasts having the lowest ratios. Patterns in $\delta^{15}N$ were exactly opposite between species, being minimal in the fronds of *G. pristoides* and the holdfasts of *H. spicifera*. C:N ratios in both algae were lowest in the fronds. Living plants of *G. pristoides* often include fronds that are senescent at the tips that demonstrated enriched $\delta^{13}C$ and depleted $\delta^{15}N$ signatures relative to healthy fronds (nitrogen only) and the holdfast (carbon and nitrogen). Although the opposite to the field results for $\delta^{13}C$, this result supports the laboratory findings and suggests that $\delta^{13}C$ changes associated with decomposition start in living plants (senescent fronds are visibly discoloured) and are completed in the water column during the formation of SPM.

4.5. Implications for SPM

Because decomposing macroalgae are likely to contribute substantially to SPM, our results suggests that SPM signatures are influenced by: the species contributing to the detrital pool and their proportions; the thallus types predominating and the ages of the detrital components. A further complication is that detritivores assimilate microflora with 50–100% efficiency, while assimilation rates of algal material are typically less than 5% (Lopez et al., 1977; Cammen, 1980), so that detritivores derive a large proportion of their nutritional requirements from the microbial component of detritus. However, the implications of this differ between elements. The bacteria associated with detritus and SPM generally supply only a small fraction (10–30%) of the carbon requirements of consumers, but a high proportion (>90%) of their nitrogen requirements (Cammen, 1980; Newell et al., 1982; Newell and Field, 1983), providing nitrogen, vitamins and essential amino acids, while the plant tissue is assimilated at lower efficiencies as a carbon and energy source (Zieman et al., 1984).

4.6. Conclusions

Our results suggest that unexplored mechanisms of carbon and nitrogen change are at work at tissue/species-specific levels. Both

Gelidium pristoides and *Hypnea spicifera* showed tissue-specific differences in values within individual plants and distinct changes in isotope and C:N ratios related to time during decomposition. It is difficult to make generalizations about the processes and rates of macroalgal decomposition in coastal areas or laboratory settings as tissue and/or species-specific differences in biochemistry and physical structure may override changes in isotopic composition. It is clear however, that detrital isotope signatures should not be used as indicators of living sources of organic material, without careful decomposition studies. Additionally, using the isotope ratios of living macroalgae to elucidate trophic relationships must be done with extreme caution due to the variable nature of algal signatures and the potential changes associated with degradation.

The general patterns in isotopic change were similar in both species, but for carbon they differed between the lab and the field, while the rates of change in signatures appear to be species specific. All this indicates a high level of complexity in the relationship between living macroalgae and SPM. Hill et al. (2006, 2008) for example, found that filter feeding bivalves acquired a large percentage of their isotopic signatures from nearshore SPM, however the composition of this SPM is not homogenous and is likely to include detritus from numerous macroalgae as well as other sources. As such, isotopic signatures of SPM are complex, showing high temporal variability (Hill et al., 2008), and clarifying the links between it and primary producers, remains a difficult task.

Acknowledgements

References

Alberts, J., Filip, Z., Price, M., Williams, D., Williams, M., 1988. Elemental composition, stable carbon isotope ratios and spectrophotometric properties of humic substances occurring in a salt marsh estuary. Organic Geochemistry 12, 455–467.

Asada, T., Warner, B., Aravena, R., 2005. Effects of the early stage of decomposition on change in carbon and nitrogen isotopes in *Sphagnum* litter. Journal of Plant Interactions 1, 229–237.

Barnes, R.S.K., Hughes, R.N., 1982. Rocky shores and kelp forests. In: An Introduction to Marine Ecology. Blackwell Scientific Publications, Oxford, pp. 119–160.

Benner, R., Fogel, M., Sprague, E., Hodson, R., 1987. Depletion of ^{13}C in lignin and its implications for stable carbon isotope studies. Nature 329, 708–710.

Benner, R., Fogel, M.L., Sprague, E., 1991. Diagenesis of belowground biomass of *Spartina alterniflora* in salt marsh sediments. Limnology and Oceanography 36, 1358–1374.

Bustamante, R., Branch, G., 1996. The dependence of intertidal consumers on kelp-derived organic matter on the west coast of South Africa. Journal of Experimental Marine Biology and Ecology 196, 1–28.

Buth, G., 1987. Decomposition of roots of three plant communities in a Dutch salt marsh. Aquatic Botany 29, 123–138.

Cammen, R., 1980. The significance of microbial carbon in the nutrition of the deposit feeding polychaete *Nereis succinea*. Marine Biology 61, 9–20.

Caraco, N.F., Lampman, G., Cole, J.J., Limburg, K.E., Pace, M.L., Fischer, D., 1998. Microbial assimilation of DIN in a nitrogen rich estuary: implications for food quality and isotope studies. Marine Ecology Progress Series 167, 59–71.

Carmichael, R., Rutecki, D., Annett, B., Gaines, E., Valiela, I., 2004. Position of horseshoe crabs in estuarine food webs: N and C stable isotopic study of foraging ranges and diet composition. Journal of Experimental Marine Biology and Ecology 299, 231–253.

Carter, A.R., 1985. Reproductive morphology and phenology, and culture studies of *Gelidium pristoides* (Rhodophyta) from Port Alfred in South Africa. Botanic Marina 28, 303–311.

Cruz-Rivera, E., Hay, M., 2000. Can quantity replace quality? Food choice, compensatory feeding, and fitness of marine mesograzers. Ecology (USA) 81, 201–219.

Currin, C., Newell, S., Paerl, H., 1995. The role of standing dead *Spartina alterniflora* and benthic microalgae in salt marsh food webs: considerations based on multiple stable isotope analysis. Marine Ecology Progress Series 121, 99–116.

De Clerck, O., Bolton, J.J., Anderson, R., Coppejans, E., 2005. Guide to the Seaweeds of KwaZulu-Natal. National Botanic Garden of Belgium, Brussels, 294 p.

Duggins, D., Simenstad, C., Estes, J., 1989. Magnification of secondary production by kelp detritus in coastal marine ecosystems. Science 245, 170–173.

Elser, J., Fagan, W., Denno, R., Dobberfuhl, D., Folarin, A., Huberty, A., Interlandi, S., Kilham, S., McCauley, E., Schulz, K., Siemann, E., Sternerl, R., 2000. Nutritional constraints in terrestrial and freshwater foodwebs. Nature 408, 578–580.

Fellerhoff, C., Voss, M., Wantzen, K., 2003. Stable carbon and nitrogen isotope signatures of decomposing tropical macrophytes. Aquatic Ecology 37, 361–375.

Fenical, W., 1975. Halogenation in the rhodophyta: a review. Journal of Phycology 11, 245–259.

Fenton, G., Ritz, D., 1988. Changes in carbon and hydrogen stable isotope ratios of macroalgae and seagrass during decomposition. Estuarine, Coastal and Shelf Science 26, 429–436.

Fogel, M.L., Tuross, N., 1999. Transformation of plant biochemicals to geological macromolecules during early diagenesis. Oecologia 120, 336–346.

Fogel, M.L., Sprague, E., Gize, A., Frey, R., 1989. Diagenesis of organic matter in Georgia salt marshes. Estuarine, Coastal and Shelf Science 28, 211–230.

Fourqurean, J.W., Schrlau, J.E., 2003. Changes in nutrient content and stable isotope ratios of C and N during decomposition of seagrasses and mangrove leaves along a nutrient availability gradient in Florida Bay, USA. Chemistry and Ecology 19, 373–390.

Fredriksen, S., 2003. Food web studies in a Norwegian kelp forest based on stable isotope ($\delta^{13}C$ and $\delta^{15}N$) analysis. Marine Ecology Progress Series 260, 71–81.

Froneman, P.W., 2001. Stable isotope ($\delta^{13}C$) study of the food web of the temperate Kariega estuary (Eastern Cape). African Journal of Aquatic Sciences 26, 49–56.

Fry, B., Sherr, E., 1984. $\delta^{13}C$ measurements as indicators of carbon flow in marine and freshwater ecosystems. Contributions in Marine Science 27, 13–17.

Gearing, J., 1991. The study of diet and trophic relationships through natural abundance ^{13}C. In: Coleman, D.C., Fry, B. (Eds.), Carbon Isotope Techniques. Academic Press Inc., San Diego, CA, pp. 201–218.

Hackney, C., De La Cruz, A., 1980. In situ decomposition of roots and rhizomes of two tidal marsh plants. Ecology (USA) 6, 226–231.

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

Haines, E.B., Montague, C.L., 1979. Food sources of estuarine invertebrates analyzed using $^{13}C/^{12}C$ ratios. Ecology 60, 48–56.

Hamilton, S., Lewis, W.J., 1992. Stable carbon and nitrogen isotopes in algae and detritus from the Orinoco River floodplain, Venezuela. Geochimica Cosmochimica Acta 56, 4237–4246.

Harvey, H.R., Tuttle, J.H., Bell, J.T., 1995. Kinetics of phytoplankton decay during simulated sedimentation: changes in biochemical composition and microbial activity under oxic and anoxic conditions. Geochimica Cosmochimica Acta 59, 3367–3377.

Hay, M., Fenical, W., 1988. Marine plant-herbivore interactions: the ecology of chemical defense. Annual Review of Ecology and Systematics 19, 111–145.

Hedges, J.L., Clark, W.A., Cowie, G.L., 1988. Fluxes and reactivities of organic matter in a coastal marine bay. Limnology and Oceanography 33, 1137–1152.

Hessen, D., 1992. Nutrient element limitation of zooplankton production. American Naturalist 140, 799–814.

Hessen, D., Bjerkeng, B., 1997. A model approach to planktonic stoichiometry and consumer-resource stability. Freshwater Biology 38, 447–471.

Hill, J.M., McQuaid, C., Kaehler, S., 2006. Biogeographic and nearshore/offshore trends in isotope ratios of intertidal mussels and their food sources around the coast of southern Africa. Marine Ecology Progress Series 318, 63–73.

Hill, J.M., McQuaid, C., Kaehler, S., 2008. Temporal and spatial variability in stable isotope ratios of SPM link to local hydrography and longer term SPM averages suggest heavy dependence of mussels on nearshore production. Marine Biology 154, 899–909.

Hillebrand, H., Kahlert, M., Haglund, A.-L., Berninger, U.-G., Nagel, S., Wickham, S., 2002. Control of microbenthic communities by grazing and nutrient supply. Ecology (USA) 83, 2205–2219.

Hughes, E.H., Sherr, E.B., 1983. Subtidal food webs in a Georgia estuary: $\delta^{13}C$ analysis. Journal of Experimental Marine Biology and Ecology 67, 227–242.

Hughes, R.N., Gliddon, C.J., Knowlton, N., Malcolm, S.B., Grubb, P.J., 1991. Marine plants and their herbivores: coevolutionary myth and precarious mutualisms. Philosophical Transactions of the Royal Society B: Biological Sciences 333, 231–239.

Kaehler, S., Pakhomov, E., McQuaid, C., 2000. Trophic structure of the marine food web at the Prince Edward Islands (Southern Ocean) determined by $\delta^{13}C$ and $\delta^{15}N$ analysis. Marine Ecology Progress Series 208, 13–20.

Kaehler, S., Pakhomov, E.A., Kalin, R.M., Davis, S., 2006. Trophic importance of kelp-derived suspended particulate matter in a through-flow sub-Antarctic system. Marine Ecology Progress Series 316, 17–22.

Kirstensen, E., 1994. Decomposition of macroalgae, vascular plants and sediment detritus in seawater: use of stepwise thermogravimetry. Biogeochemistry 26, 1–24.

Lehmann, M.F., Bernasconi, S.M., Barbieri, A., McKenzie, J.A., 2002. Preservation of organic matter and alteration of its carbon and nitrogen isotope composition during simulated and in situ early sedimentary diagenesis. Geochimica Cosmochimica Acta 66, 3573–3584.

Levinton, J., Ward, J., Shumway, S., 2002. Feeding responses of the bivalves Crassostrea gigas and Mytilus trossulus to chemical composition of fresh and aged kelp detritus. Marine Biology 141, 367–376.

Lopez, G., Levinton, J., Slobodkin, L., 1977. The effects of grazing by the detritivore Orchestia grillus on Spartina litter and its associated microbial community. Oecologia 30, 111–127.

Machás, R., Santos, R., Peterson, B., 2006. Elemental and stable isotope composition of Zostera noltii (Horneman) leaves during the early phases of decay in a temperate mesotidal lagoon. Estuarine, Coastal and Shelf Science 66, 21–29.

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

Mann, K., 1988. Production and use of detritus in various freshwater, estuarine and coastal marine ecosystems. Limnology and Oceanography 33, 910–930.

McQuaid, C., 1996. Biology of the gastropod family littorinidae. II. Role in the ecology of intertidal and shallow marine ecosystems. In: Ansell, A., Gibson, R., Barnes, M. (Eds.), Oceanography and Marine Biology. An Annual Review. UCL Press, pp. 263–302.

McQuaid, C., Branch, G., 1985. Trophic structure of rocky intertidal communities: response to wave action and implications for energy flow. Marine Ecology Progress Series 22, 153–161.

Michener, R., Schell, D., 1994. Stable isotope ratios as tracers in marine aquatic food webs. In: Lajtha, K., Michener, R. (Eds.), Stable Isotopes in Ecology and Environmental Science. Blackwell Scientific, Oxford, England, pp. 138–157.

Müller, P.J., 1977. C/N ratios in Pacific deep-sea sediments: effect of inorganic ammonium and organic nitrogen compounds sorbed by clays. Geochimica Cosmochimica Acta 41, 765–776.

Newell, R., Field, J.G., 1983. The contribution of bacteria and detritus to carbon and nitrogen flow in a benthic community. Marine Biology Letters 4, 23–36.

Newell, R., Lucas, M., 1981. The quantitative significance of dissolved and particulate organic matter released during fragmentation of kelp in coastal waters. In: Rheinheimer, G., Fluegel, H., Lenz, J., Zeitschel, B. (Eds.), Lower Organisms and Their Role in the Food Web: Proceedings of the 15th European Marine Biology Symposium, vol. 5. Institut für Meereskunde an der Universität Kiel, Kiel, Germany, pp. 356–369.

Newell, R., Field, J., Griffiths, C., 1982. Energy balance and significance of micro-organisms in a kelp bed community. Marine Ecology Progress Series 8, 103–113.

Norderhaug, K., Fredriksen, S., Nygaard, K., 2003. Trophic importance of Laminaria hyperborea to kelp forest consumers and the importance of bacterial degradation to food quality. Marine Ecology Progress Series 255, 135–144.

Pagiro, T.A., Thomaz, S.M., 1999. Decomposition of Eichhornia azurea from limnologically different environments of the Upper Parana River floodplain. Hydrobiologia 411, 45–51.

Paterson, A., Whitfield, A., 1997. A stable carbon isotope study of the food-web in a freshwater-deprived South African estuary, with particular emphasis on the ichthyofauna. Estuarine, Coastal and Shelf Science 45, 705–715.

Reis, R.P., Yoneshigue-Valentin, Y., 2000. Phenology of H. musciformis (Wulfen) Lamouroux (Rhodophyta,Gigartinales) in three populations from Rio de Janeiro State, Brazil. Botanica Marina 43, 299–304.

Russel-Hunter, W.D., 1970. Aquatic Productivity: an Introduction to Some Basic Aspects of Biological Oceanography and Limnology. MacMillan Co., New York, NY, 352 p.

Schweizer, M., Fear, J., Cadisch, G., 1999. Isotopic (^{13}C) fractionation during plant residue decomposition and its implications for soil organic matter studies. Rapid Communications in Mass Spectrometry 13, 1284–1290.

Sjøtun, K., Fredriksen, S., Rueness, J., 1996. Seasonal growth and carbon and nitrogen content in canopy and first-year plants of Laminaria hyperborea (Laminariales, Phaeophyceae). Phycologia 35, 1–8.

Stephenson, R., Tan, F., Mann, K., 1984. Stable carbon isotope variability in marine macrophytes and its implications for food web studies. Marine Biology 81, 223–230.

Stephenson, R., Tan, F., Mann, K., 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.

Stuart, V., Lucas, M., Newell, R., 1981. Heterotrophic utilization of particulate matter from the kelp Laminaria pallida. Marine Ecology Progress Series 4, 337–348.

Šyvokeine, J., Sineviciene, D., Salcuite, B., 1988. Peculiarities of group composition of symbiotic microflora of Unionacea digestive tract depending on their nutrition. Acta Hydrobiologica Lituanica 7, 69–76.

Taylor, D., Allanson, B., 1995. Organic carbon fluxes between a high marsh and estuary, and the inapplicability of the outwelling hypothesis. Marine Ecology Progress Series 120, 263–270.

Thornton, S.F., McManus, J., 1994. Application of organic carbon and nitrogen stable isotope and C/N ratios as source indicators of organic matter provenance in estuarine systems: evidence from the Tay Estuary, Scotland. Estuarine, Coastal and Shelf Science 38, 219–233.

Valiela, I., Teal, J., Allen, S., 1985. Decomposition in salt marsh ecosystems: the phases and major factors affecting disappearance of above-ground organic matter. Journal of Experimental Marine Biology and Ecology 89, 29–54.

Williams, G., 1993. Seasonal variation in algal species richness and abundance in the presence of molluscan herbivores on a tropical rocky shore. Journal of Experimental Marine Biology and Ecology 167, 261–275.

Wooller, M., 2003. A taphonomic study of δ^{13}C and δ^{15}N values in Rhizophora mangle leaves for a multi-proxy approach to mangrove palaeoecology. Organic Geochemistry 34, 1259–1275.

Zieman, J., Macko, S., Mills, A., 1984. Role of seagrass 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.