Trophic ecology in a Northern Brittany (Batz Island, France) kelp (*Laminaria digitata*) forest, as investigated through stable isotopes and chemical assays

Gauthier Schaal [a,b,*], Pascal Riera [a,b], Cédric Leroux [b,c]

[a] UPMC Univ Paris 06, UMR 7144 AD2M, Equipe Ecologie Benthique, France
[b] CNRS, Station Biologique de Roscoff, Place Georges Teissier, 29682 ROSCOFF, France
[c] UPMC Univ Paris 06, FR 2424, France

ARTICLE INFO

ABSTRACT

This study aimed at characterizing the relationships between the food web's structure and the nutritive value of basal food sources in a Northern Brittany (France) *Laminaria digitata* bed. Stable isotopes were used to identify the food sources consumed by benthic invertebrates, and the nutritive value of primary producers was assessed according to four descriptors (total organic matter, C/N ratio, proteins content, lipids content). Although the food web appeared to be based on a wide diversity of food sources, only Rhodophyta (red algae) and biofilms (epilithic and epiphytic) were heavily consumed by grazers. In contrast, Phaeophyta (brown algae), which are dominant in this habitat, have no specialized grazer (with the exception of *Helcion pellucidum*, specialized grazer of *Laminaria digitata*). This selective consumption may be related to the higher protein content and lower C/N ratio of Rhodophyta and biofilms, in comparison with Phaeophyta. Fresh brown algae are thus of poor nutritive value, but processes associated with their degradation are likely to improve this nutritive value, leading in the assimilation of detritus by filter-feeders, revealed by high δ^{13}C in these consumers. Our results thus suggest that the nutritive value of basal food sources may be an important factor involved in the structuration of kelp-associated food webs.

© 2009 Elsevier B.V. All rights reserved.

1. Introduction

Kelps form extensive beds around temperate and polar coasts in all the oceans of the world. Their importance for global biogeochemical cycles is recognised, since this kind of environment is considered to be one of the most productive of the world (Mann 1973). In European coastal areas, kelp beds are dominated by the genus *Laminaria*. In Brittany, kelp forests occur along rocky coasts from the mean low waters at spring tides to a depth of about 30 m (Arzel, 1998). The three-dimensional structure created by these algae forms in a large variety of microhabitats, resulting in a high algal and animal diversity characterizing these environments (Christie et al., 2003).

In spite of their ecological importance, few are known about the functioning of the food web associated to these habitats (but see Fredriksen, 2003). Particularly, the major trophic resources of consumers inhabiting kelp beds remain unclear yet. Indeed, although kelps represent an important potential food supply for consumers inhabiting these environments, most of kelp species are characterized by low nitrogen content and the presence in their tissues of herbivore-deterrent secondary metabolites (Duggins and Eckman, 1997; Norderhaug et al., 2003). These characteristics result in a low nutritive

value of kelps, which prevents them to be intensively grazed, as previously observed for various grazers fed *Laminaria hyperborea* (Norderhaug et al., 2003). However, coevolution processes may result in an adaptation of these grazers to a low nutritive value food (Steinberg et al., 1995). In this way, there are several evidences of kelp consumption by various consumers such as sea urchins (Lawrence, 1975) or gastropods (Fredriksen, 2003; Norderhaug et al., 2003). Moreover, some studies have shown that kelps could be consumed through the detrital pathway by various consumers, such as amphipods (Norderhaug et al., 2003) or bivalves (Duggins and Eckman, 1997). Microbial processes occurring during the degradation of kelp-derived organic matter are responsible of an enrichment in nitrogen of the detritus (Norderhaug et al., 2003), which makes these detrital complexes readily available for several consumers. The role of kelps as a food supply for consumers inhabiting kelp forests is thus still not clear. In addition, these habitats host a large diversity of understory algae. In *L. digitata* beds, the dominant algae under the canopy-forming kelps are *Fucus serratus* and *Himanthalia elongata* for Phaeophyta, *Mastocarpus stellatus*, *Chondrus crispus* and *Corallina elongata* for Rhodophyta, as described by Castric-Fey et al. (1997). Other species, such as the Rhodophyta *Palmaria palmata*, may be seasonally important (G. Schaal, pers. obs.). These understory algae are also likely to be exploited by grazers inhabiting kelp forests, as reported by Fredriksen (2003). The relative importance of kelps and understory algae as a basis for the food web is unclear yet.

* Corresponding author: UPMC Univ Paris 06, UMR 7144 AD2M, Equipe Ecologie Benthique, France. Tel.: +33 2 98 29 23 77; fax: +33 2 98 29 23 24.
E-mail address: schaal@sb-roscoff.fr (G. Schaal).

Factors affecting the consumption of food sources in benthic invertebrates remain to date unclear. Several studies have investigated this issue through food choice laboratory experiments (Pavia et al., 1999; Cruz-Rivera and Hay, 2000; Karez et al., 2000). Although providing precious data on factors susceptible to affect herbivores' food choice, the conclusions of these studies appear sometime contradictory and difficult to extrapolate to natural conditions. For example, Pavia et al. (1999) found that the amphipod *Gammarus locusta* preferred to feed on *Ascophyllum nodosum* macroepiphytes rather than on the host, while Karez et al. (2000) found the contrary. Some studies suggested that herbivores consume preferentially the more nutritive food source (Cruz-Rivera and Hay, 2000), while others found that herbivores prefer to consume a temporally perennial food source rather than a more nutritive, but seasonal one (Paine and Vadas, 1969). Several mesograzers have been observed to choose low nutritive value seaweeds to avoid predation by omnivorous fishes (Hay et al., 1987). Finally, food choice experiment studies have contributed to a strong background in the knowledge of factors which affect herbivore's consumption of food sources. However, generalising results from laboratory experiments to processes occurring in the wild is not straightforward, and a more thorough understanding of the relative importance of these factors in the consumption of coexisting sources requires necessarily field approaches. In European kelp forests, the important diversity of potential food sources (macroalgae, epiphytic and epilithic biofilms, suspended particulate organic matter) associated to the diversity of consumers makes these issues challenging and relevant.

In this context, stable isotopes provide promising insights. This method provides a time-integrated measure of really ingested food, and is thus complementary with food choice experiments. Due to the low carbon isotope fractionation occurring from a food source to its consumer, $\delta^{13}C$ is often used to assess basal sources in food webs, while $\delta^{15}N$ is an indicator of trophic level and food webs length, due to the higher trophic fractionation occurring at each trophic transfer (Peterson, 1999). Numerous examples have highlighted that rocky shore's macroalgae were efficiently discriminated by their $\delta^{13}C$ and $\delta^{15}N$ (Fredriksen, 2003; Bode et al., 2006; Kang et al., 2008). Due to the possibility to clearly identify macroalgal food sources with stable isotopes, this method appears to be a powerful tool for the study of rocky shores' food webs.

The present study aimed thus at (1) describing the general features of the food web in a *L. digitata* forest (2) identifying the main herbivores and their food sources (3) assessing the nutritive value of the food sources potentially available for the consumers inhabiting this habitat and (4) linking the biochemical characteristics of primary producers to their utilisation in the food web.

2. Material and methods

2.1. Study site

Sampling was carried out in a granite boulder field of Western Batz Island (Brittany, France) (Fig. 1). Although the intertidal shore is mainly unvegetated, a dense *L. digitata* forest occurs from the mean low water spring tides. This site is characterized by a high hydrodynamism exposure, due to water motion and the intensity of tide currents along the northern coast of Brittany (Castel et al., 1997). The English Channel, where our sampling site is located, is a megatidal sea. The maximal tidal amplitude at Roscoff is higher than 9 m. Due to the absence of city close to this study site and the high hydrodynamic exposure, this site can be considered as totally preserved from anthropogenic perturbations.

2.2. Samples processing

Sampling was carried out during spring tides in October 2006 and May 2007. At each sampling date, the most representative food sources (i.e. algae, epiphytic and epilithic biofilms, suspended particulate organic matter (SPOM)) and the most abundant consumers were sampled by hand. In May 2007, detrital drifted macroalgae were also opportunistically sampled floating on the surface of the water at the study site. The water sample was carried
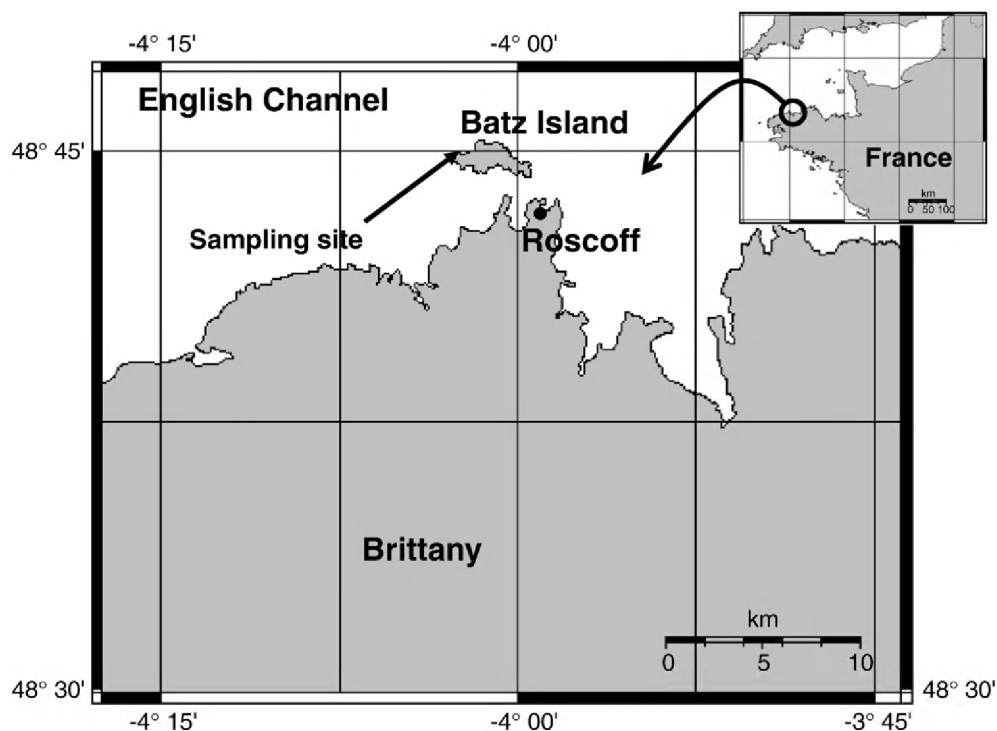

Fig. 1. Localization of the sampling site in the Eastern English Channel.

out more than 5 km offshore in order to avoid the presence of macrophytes detritus in the water column, and to obtain an isotopic signature of a SPOM mainly dominated by phytoplankton. Zooplankton was sampled using a plankton net (mesh 200 μm). Rocks and algae were gently scrapped with a thin brush to collect epiphytic and epilithic biofilms, and then cautiously resuspended in filtered (0.45 μm) seawater.

Once back to the laboratory, algae were cleaned of their eventual epiphytes, briefly acidified (HCl, 1 N), rinsed with distilled water, and dried (48 h, 60 °C). They were then ground into a fine and homogenous powder and stored frozen (−20 °C) until subsequent analysis. Animals were kept alive overnight to allow the evacuation of their stomach content. They were then killed by freezing and stored frozen until subsequent analysis. The fleshes were then extracted from their shell (molluscs) or their cuticle (crustaceans), briefly acidified (HCl, 1 N) to remove any carbonates debris, rinsed with distilled water and then processed as previously described for algae. SPOM was obtained by filtering until clogging seawater on a precombusted (520 °C, 3 h) GF/F filter. The filter was then quickly acidified, rinsed with distilled water, dried (24 h, 60 °C) and stored frozen until isotopic analysis. Epiphytic and epilithic biofilms samples were pre-filtered on a 63 μm mesh to remove meiofaunal organisms and large detritus, and then processed as described for SPOM.

2.3. Biochemical characterisation of food sources

Several biochemical analyses were conducted to estimate the respective nutritive value of food sources. Algae were freeze dried and ground into a fine and homogenous powder using a mortar and a pestle. All measurements were realized on triplicates. Total organic matter content (TOM) was measured by weight loss after combustion (5 h, 520 °C) in a muffle furnace. Organic carbon and nitrogen contents were measured using a Flash EA CN analyser. Total lipids contents were measured colorimetrically according to the Barnes and Blackstock (1973) procedure. Protein content was obtained according to Kjeldahl's method (AOAC, 1990), using an N-prot conversion factor of 6.25. Although we acknowledge that this factor may vary according to the species considered (Lourenço et al., 2002), the variations induced by this variability are likely too weak to contradict our conclusions.

2.4. Stable isotope measurements

Carbon and nitrogen isotope ratios were determined using a Flash EA CN analyser coupled with a Finnigan Delta Plus mass spectrometer, via a Finnigan Con-Flo III interface. Data are expressed in the standard δ unit.

$$\delta X = [(R_{sample} / R_{reference}) - 1] \times 10^3$$

With $R = {}^{13}C/{}^{12}C$ for carbon and ${}^{15}N/{}^{14}N$ for nitrogen. These abundances were calculated in relation to the certified reference materials Vienna Pee Dee Belemnite-limestone (V-PDB) and atmospheric dinitrogen (at-air). The VPDB and at-air scales were achieved using in-house protein standards, calibrated against NBS-19 and IAEA N3 reference materials. The standard deviation of repeated measurements of $\delta^{13}C$ and $\delta^{15}N$ values of a laboratory standard was 0.10‰ versus V-PDB and 0.05‰ versus at-air, respectively.

2.5. Trophic level estimation

The trophic level of each species was calculated on the basis of their mean $\delta^{15}N$, which has been proven to be a valuable indicator of trophic position (Post et al., 2000). We considered a mean trophic isotopic fractionation of 3.4‰ between a food source and its consumer

(DeNiro and Epstein, 1978). The following formula was used for the calculation of trophic levels (TL):

$$TL_i = [(\delta^{15}N_i - \delta^{15}N_G) / 3.4] + 2$$

where:

TL_i	trophic level of species i
$\delta^{15}N_i$	mean $\delta^{15}N$ of species i
$\delta^{15}N_G$	mean $\delta^{15}N$ of grazers
3.4	mean trophic enrichment occurring from primary consumers

Grazers were chosen for the basis of TL calculations because they displayed very close and low $\delta^{15}N$ values, consistent with a primary consumer status. Moreover, the formula used in this study has the advantage to be independent of the trophic fractionation occurring between primary producers and primary consumers. This fractionation coefficient is known to be more variable than those occurring in upper TL (Vander Zanden and Rasmussen, 2001).

3. Results

3.1. Biochemical composition of macroalgae

The results of the biochemical characterisation of potential food sources are presented Table 1. Five of the ten macroalgae species considered were present both in October and in May (*M. stellatus*, *P. palmata*, *L. digitata*, *H. elongata*, *F. serratus*), four were present only in October (*C. crispus*, *C. elongata*, *Lithotamnion incrustans*, *Cladophora rupestris*) and one was present only in may (*Laurencia pinnatifida*). The total organic matter content (TOM) displayed low variation among algae (between 65% and 85%), except for the calcareous algae, *C. elongata* and *L. incrustans*, whose organic content was very low (around 15% of the total dry weight). The C/N ratio was more discriminating among the different species, being higher in brown algae than in green and red algae (Mann–Whitney (MW) test, $p < 0.001$). In the same way, brown algae were less protein-rich than green and red algae (MW test, $p < 0.001$), except the encrusting algae *L. incrustans*, whose protein content was low (6.9% of dry weight). The

Table 1
Biochemical characteristics (±S.D.) of Rhodophyta, Phaeophyta and Chlorophyta sampled in October 2006 and May 2007 in Batz Island.

Species	TOM (%)		C/N		Proteins (% DW)		Lipids (mg g^{-1})	
	October 2006	May 2007	October 2006	May 2007	October 2006	May 2007	October 2006	May 2007
Chondrus crispus	75.6 (1.4)		11.1 (0.4)		17.7 (1.5)		9 (1.6)	
Mastocarpus stellatus	74.9 (1)	81.9 (0.7)	10.4 (0.8)	9.2 (1.1)	18.8 (2.2)	23.5 (4.3)	20.9 (2.2)	12.8 (1.3)
Palmaria palmata	85.7 (2.1)	73 (3.6)	11.3 (0)	9.3 (0.4)	21.4 (2.7)	29.8 (1.2)	22 (2)	16.6 (1.9)
Corallina elongata	16.9 (0.5)		7.1 (0.4)		30.9 (2.5)		18.6 (0.8)	
Lithothamnion incrustans	14.1 (2.9)		15.5 (0.7)		6.9 (0.8)		11.1 (1.8)	
Laurencia pinnatifida		77.9 (4.6)		8 (0.1)		30 (2.7)		45.6 (3.6)
Laminaria digitata	73 (0.6)	71.1 (3.5)	20.7 (0.5)	22.5 (1.8)	10.2 (2)	9.3 (0.6)	59.6 (3.2)	48.3 (8.9)
Himanthalia elongata	69.8 (1.2)	65.9 (1.3)	15.2 (0.2)		13 (0.5)	15.8 (4.7)	66.2 (4.7)	63.6 (2.7)
Fucus serratus	76.8 (0.9)	77.6 (2.1)	20.6 (1.2)	16.4 (2.8)	11.9 (1.1)	15.5 (2.4)	179.4 (15.8)	
Cladophora rupestris	62.6 (1.7)		8.7 (0.3)		29.2 (1.4)		48 (0.6)	

opposite pattern was observed concerning the total lipid content, with brown algae being richer than green and red algae (MW test, $p < 0.001$).

The temporal variations did not show any clear trend, most of the variation being not statistically significant. Indeed, only two species displayed significant variation. Firstly, the TOM of *M. stellatus* rose from 74.9% to 81.9% (MW test, $p = 0.049$), while its lipid content decreased from 20.9 mg g^{-1} to 12.8 mg g^{-1} (MW test, $p = 0.049$). Secondly, the temporal variation of the nutritive characteristics of *P. palmata* decreased significantly for the TOM, C/N ratio and lipid content (MW test, $p = 0.049$), while its protein content rose from October 2006 to May 2007 (MW test, $p = 0.049$).

A principal component analysis (PCA) was carried out to synthesize the information provided by the nutritional evaluation of macroalgae (Fig. 2). Data were standardized prior to analysis to account for differences in the units of the descriptors (TOM, C/N ratios, lipids content, proteins content). This ordination synthesizes 82% of the total variance on the two first factorial axes. Red and green algae are clearly opposed to brown algae on the first axis (55% total variance), which is mainly explained by nitrogen-related descriptors (i.e. C/N ratio, protein content), and calcified versus non-calcified algae on the second axis (27% total variance). The TOM content is the main factor contributing to the second axis.

3.2. $\delta^{13}C$ and $\delta^{15}N$ of food sources

The results of the isotopic characterisation of the potential food sources are presented in Table 2. In both October and May, food sources were largely more discriminated by $\delta^{13}C$ than by $\delta^{15}N$ ($\delta^{13}C$ range = 8.8‰ and 12‰ and $\delta^{15}N$ range = 1.3‰ and 2.7‰ for October and May, respectively). The $\delta^{15}N$ of food sources ranged from 4.3‰ (SPOM) to 7.3‰ (*C. rupestris*). SPOM was the more ^{13}C-depleted source and displayed unchanged $\delta^{13}C$ value of about −24‰, while its $\delta^{15}N$ value decreased from 6.4‰ in October to 4.3‰ in May. Among algae, the more ^{13}C-depleted were red algae (*C. crispus*, *M. stellatus*), while the more ^{13}C-enriched were brown algae (*L. digitata*, *H. elongata*). In particular, *L. digitata* was the most ^{13}C-enriched food sources in May (−12.3‰) and in October (−15.2‰) together with *P. palmata* (−15.1‰ in October and −16.5‰ in May). The $\delta^{13}C$ of *L. digitata* in October was in the same range than this reported by Raven et al. (2002). In both October 2006 and May 2007, epilithic and epiphytic biofilms displayed $\delta^{13}C$ intermediate between red algae and *L. digitata* (−18.7‰ and −21.2‰ for epilithic biofilms in October and May, respectively, and −18.1‰ and −19.5‰ for epiphytes in October and May, respectively). $\delta^{15}N$ temporal variations for the sources considered were below 1‰, excepted for SPOM (^{15}N-depletion of 2.1‰). $\delta^{13}C$ temporal variations were much more important, up to 3.3‰ (*M. stellatus*), excepted for SPOM (^{13}C-depletion of 0.4‰). No difference was observed between fresh algae and detritus for the three species (*P. palmata*, *F. serratus*, *H. elongata*) analyzed (MW tests, $p > 0.05$) (Table 3).

3.3. $\delta^{13}C$ and $\delta^{15}N$ of consumers

A total of 58 species were sampled for October and May (29 in October, 48 in May). Each species was assigned to a trophic group (filter-feeders, deposit-feeders, grazers, carnivores) according to the bibliography (Table 4). Among these species, nine were reported to be grazer species, whereas most of them were reported to be predators (22 species) or filter-feeders (19 species).The $\delta^{13}C$ and $\delta^{15}N$ measured for these consumers are presented in Table 2 and Fig. 3. The range of $\delta^{13}C$ displayed by the consumers was similar for the two dates (6.3‰ in October vs. 6.5‰ in May), while the range of $\delta^{15}N$ increased slightly from October (5.4‰) to May (7.1‰). The trophic level estimations of each consumer are presented Table 4. From these calculations, among the 58 species, only 4 (*Asterina gibbosa*, *Urticina felina*, *Henricia sanguinolenta*, *Cancer pagurus*) displayed TL at least equal to 3, which indicated a strictly carnivorous diet. Most of the consumers displayed TL comprised between 2 and 3, which indicated a dominance of omnivory within this food web.

Grazers displayed a wide variety of $\delta^{13}C$ values, including *H. pellucidum*, which was the more ^{13}C-enriched consumer both in October and May. At the opposite, the isopod *Idotea pelagica* and the abalone *Haliotis tuberculata* were the more ^{13}C-depleted grazers in October and in May, respectively. Contrarily to grazers, filter-feeders and predators presented more heterogeneous $\delta^{15}N$ values (Fig. 4). On the contrary, deposit-feeders displayed isotopic ratios similar to grazers, with a wide $\delta^{13}C$ range and a restricted $\delta^{15}N$ range.

Based on these results, the contribution of macroalgae-derived organic matter to the diet of filter-feeders was assessed using a two-source mixing model on the basis of $\delta^{13}C$ values. The two end-
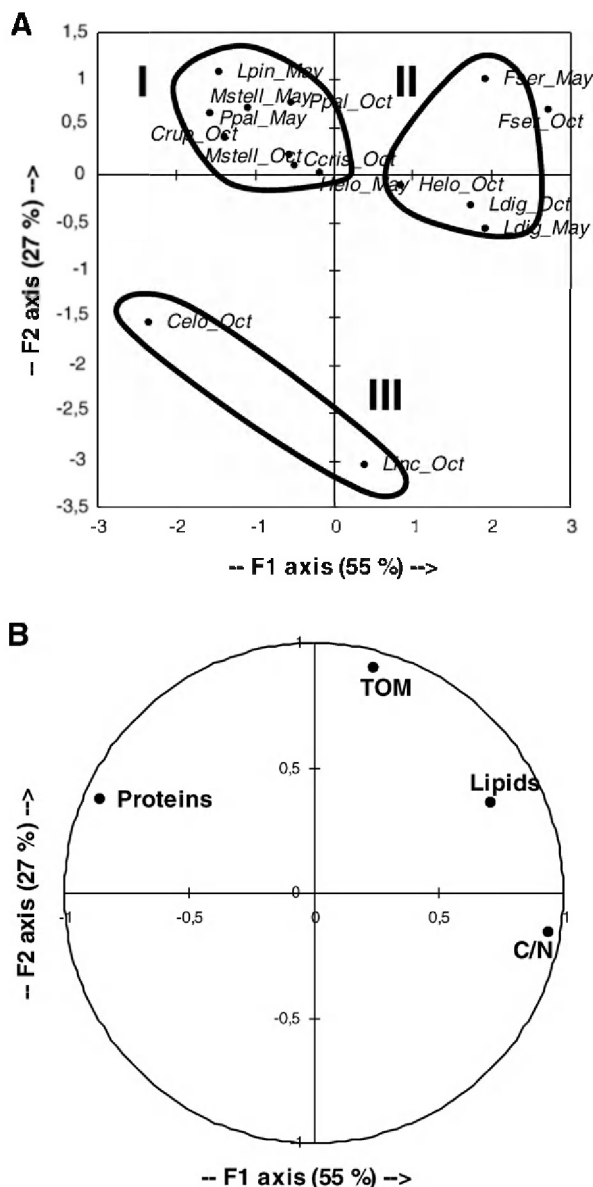

Fig. 2. Principal component analysis (PCA) realized on standardized biochemical characteristics of food sources. A: individuals B: variables. Groups of points represent I: red and green non-calcified algae, II: brown algae, III: calcified red algae. Ccris = *Chondrus crispus*, Mstell = *Mastocarpus stellatus*, Ppal = *Palmaria palmata*, Celo = *Corallina elongata*, Linc = *Lithothamnion incrsutans*, Lpin = *Laurencia pinnatifida*, Ldig = *Laminaria digitata*, Helo = *Himanthalia elongata*, Fser = *Fucus serratus*, Crup = *Cladophora rupestris*.

Table 2
$\delta^{13}C$ and $\delta^{15}N$ (overall mean ± S.D.) of potential food sources and consumers sampled in October 2006 and May 2007 in Batz Island.

Code	Species	October 2006		n	May 2007		n
		$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)		$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	
Sources							
SPOM	SPOM	6.4 ± 0.22	−23.9 ± 0.32	3	4.3 ± 0.29	−24.3 ± 0.93	2
SOM	SOM				6 ± 0	−19.5 ± 0	1
El	Epilithon	6.2 ± 0	−18.7 ± 0	1	6.5 ± 0	−21.2 ± 0	1
Ep	Epiphytes	6.6 ± 0.18	−18.1 ± 0.14	3	7 ± 0	−19.5 ± 0	1
Ent	*Enteromorpha sp.*				6.2 ± 0.52	−17.7 ± 0.52	3
Cr	*Cladophora rupestris*	7.3 ± 0.27	−19.4 ± 0.53	3			
Ce	*Corallina elongata*	6.9 ± 0.21	−20.3 ± 0.59	3			
Cc	*Chondrus crispus*	6.2 ± 0.17	−23.2 ± 1.09	3			
Ms	*Mastocarpus stellatus*	6.5 ± 0.36	−22.3 ± 0.62	3	6.6 ± 0.18	−19.1 ± 1.29	3
Pp	*Palmaria palmata*	6 ± 0.09	−15.1 ± 0.71	3	5.5 ± 0.24	−16.5 ± 0.91	3
Lp	*Laurencia pinnatifida*				6.2 ± 0.56	−14.5 ± 0.29	3
Fs	*Fucus serratus*	6.2 ± 0.06	−19.1 ± 0.88	3	5.3 ± 0.2	−17.1 ± 0.34	3
He	*Himanthalia elongata*	6.7 ± 0.20	−16.9 ± 0.51	3	6.8 ± 0.41	−13.9 ± 0.6	3
Ld	*Laminaria digitata*	7 ± 0.08	−15.2 ± 0.25	3	6.6 ± 0.81	−12.3 ± 0.01	3
Consumers							
Porifera							
1	*Halichondria panicea*	9.2 ± 0.35	−18.8 ± 0.35	3	7.3 ± 0.10	−18.6 ± 0.19	
2	*Hymeniacidon sanguinea*	9.2 ± 0.24	−19.4 ± 0.45	4	5.9 ± 0.65	−19.9 ± 0.93	3
3	*Pachymatisma johnstonia*				9.4 ± 0.28	−17.2 ± 0.33	3
Cnidaria							
4	*Anemonia viridis*	9.9 ± 0	−17.4 ± 0	1			
5	*Urticina felina*				12 ± 0.49	−16.4 ± 0.21	3
6	*Bunodactis verrucosa*				11 ± 0	−18 ± 0	1
Annelida							
7	*Sabella pavonina*				5.3 ± 0	−18 ± 0	1
8	*Amphiglenia mediterranea*				7.4 ± 0.26	−18.2 ± 0.47	3
9	*Amphitrite gracilis*				8.3 ± 0.04	−17 ± 0.22	2
10	*Platynereis dumerilii*	8.1 ± 0.05	−19.7 ± 0.22	2	8 ± 0.43	−18 ± 0.67	3
11	*Harmothoe sp.*	11 ± 0.10	−18.1 ± 0.35	2			
12	*Perinereis cultrifera*	9.9 ± 1.55	−18.1 ± 1.45	3	8.7 ± 0.89	−18.9 ± 1.883	3
13	*Syllis hyalina*				9.9 ± 0.56	−17.6 ± 0.06	3
Crustacea							
14	*Elminius modestus*				8.2 ± 0.3	−18 ± 0.65	3
15	*Chthamalus montagui*				9.4 ± 0.02	−18.3 ± 0.56	3
16	*Balanus perforatus*	9.9 ± 0.21	−19.9 ± 0.76	2	10.7 ± 0.42	−18.1 ± 0.83	3
17	*Gammarus locusta*	7.3 ± 0.31	−16 ± 0.50	5			
18	*Ampithoe ramondi*				5.3 ± 0.04	−17.9 ± 0.13	3
19	*Elasmopus rapax*				8.4 ± 0.08	−17.6 ± 0.23	4
20	*Paragnathia formica*				10.6 ± 0	−17.1 ± 0	1
21	*Dynamene bidentata*				7.6 ± 0.65	−20.1 ± 1	4
22	*Idotea pelagica*				6.6 ± 0.63	−20.1 ± 1.29	4
23	*Pisidia longicornis*	8.2 ± 0.24	−18.7 ± 0.04	3	6.2 ± 0.55	−18.4 ± 0.09	3
24	*Porcellana platycheles*	8.8 ± 0.10	−16.1 ± 0.35	6	8.4 ± 0.19	−18.41 ± 0.63	3
25	*Galathea squamifera*	8.1 ± 0.12	−17.1 ± 0.28	1	7.6 ± 0	−18.3 ± 0	1
26	*Anapagurus hyndmanni*				6.6 ± 0	−18 ± 0	1
27	*Pagurus bernhardus*	9.7 ± 0.55	−16.6 ± 0.35	2	10.1 ± 0.26	−17 ± 0.56	3
28	*Xantho pilipes*	9.3 ± 0	−17.8 ± 0	1	9.4 ± 0.29	−17.4 ± 0.51	2
29	*Xantho incisus*	8.3 ± 0.09	−17.1 ± 0.18	2	8.8 ± 0.39	−16.6 ± 0.54	3
30	*Cancer pagurus*				12.4 ± 0.45	−15.2 ± 0.23	3
31	*Pilumnus hirtellus*	9.5 ± 0.67	−17.4 ± 0.33	2	9.7 ± 0.4	−17.2 ± 0.45	6
32	*Liocarcinus arcuatus*	9.9 ± 0	−17.5 ± 0	1			
33	*Necora puber*				10.5 ± 0.06	15.3 ± 0.14	2
Mollusca							
34	*Leptochiton cancellatus*				10.7 ± 0	−18.3 ± 0	1
35	*Acanthochitona crinitus*				10.2 ± 0.73	−17.4 ± 1.29	2
36	*Patella vulgata*	8 ± 0.32	−17.7 ± 1.45	5	8.8 ± 0.34	−18.1 ± 0.58	3
37	*Helcion pellucidum*	8.3 ± 0.20	−14.1 ± 1.51	5	7.6 ± 0.86	−13.6 ± 0.72	3
38	*Haliotis tuberculata*	7.1 ± 0.05	−20.4 ± 0.03	2			
39	*Gibbula pennanti*	8.7 ± 1.02	−16.8 ± 0.39	4	8.8 ± 0.22	−16.5 ± 0.7	6
40	*Gibbula cineraria*	8.4 ± 0.26	−16.2 ± 0.39	6	9.1 ± 0.64	−16 ± 0.29	5
41	*Calliostoma zizyphinum*	11.2 ± 0.28	−16.7 ± 0.38	7	11.2 ± 0.57	−16.3 ± 0.05	3
42	*Littorinids juveniles*				7.1 ± 0.39	−17.9 ± 0.66	3
43	*Nassarius incrassatus*				10.6 ± 0.26	−16.5 ± 0.05	2
44	*Nucella lapillus*				11.1 ± 0.45	−16.6 ± 0.63	6
45	*Ocenebra erinacea*	10.1 ± 0.16	−17.2 ± 0.35	6	10.4 ± 0.45	−17.2 ± 0.33	6
46	*Trivia monacha*	9.7 ± 0.35	−17.9 ± 0.79	4			
47	*Mytilus edulis*				6.1 ± 0.32	−17.8 ± 0.10	3
Bryozoa							
48	*Electra pilosa*	6.3 ± 0	−18.9 ± 0	1			
49	*Flustrellidra hispida*	6.6 ± 0.11	−19.4 ± 0.77	2			
50	*Schizoporella sp.*				6.7 ± 0.23	−16.5 ± 0	2

Table 2 (continued)

Code	Species	October 2006		n	May 2007		n
		δ^{15}N (‰)	δ^{13}C (‰)		δ^{15}N (‰)	δ^{13}C (‰)	
Consumers							
Echinodermata							
51	Asterina gibbosa				11.8 ± 0.32	− 18 ± 1.54	3
52	Henricia sanguinolenta	11.7 ± 0.03	− 14.2 ± 0.44	3			
53	Ophiotrix fragilis				7.3 ± 0.96	− 18.8 ± 0.84	2
Chordata							
54	Botryllus schlosseri	7.3 ± 0.79	− 18.3 ± 0.42	2	6.4 ± 0.46	− 18.7 ± 0.22	3
55	Aplidium pallidum	8.3 ± 0	− 19.5 ± 0	1			
56	Didemnum spp.				6.5 ± 0.51	− 19.8 ± 0.32	3
57	Dendrodoa grossularia				8.3 ± 0.35	− 19.6 ± 0.08	3
58	Lepadogaster lepadogaster				11.6 ± 0.24	− 16 ± 0.08	4
59	Zooplankton	8.2 ± 0	− 19.9 ± 0	1	8.2 ± 0.03	− 19.8 ± 0.65	3

n = sample size.

members used for this calculation were (1) phytoplanktonic-dominated SPOM and (2) macroalgae. Since the isotopic value of the pool of macroalgal detritus is unknown, we considered two possibilities to estimate the contribution of such detritus to the diet of filter-feeders: (1) the pool of macroalgal detritus is only constituted of kelp detritus (2) this pool is constituted of constituted of a mix of all co-occurring macroalgae, in equal proportions. The results (Table 5) show that even considering the lowest estimation, filter-feeders derive at least 30% of their diet from macroalgae-derived organic matter.

The δ^{13}C and δ^{15}N temporal variation between October and May was non-significant for most of the consumers sampled (MW test, $p > 0.05$ for the different species). However, a significant δ^{15}N decrease was observed for filter-feeders (MW test, $p = 0.002$).

4. Discussion

4.1. Nutritional value of food sources

The results observed for the different co-occurring algae are contrasted according to the different descriptors of the nutritive value considered. This highlights the necessity of considering several descriptors for studying the nutritive value of food sources. Here, four of the most commonly used descriptors were used.

The TOM reflects the relative proportions of the organic fraction, likely to provide essential nutrients to consumers, and the mineral fraction, indigestible although providing essential minerals (Rupérez, 2002). Here, only two species differed strongly from the other (the calcified red algae Lithothamnion incrustans and C. elongata). Their very low organic content is likely to be responsible of a poor nutritive value. Hence, a consumer feeding on these algae will have an energetic cost more elevated than when feeding on non-calcified algae.

The C/N ratio is one of the most commonly used descriptors of food source nutritive value. For Norwegian amphipods inhabiting L. hyperborea forests, this ratio has been observed to be important for the suitability of kelps as food source (Norderhaug et al., 2003, 2006). A limit value of 17 is usually adopted as the limit over which a food source is not nutritive enough to meet the nutritional requirement of

a consumer (Russel-Hunter, 1970). Indeed, if N is not abundant enough in a food source, the imbalance between the N content of alga and grazers causes carbon to be less efficiently used by the consumer,

Table 4

Trophic levels calculated for filter-feeders (F), deposit-feeders (DF) and carnivores (C) sampled in October 2006 and May 2007.

Species	Feeding mode	Trophic levels	
		October 2006	May 2007
Sabella pavonina	F	–	1.1
Mytilus edulis	F	–	1.3
Didemnum spp.	F	–	1.4
Electra pilosa	F	1.5	–
Schizoporella sp.	F	–	1.5
Flustrellidra hispida	F	1.6	–
Ophiotrix fragilis	F	–	1.7
Botryllus schlosseri	F	1.8	1.4
Elminius modestus	F	–	1.9
Pisidia longicornis	F	2	1.3
Dendrodoa grossularia	F	–	2
Applidium pallisum	F	2.1	–
Porcellana platycheles	F	2.2	2
Hymeniacidon sanguinea	F	2.3	1.2
Halichondria panicea	F	2.3	1.7
Chthamalus montagui	F	–	2.3
Pachimatisma johnstonia	F	–	2.3
Balanus perforatus	F	2.5	2.7
Ampithoe ramondi	DF	–	1
Anapagurus hyndmanni	DF	–	1.4
Dynamene bidentata	DF	–	1.7
Gammarus locusta	DF	1.8	–
Amphitrite gracilis	DF	–	1.9
Elasmopus rapax	DF	–	2
Galathea squamifera	DF	2	1.7
Platynereis dumerilii	DF	2	1.8
Xantho incisus	C	2.1	2.1
Xantho pilipes	C	2.3	2.3
Pilumnus hirtellus	C	2.4	2.3
Syllis hyalina	C	–	2.4
Trivia monacha	C	2.5	–
Pagurus bernhardus	C	2.5	2.5
Anemonia viridis	C	2.5	–
Perinereis cultrifera	C	2.5	2.1
Liocarcinus arcuatus	C	2.5	–
Necora puber	C	–	2.6
Nassarius incrassatus	C	–	2.6
Paragnathia formica	C	–	2.6
Ocenebra erinacea	C	2.6	2.6
Bunodactis verrucosa	C	–	2.7
Harmothoe sp.	C	2.8	–
Nucella lapillus	C	–	2.8
Calliostoma zizyphinum	C	2.9	2.8
Lepadogaster lepadogaster	C	–	2.9
Asterina gibbosa	C	–	3
Urticina felina	C	–	3
Henricia sanguinolenta	C	3.1	–
Cancer pagurus	C	–	3.1

Table 3

Comparison of δ^{13}C vs. δ^{15}N (± S.D.) between fresh and detrital macroalgae for three species sampled in May 2007.

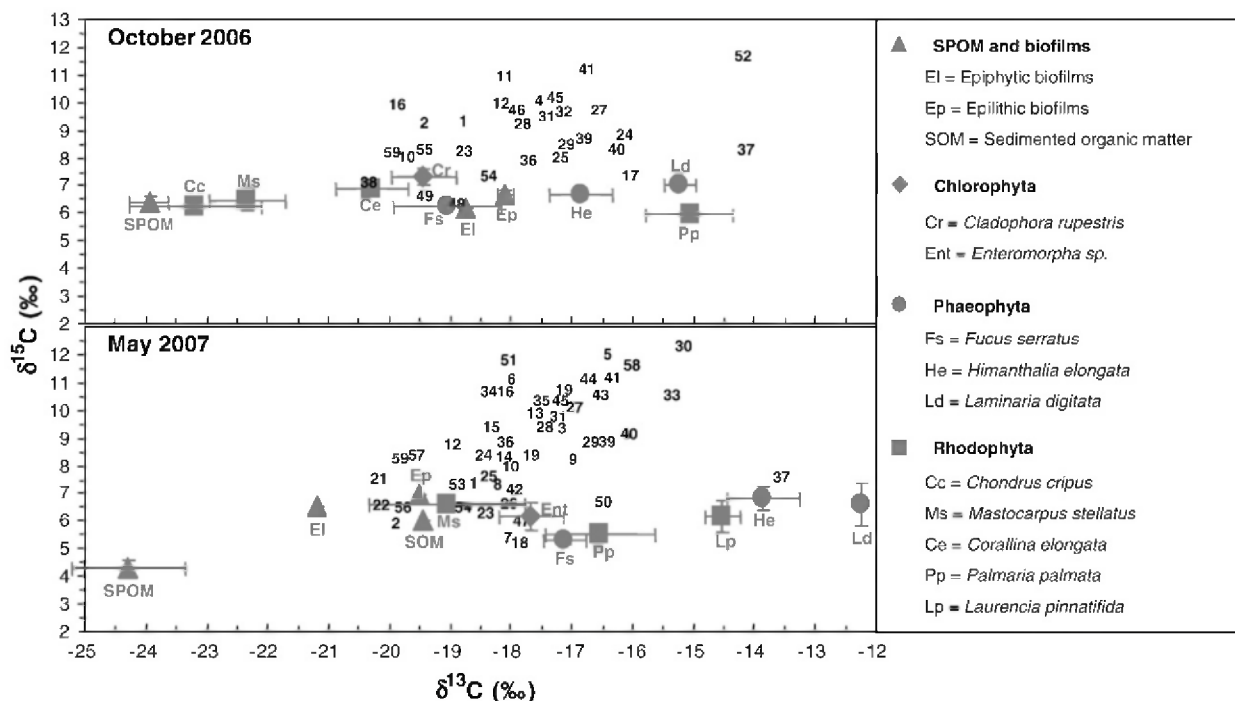| Species	δ^{13}C		δ^{15}N	
	Fresh	Detritus	Fresh	Detritus
Palmaria palmata	− 16.5 ± 0.91	− 16.9 ± 2.84	5.5 ± 0.24	7.5 ± 1.44
Himanthalia elongata	− 13.9 ± 0.6	− 13.7 ± 0.21	6.8 ± 0.41	5.6 ± 1.16
Fucus serratus	− 17.1 ± 0.34	− 16.8 ± 1.28	5.3 ± 0.20	5.5 ± 0.88

Fig. 3. δ^{13}C and δ^{15}N ($\pm$ S.D.) of potential food sources and consumers sampled in October 2006 and May 2007. Legends for consumers are given in Table 2. Sources: SPOM = Suspended Particulate Organic Matter, SOM = Sedimented Organic Matter, El = Epilithic biofilm, Ep = Epiphytic biofilm, Cr = *Cladophora rupestris*, Ent = *Enteromorpha* sp., Cc = *Chondrus crispus*, Ms = *Mastocarpus stellatus*, Pp = *Palmaria palmata*, Lp = *Laurencia pinnatifida*, Ce = *Corallina elongata*, Fs = *Fucus serratus*, He = *Himanthalia elongata*, Ld = *Laminaria digitata*.

resulting in a lower nutritive value (Sterner and Hessen, 1994). From our results, it appears clearly that brown algae display too high C/N ratios to be considered as highly nutritive food sources. Indeed, *L. digitata* has the highest C/N ratio of the macroalgae considered both in October (20.7) and May (22.5), and *F. serratus* and *H. elongata* have

also C/N ratios ranging from 15.2 to 20.6. On the contrary, red and green algae display values around 10, which is consistent with a higher nutritive value of these algae.

The protein content of food sources has been observed to be one of the most important characteristics of the nutritive value (Bowen et al.,
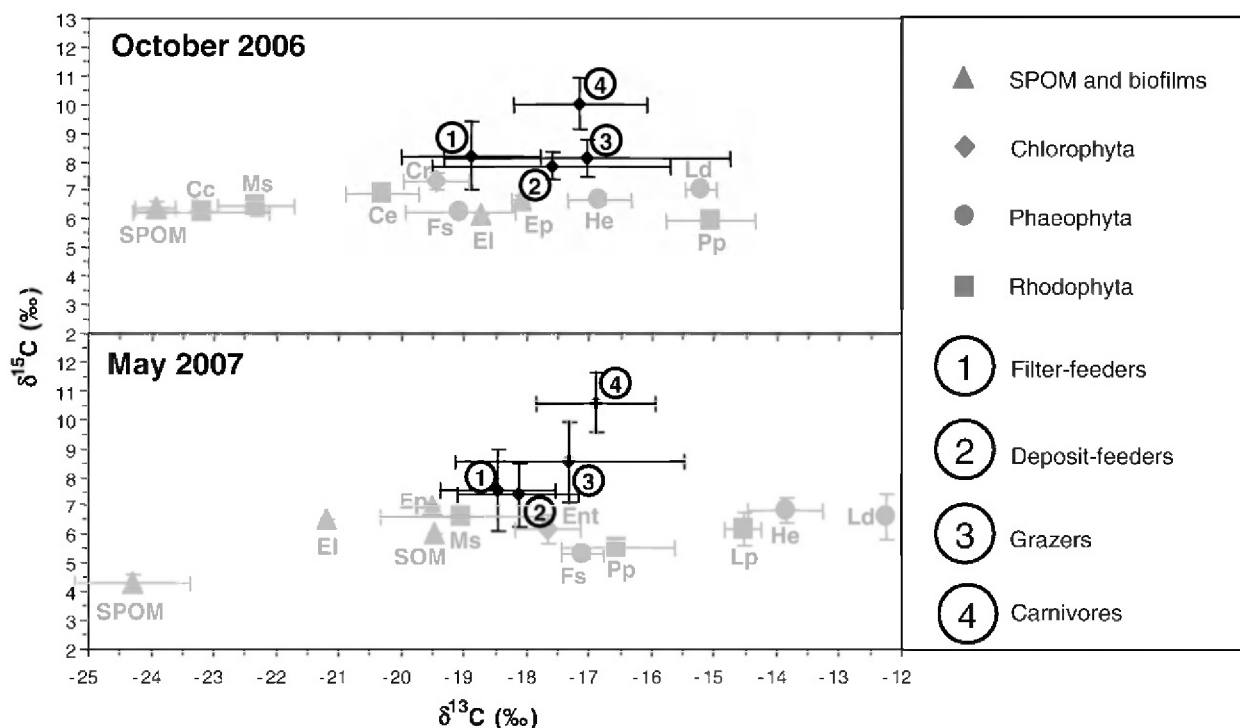

Fig. 4. δ^{13}C and δ^{15}N ($\pm$ S.D.) of filter-feeders, deposit-feeders, grazers and carnivores sampled in October 2006 and May 2007. Sources: SPOM = Suspended Particulate Organic Matter, SOM = Sedimented Organic Matter, El = Epilithic biofilm, Ep = Epiphytic biofilm, Cr = *Cladophora rupestris*, Ent = *Enteromorpha* sp., Cc = *Chondrus crispus*, Ms = *Mastocarpus stellatus*, Pp = *Palmaria palmata*, Lp = *Laurencia pinnatifida*, Ce = *Corallina elongata*, Fs = *Fucus serratus*, He = *Himanthalia elongata*, Ld = *Laminaria digitata*.

Table 5
Contribution of macroalgae-derived organic matter (%) to the diet of filter-feeders sampled in October 2006 and May 2007.

Species	Macroalgal contribution (% of diet)			
	October 2006		May 2007	
	(1)	(2)	(1)	(2)
Halichondria panicea	47	82	39	56
Hymeniacidon sanguinea	40	70	29	41
Pachimatisma johnstonia			51	73
Sabella pavonina			45	64
Elminius modestus			44	63
Chthamalus montagui			42	60
Balanus perforatus	35	60	43	62
Pisidia longicornis	48	83	41	58
Porcellana platycheles	78	100	41	58
Mytilus edulis			46	66
Electra pilosa	46	80		
Schizoporella sp.			57	82
Flustrellidra hispida	41	71		
Ophiotrix fragilis			37	53
Didemnum spp.			30	42
Applidium pallidum	39	69		
Botryllus schlosseri	53	92	38	55
Dendrodoa grossularia			31	44

These contributions are calculated based on two configurations, (1) considering a pool of suspended detritus only constituted of kelp detritus (2) considering a pool of detritus constituted of a mix of all co-occurring macroalgae.

1995). Indeed, consumers derive most of their nitrogen from dietary proteins, which are composed of several essential amino acids that invertebrates are incapable to synthesize *de novo*. In the present kelp forest, the protein contents of macroalgae are in accordance with those observed in the literature, the brown algae being less protein-rich than red and green algae (Fleurence, 1999). In particular, two of the most abundant understory red algae (i.e. *M. stellatus*, *P. palmata*) are among the more nutritive food sources concerning proteins.

Our total lipid content measurements are in agreement with those previously observed (Fleurence et al., 1994; Herbreteau et al., 1997), indicating that brown algae are most enriched in lipids than red and green algae. Notably, *F. serratus* exhibited much higher total lipid content than measured by these authors. This discrepancy may be the result of the seasonal variation in the lipid content of algae but may also result from the presence of lipid-rich reproductive organs on the thalli of algae.

The PCA realized on the different descriptors of the nutritive value of food sources differentiates three groups of algae (Fig. 2). The first axis (55% of total variance) is representative of nitrogen-related descriptors (C/N, protein content). According to this axis, total lipids are negatively correlated to proteins, and red algae are opposed to brown algae. C/N, protein content and total lipid content have all been reported to be paramount in determining the nutritive value of food sources (Bowen et al., 1995; Grémare et al., 2003; Norderhaug et al., 2006). Consequently, based on these chemical descriptors, it appears difficult to attribute a more or less elevated nutritive value to one of these two groups. Indeed, a given food source can be of acceptable nutritive value for a consumer, while it may be unable to support the growth of others (Norderhaug et al., 2003). The nutritive value of a food source really undergone by consumers can thus only be inferred from its consumption or not by consumers.

4.2. $\delta^{13}C$ and $\delta^{15}N$ of food sources

In both October 2006 and May 2007, the potential food sources displayed close $\delta^{15}N$ values, while their $\delta^{13}C$ values were distributed over a wide range. This pattern seems to be the most commonly observed among co-occurring macroalgae in temperate coastal environments (Fredriksen, 2003; Bode et al., 2006; Kang et al.,

2008). In fact, several factors can influence the $\delta^{13}C$ of algae, such as the fixation of HCO_3^- rather than dissolved CO_2 or the use of inorganic carbon of different origins (reviewed in Raven et al., 2002). It results in the wide range of $\delta^{13}C$ displayed by marine algae. On the contrary, although factors affecting nitrogen isotope discrimination in algae are fewly studied (but see Pennock et al., 1996), this discrimination appears here to vary fewly among these co-occurring macroalgae. The $\delta^{15}N$ observed here for macroalgae are relatively low, as compared to those observed in areas submitted to anthropogenic loading (Camusso et al., 1998; McClelland et al., 1997; Riera et al., 2000). These values are in accordance with the absence of anthropogenic nitrogen sources in this site, which would have resulted in ^{15}N-enrichment in primary producers (McClelland et al., 1997).

The pattern of $\delta^{13}C$ observed here among algae appears consistent in the time, with red algae such as *M. stellatus* or *C. crispus* being the more ^{13}C-depleted, while brown algae (i.e. *H. elongata*, *L. digitata*) are the more ^{13}C-enriched among the co-occurring algae. This result is in accordance with the observations of Fredriksen (2003) in a *L. hyperborea* forest. This author found a group of red algae departing from the others through their ^{13}C enrichment, while kelps were the more ^{13}C-depleted among co-occurring algae.

The $\delta^{13}C$ observed for *L. digitata* is comparable in October to the $\delta^{13}C$ of −15.49‰ reported by Raven et al. (2002). The temporal variation in the $\delta^{13}C$ of this kelp (^{13}C enrichment in May compared to October) may result from its growth cycle, which is known to be strongly seasonal (Schaffelke and Lüning, 1993). Indeed, the sampling of May corresponds to the end of the growth period of *L. digitata* (Pérez, 1969). The tissues analyzed are thus mainly newly formed tissues, which have been observed to be more ^{13}C-depleted than old tissues (Fredriksen, 2003). The temporal variation observed here highlights the importance of the growth cycle of algae in their isotopic ratios. Since the isotopic signature of kelp frond is known to be variable within a single individual (Fredriksen, 2003), we analyzed both basal and distal parts of the kelps fronds, in order to estimate the variability occurring within individuals. In contrast to the previously cited study, the intra-individual $\delta^{13}C$ variability observed here was relatively low for *L. digitata*.

The $\delta^{13}C$ of SPOM was around −24‰ both in October and in May, which is close to values previously measured in the same site (Riera and Hubas, 2003) and around the French Atlantic coast (Riera and Richard, 1996; Grall et al., 2006; Dubois et al., 2007). Due to the sampling of SPOM, this value seems to be associated to a phytoplankton-dominated SPOM. In addition, the presence of detritus from macroalgae in the water column has been reported in the literature to be associated with a ^{13}C-enrichment of SPOM (Kaehler et al., 2000).

4.3. Food web structure of the L. digitata forest

The food web of this habitat was relatively stable in time (Fig. 3). In October 2006 and May 2007, the range of $\delta^{13}C$ displayed by the consumers was large as compared to the range of $\delta^{15}N$. These important $\delta^{13}C$ ranges for sources and consumers clearly suggest that consumers inhabiting this ecosystem exploit a diversity of food sources rather than a unique one, as it has been recently observed in other subtidal environments (Grall et al., 2006; Carlier et al., 2007; Le Loc'h et al., 2008). Consequently, this habitat likely contains a diversity of major trophic pathways, which seems to be a characteristic of natural and artificial rocky shore (Fredriksen, 2003; Bode et al., 2006; Kang et al., 2008; Schaal et al., 2008).

Due to the large nitrogen trophic fractionation occurring between a consumer and its food source, $\delta^{15}N$ has often been used for the determination of consumers' trophic levels (Post et al., 2000). In the present study, trophic groups reported to be mainly primary consumers (i.e. grazers, deposit-feeders) displayed close and low $\delta^{15}N$ (Fig. 4), which is consistent with a strict primary consumer status. The range of $\delta^{15}N$ displayed by grazers in May 2007 was

slightly higher due to the presence of two chitons whose $\delta^{15}N$ was higher than other grazers. These low and homogeneous $\delta^{15}N$ makes grazers and deposit-feeders suitable for the establishment of trophic levels (Table 4). The trophic levels scale points out the shortness of this benthic food web, since top predators only displayed a TL of 3.1, which reveals a first order predator level. This is comparable to other recent findings in comparable rocky environments (Fredriksen, 2003; Kang et al., 2008), but shorter than what can be observed in other marine ecosystems (see Vander Zanden and Fetzer, 2007). As a consequence, most of species reported in the literature to be predators are considered as omnivores in this ecosystem because displaying TL between 2 and 3. The strong degree of omnivory in the *L. digitata* habitat is consistent with the conclusions of Thompson et al. (2007). Based on a review of 10 marine ecosystems, these authors concluded that above the TL 2, food webs are characterized by a "tangled web of omnivores", which likely plays a role in the resilience capacities of ecosystems against perturbations.

4.4. Trophic resources of filter-feeders

In the present study, considering a mean $\delta^{13}C$ trophic fractioning of 1‰, the theoretical food source of filter-feeders is ^{13}C-enriched of at least 4‰ as compared with SPOM. This indicates the use of other sources than phytoplankton. Indeed, it seems unlikely that phytoplankton from the study site differed significantly from phytoplankton sampled 5 km offshore, considering the motion of water masses due to tide currents in the English Channel (Castel et al., 1997). Filter-feeders assimilate thus a ^{13}C-enriched fraction of the local SPOM. Considering the stable isotope values of macroalgae and the extent of kelp beds along the Northern Coast of Brittany (Arzel, 1998), this fraction corresponds likely to detritus derived from macroalgae. The modification of macroalgae isotopic signature during decomposition is not a clearly identified pattern. However, previous experimental studies indicated that the $\delta^{13}C$ of macroalgae does not vary significantly during decomposition (Stephenson et al., 1986; Fellerhoff et al., 2003; Machás et al., 2006). The $\delta^{13}C$ of the three species of drifted macroalgae (i.e. *P. palmata*, *H. elongata*, *F. serratus*) sampled in May supports this hypothesis, since it does not differ significantly from those of fresh algae (Table 3). Moreover, in a rocky intertidal site close to our sampling area, and characterized by an important algal canopy, SPOM exhibited a $\delta^{13}C$ of − 15.1‰ (Golléty, 2008), which supports the importance of macroalgal detritus in the water surrounding rocky shores.

The use of detritus derived from macroalgae was previously reported in the southeastern Atlantic (Bustamante and Branch, 1996) and for *Mytilus* spp. living near kelp beds (Duggins et al., 1989). The origin of these detritus is variable, but kelps are likely to be the main contributor to this pool of suspended detritus because *L. digitata* is largely dominant on the study site. Moreover, an important amount of dissolved and particulate organic matter is released by kelps during their growth (Mann, 1972). However, considering the diversity and abundance of other co-occurring macroalgae, it seems very unlikely that only *L. digitata* contributes to the pool of suspended macroalgae-derived detritus. Thus, to obtain a more realistic estimation of the contribution of macroalgae to the diet of filter-feeders, we calculated a range of contribution based on two configurations, (1) considering a pool of suspended detritus only constituted of kelp detritus (2) considering a pool of detritus constituted of a mix of all co-occurring macroalgae which likely contribute to the detritic organic matter pool available to filter-feeders. From these estimations, the filter-feeders derive at least between 35% and 50% of their diet from macroalgal-derived organic matter (Table 5). These results are in accordance with those previously reported by Kaehler et al. (2006), who found that the filter-feeder brachiopod *Magellania kerguelenensis* assimilated 41% of kelp-derived organic matter. In addition, Fredriksen (2003) reported that most of the filter-feeders inhabiting a *L. hyperborea* forest derived

around 50% of the carbon from kelp-derived organic matter. It should be noted that these authors based their estimations of kelp contribution considering a pool a detritus only constituted of kelp-derived detritus, which makes it difficult to compare with the higher limits of our range. Nevertheless, our results suggest that previous estimations underestimate the trophic importance of macroalgal carbon in the diet of coastal filter-feeders. Hence, the present results point out the ability of filter-feeders inhabiting *L. digitata* forests to significantly derive their food source from macroalgae-derived detritus.

The degradation of kelp detritus has been shown to be associated with a nitrogen-enrichment and a loss of herbivore-deterrent phenolic compounds (Norderhaug et al., 2003). This results in a nutritive enrichment of the detritus leading to its assimilability by filter-feeders. Considering the fact that a great part of the diversity in kelp forest is represented by filter-feeding species, it appears thus that a great part of *L. digitata* primary production is transferred in the food web through the detrital pathway. Since this trophic pathway necessitates the resuspension in the water column of kelp detritus, advective transfers of organic matter are thus favoured and these environments are likely to be widely implicated in sustaining trophically adjacent environments (Bustamante and Branch, 1996; Kaehler et al., 2006), as it has been recently observed by Vanderklift and Wernberg (2008).

Contrarily to grazers and deposit-feeders, filter-feeders displayed a wide range of $\delta^{15}N$, which suggests a differential assimilation of more or less ^{15}N-enriched components of SPOM. The highest TL filter-feeder (*Balanus perforatus*) displayed a TL of 2.7, which suggests that most of the barnacles' diet is constituted of animal items. The lowest TL of filter-feeders were mainly occupied by bryozoans, which appear thus to be strictly primary consumers. The resource appears thus to be partitioned among filter-feeders, as already observed in maerl communities (Grall et al., 2006). Our results do not allow us to detect if this resource partitioning is related to an assimilation of different size classes of detritus or to the use of detritus of different origins.

4.5. Trophic resources of grazers

The range of $\delta^{13}C$ displayed by grazers is comparable to those displayed by the food sources, which indicates that there is no privileged unique source for grazers in this environment. This suggests rather a resource partitioning among grazers, which do not base their diet on the quantitatively dominant source (*L. digitata*). Interestingly, in this study the ^{13}C-enrichment observed for *L. digitata* as compared to other primary producers provides an opportunity to assess the role of the dominant alga as support for the associated food web.

Contrarily to their wide $\delta^{13}C$ range, the range of $\delta^{15}N$ displayed by grazers was much narrow, which is consistent with a primary consumer trophic status. This can appear contradictory with the conclusions of Camus et al. (2008). These authors found more than 40% of animal items in the gut of the 29 herbivore species inhabiting a Chilean rocky shore. Our results suggest that invertebrates ingested by grazers are not significantly assimilated in the tissues of these consumers.

Considering a mean $\delta^{15}N$ trophic enrichment of 2.5‰ between primary producers and their consumers, the $\delta^{15}N$ of several grazers (*H. pellucidum*, *H. tuberculata*, *Patella vulgata*) corresponds to the value of a food source whose $\delta^{15}N$ is lower than the lowest $\delta^{15}N$ observed for the food sources (Fig. 3). In fact, consumers feeding on nitrogen-poor sources often display lower-than-average nitrogen trophic fractionation due to a lower discrimination between isotopes (Adams and Sterner, 2000). Considering the C/N ratios displayed by most of food sources, whose C/N is higher than 10, this may be the reason of the low $\delta^{15}N$ values observed for some grazers. Such lower-

than-average trophic fractionation seems to be common in rocky environments where algae are an important food source, since a proximity between the $\delta^{15}N$ of grazers and their respective algal food sources has already been observed (Lepoint et al., 2000; Pinnegar and Polunin, 2000; Fredriksen, 2003). Consequently, due to 1) the high variability induced by these low algal nitrogen contents in the trophic fractionation of nitrogen, and 2) the low $\delta^{15}N$ range observed in food sources, we considered only $\delta^{13}C$ values to infer the food sources of grazers.

In this habitat, *H. pellucidum* is the only species directly specialized on the consumption of *L. digitata* (Fig. 3). *H. pellucidum* is a highly specialized gastropod, which is found exclusively on *L. digitata*, excepted for juveniles also often found in *M. stellatus* and *C. crispus* (G. Schaal pers. obs.). This specialisation may have allowed adaptations in this species relative to the low nitrogen and protein content of its food source, as it was previously described for sea urchins by Steinberg et al. (1995). In contrast, other grazers are too ^{13}C-depleted to base most of their diet on kelps. This relative absence of consumption of living *L. digitata* may be related to its low protein content and its high C/N ratio, making this alga a food source difficult to use directly by most invertebrates.

The $\delta^{13}C$ of the two top-shells (*Gibbula penanti*, *Gibbula cineraria*), close to the $\delta^{13}C$ of epiphytic biofilms both in October and May, suggests that epiphytes are their privileged food source. This is consistent with their rhipidoglossan radula, which is not considered to allow them feeding on algae, but rather brushing microorganisms living on the thalli of algae (Steneck and Watling, 1982). However, these two grazers are slightly ^{13}C-enriched relative to the epiphytes, which implies the consumption of carbon derived from a more ^{13}C-enriched source. It can be hypothesised that these grazers assimilate a fraction of their carbon from *L. digitata*, on the frond of which they were collected. Since top-shells appear to be unable to feed on leathery macrophytes such as kelps, the assimilation of kelp-derived matter may be through the mediation of microorganisms assimilating DOC from kelps. This would imply that kelp production is also transferred in the local food web through the consumption of their dissolved exudates. This consumption of DOC from macroalgae has already been observed for filter-feeders (Alber and Valiela, 1995) but, to our knowledge, it is the first time that this is suggested for grazers.

The $\delta^{13}C$ of the limpet *P. vulgata* is consistent with the consumption of epilithic biofilms, already reported to be their main food source (Hawkins et al., 1989). Since *G. penanti*, *G. cineraria* and *P. vulgata* are among the most abundant species of this habitat (G.S. pers. obs.), the epilithic and epiphytic biofilms appears thus to be a privileged resource for communities associated to this kelp bed. For technical reasons, we were unable to sample enough of these sources that perform biochemical assays to quantify their nutritive value. Biofilms are constituted of living microalgae and bacteria embedded in a matrix of polysaccharides (Hawkins et al., 1989). Previous studies investigating the nutritive value of bacteria and microalgae have reported high protein content and lipid content similar to those measured for red algae in the present study (Brown et al., 1996; 1997).

The ^{13}C-depletion of the abalone *H. tuberculata* suggests the assimilation of *C. crispus* and/or *M. stellatus*, which are the only sources whose $\delta^{13}C$ is likely to explain such depletion. However, other sources are assimilated by *H. tuberculata*, as revealed by the ^{13}C-enrichment compared to the algae previously cited. For example, *P. palmata* is likely to be also consumed, which is confirmed by field observations of abalones grazing on this alga. The isopod *I. pelagica* displays a $\delta^{13}C$ which suggests a diet mostly based on *M. stellatus*, where this isopod can be regularly found in this area. The significant utilisation of these 3 red algae (*M. stellatus*, *C. crispus*, *P. palmata*) is coherent with their high nutritive value, with respect to the C/N ratio and protein content.

Except the case of *H. pellucidum*, this study points out that *L. digitata* kelps and more generally brown algae are poorly consumed directly by grazers. On the contrary, our results indicate a more readily utilisation of red algae in spite of their much lower abundance. Epilithic and epiphytic biofilms, which are likely to display a nutritive composition close to red algae, are also observed to be highly consumed. Hence, the consumption of the different coexisting algae seems in accordance with the nutritive value associated to these sources (as evaluated through chemical assays), which indicated a clear opposition between brown algae and other species, in relation with nitrogen-related biochemical descriptors (C/N ratio, protein content) (Fig. 2). The total lipid content appears thus to be of less importance than C/N and protein content for the food choice of consumers studied here. These results thus show that the nutritive value of food sources is likely to be an important factor affecting the structure of the food web in kelp forests communities.

5. Conclusions

This study is to our knowledge the first to assess simultaneously the structure and functioning of the whole food web together with the nutritive value of food sources. The results of the present study are sensibly different to what was previously described for the extensively studied northeastern Pacific kelp forests habitat, were the giant kelp *Macrocystis pyrifera* supports most of the food web associated with this habitat (Graham, 2004). Here, the dominant kelp (i.e. *L. digitata*) does not contribute preferentially to the diet of grazers, although its trophic contribution via the detrital pathway for filter-feeders appears important. This food web appears rather to be based on a diversity of food sources.

The use of a combined approach provided new perspectives, suggesting that the respective nutritive value of food sources may be an important factor controlling the diversity of food sources used in a food web. This concordance between these two parallel approaches suggests that assessing the nutritive value of food sources is of particular relevance when studying food webs. Our results thus suggest that the nutritive value of food sources may be an important factor involved in the structuration of European kelp forests communities.

Unfortunately, in the present study we did not consider phenols as descriptors of nutritive value, as previously done by several authors (Granado and Caballero, 2001; Norderhaug et al., 2003). Phenols are the main secondary metabolites of brown algae, and have been reported to deter herbivores (Granado and Caballero, 2001). Several other molecules have also been reported to act as deterrents, such as halogenated compounds, terpenoids and acetogenins (Hay and Fenical, 1988). Although the secondary metabolite content is often not related to food choice in coastal invertebrates (Steinberg et al., 1995), a more thorough approach based on these compounds in co-occurring macroalgae represents a promising way to address food web issues in kelp forests ecosystems.

References

Adams, T., Sterner, R., 2000. The effect of dietary nitrogen content on trophic level ^{15}N enrichment. Limnol. Oceanogr. 45, 601–607.

Alber, M., Valiela, I., 1995. Organic aggregates in detrital food webs: incorporation by bay scallop *Agropecten irradians*. Mar. Ecol. Prog. Ser. 121, 117–124.

Arzel, P., 1998. Les laminaires sur les côtes bretonnes. Evolution de l'éxploitation et de la flotille de pêche, état actuel et perspectives. Ifremer, Plouzané.

Association of Official Analytical Chemists—AOAC, 1990. Official Methods of Analysis, 15th Edn. AOAC, Washington, D.C, p. 556.

Barnes, H., Blackstock, J., 1973. Estimation of lipids in marine animals and tissues: detailed investigations of the sulphophosphovanillin method for "total" lipids. J. Exp. Mar. Biol. Ecol. 12, 103–118.

Bode, A., Alvarez-Ossorio, M.T., Varela, M., 2006. Phytoplankton and macrophyte contributions to littoral food webs in the Galician upwelling estimated from stable isotopes. Mar. Ecol. Prog. Ser. 318, 89–102.

Bowen, S.H., Lutz, E.V., Ahlgren, M.O., 1995. Dietary protein and energy as determinants of food quality: trophic strategies compared. Ecology 76, 899–907.

Brown, M.R., Barrett, S.M., Volkman, J.K., Nearhos, S.P., Nell, J.A., Allan, G.L., 1996. Biochemical composition of new yeasts and bacteria evaluated as food for bivalve aquaculture. Aquaculture 143, 341–360.

Brown, M.R., Jeffrey, S.W., Volkman, J.K., Dunstan, G.A., 1997. Nutritional properties of microalgae for mariculture. Aquaculture 151, 315–331.

Bustamante, R., Branch, G., 1996. The dependence of intertidal consumers on kelp-derived organic matter on the west coast of South Africa. J. Exp. Mar. Biol. Ecol. 196, 1–28.

Camus, P., Daroch, K., Opazo, L.F., 2008. Potential for omnivory and apparent intraguild predation in rocky intertidal herbivore assemblages from northern Chile. Mar. Ecol. Prog. Ser. 361, 35–45.

Camusso, M., Martinotti, W., Balestrini, R., Guzzi, L., 1998. C and N stable isotopes and trace metals in selected organisms from the river Po delta. Chemosphere 37, 2911–2920.

Carlier, A., Riera, P., Amouroux, J., Bodiou, J., Grémare, A., 2007. Benthic trophic network in the Bay of Banyuls-sur-Mer (northwest Mediterranean, France): an assessment based on stable carbon and nitrogen isotopes analysis. J. Exp. Mar. Biol. Ecol. 72, 1–15.

Castel, J., Dauvin, J.-C., Glémarec, M., 1997. Les conditions générales en Atlantique, Manche et Mer du Nord. In: Dauvin, J.-C. (Ed.), Les biocénoses marines et littorales françaises des côtes Atlantique, Manche et Mer du Nord. Synthèse, menaces et perspectives. Museum National d'Histoire Naturelle, Paris, pp. 6–16.

Castric-Fey, A., Girard-Descatoire, A., Gentil, F., Davoult, D., Dewarumez, J.-M., 1997. Macrobenthos des substrats durs intertidaux et subtidaux. In: Dauvin, J.-C. (Ed.), Les biocénoses marines et littorales françaises des côtes Atlantique, Manche et Mer du Nord. Synthèse, menaces et perspectives. Museum National d'Histoire Naturelle, Paris, pp. 83–96.

Christie, H., Jorgensen, N.M., Norderhaug, K.M., Waage-Nielsen, E., 2003. Species distribution and habitat exploitation of fauna associated with kelp (*Laminaria hyperborea*) along the Norwegian coast. J. Mar. Biol. Ass. U.K. 83, 687–699.

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

DeNiro, M.J., Epstein, S., 1978. Influence of diet on the distribution of carbon isotopes in animals. Geochim. Cosmochim. Acta 42, 495–506.

Dubois, S., Orvain, F., Marin-Léal, J.-C., Ropert, M., Lefebvre, S., 2007. Small-scale spatial variability of food partitioning between cultivated oysters and associated suspension-feeding species, as revealed by stable isotopes. Mar. Ecol. Prog. Ser. 336, 151–160.

Duggins, D.O., Eckman, J.E., 1997. Is kelp detritus a good food for suspension feeders? Effects of kelp species, age and secondary metabolites. Mar. Biol. 128, 489–495.

Duggins, D.O., Simenstad, C., Estes, J.A., 1989. Magnification of secondary production by kelp detritus in coastal marine ecosystems. Science 245, 270–273.

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

Fleurence, J., 1999. Seaweed proteins, nutritional aspects and potential uses. Trends Food. Sci. Tech. 10, 25–28.

Fleurence, J., Gutbier, G., Mabeau, S., Leray, C., 1994. Fatty acids from 11 marine macroalgae of the French Brittany coast. J. Appl. Phycol. 6, 527–532.

Fredriksen, S., 2003. Food web studies in a Norwegian kelp forest based on stable isotope (δ^{13}C and δ^{15}N) analysis. Mar. Ecol. Prog. Ser. 260, 71–81.

Golléty, C., 2008. Fonctionnement (métabolisme et réseau trophique) d'un système intertidal rocheux abrité, la zone à *Ascophyllum nodosum*, relation avec la biodiversité algale et animale. Ph.D Thesis. Université Pierre et Marie Curie, Paris, France.

Graham, M.H., 2004. Effects of local deforestation on the diversity and structure of southern California giant kelp forest food webs. Ecosystems 7, 341–357.

Grall, J., Le Loc'h, F., Guyonnet, B., Riera, P., 2006. Community structure and food web based on stable isotopes (δ^{15}N and δ^{13}C) analysis of a North Eastern Atlantic maerl bed. J. Exp. Mar. Biol. Ecol. 338, 1–15.

Granado, I., Caballero, P., 2001. Feeding rates of *Littorina striata* and *Osilinus atratus* in relation to nutritional quality and chemical defenses of seaweeds. Mar. Biol. 138, 1213–1224.

Grémare, A., Medernach, L., DeBovee, F., Amouroux, J.-M., Charles, F., Dinet, A., Vetion, G., Albert, P., Colomines, J.-C., 2003. Relationship between sedimentary organic matter and benthic fauna within the Gulf of Lion: synthesis on the identification of new biochemical descriptors of sedimentary organic nutritional value. Oceanol. Acta 26, 391–406.

Hawkins, S.J., Watson, D.C., Hill, A.S., Harding, S.P., Kyriakides, M.A., Hutchinson, S., Norton, T.A., 1989. A comparison of feeding mechanisms in microphagous, herbivorous, intertidal prosobranch in relation to resource partitioning. J. Molluscan. Stud. 55, 151–165.

Hay, M.E., Fenical, W., 1988. Marine plant–herbivor interactions: the ecology of chemical defense. Annu. Rev. Ecol. Syst. 19, 111–145.

Hay, M.E., Duffy, J.E., Pfister, C.A., Fenical, W., 1987. Chemical defences against marine herbivores: are amphipods insects equivalents? Ecology 68, 233–245.

Herbreteau, F., Coiffard, J.-M., Derrien, A., De Roeck-Holtzhauer, Y., 1997. The fatty acid composition of five species of macroalgae. Bot. Mar. 40, 25–27.

Kaehler, S., Pakhomov, E., McQuaid, C., 2000. Trophic structure of the marine food web at the Prince Edward Islands (Southern Ocean) determined by δ^{13}C and δ^{15}N analysis. Mar. Ecol. Prog. Ser. 208, 13–20.

Kaehler, S., Pakhomov, E., Kalin, R.M., Davis, S., 2006. Trophic importance of kelp-derived suspended particulate matter in a through-flow sub-Antarctic system. Mar. Ecol. Prog. Ser. 316, 17–22.

Kang, C.-K., Choy, E.J., Son, Y., Lee, J.-Y., Kim, J.K., Kim, Y., Lee, K.-S., 2008. Food web structure of a restored macroalgal bed in the eastern Korean peninsula determined by C and N stable isotope analyses. Mar. Biol. 153, 1181–1198.

Karez, R., Engelbert, S., Sommer, U., 2000. Co-consumption and protecting coating: two new proposed effects of epiphytes on their macroalgal hosts in mesograzer–epiphyte–host interactions. Mar. Ecol. Prog. Ser. 205, 85–93.

Lawrence, J.M., 1975. On the relationship between marine plants and sea urchins. Oceanogr. Mar. Biol. Annu. Rev. 13, 213–286.

Le Loc'h, F., Hily, C., Grall, J., 2008. Benthic community and food web structure on the continental shelf of the Bay of Biscay (North Eastern Atlantic) revealed by stable isotope analysis. J. Mar. Syst. 72, 17–34.

Lepoint, G., Nyssen, F., Gobert, S., Dauby, P., Bouquegneau, J., 2000. Relative impact of a seagrass bed and its adjacent epilithic algal community in consumer diets. Mar. Biol. 136, 513–518.

Lourenço, S.O., Barbarino, E., De Paula, J.C., Pereira, L.O., Lanfer Marquez, U.M., 2002. Amino acid composition, protein content and calculation of nitrogen-to-protein conversion factors for 19 tropical seaweeds. Phycol. Res. 50, 233–241.

Machás, R., Santos, R., Peterson, B., 2006. Elemental and stable isotopes composition of *Zostera noltii* (Horneman) leaves during the early phases of decay in a temperate mesotidal lagoon. Est. Coast. Shelf Sci. 66, 21–29.

Mann, K.H., 1972. Macrophyte production and detritus food chains in coastal waters. Mem. del Ist. Ital. Idrobiol. 29, 353–383.

Mann, K.H., 1973. Seaweeds: their productivity and strategy for growth. Science 182, 975–981.

McClelland, J.W., Valiela, I., Michener, R.H., 1997. Nitrogen-stable isotope signature in estuarine food webs: a record of increasing urbanisation in coastal watersheds. Limnol. Oceanogr. 42, 930–937.

Norderhaug, K.M., Fredriksen, S., Nygaard, K., 2003. Trophic importance of *Laminaria hyperborea* to kelp forest consumers and the importance of bacterial degradation to food quality. Mar. Ecol. Prog. Ser. 255, 135–144.

Norderhaug, K.M., Nygaard, K., Fredriksen, S., 2006. Importance of phlorotanins content and C:N ratio of *Laminaria hyperborea* in determining its palatability as food for consumers. Mar. Biol. Res. 2, 367–371.

Paine, R.T., Vadas, R.L., 1969. The effect of grazing by sea urchin *Strongylocentrotus spp.* on benthic algal populations. Limnol. Oceanogr. 14, 710–719.

Pavia, H., Carr, H., Aberg, P., 1999. Habitats and feeding preferences of crustacean mesoherbivores inhabiting the brown seaweed *Ascophyllum nodosum* (L.) Le Jol. and its epiphytic macroalgae. J. Exp. Mar. Biol. Ecol. 236, 15–32.

Pennock, J.R., Velinsky, D.J., Ludlam, J.M., Sharp, J.H., 1996. Isotopic fractionation of ammonium and nitrate during uptake by *Skeletonema costatum*: implications for δ^{15}N dynamics under bloom conditions. Limnol. Oceanogr. 41, 451–459.

Pérez, R., 1969. Croissance de *Laminaria digitata* (L.) Lamouroux étudiée sur trois années consécutives. Proc. Int. Seaweed Symp 6, 329–344.

Peterson, B., 1999. Stable isotopes as tracers of organic matter input and transfer in benthic food webs: a review. Acta. Oecol. 20, 479–487.

Pinnegar, J.K., Polunin, N., 2000. Contributions of stable isotope data to elucidating food webs of Mediterranean rocky littoral fishes. Oecologia 122, 399–409.

Post, D., Pace, M.L., Hairston Jr, N.G., 2000. Ecosystem size determines food-chain length in lakes. Nature 405, 1047–1049.

Raven, J.A., Johnston, A.M., Kübler, J.E., Korb, R., McInroy, S.G., Handley, L.L., Scrimgeour, C.M., Walker, D.I., Beardall, J., Vanderklift, M.A., Fredriksen, S., Dunton, K.H., 2002. Mechanistic interpretation of carbon isotope discrimination by marine macroalgae and seagrasses. Func. Plant Biol. 29, 355–378.

Riera, P., Hubas, C., 2003. Trophic ecology of nematodes from various microhabitats of the Roscoff Aber Bay (France): importance of stranded macroalgae evidenced through δ^{13}C and δ^{15}N. Mar. Ecol. Prog. Ser. 260, 151–159.

Riera, P., Richard, P., 1996. Isotopic determination of food sources of *Crassostrea gigas* along a trophic gradient in the estuarine bay of Marennes-Oléron. Est. Coast. Shelf Sci. 42, 347–360.

Riera, P., Stal, L.J., Nieuwenhuize, J., 2000. Heavy δ^{15}N in intertidal benthic algae and invertebrates in the Scheldt estuary (The Netherlands): effects of river nitrogen inputs. Est. Coast. Shelf Sci. 51, 365–372.

Rupérez, P., 2002. Mineral content of edible marine seaweeds. Food Chem. 79, 23–26.

Russel-Hunter, W.D., 1970. Aquatic Productivity. Macmillan, New York.

Schaal, G., Riera, P., Leroux, C., 2008. Trophic coupling between two adjacent benthic food webs within a man-made intertidal area: a stable isotopes evidence. Est. Coast. Shelf Sci. 77, 523–534.

Schaffelke, B., Lüning, K., 1993. A circannual rythm controls seasonal growth in the kelps *Laminaria hyperborea* and *L. digitata* from Helgoland (North Sea). Eur. J. Phycol. 29, 49–56.

Steinberg, P.D., Estes, J.A., Winter, F.C., 1995. Evolutionary consequences of food chain length in kelp forest communities. Proc. Natl. Acad. Sci. U. S. A. 92, 8145–8148.

Steneck, R., Watling, L., 1982. Feeding capabilities and limitations of herbivorous molluscs: a functional group approach. Mar. Biol. 68, 299–319.

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. Mar. Ecol. Prog. Ser. 30, 1–7.

Sterner, R., Hessen, D.O., 1994. Algal nutrient limitation and the nutrition of aquatic herbivores. Annul. Rev. Ecol. Syst. 25, 1–29.

Thompson, R.M., Hemberg, M., Starzomski, B.M., Shurin, J.B., 2007. Trophic levels and trophic tangles: the prevalence of omnivory in real food webs. Ecology 88, 612–617.

Vander Zanden, M.J., Fetzer, W.W., 2007. Global patters of aquatic food chain length. Oikos 116, 1378–1388.

Vander Zanden, M.J., Rasmussen, J.B., 2001. Variation in δ^{15}N and δ^{13}C trophic fractionation. Implications for aquatic food web studies. Limnol. Oceanogr. 46, 2061–2066.

Vanderklift, M.A., Wernberg, T., 2008. Detached kelps from distant sources are food subsidy for sea urchins. Oecologia 157, 327–335.