Intraspecific diet shift in *Talitrus saltator* inhabiting exposed sandy beaches

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ABSTRACT

Talitrid amphipods are the most abundant herbivores on exposed sandy beaches. Despite their important role as trophic intermediates between macrophytes and higher levels (i.e. insect and bird) of beach food webs, very little information is available on their feeding patterns. The main aim of this study was to investigate intraspecific differences in the feeding behaviour of *Talitrus saltator*. We tested the hypotheses that: (1) adult females and males showed different isotope signatures and therefore relied on different sources of food; and (2) patterns of variation of isotope signatures of juveniles differed from those of adult specimens, evidencing a diet shift during the development. We used stable isotope signatures and tested for differences upon the level on the shore, times of the year and beaches experiencing similar morpho-dynamic and environmental conditions. Finally, we investigated the trophic significance of macrophyte detritus in the diet of males, females and juveniles. Results showed that adult males had a more variable diet than females and juveniles (inferred from δ^{13}C and δ^{15}N values). Dual-isotope graphs suggested that *Sargassum muticum* and *Cystoseira baccata* wrack could be among the main food sources for both juvenile and adult stage.

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1. Introduction

On exposed sandy beaches of temperate regions, the major sources of food are often marine macrophytes (macroalgae, seagrasses) because little primary production occurs on the beach itself (e.g. Brown and McLachlan, 1990; Dugan et al., 2003). Hence, macrofaunal assemblages of exposed sandy beaches depend largely upon allochthonous inputs from the coastal ocean that arrive as stranded wrack (e.g. Kirkman and Kendrick, 1997; Colombini and Chelazzi, 2003; Lastra et al., 2008). This benefit may also extend beyond the beach itself. For example, wrack deposited along shorelines is consumed by detritivores and scavengers, which in turn are important food sources for vertebrate predators such as shorebirds, seabirds, lizards, rodents, marine mammals, and fishes (Polis et al., 1997). Despite the role of wrack as food subsidy for the macrofauna inhabiting sandy beaches, its trophic significance has proved difficult to evaluate in the field (e.g. Griffiths and Stenton-Dozey, 1981; Jedrzejczak, 2002).

Talitrid amphipods are some of the most abundant sand inhabitants on exposed beaches (e.g. Dugan et al., 2003; Lastra et al., 2006) and important consumers of wrack (Dugan et al., 2003). Their rate of consumption may be affected by several processes, such as competition with other consumers, physico-chemical and morphological characteristics of the macrophyte species, and availability of wrack (Van Alstyne et al., 2001; Orr et al., 2005). For instance, abundance, and identity of the consumers and the spatial and temporal variability of wrack composition could influence the processing and availability of macrophyte wrack and as a consequence, abundance and feeding behaviour of amphipods (Dugan et al., 2003).

Accumulating evidences suggest that benthic invertebrates might change their diet as they grow (Bergman and Greenberg, 1994; Polis et al., 1997; Hentschel, 1998; Rossi et al., 2004). Onto-genetic shifts in diets can have dramatic effects on the structure and composition of benthic assemblages (e.g. Cohen et al., 1993; Hentschel, 1998). For example, size-specific competition for food resources may limit population densities of deposit-feeding species (Hentschel and Jumars, 1994). Thus when an ontogenetic change in diet occurs, descriptions of food webs should distinguish among the size classes or life cycle stages on that species (Cohen et al., 1993). In addition, sex-related differences in diet have been reported as a fairly common phenomenon in marine organisms

(e.g. Forero et al., 2002; Guerra-García et al., 2004; Bearhop et al., 2006; Schmidt et al., 2006; Hoeinghaus and Davis, 2007). Thus, female and male of the same consumer species may show resource repartition and different feeding behaviour.

Exposed sandy beaches on the Galician coast (northwestern Spain) support a high diversity of species and rich assemblages (Lastra et al., 2006). High productivity linked to phytoplankton biomass and wrack deposits are likely to be in part responsible for such patterns (Lastra et al., 2006). Despite the importance of wrack on sandy beaches very little is known about composition of assemblages or trophic interactions among species colonizing wrack deposits (but see Olabarria et al., 2007; Rodil et al., 2008). This information is essential to a better understanding of processes shaping structure of macrofaunal assemblages on exposed sandy beaches along the Galician coast.

This study investigated whether there were differences in the feeding behaviour of *Talitrus saltator* related to the sex and the development stage. We used stable isotopes to examine whether: (1) adult females and males showed different patterns of variation of isotope signatures and therefore relied on different sources of food; and (2) patterns of variation of isotope signatures of juveniles differed from those of adult specimens, evidencing a diet shift during ontogeny. We tested for differences upon the levels on the shore, times of the year and beaches experiencing similar morpho-dynamic and environmental conditions. Finally, we explored the importance of macrophyte detritus for the diet of males, females and juveniles. We employed stable isotope methods, since they have been revealed as a useful tool for identifying the diet of consumers inhabiting sandy beaches (e.g. Adin and Riera, 2003; Ince et al., 2007).

2. Materials and methods

2.1. Study site

Samples were collected from two exposed sandy beaches, Barra (42° 15′ N, 8° 51′ W) and Viñó (42° 15′ N, 8° 50′ W) located on the Galician coast, northwestern Atlantic coast of Spain. These beaches, about 400 m apart, were randomly chosen from a pool of six beaches (located in an area of approximately 7 km) with similar morpho-dynamic conditions and where talitrid amphipods were quite abundant. Both are backed by dunes and separated by a rocky platform. The beaches are ∼1 km in length and experience mixed semi-diurnal tides with an approximate range of 2–4 m. The intertidal width (dune base to low swash level) ranges from ∼30 to 80 m. Although the composition of wrack is very variable spatially and temporally both beaches receive mainly large inputs of brown algae such as *Fucus vesiculosus*, *Sargassum muticum* and *Laminaria ochroleuca* and the seagrass *Zostera marina*, as well as small quantities of other brown, red and green algae species from rocky shores located just offshore (personal observation).

2.2. Sampling collections

Animals were collected at two different levels on the shore, i.e. the strandline and 3 m above the strandline (hereafter low and upper zone, respectively) in December 2007, March and May 2008. In each zone, eight pitfall traps about 2 m apart were placed at both levels on the shore and beaches in the afternoon the day before collection. At least four adults and four juvenile individuals of *Talitrus saltator* were picked randomly from four chosen pitfalls at each sampling date. Detrital macroalgae and seagrass were collected by hand within an area of ∼30 cm around each pitfall trap. Additional detrital material deposited on the beach and located outside this area was also collected.

In the laboratory, animals were sorted alive and cleaned after relaxation with 10% MgCl. Animals were divided into juveniles, adults, females and males, based on their size (Scapini et al., 1997) and morphology (Lincoln, 1979), respectively. We considered juveniles the individuals smaller than 5 mm. Animals were dried at 60 °C for 48 h and then frozen (−20 °C). Adults were large enough to be analyzed individually, but juveniles had to be pooled into groups of two to three individuals to obtain sufficient tissue for chemical analyses. The detrital macroalgae and seagrass were rinsed with filtered seawater to clean off epibionts and then dried (60 °C) for 48 h. Subsequently, samples were ground into fine powder using a mortar and pestle, and then kept frozen (−20 °C) until isotopes analyses. Prior to analyses all samples were acidified with 10% HCl. The presence of inorganic carbon in amphipods can alter the carbon isotopic signature. The process of acidification is, therefore, necessary in sample preparation, mainly working with small organisms where it is not possible to dissect and remove the carbon structures (Vizzini and Mazzola, 2003).

2.3. Analytical technique

The carbon and nitrogen isotopic composition of the samples was determined using a MAT 253 stable isotope ratio mass spectrometer. The carbon and nitrogen isotope ratios are expressed in the delta notation $\delta^{13}C$ and $\delta^{15}N$, where

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

with $R = {}^{13}C/{}^{12}C$ for carbon and $^{15}N/^{14}N$ for nitrogen, and reported relative to the Vienna Pee Dee Belemnite standard (PDB) for carbon and to air N_2 for nitrogen. Precision in the overall preparation and analysis was ±0.13% for both $\delta^{13}C$ and $\delta^{15}N$.

2.4. Analysis of data

To test the hypothesis that adult females and males as well as juveniles of *Talitrus saltator* showed different spatio-temporal patterns of variation in their diets, the isotopic composition $\delta^{13}C$ and $\delta^{15}N$ were analysed separately with three-factor mixed model of analyses of variance (ANOVA). Level on the shore (two levels: low and upper zone) was a fixed factor and beach (two levels: Barra and Viñó) and date (three levels: December 07, March 08 and May 08) were random factors. In order to have a balanced design, individuals from the four pitfall traps were pooled and then, ten adults and six juveniles were randomly chosen from each beach, level on the shore and date of sampling. Data were not transformed whether or not variances were heterogeneous because the analysis of variance is robust to heterogeneous variance when there are balanced data and number of replicates is relatively large (Underwood, 1997). When significant differences among treatments and their interactions were found, Student-Newman–Keuls (SNK) test was done as a posteriori comparison (Underwood, 1997). When ANOVA analyses showed significant patterns, i.e. stable carbon and nitrogen isotope values of males, females or juveniles varied spatially or temporally, Spearman's rank correlations were used to test whether there were relationships between patterns of male and female adults, and adults and juveniles. We would expect non-significant correlation if isotopic signatures of different stages or sexes were independent from each other (e.g. they feed on different sources).

To investigate the role of wrack species as food sources for adults and juveniles, we plotted data of $\delta^{13}C$ and $\delta^{15}N$ for consumers and wrack species in a dual-isotope graph and considered a mean trophic enrichment of 1‰ for $\delta^{13}C$ (Rau et al., 1983;

Adin and Riera, 2003) and of 3.4‰ for δ^{15}N (Minagawa and Wada, 1984; Owens, 1987; Adin and Riera, 2003). Although fractionation has been accepted to be relatively constant at each trophic level, amphipods and herbivores in general may show great variability (Vander Zanden and Rasmussen, 2001). In this case, we used the well-accepted positive fractionation values. In addition, these values better identified wrack species as food sources for this amphipod species than other values recently proposed in the literature (Rossi et al., submitted for publication).

3. Results

3.1. δ^{13}C and δ^{15}N of Talitrus saltator

There were marked differences between beaches and zones in the δ^{13}C composition of tissues for males, but these differences were not consistent over time (i.e. significant interaction date × beach × zone, $F_{2,108} = 3.62$, $P < 0.05$; Fig. 1a). There was a similar trend for females, but non-significant marginally ($F_{2,108} = 2.89$, $P = 0.059$; Fig. 1b). For males, the δ^{13}C values were more enriched at Viñó than at Barra, but only on the lower zone in December 2007 (Viñó: -16.3 ± 0.3; Barra: -17.4 ± 0.2‰) [mean - $\pm$ SE] and March 2008 (Viñó: -16.1 ± 0.2‰; Barra: -17.5 ± 0.6‰) (SNK, $P < 0.05$; Fig. 1a). In addition, males from the upper zone of Viñó in December 2007 were the most δ^{13}C-depleted (-17.7 ± 0.4‰) (SNK, $P < 0.05$; Fig. 1a). Males presented marked temporal differences in δ^{15}N composition of tissues (i.e. significant effect of date, $F_{2,2} = 119.11$; $P < 0.001$; SNK, $P < 0.05$; Fig. 2a). The δ^{15}N values were lower in December 2007 (8.3 ± 0.3‰) than in March and May 2008 (9.8 ± 0.3, 9.6 ± 0.2‰, respectively) (Fig. 2a). Males also presented spatial differences in δ^{15}N composition of tissues (i.e. significant effect of beach, $F_{1,2} = 306.83$; $P < 0.01$; SNK, $P < 0.05$; Fig. 2a) with the highest values at Barra (9.3 ± 0.2‰). Females showed neither spatial nor temporal significant variation in δ^{15}N values (i.e. non-significant main effects or interactions ($P > 0.05$). Patterns of variation of males and females were independent since the isotopic signatures of males and females were not correlated or the correlation was very low (i.e. δ^{15}N; $R = 0.23$; $P < 0.05$, $n = 120$).

Juveniles only showed significant temporal variation in δ^{15}N composition of tissues (i.e. significant effect of date, $F_{2,2} = 31.13$, $P < 0.05$, SNK, $P < 0.05$; Fig. 2b) with the highest values in March 2008 (10.3 ± 0.1‰). Patterns of variation of juveniles and adult females were also independent (i.e. non-significant correlations). There was only a significant correlation between the δ^{15}N

composition of juveniles and adult males, but this correlation was very low ($R = 0.33$; $P < 0.05$, $n = 72$).

3.2. Stranded algae as potential food sources

A total of 14 species of stranded algae and one species of stranded seagrass, Zostera marina, were identified as potential food sources (Table 1). The most common and abundant species were Sargassum muticum, Z. marina, Fucus vesiculosus, Ascophyllum nodosum and Cystoseira baccata (personal observation). The δ^{13}C and δ^{15}N values varied among species (Fig. 3). The mean macro-algae and seagrass δ^{13}C values ranged from -32.0‰ (Plocamium cartilagineum) to -8.3‰ (Enteromorpha intestinalis) on the upper zone of Viñó in March, while the δ^{15}N mean values ranged from 4.5‰ (S. muticum) to 9.8‰ (Fucus spiralis) on the lower zone of Viñó in December.

Some species such as Sargassum muticum and Cystoseira baccata had similar δ^{13}C and δ^{15}N values in most cases (Fig. 3). The δ^{13}C and δ^{15}N values for Zostera marina and Himanthalia elongata were also closed, being Z. marina the species that showed the highest values of δ^{13}C (ranged from -11.2 to -9.3‰; Table 1, Fig. 3). The δ^{13}C and δ^{15}N values for Fucus vesiculosus were close to those of Ascophyllum nodosum, although the highest similarity between the isotopic values for the two species was found on the upper zone of Barra in March 2008 (Fig. 3).

Although the δ^{13}C and δ^{15}N values of macrophytes were, in general, pretty constant between beaches, shore levels and over time, there were some exceptions. For example, the alga Sargassum muticum was more δ^{13}C-depleted in March (average of -19.5‰) than in May and in December (average of -18.3 and -17.7‰, respectively), whereas it was more δ^{15}N-enriched in March (average of 7.7‰) than in May and in December (5.9 and 5.2‰, respectively). The δ^{13}C and δ^{15}N values for Zostera marina and Fucus vesiculosus also varied spatio-temporally. The seagrass Z. marina was more δ^{13}C-depleted on the upper zone of Barra in March 2008 (average of -11‰), whereas it was more δ^{15}N-enriched at the same site in December 2007 and May 2008 (average of 7.8‰). In contrast, F. vesiculosus was more δ^{13}C-depleted on the lower zone of Barra in March 2008, while it was more δ^{15}N-enriched on the lower zone of Viñó and Barra, i.e. similar values, in December 2007 and March 2008, respectively.

The dual plots evidenced that the isotopic composition of both adults and juveniles corrected for discrimination approached to the isotopic composition of Sargassum muticum and/or Cystoseira baccata at any time of sampling, beach and level on the shore, except in Viñó
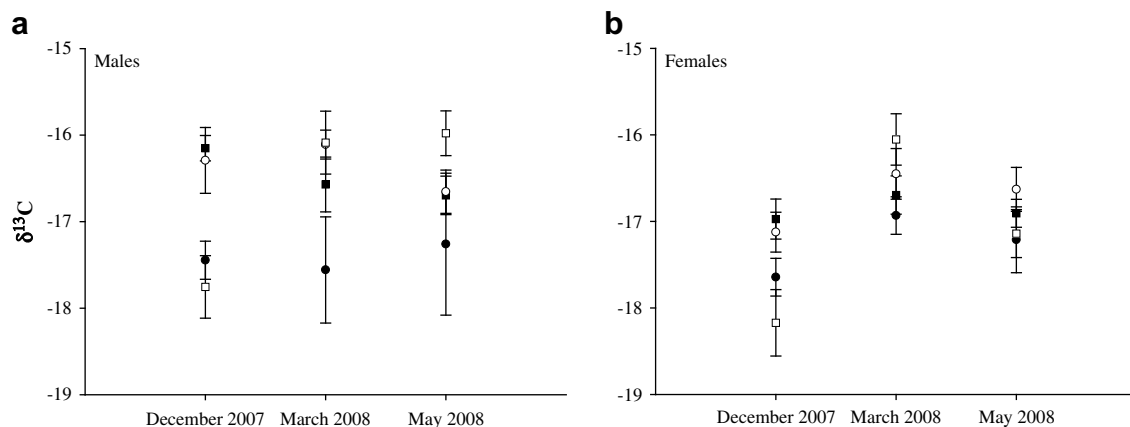

Fig. 1. Temporal variation (mean ± SE) in the carbon isotope signatures of adult males and females of *Talitrus saltator* collected at two levels on the shore at two beaches over the three sampling dates. Square: upper zone, circle: lower zone. Black symbols: Barra, white symbols: Viñó.
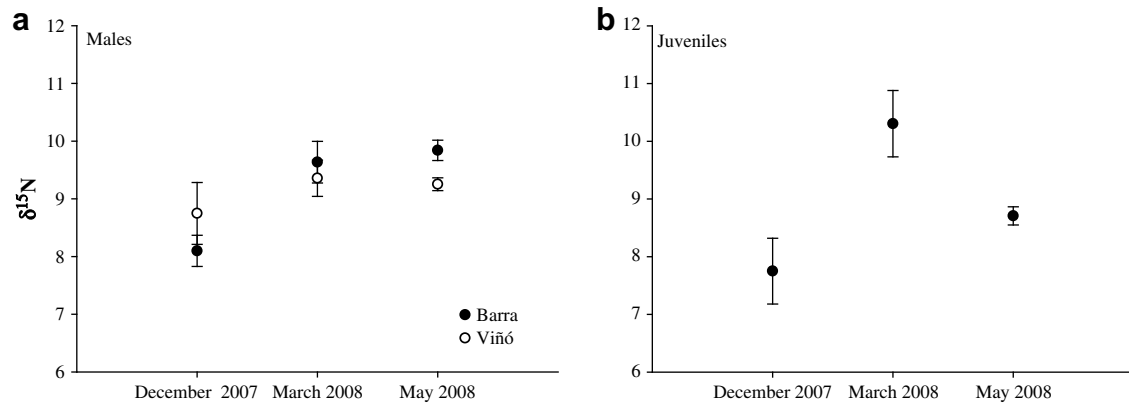
Fig. 2. Temporal variation (mean ± SE) in the nitrogen isotope signatures of adult males and juveniles of *Talitrus saltator*. For juveniles the average value of Barra and Viñó was represented because the main factor "Beach" was not significant.

beach lower on the shore in May and upper on the shore in March, where *Talitrus saltator* was close to other macroalgae (Fig. 3). It is therefore possible that females, males and juveniles exploited similar food resources in most cases. In general, a diet based on *S. muticum* (e.g. lower zone at Barra and Viñó in December 2007) or *C. baccata* (e.g. lower zone at Viñó in March 2008) or a mixing diet of both algae species (e.g. upper zone at Barra in March 2008 and upper zone at Viñó in May 2008) could be hypothesized to explain the isotopic composition of *T. saltator*. Nonetheless, the isotopic composition of females, males and juveniles was differentiated (Fig. 3). Major differences were found between juveniles and adults at the upper zone of Barra and Viñó in December 2007 and at the lower zone of Barra in March 2008. Females and juveniles were instead very close at the upper zone of Viñó beach in May (Fig. 3). Such variation evidenced that adults and juveniles had different patterns of spatial and temporal variability in their isotopic composition.

4. Discussion

Males and females presented different patterns of variation of stable isotope signatures in the area of study. Males showed significant dietary shift (δ^{13}C) at small scale (from a few metres to few hundred metres apart). In contrast, females did not show any significant spatial and temporal variability. Stable isotope differences can indicate differences in diet between sexes (e.g. Guerra-García et al., 2004; Bearhop et al., 2006; Schmidt et al., 2006). The differences in the pattern of variation might thus indicate that females and males use different pools of carbon and nitrogen. It is possible that females and males derived carbon and nitrogen from different wrack species or foraged on the same wrack species, but consumed and assimilated the carbon and the nitrogen from different tissues having different isotopic signatures.

The changes in diet of some macrobenthic species often occur at scales ranging from metres to tens of kilometres and indicate shifts in feeding habits (i.e. feeding mode and food selectivity) in response to species interactions or food availability (e.g. Wainright et al., 2000; Doi et al., 2005; Kanaya et al., 2007). In vertebrates and invertebrates sex-related differences in diet and a larger feeding area of males than females may be due to differences in feeding behaviour, following the different morphology and function of males and females (e.g. Forero et al., 2002; Hoeinghaus and Davis, 2007). For example, there can be segregation in foraging habitats during reproduction when females cannot go away from their offspring. In addition, the different body size may increase the predation risk of one sex compared to the other (e.g. Forero et al., 2002; Bearhop et al., 2006; Hoeinghaus and Davis, 2007). To our

knowledge, sex-related shifts in the diet of *Talitrus saltator* and other amphipod species have not been previously investigated. It is however well known that males and females of *T. saltator* present different morphology (Lincoln, 1979) and body size, with males being larger than females. We may thus suggest that the smaller female size might prevent females to change feeding behaviour. Males, but not females might move further to forage in response to external cues such as increased competition or food availability. Appropriate experiments must be done to test hypotheses related to intraspecific variability in the diet of this species.

Patterns of variation of stable isotopes signatures in juveniles varied from those observed in male and female adults. On one hand, stage of the life cycle of individuals has been reported as an important factor contributing to variation in diet within species (Hoeinghaus and Davis, 2007) and ontogenetic shifts in diet may occur in order to overcome physiological constraints (Hentschel, 1998; Rossi et al., 2004). For instance, when juveniles have physiological limitation in the maximum rate of food uptake, they might rely on higher quality sources of food to minimize the amount of food and maximize energy uptake (Hentschel, 1998). On the other hand, juveniles and adults could assimilate diet components with varying efficiency. As a consequence, rapidly growing juveniles might incorporate new carbon and nitrogen more rapidly than adults and they might also fractionate isotopes and allocate them differently in the tissues (Hentschel, 1998). As a result, isotope variations between adults and juveniles might indicate an artefact due to size-dependent rates of nutrient turnover, rather than ontogenetic changes in diet. Nevertheless, amphipods have turnover rates of few weeks (Crawley et al., 2007), and the temporal scale in this study was "months". Thus, although we cannot discard this explanation, the chances of having such artefact are relatively low. In this study, juveniles did not show any significant variation in their δ^{13}C signatures, but they did show a δ^{15}N enrichment in March respect to December and May of 2.6 and 1.6‰, respectively. These differences were lower than the expected 3–4‰ increase per trophic level (Minagawa and Wada, 1984) suggesting that this variation may be due more to differences in the type of macroalgae ingested, probably with higher C:N ratios (see Michener and Lajtha, 2007), rather than from feeding at a higher trophic level. Different growth rates of *Talitrus saltator*, i.e. it grows faster during spring and summer (Marques et al., 2003), may also provoke a more rapid turnover rate of isotopes (Hesslein et al., 1993) and subsequently, differences in signature values. Alternatively, an enrichment of δ^{15}N in tissues might be due to a greater consumption of wrack-colonizing micro biota or to changes in composition of macroalgae (i.e. some species showed a δ^{15}N enrichment in March; Table 1).

Table 1
Carbon and nitrogen isotopic compositions (‰) of the potential food sources of *Talitrus saltator* at two levels on the shore (lower and upper zone) at two beaches (Barra and Viñó) over the three sampling dates. The mean ± SE values are given with the number of samples between brackets.

	December 2007		March 2008		May 2008	
	δ^{15}N	δ^{13}C	δ^{15}N	δ^{13}C	δ^{15}N	δ^{13}C
Beach: Barra						
Zone: lower zone						
Seaweeds						
Green algae						
Ulva rigida	7.6 ± 0.41	−14.5 ± 0.97 (2)				
Codium sp.	6.9	−13.6 (1)				
Brown algae						
Ascophyllum nodosum			8.0 ± 0.47	−17.4 ± 0.92 (3)		
Fucus vesiculosus	8.6	−14.3 (1)	9.6 ± 0.69	−16.2 ± 0.73 (2)		
Himanthalia elongata					7.2 ± 0.35	−13.4 ± 0.58 (2)
Cystoseira baccata			6.28.0 ± 1.29	−19.0 ± 0.64 (3)		
Sargassum muticum	5.2 ± 0.09	−17.7 ± 0.52 (4)	7.8 ± 0.26	−20.4 ± 0.31 (4)	5.9 ± 0.35	−18.7 ± 0.61 (4)
Red algae						
Plocamium cartilagineum	7.4	−22.9 (1)				
Seagrass						
Zostera marina	6.8 ± 1.05	−10.3 ± 0.76 (4)	6.3	−11.2 (1)	7.0 ± 0.25	−9.6 ± 0.42 (5)
Beach: Barra						
Zone: upper zone						
Seaweeds						
Brown algae						
Ascophyllum nodosum	7.2 ± 0.01	−14.6 ± 0.98 (2)	8.7 ± 0.28	−16.9 ± 0.33 (8)	8.5	−16.6 (1)
Fucus vesiculosus			8.4 ± 0.24	−15.7 ± 0.38 (7)	7.4 ± 0.72	−15.5 ± 0.87 (2)
Fucus spiralis					8.2	−17.1
Cystoseira baccata			5.6 ± 0.69	−19.0 ± 0.54 (6)	7.0 ± 0.48	−18.9 ± 0.50 (2)
Sargassum muticum	4.9 ± 0.45	−17.9 ± 0.94 (4)	7.1 ± 0.33	−19.2 ± 0.20 (8)	5.6 ± 0.39	−17.7 ± 0.46 (3)
Red algae						
Ceramium rubrum	8.2	−18.6 (1)				
Seagrass						
Zostera marina	7.8 ± 0.23	−9.3 ± 0.61 (4)	6.0 ± 0.27	−11.0 ± 0.51 (4)	7.8 ± 0.13	−10.3 ± 0.20 (3)
Beach: Viñó						
Zone: lower zone						
Seaweeds						
Green algae						
Ulva rigida	7.3 ± 0.14	−14.0 ± 1.96 (3)	8.1	−16.5 (1)		
Brown algae						
Colpomenia peregrina			6.7 ± 0.48	−13.0 ± 0.35 (3)		
Ascophyllum nodosum					5.0	−17.4 (1)
Fucus vesiculosus	9.8	−14.2 (1)				
Fucus spiralis					6.6	−14.9 (1)
Himanthalia elongata					6.0	−10.3 (1)
Cystoseira baccata			5.8 ± 0.60	−18.7 ± 0.83 (2)	6.8 ± 0.16	−18.7 ± 1.27 (4)
Sargassum muticum	4.5 ± 0.15	−19.0 ± 0.25 (4)	8.0 ± 0.18	−19.3 ± 0.33 (4)	6.7 ± 0.25	−18.2 ± 0.84 (5)
Sacchoriza polyschides	7.2	−13.5 (1)				
Red algae						
Ceramium rubrum			7.5	−8.4 (1)		
Seagrass						
Zostera marina	7.7 ± 0.30	−10.1 ± 0.66 (4)				
Beach: Viñó						
Zone: upper zone						
Seaweeds						
Green algae						
Enteromorpha intestinalis			8.6	−8.3 (1)		
Ulva rigida			6.7	−17.6 (1)		
Brown algae						
Colpomenia peregrina			6.7 ± 0.05	−11.0 ± 0.30 (5)		
Fucus vesiculosus	5.9	−15.2 (1)			6.2	−12.3 (1)
Himanthalia elongata					6.5	−10.5 (1)
Cystoseira baccata			6.6	−20.9 (1)	5.5 ± 0.41	−17.7 ± 0.57 (4)
Sargassum muticum	5.3 ± 0.17	−19.1 ± 0.55 (4)	7.8 ± 0.3	−19.1 ± 0.43 (5)	5.4 ± 0.48	−18.5 ± 1.00 (3)
Red algae						
Plocamium cartilagineum			6.7	−32.4 (1)		
Ceramium rubrum			6.4	−16.7 (1)		
Lomentaria clavellosa			6.6	−29.9 (1)		
Seagrass						
Zostera marina	6.9	−10.1 (1)			7.0 ± 0.31	−10.1 ± 0.13 (5)

Dual-isotope graphs showed that the range of δ^{13}C was wider for potential food sources than for *Talitrus saltator* and only the brown algae *Sargassum muticum* and *Cystoseira baccata* isotopically resembled adult and juvenile diet of *T. saltator*. Thus, both algae might be the most important food sources for both male and female adults and juveniles of *Talitrus*. Although we did not take any quantitative measurement of the biomass of different algae, along the south Galician coast *C. baccata* wrack is more abundant in mid-spring
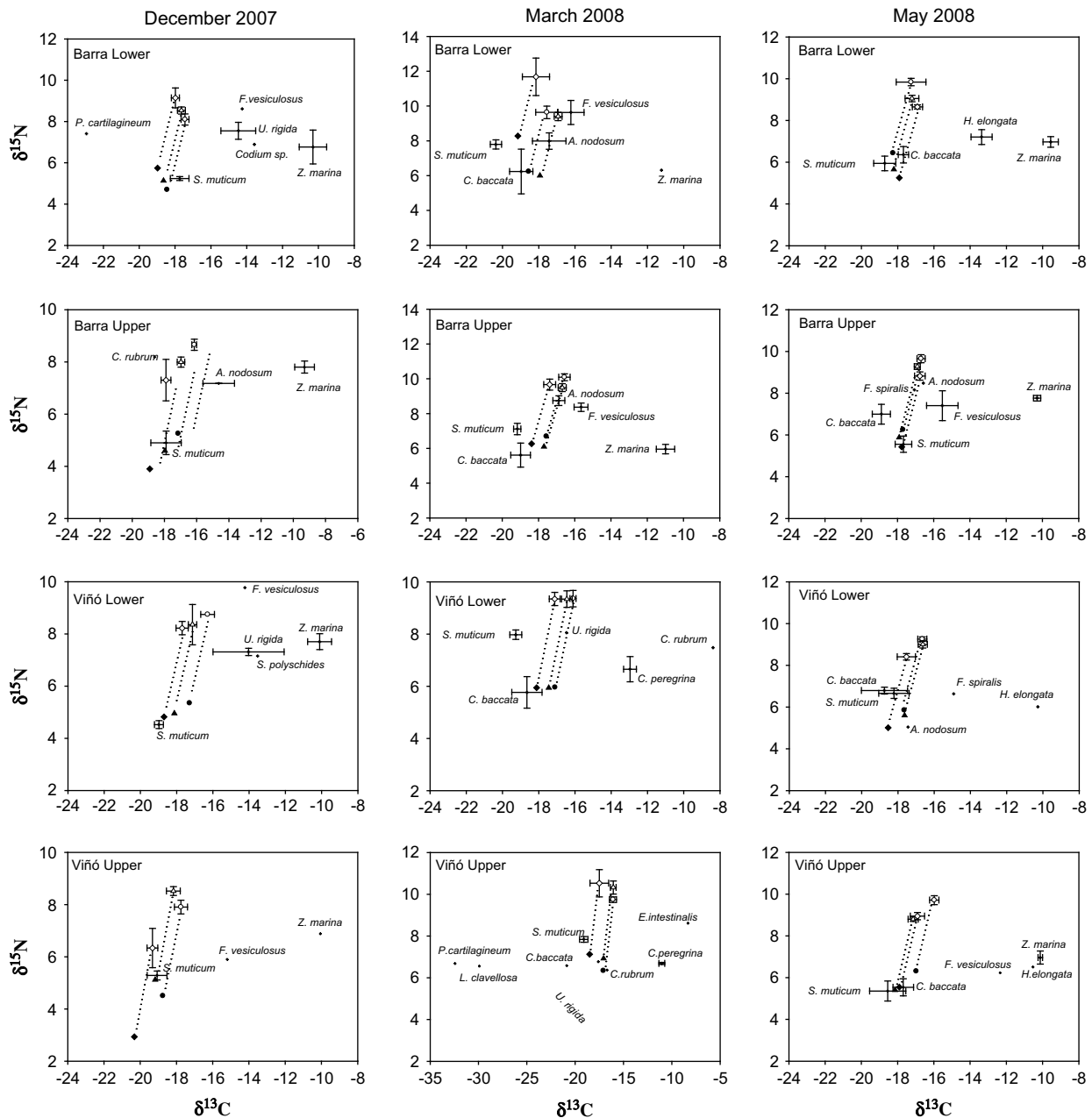
Fig. 3. Dual isotopic composition (mean ± SE) of adults (males and females) and juveniles of *Talitrus saltator* and its potential food sources at the two levels on the shore at two beaches over the three sampling dates. Stable isotope data of *T. saltator* were corrected to account for different diet-tissue isotopic fractionation and determine the position of the expected diet at the lower end of the dashed line. Correction factors were 1 and 3.4‰ for carbon and nitrogen, respectively (see Section 1). Circle: males, triangle: females and diamond: juveniles.

whereas *S. muticum* is more abundant in summer–autumn (personal observations). We indeed observed that *S. muticum* was more important in December, whereas *C. baccata* became more important in spring. Our results were consistent with previous studies that have reported brown algae as common food sources for *T. saltator*. For example, Adin and Riera (2003), using natural stable isotope analyses and the same trophic enrichment factor that we used, found that *Fucus serratus* was the preferential food source for this species. In controlled field and laboratory experiments Lastra et al. (2008) also found that *T. saltator* preferred *Sacchorriza polyschides* to *Fucus spiralis* and *Ulva rigida*. In addition, several studies have found brown algae to be preferential food for other amphipod species (e.g. Karez et al., 2000; Pennings et al., 2000; Crawley and Hyndes, 2007), despite brown algae have lower nutritive value (Boettcher and Targett, 1993)

than red and green algae (Buchsbaum et al., 1991). It is possible that the aging brown algae compensate for low nutritional value with low decomposition rates that allow a longer stay on the beach and a more prolonged availability to consumers (see Buchsbaum et al., 1991). Nevertheless, these results must be interpreted with caution because stable isotope discrimination may be more variable than generally assumed. Recent studies have, in fact, found that the degree of fractionation is quite variable between food sources and within and among species (e.g. Vander Zanden and Rasmussen, 2001; Vanderklift and Ponsard, 2003; Yokoyama et al., 2005; Crawley et al., 2007). Therefore, a stable isotope approach to diet analysis can indicate potential diet items and orientate further experimental work, including controlled studies examining the trophic fractionation of each species.

Using the isotopic approach, we have described for the first time spatial and temporal patterns of intraspecific variation of $\delta^{13}C$ and $\delta^{15}N$ values in adult males and females, and juveniles of *Talitrus saltator*. Adult males showed more variation of $\delta^{13}C$ and $\delta^{15}N$ values than adult females and juveniles. Sex and age-related diet shifts must therefore be considered when evaluating the role of *T. saltator* in food webs of sandy beaches. *Sargassum muticum* and/or *Cystoseira baccata* could provide the largest proportion of the carbon and nitrogen that this species assimilated from wrack during both juvenile and adult stages.

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