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Exploitation of natural food sources by two sympatric, invasive suspension-feeders: *Crassostrea gigas* and *Crepidula fornicata*

Priscilla Decottignies[1,*], Peter G. Beninger[1], Yves Rincé[1], Richard J. Robins[2], Pascal Riera[3]

[1]Université de Nantes, Laboratoire de Biologie Marine, UPRES EA 2663, Faculté des Sciences et des Techniques, 2 Rue de la Houssinière, Nantes 44322, France

[2]Université de Nantes, Laboratoire d'Analyse Isotopique et Électrochimique des Métabolismes, Faculté des Sciences et des Techniques, 2 Rue de la Houssinière, Nantes 44322, France

[3]Université de Paris 6, Centre d'Etudes d'Océanographie et de Biologie Marine, UMR 7127, Station Biologique de Roscoff, Place Georges-Teissier, Roscoff 29682, France

ABSTRACT: The natural diets of the introduced suspension-feeders *Crassostrea gigas* (Thunberg) and *Crepidula fornicata* (L.) were determined at a mid-latitudinal oyster-farming site within their European range (Bourgneuf Bay, France). Carbon and nitrogen stable isotope deviations of Pacific oysters and slipper limpets were compared with potential food sources on 3 sampling dates (March, July and November 2003). Four end-members were assimilated by the 2 species: C_3 angiosperm detritus, macroalgae-C_4 plant detritus, marine phytoplankton and benthic diatoms. Given the lack of source digestibility data for suspension-feeders, and these 2 species in particular, extreme feasible combinations of relative end-member contributions were calculated according to 2 assimilation scenarios, using either IsoSource software or a concentration-dependent model. For both *Crassostrea gigas* and *Crepidula fornicata*, benthic and planktonic microalgae dominated diets on the 3 sampling dates. Planktonic microalgae were ingested in greater proportions than benthic species in July and November; however, benthic diatoms also formed a constant and significant part of diets in these months, and were consumed in greater proportions than planktonic species in March. Plant (especially macroalgal) detritus played a major role in the diets of the 2 suspension-feeders, notably in March 2003 when it became the principal ingested source. The substantial contribution of plant detritus to the natural diets of these species has not previously been reported. Although *Crassostrea gigas* and *Crepidula fornicata* showed significantly different isotopic deviations in March and July 2003, trophic niches of *Crassostrea gigas* and *Crepidula fornicata* overlapped on all 3 sampling dates, with a greater ingestion of identical sources in November. These 2 invasive species could therefore be trophic competitors in the context of end-member supply limitation. Contrary to previous analyses conducted on these 2 species in Europe, this study reports significant dietary overlap. Ecosystem-specific diet studies of invasive species are thus necessary in order to understand trophic overlap/competition as a function of the diversity and availability of local food sources.

KEY WORDS: Slipper limpet · Oyster · Diet · Stable isotopes · IsoSource · $\delta^{13}C$ · $\delta^{15}N$

INTRODUCTION

The ecological and evolutionary consequences of invasive species (sensu Davis & Thompson 2000) within coastal marine systems have only become the focus of scientific investigation relatively recently (see Grosholz 2002). To date, the approach has generally been to study the effects of invasive species relative to indigenous communities. European oyster rearing sites represent a special situation, because an accidentally

*Email: priscilla.decottignies@univ-nantes.fr

introduced suspension-feeder *Crepidula fornicata* L. is sympatric with the intentionally established species *Crassostrea gigas* Thunberg, and trophic competition between the two is suspected (Blanchard 1997). Moreover, *C. gigas* shows signs of proliferation outside of oyster rearing sites, making it both a 'desired' species within farms and a 'pest' species (Ruesink et al. 2005).

Despite long-standing concerns over the potential effects of the slipper limpet *Crepidula fornicata* on indigenous communities, there was no research interest in this species until quite recently, and this was largely motivated by problems encountered in regions of high density where shellfish are also exploited, in particular the introduced Pacific oyster *Crassostrea gigas*. Multiple ecosystem impacts have since been documented: sediment transformation (Ehrhold et al. 1998), macrozoobenthos modifications (de Montaudouin et al. 1999, Le Pape et al. 2004) and changes in food web structure (Chauvaud et al. 2000).

Concerns over the impact of slipper limpets on oyster production were reinforced by observations of simultaneous larval presence and juvenile recruitment (Cole & Hancock 1956), oyster larvae consumption (Korringa 1951), feeding mode and qualitative analyses of stomach contents (Orton 1912). More recent studies (e.g. de Montaudouin et al. 1999, Riera et al. 2002) raised the possibility that a partial overlap of trophic niches exists, and stressed the importance of taking into account the specific trophic dynamics of each ecosystem occupied by both *Crepidula fornicata* and *Crassostrea gigas*. Furthermore, although both suspension-feeders were found to have equivalent biomass and the same filtration impact in Bourgneuf Bay, France (Barillé et al. 2006), it is necessary to determine the particle types consumed by each species in order to estimate the eventual degree of trophic competition.

Apart from early qualitative studies (Orton 1912), the diets of sympatric cultivated oysters and slipper limpets are largely unknown. Currently, multiple natural stable isotope analyses are widely used in food web studies, notably to determine the food sources of suspension-feeders (e.g. Kang et al. 1999, Page & Lastra 2003), which are exposed to seasonal variations in food availability. Such an approach was successfully used to compare the nutritional resources of *Crepidula fornicata* and *Crassostrea gigas* in a Northern European coastal ecosystem (Riera et al. 2002).

Here we present a stable isotope study that determined the sources of carbon and nitrogen assimilated by *Crepidula fornicata* and *Crassostrea gigas* on 3 seasonal sampling dates in a high-turbidity oyster-farming site, which is characterized by decreasing yields concomitant with slipper limpet proliferation (Barillé-Boyer et al. 1997). The site chosen was Bourgneuf Bay, a mid-latitudinal point in the European distribution of these species.

MATERIALS AND METHODS

Study area. The 34 000 ha of Bourgneuf Bay, located south of the Loire Estuary on the French Atlantic coast (Fig. 1), is comprised of 10 000 ha of intertidal area (mean tidal range: 4 m). The large northern opening into the Atlantic Ocean allows considerable mixing of bay and oceanic waters, whereas the southern narrows restricts water exchange. Similarly, the deeper (mean: 10 m) northern part of the bay is strongly influenced by W and SW winds, swells and gyratory currents. These hydrodynamic characteristics transport resuspended sediment from mudflats, resulting in particularly high turbidity in the northern reaches of the Bay (annual mean: 150 mg l^{-1}; Barillé-Boyer et al. 1997). The bay is also variably affected by the Loire estuary, depending on discharge rates and prevailing winds.

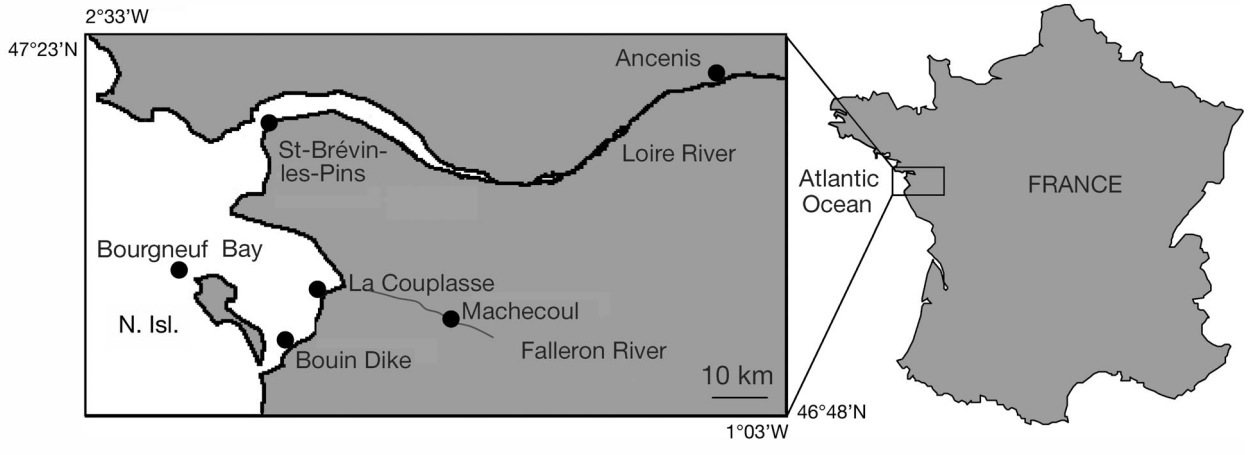

Fig. 1. Location of sampling sites (●) in Bourgneuf Bay, Loire Estuary and River. N. Isl.: Noirmoutier Island

The sampling site for oysters and slipper limpets was an oyster farm at La Couplasse (Fig. 1), where a high biomass of slipper limpets *Crepidula fornicata* (L. Barillé pers. comm.) is located directly adjacent to cultured oyster *Crassostrea gigas* stocks. This area is characterized by high residence times, which increase the influence of freshwater input from the Falleron River (Barillé-Boyer et al. 1997), and by an important diatom-dominated microphytobenthos proliferation on mudflats, which covers 19 to 25% of the surface (Méléder et al. 2003).

Sampling plan. Five Pacific oysters (mean shell length ± SD: 9.7 ± 1.4 cm) and 5 slipper limpets (mean linear shell length ± SD: 3.2 ± 0.5 cm) were collected at low spring tide at La Couplasse in March, July and November 2003. All possible sources of organic matter available to suspension-feeders at this site were collected concomitantly: freshwater, brackish water, oceanic microplankton, microphytobenthos, macroalgal and higher plant detritus.

Sub-surface oceanic water samples were taken during sampling cruises off Noirmoutier Island. Brackish water was collected at the Bouin seawall and from the Loire estuary at St-Brévin-les-Pins. Freshwater was sampled upriver from the Loire estuary at Ancenis, and from the Falleron River at Machecoul (Fig. 1); 20 l of each water sample were collected in plastic containers.

Microphytobenthos was sampled at La Couplasse. The upper 5 mm of a 1 m^2 mudflat area with dense brown microphytobenthic covering was collected with a spatula. Macroalgal thalli and marine angiosperm leaves were collected at La Couplasse and Bouin, and terrestrial angiosperm leaves at Ancenis and Machecoul (Fig. 1). The selected plant species were common at the different sampling sites: *Enteromorpha* sp., *Fucus serratus* and *Fucus vesiculosus* represented macroalgae; *Halimione portulacoides*, *Salicornia* sp. and *Spartina* sp. represented marine angiosperms; and *Alnus* sp., *Populus* sp. and *Robinia pseudoacacia* represented terrestrial angiosperms. As *Alnus* sp. and *Populus* sp. leaves were not present on the March sampling date, data for these potential food sources were limited to June and November.

Sample preparation. Sampled individuals were cleaned of epibionts in the laboratory and gut contents were purged in 0.2 µm filtered seawater from the sampling site for 12 to 24 h. The individuals were killed by freezing, and then soft tissues were separated from the shells, bathed for 1 to 2 min in 1 M HCl in order to eliminate trace shell carbonates, rinsed in Milli-Q ultrapure water and then homogenized with an Ultra-Turrax blender. Samples were stored at −80°C, freeze-dried, and reduced to a powder with a mortar and pestle prior to analysis.

Triplicate water samples were vacuum-filtered onto 47 mm or 25 mm GF/F pre-combusted (500°C, 4 h) filters until clogged. The retentate was acidified with several drops of 1 M HCl in order to remove carbonates, rinsed with ultrapure water, and stored at −80°C prior to freeze-drying.

Benthic microalgae, mainly motile diatoms, were extracted as per Riera & Richard (1996). The sediment sample was spread onto a tray to a thickness of about 1 cm, covered with a 63 µm mesh nylon net, and then with a thin layer of pre-combusted (500°C, 4 h) fine (150 to 300 µm) silicious sand. The size of the mesh was chosen to include all migrating pennate forms, whose widths are smaller than the mesh size. The tray contents were regularly wetted with vaporized (0.2 µm-filtered) seawater from the sample site and illuminated until the following diurnal tide, because benthic diatoms maintain an endogenous rhythm (Mitbavkar & Anil 2004). After migration of the diatoms to the sand layer, visible as a brownish coloration, the superficial layer was then carefully removed with a spatula, placed in a 63 µm mesh screen and rinsed with filtered seawater. The resulting liquid was then processed as above for water samples.

When present, epibionts were discarded from plant samples, which were then acidified and treated as above for mollusks.

Stable isotope and % organic C and N. Duplicate powdered samples or pieces of filter were sealed in ultraclean tin capsules and analyzed for nitrogen and carbon content (% dry weight) and composition ($\delta^{13}C$ and $\delta^{15}N$) using a Carlo-Erba NA 2100 elemental analyzer coupled (via a Thermo Finnigan CONFLO II interface) with a Thermo Finnigan Delta S mass spectrometer. Carbon and nitrogen isotope compositions were expressed in standard delta notations (‰ deviations from a reference): $\delta X = [(R_{sample} \times R_{reference}{}^{-1}) - 1] \times 10^3$, where X is either ^{13}C or ^{15}N and R is the corresponding $^{13}C{:}^{12}C$ or $^{15}N{:}^{14}N$ ratio. Results were referred to Vienna Pee Dee Belemnite for carbon and to atmospheric N_2 for nitrogen. A regularly calibrated working reference (glutamic acid) was run every 11 samples. The SD of 200 $\delta^{13}C$ and $\delta^{15}N$ working reference measurements was 0.2 and 0.4‰ respectively. Isotope readings were validated when the difference between duplicate capsules of the same sample was less than 0.4 and 0.5‰ for carbon and nitrogen deviations respectively; otherwise, samples were analyzed a second time.

As described above, microphytobenthos and marine particulate organic matter (POM) were obtained by GF/F filtration. Insufficient material was collected to allow removal from filters without including glass fibers. Consequently, it was not possible to calculate the carbon and nitrogen content of the masses sealed

in capsules; we therefore calculated mean C:N ratios and assigned %C and %N content according to data from fiber-free samples available in the literature (Abed-Navandi & Dworschak 2005).

Statistics. R freeware (R Development Core Team 2005) was used for all statistical computing. Data normality was verified using the Shapiro-Wilks test, and heteroscedasticity checked with either an *F*-test (2 samples) or a Bartlett's test (>2 samples) before choosing parametric or non-parametric statistical analysis.

For each source, isotope composition according to sampling date was analysed with a 1-way parametric or non-parametric (Kruskal-Wallis) ANOVA as appropriate. As data for *Alnus* sp. and *Populus* sp. were only available from the June and November samples, their carbon and nitrogen isotope deviations were tested using a Welch 2-sample *t*-test or Wilcoxon rank sum test, as appropriate. Two-way ANOVAs, followed by Tukey's honestly significant difference (HSD) test, were performed to compare oyster and slipper limpet isotope compositions, with sampling dates and species as factors.

Dietary analysis. A certain amount of debate has been generated concerning the enrichment factors to be used in diet calculations (e.g. Gannes et al. 1997, Post 2002). The most widely-used values, specifically recommended since the inception of the stable isotope technique, are 1 and 3.4‰ for carbon and nitrogen deviations respectively (DeNiro & Epstein 1978, Post 2002). These enrichment factors have recently been corroborated for bivalve soft tissues (Yokoyama et al. 2005). Alternate values of 0.5 and 2‰ were proposed (McCutchan et al. 2003), and we also completed our calculations using these values; however, as this did not affect our main results or conclusions, the more widely-accepted values of 1 and 3.4‰ were maintained in the present study.

After determination of the food sources implicated in *Crepidula fornicata* and *Crassostrea gigas* diets (see 'Results'), isotopic values were combined *a priori* to characterize source types according to functional considerations, e.g. the C_3 saltmarsh angiosperm group. The squared nearest neighbor distances (NND^2) (Lubetkin & Simenstad 2004) were then calculated to determine whether the isotopic deviations of these source types were distinct or required pooling. The number of source types (> n isotopes + 1) precluded the use of linear mixing models, and hence calculation of the exact proportional contributions of each end-member. We therefore used IsoSource 1.2 software (Phillips & Gregg 2003) to estimate the ranges of biomass contributions of each end-member to the oyster and slipper limpet diets. We also wrote a Scilab 3.1.1 program (INRIA, ENPC) to estimate elemental concentration-weighted biomasses (Newsome et al. 2004). These models determined all feasible end-member combinations by successively incrementing each source proportion by 1% from 0 to 100%. A combination was considered feasible if the calculated mixture composition was equal to the measured composition or within a mass balance tolerance of 0.2‰. This tolerance greatly exceeded the recommended minimum (0.5 × source increment × maximum difference between sources, i.e. 0.09‰ for our data; Phillips & Gregg 2003), given the observed sample variability.

The range of feasible solutions was used in all subsequent representations (frequency distributions and bivariate graph matrices) of feasible dietary contributions. However, means were used in calculations of trophic niche parameters, because single values are required and it was not possible to associate oyster and slipper limpet combinations. Niche breadths of *Crassostrea gigas* and *Crepidula fornicata* were compared using Levins' standardized measure (Krebs 1999)

$$B_A = \frac{B-1}{n-1}$$
$$B = \frac{1}{\sum_1^n p_j^2} \quad (1)$$

where B_A is Levins' standardized niche breadth, B is Levins' measure of niche breadth, n is the number of possible source types and p_j is the fraction of food category j in the diet (mean of IsoSource simulations).

Niche overlap (Krebs 1999) between *Crassostrea gigas* and *Crepidula fornicata* was determined as Pianka's measure

$$O_{jk} = \frac{\sum_i^n p_{ij} p_{ik}}{\sqrt{\sum_i^n p_{ij}^2 \sum_i^n p_{ik}^2}} \quad (2)$$

where O_{jk} is Pianka's measure of niche overlap between species j and k, and p_{ij} and p_{ik} are the proportions of food source i (mean of IsoSource simulations) of the total sources used by species j and k.

RESULTS

Source $\delta^{13}C$ and $\delta^{15}N$

Mean isotopic deviations of the source samples and results of statistical tests among dates are presented in Tables 1 & 2 respectively. The $\delta^{13}C$ deviations of river freshwater POM of the Loire and Falleron were similar at approx. −30‰, which is characteristic of

terrestrial inputs. Estuarine POM carbon isotope values (approx. –25‰) were intermediate between freshwater POM and open sea phytoplankton (approx. –22‰). The bay POM $\delta^{13}C$ was clearly marine, with values close to –23‰. The most depleted carbon deviations of POM were encountered in March 2003, and the most-enriched in November (in most cases). Although the origin of this trend is not clear, similar results were reported by Sará et al. (2003), one of the few marine studies dealing with seasonal changes.

Alnus sp., *Fraxinus* sp., *Populus* sp. and *Robinia pseudoacacia* displayed carbon deviations between –26 and –29‰, typical of terrestrial C_3 angiosperms. *Spartina* sp. presented characteristic C_4 $\delta^{13}C$ values (approx. –14‰), which were ^{13}C-enriched relative to the two C_3 marsh angiosperms, *Salicornia* sp. and *Halimione portulacoides* that presented carbon isotope deviations of about –26‰. Macroalgae also exhibited typical carbon values (approx. –16‰). In marine angiosperms and macroalgae, the most ^{13}C-enriched compositions were measured in March 2003, especially for *Enteromorpha* sp., which presented an extremely high $\delta^{13}C$ value (–6.4‰). The majority of $\delta^{13}C$ values, as well as $\delta^{15}N$ (7 to 11‰), were within the ranges measured in the same species elsewhere, e.g. in France and in the Netherlands (Riera et al. 1996, 2002).

Saltmarsh plant (genera *Halimione*, *Salicornia* and *Spartina*) nitrogen deviations (8.3 to 10.8‰ and one outlier of 17.3‰; Table 1) were relatively ^{15}N-enriched compared with previous studies conducted in France or elsewhere, with values of 3 to 9‰ (e.g. Currin et al. 1995, Créach et al. 1997). This could be related to the incorporation of ^{15}N-rich ammonium and nitrate either from anthropogenic inputs or more probably from nitrification or denitrification processes in marsh sediments (Wada & Hattori 1991).

Benthic diatom $\delta^{13}C$ and $\delta^{15}N$ values were within the ranges previously measured in Oosterschelde (The Netherlands) and in Marennes-Oléron Bay (France) (–14.5 to –11.3‰ and 4.6 to 6.6‰ respectively; Riera et al. 1996, 2002).

Table 1. Bourgneuf Bay: $\delta^{13}C$ and $\delta^{15}N$ (mean ‰ ± SD) of sampled sources; and (in **bold**) end-member input data (mean $\delta^{13}C$ and $\delta^{15}N$ [‰] and mean C and N [g 100 g dry matter^{-1}]) for mixing programs. –: not sampled, n: no. of samples analyzed

	March 2003					July 2003					November 2003				
	$\delta^{13}C$	$\delta^{15}N$	C	N	n	$\delta^{13}C$	$\delta^{15}N$	C	N	n	$\delta^{13}C$	$\delta^{15}N$	C	N	n
Microphytobenthic material															
Benthic diatoms	**–13.1** ± 0.2	**5.3** ± 0.2	**5.0**	**0.9**	3	**–13.8** ± 0.1	**4.6** ± 0.8	**5.0**	**0.9**	3	**–11.9** ± 0.1	**5.9** ± 0.2	**5.0**	**0.9**	3
Particulate organic matter															
Falleron (freshwater)	–30.4 ± 0.3	4.0 ± 0.9			3	–30.4 ± 0.6	1.7 ± 1.6			4	–28.1 ± 0.2	3.7 ± 0.6			4
Loire (freshwater)	–30.7 ± 0.0	2.4 ± 0.3			3	–30.8 ± 0.1	6.5 ± 0.4			3	–28.8 ± 0.4	6.3 ± 0.8			4
Loire (estuarine)	–26.0 ± 0.0	1.5 ± 1.2			3	–24.7 ± 0.5	2.4 ± 1.5			3	–24.8 ± 0.3	2.2 ± 1.2			4
Bay (brackish)	–23.3 ± 0.3	–0.7 ± 1.1			3	–22.8 ± 0.3	2.6 ± 0.6			3	–22.3 ± 0.7	3.8 ± 0.2			4
Marine (open sea)	**–22.9** ± 0.2	**1.7** ± 1.7	**18.0**	**2.5**	3	**–21.4** ± 0.4	**4.0** ± 1.7	**18.0**	**2.5**	3	**–22.0** ± 0.2	**4.2** ± 0.9	**18.0**	**2.5**	4
Macroalgae and C_4 marine angiosperms	**–14.7**	**8.5**	**36.6**	**1.9**		**–15.3**	**8.1**	**34.4**	**1.8**		**–16.0**	**8.5**	**38.1**	**2.5**	
Macroalgae *Fucus serratus*	–13.7 ± 0.1	8.4 ± 1.6			4	–14.2 ± 0.1	7.0 ± 1.3			4	–17.3 ± 0.1	7.6 ± 1.0			4
Fucus vesiculosus	–16.7 ± 0.3	8.1 ± 0.5			4	–18.0 ± 0.5	7.0 ± 1.7			4	–17.0 ± 0.2	10.2 ± 0.9			4
Enteromorpha sp.	–6.4 ± 0.2	8.3 ± 0.9			4	–14.9 ± 0.2	10.8 ± 1.4			4	–15.5 ± 0.2	7.8 ± 0.8			4
C_4 angiosperms *Spartina* sp.	–13.7 ± 0.0	9.1 ± 1.2			4	–14.1 ± 0.1	8.6 ± 0.8			4	–14.2 ± 0.2	8.3 ± 0.4			4
C_3 marine angiosperms	**–25.9**	**9.2**	**33.0**	**2.2**		**–26.9**	**9.4**	**28.9**	**2.1**		**–26.4**	**13.1**	**32.4**	**1.8**	
Halimione portulacoides	–24.8 ± 0.2	9.2 ± 1.0			4	–25.9 ± 0.1	10.8 ± 0.8			4	–25.5 ± 0.3	17.3 ± 2.0			4
Salicornia sp.	–26.5 ± 0.3	9.2 ± 1.4			8	–27.4 ± 0.1	8.7 ± 1.1			8	–26.9 ± 0.4	10.8 ± 2.1			8
Terrestrial angiosperms															
Alnus sp.	–	–				–26.1 ± 0.2	–2.3 ± 1.7			4	–28.3 ± 0.2	–1.3 ± 0.5			4
Fraxinus sp.	–	–				–28.7 ± 1.1	7.9 ± 1.9			8	–	–			
Populus sp.	–	–				–27.8 ± 0.1	2.0 ± 1.2			4	–28.7 ± 0.4	0.5 ± 1.2			4
Robinia pseudoacacia	–	–				–	–				–29.1 ± 0.2	2.1 ± 0.3			4

Table 2. Statistical tests of differences in $\delta^{13}C$ and $\delta^{15}N$ values of sampled sources among months. For 2-way ANOVAs, degrees of freedom (df) between and within groups (= error or residual) are indicated by the first and second number respectively. Test statistics are values of F, χ^2, W and t for ANOVAs, Kruskal-Wallis ANOVAs (KW), Wilcoxon tests (W) and t-tests respectively. MS: mean squares. *: $p \leq 0.05$, **: $p \leq 0.01$, ***: $p \leq 0.001$, ns: not significant

	$\delta^{13}C$						$\delta^{15}N$					
	Test	df	MS	Test statistic	p		Test	df	MS	Test statistic	p	
Benthic diatoms	ANOVA	2, 6	2.823, 0.017	161.130	6×10^{-6}	***	ANOVA	2, 6	1.246, 0.244	5.112	0.051	ns
Falleron (freshwater)	KW	2		7.053	0.029	*	ANOVA	2, 8	6.018, 1.304	4.614	0.046	*
Loire (freshwater)	KW	2		6.564	0.038	*	KW	2		5.982	0.050	ns
Loire (estuarine)	ANOVA	2, 7	1.514, 0.093	16.230	0.002	**	ANOVA	2, 6	0.758, 1.677	0.452	0.656	ns
Bay (brackish)	ANOVA	2, 7	0.811, 0.246	3.291	0.098	ns	ANOVA	2, 7	18.187, 0.467	38.945	2×10^{-4}	***
Marine (open sea)	ANOVA	2, 7	1.686, 0.065	26.087	6×10^{-4}	***	ANOVA	2, 7	6.433, 2.100	3.064	0.111	ns
Fucus serratus	KW	2		9.846	0.007	**	ANOVA	2, 9	1.914, 1.742	1.099	0.374	ns
Fucus vesiculosus	ANOVA	2, 9	1.669, 0.136	12.240	0.003	**	ANOVA	2, 9	10.473, 1.316	7.959	0.010	*
Enteromorpha sp.	KW	2		9.846	0.007	**	ANOVA	2, 8	8.377, 1.100	7.618	0.014	*
Spartina sp.	KW	2		7.538	0.023	*	ANOVA	2, 8	0.656, 0.733	0.895	0.446	ns
Halimione portulacoides	ANOVA	2, 9	1.270, 0.049	25.954	2×10^{-4}	***	ANOVA	2, 8	68.332, 1.902	35.923	1×10^{-4}	***
Salicornia sp.	KW	2		17.165	2×10^{-4}	***	ANOVA	2, 20	8.706, 2.420	3.597	0.046	*
Alnus sp.	W			16.000	0.029	*	W			2	0.400	ns
Populus sp.	W			16.000	0.029	*	t-test	5		1.688	0.159	ns

Stable isotope deviations of oysters and slipper limpets

Compared with the only previously recorded values of *Crepidula fornicata* (Riera et al. 2002), our results (Figs. 2 & 7) are depleted in both carbon and nitrogen heavy isotopes by about 1‰. However, the deviations measured in the present study for *Crassostrea gigas* are within the ranges previously reported in the more extensive literature for this species (e.g. Riera 1998, Hsieh et al. 2000).

Two-way ANOVAs revealed significant differences between species for carbon and nitrogen deviations (df = 1, $F = 22.93$, $p \leq 0.001$ and $F = 6.872$, $p = 0.015$ respectively). Significant ^{13}C-enrichment in slipper limpets (approx. 0.9‰) relative to values of oysters was observed in March and July 2003 (Tukey's HSD: $p = 0.007$ and $p = 0.002$ respectively). Nitrogen deviations of oysters were significantly higher (approx. 1.1‰) than those of slipper limpets in March 2003 (Tukey's HSD: $p < 0.001$). No significant differences were observed in November samples (Tukey's HSD: $p = 0.101$ for $\delta^{13}C$; $p = 0.552$ for $\delta^{15}N$).

Although carbon deviations of *Crepidula fornicata* tissues did not differ significantly among the 3 sampling dates (2-way ANOVA: df = 2, $F = 2.829$, $p = 0.079$; Tukey's HSD: $p > 0.05$), the nitrogen deviations were significantly different (2-way ANOVA: df = 2, $F = 37.904$, $p \leq 0.001$), with a ^{15}N depletion of approx. 0.8‰ in November compared with March (Tukey's HSD: $p = 0.021$). The $\delta^{15}N$ values of oysters sampled in March were also significantly higher relative to July and November (approx. 2 and 1.7‰ respectively; 2-way ANOVA: df = 2, $F = 37.904$, $p \leq 0.001$; Tukey's HSD: $p < 0.001$). There was no significant difference in carbon deviations of *Crassostrea gigas* among the 3 sampling dates (2-way ANOVA: df = 2, $F = 2.829$, $p = 0.079$; Tukey's HSD: $p > 0.05$).

Determination of *Crepidula fornicata* and *Crassostrea gigas* food sources

Dual plot graphs of $\delta^{15}N$ versus $\delta^{13}C$ (Fig. 2) allowed us to determine the food sources of *Crassostrea gigas* and *Crepidula fornicata*. On the 3 sampling dates, calculated diets had values of –19.5 to –18.3‰ carbon and 5.4 to 7.4‰ nitrogen.

Nearshore POM is a complex mixture of marine phytoplankton, plant organic detritus and various other components (Heip et al. 1995); owing to the heterogeneous nature of such POM, it was thus not possible to include Bourgneuf Bay POM as a dietary component. However, since the bay isotopic composition was clearly not influenced by the continental Loire and Falleron discharges (in contrast to estuarine POM), we could exclude freshwater seston and terrestrial plants from the diets.

The isotopic values of the various source diet components assimilated by oysters and slipper limpets in Bourgneuf Bay (i.e. sources within polygons, Fig. 2) were pooled according to ecological categories: C_3 saltmarsh angiosperms (*Salicornia* sp. and *Halimione portulacoides*), C_4 shore angiosperms (*Spartina* sp.), macroalgae (*Fucus serratus*, *Fucus vesiculosus* and/or *Enteromorpha* sp.), marine POM and benthic diatoms.

The $\delta^{13}C$ values indicated a dominance of the latter 4 sources. However, the macroalgae and C_4 angiosperms were not isotopically distinct, because the total NND^2 of their carbon and nitrogen isotope values was less than 0.1. Hence, 4 end-members (Table 1) were used in diet contribution estimations: macroalgae and C_4 angiosperms, C_3 angiosperms, marine POM and benthic diatoms. They presented distinct ($NND^2 > 0.1$) and significantly different (Kruskal-Wallis: $p < 0.001$) isotopic compositions.

End-member elemental content and dietary contributions

End-members implicated in both diets differed in elemental content (Table 1). The plant sources of the present study were typically low in N. Benthic diatoms and phytoplankton exhibited the lowest C:N ratios (5.6 and 7.2 respectively), whereas the highest ratios were observed in macroalgae-C_4 angiosperms (15.2 to 19.4). The C:N ratios of benthic diatoms and phytoplankton were similar to the published values of diatom mats and plankton (Abed-Navandi & Dworschak 2005) on which we based our calculation of carbon and nitrogen content (see 'Materials and methods').

The IsoSource program assumes similar carbon and nitrogen concentrations and equal digestibility of each end-member (Newsome et al. 2004). Because we were interested in elucidating ecological relationships (food proportions ingested), 2 potential error sources were evident in our dietary contribution estimations: the different proportions of C and N in microalgae and plant detritus, and the potential differential assimilation of these sources (which contain different amounts of refractory carbon). However, combined use of IsoSource and of the elemental concentration-dependent model allowed us to estimate the extreme feasible contributions of end-members according to 2 alternate scenarios.

(1) The elemental assimilation efficiencies of both *Crepidula fornicata* and *Crassostrea gigas* were similar for microalgae and plant detritus; hence, it was necessary to distinguish between the C and N content of these sources. The biomasses ingested from the 4 end-members were therefore estimated by the elemental concentration-dependent model.

(2) The elemental assimilation efficiencies of both species were lower for plant detritus than for microalgae (as might occur if putatively high plant refractory carbon was poorly-assimilated). Even if the C and N content of these sources differed, similar C and N concentrations could be assimilated. The biomasses ingested from the 4 end-members were therefore estimated using the IsoSource model.

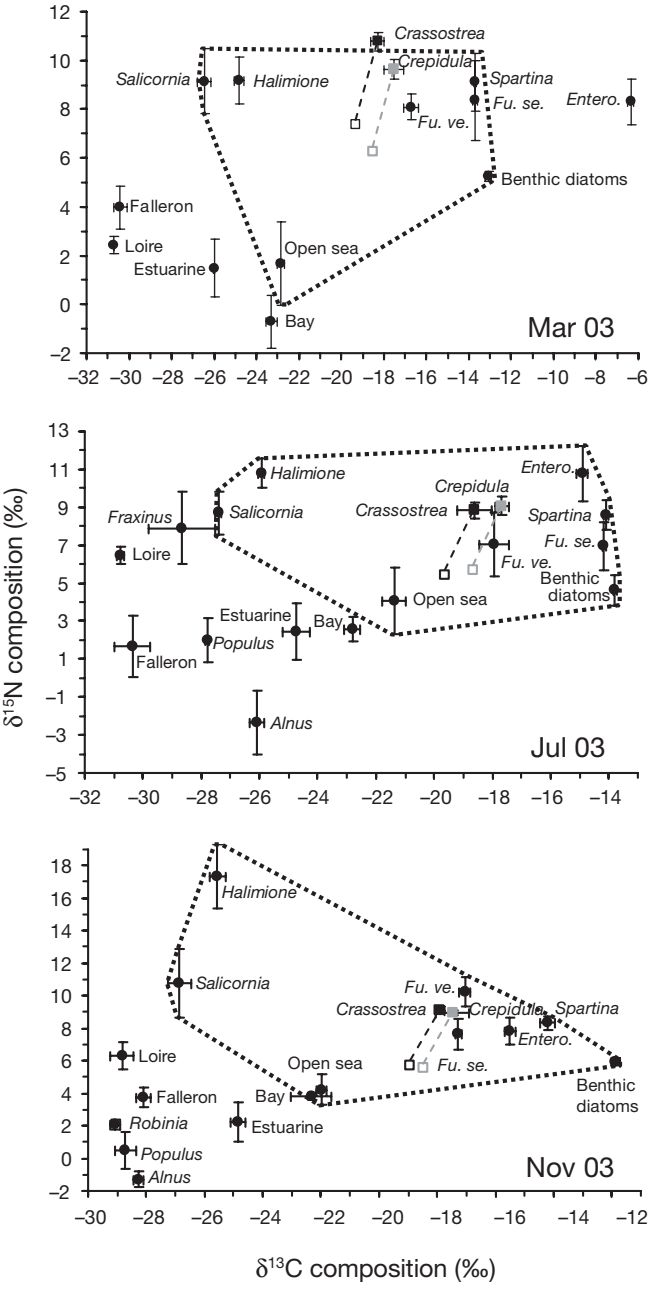

Fig. 2. Dual $\delta^{13}C$ and $\delta^{15}N$ isotopic compositions (mean ‰ ± SD) of 2 suspension-feeders (■,■) and food sources (●) in March, July and November 2003; $\delta^{13}C$ and $\delta^{15}N$ values of calculated diets (□,□) of *Crepidula fornicata* (grey) and *Crassostrea gigas* (black) were determined by subtracting trophic enrichments (dashed lines) of 1 and 3.4‰, respectively, from $\delta^{13}C$ and $\delta^{15}N$ values of both species. Dotted polygons delimit sources implicated in the dietary mixture; these sources are pooled to constitute 4 end-members in subsequent analyses. *Entero.*: *Enteromorpha* sp.; *Fu. se.*: *Fucus serratus*; *Fu. ve.*: *Fucus vesiculosus*. POM samples are: Falleron, Loire (freshwater), estuarine, bay (brackish) and open sea. *Enteromorpha* sp. was discarded from the mixing polygon in March 2003 owing to extreme carbon deviation, which disagreed with other macroalgae and with values from the other 2 sampling events and from previous studies

IsoSource mixing model

For each end-member on each sampling date, IsoSource provided distributions of feasible contribution ranges to the 2 consumer diets (Fig. 3). The distributions were quite dispersed and included zero contributions in most cases. Nevertheless, dietary proportions differed among sampling dates. The greatest ranges of feasible end-member contributions were observed in March 2003, whereas the smallest ranges were found in November 2003, especially for marine POM and C_3 angiosperms. Both suspension-feeders consumed more phytoplankton in November than in March (50 to 61% vs. 0 to 36%). At the same time, *Crassostrea gigas* and *Crepidula fornicata* ingested lower macrophyte proportions (0 to 37% vs. 0 to 59%, and 0 to 12% vs. 6 to 50%, respectively, for macroalgae-C_4 angiosperms and C_3 angiosperms). Thus, phytoplankton contributed 52 to 61% to the diet of oysters and 50 to 59% to the diet of slipper limpets in November, whereas 0 to 22% and 7 to 36% were assimilated by the 2 species, respectively, in March 2003. C_3 angiosperms represented 0 to 12% of the diet of oysters and 0 to 10% of the diet of slipper limpets in November, compared with 24 to 50% and 7 to 37% in March. In July, intermediate ranges were observed for both species. The significant difference in isotope compositions between *C. gigas* and *C. fornicata* in March and July thus appeared to be a result of differential utilization of macrophytes and phytoplankton. The major food source of both species was microalgae (marine phytoplankton and microphytobenthos) in July and November 2003 (but to a lesser extent for slipper limpets in July).

In contrast to the depiction provided in Fig. 3, bivariate graph matrices (Phillips & Gregg 2003; see Fig. 5) allowed us to examine all feasible end-member contribution combinations. As the contributions summed to 100%, the contribution of 1 trophic source constrains the possible contributions of other sources. Approximately 92% of feasible sets of solutions implicated 4 sources in all simulations, i.e. the trophic niches of both species overlapped for 4 food resources. In March, only 16 and 15% of feasible combinations made identical contributions to both species' diets: benthic diatoms and macroalgae-C_4 angiosperms, and marine POM and C_3 angiosperms respectively. In November, higher overlaps were observed: up to 40% of feasible combinations contributed equally to slipper limpet and oyster diets. However, no combination showed more than 2 identical percent contributions between diets of either species.

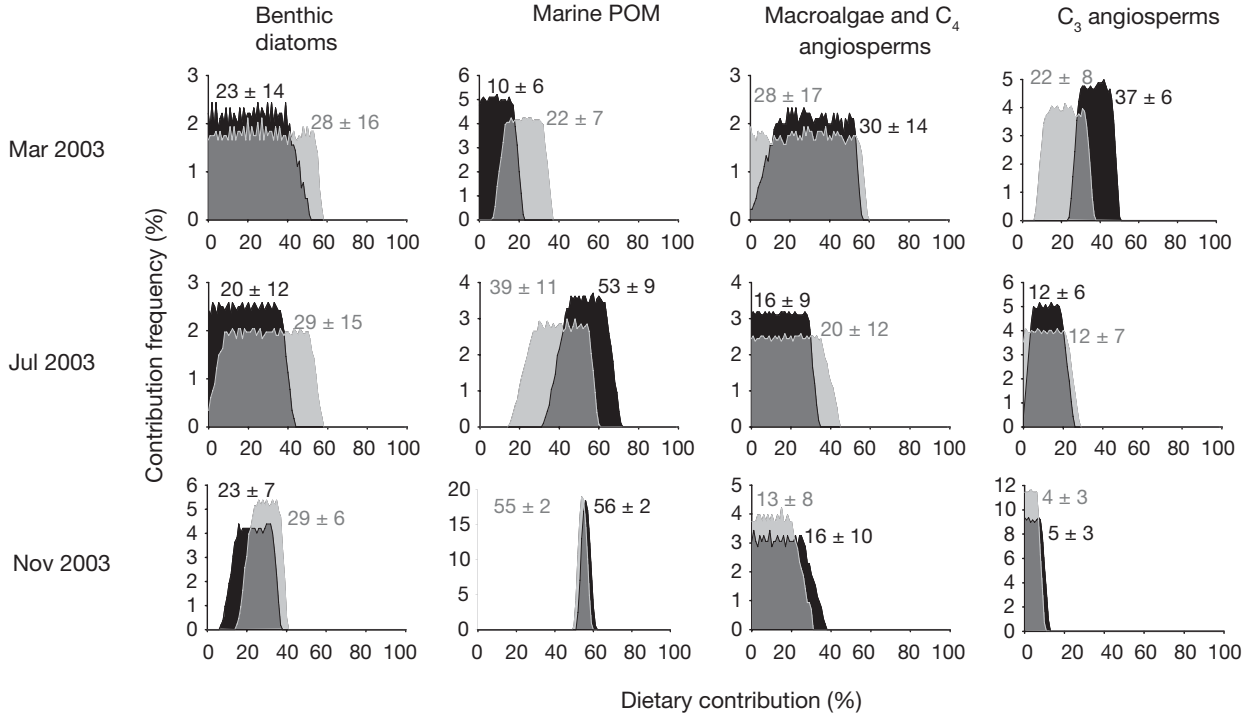

Fig. 3. Distribution of feasible end-member contributions to the diets of *Crassostrea gigas* (black shaded areas) and *Crepidula fornicata* (light grey shaded areas) calculated using IsoSource. Mid-grey shading represents overlapping areas. Mean (% ± SD) contributions are indicated

Elemental concentration-dependent program

The concentration-dependent model (Figs. 4 & 5) gave larger ranges of feasible microalgae contributions and smaller ranges of C_3 angiosperm contributions to *Crepidula fornicata* and *Crassostrea gigas* diets. The tissue production of these 2 species may thus derive more from benthic diatoms than was estimated by the IsoSource program. C_3 angiosperms contributed 20 to 36% and 10 to 22% to the diet of oysters and slipper limpets, respectively, in March 2003; *Crepidula fornicata* appeared to derive its biomass mainly from microalgae, whereas *Crassostrea gigas* depended more on macrophytes.

Feasible combinations (Fig. 5) of oyster and slipper limpet diets were more similar in March and July than estimated by IsoSource. However, as noted above for the IsoSource estimations, the concentration-dependent model did not estimate more than 2 identical percent contributions in dietary combinations to either species. Four sources also contributed up to 90% to feasible sets of dietary solutions, i.e. the trophic niches of both species overlapped for all 4 sources using this concentration-dependent program, as was observed using IsoSource.

Characteristics of trophic niches

Using results from IsoSource, Levins' standardized niche breadth of *Crepidula fornicata* was wider than that of *Crassostrea gigas* in March and July 2003. Calculated slipper limpet niche breadth varied from 0.50 to 0.98, whereas that of oysters varied between 0.51 and 0.81. From March 2003 onwards, the 2 mollusks displayed a progressive reduction in niche breadth, attaining the same minimal value in November (~0.5). Over the same period, Pianka's measure exhibited a progressively increasing niche overlap, from 92 to 99% (Fig. 6). The niche breadths of *Crepidula fornicata* and *Crassostrea gigas* calculated from the concentration-dependent model were more similar, showing the same reduction from March to November. Niche overlap was also slightly greater in July and November.

DISCUSSION

Food sources of *Crassostrea gigas* and *Crepidula fornicata*

The food supply available to suspension-feeders in mudflat ecosystems such as Bourgneuf Bay is conven-

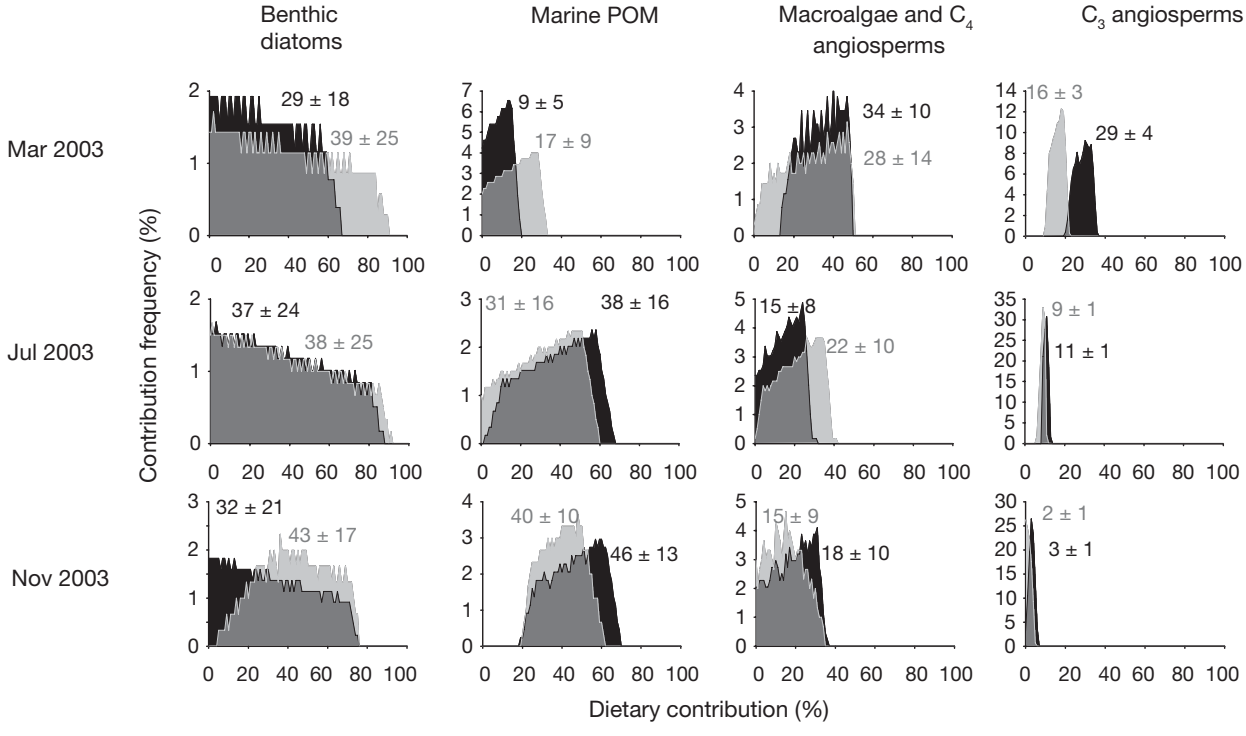

Fig. 4. Distribution of feasible end-member contributions to the diets of *Crassostrea gigas* (black shaded areas) and *Crepidula fornicata* (light grey shaded areas) satisfying concentration-dependent conditions. Mid-grey shading represents overlapping areas. Mean (% ± SD) contributions are indicated

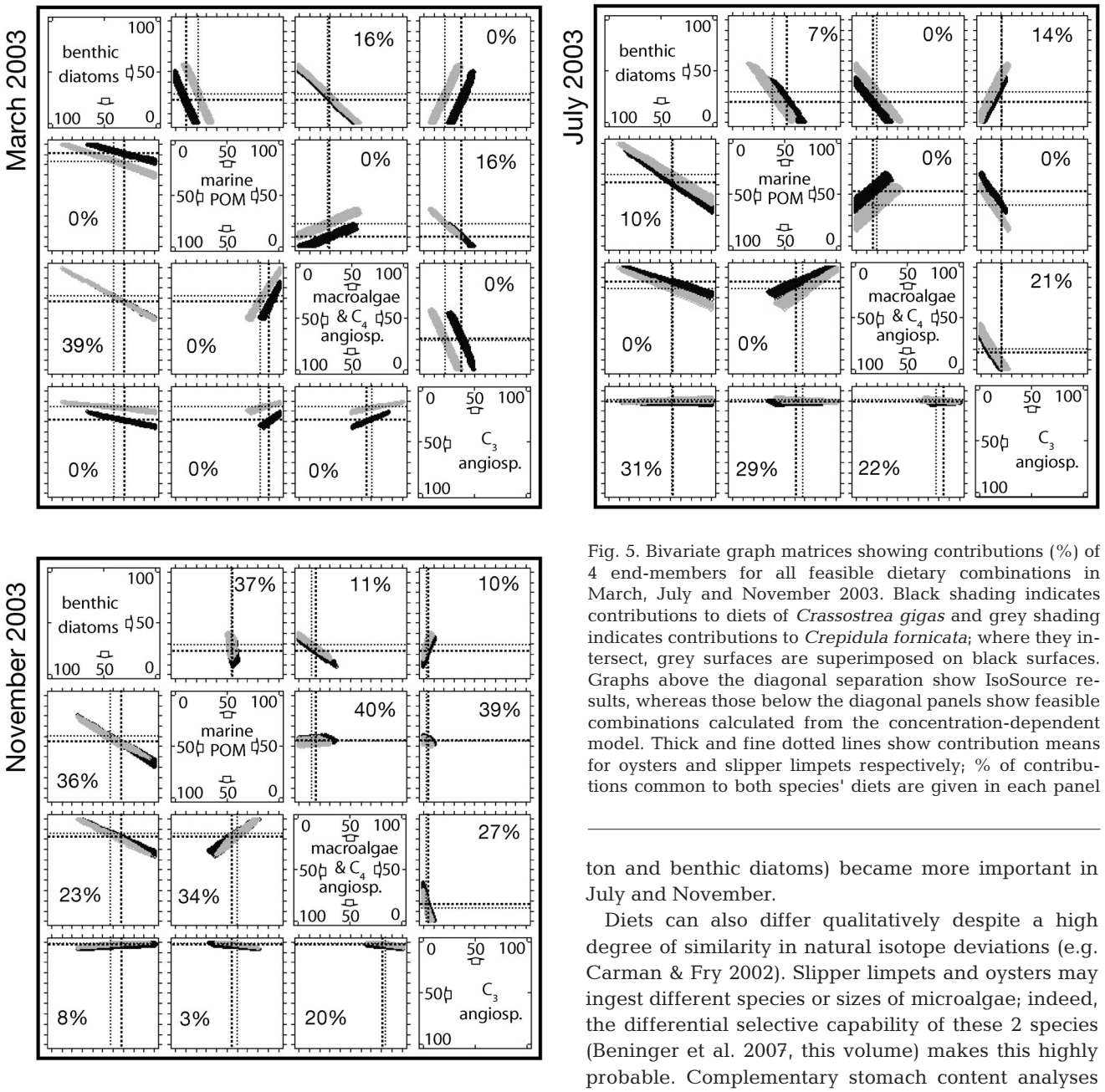

Fig. 5. Bivariate graph matrices showing contributions (%) of 4 end-members for all feasible dietary combinations in March, July and November 2003. Black shading indicates contributions to diets of *Crassostrea gigas* and grey shading indicates contributions to *Crepidula fornicata*; where they intersect, grey surfaces are superimposed on black surfaces. Graphs above the diagonal separation show IsoSource results, whereas those below the diagonal panels show feasible combinations calculated from the concentration-dependent model. Thick and fine dotted lines show contribution means for oysters and slipper limpets respectively; % of contributions common to both species' diets are given in each panel

tionally considered to be chiefly composed of benthic diatoms (Riera & Richard 1996, Leguerrier et al. 2003). However, our comparison of isotopic compositions of the potential food sources and tissues of *Crepidula fornicata* and *Crassostrea gigas* revealed that 4 primary production sources were predominantly assimilated: suspended POM (essentially phytoplankton; up to 70%), benthic diatoms (up to 90%), angiosperms and macroalgae (up to 60%). The relative contribution of each component differed according to sampling date (more markedly for oysters): macrophytes were more important in March 2003, and microalgae (phytoplankton and benthic diatoms) became more important in July and November.

Diets can also differ qualitatively despite a high degree of similarity in natural isotope deviations (e.g. Carman & Fry 2002). Slipper limpets and oysters may ingest different species or sizes of microalgae; indeed, the differential selective capability of these 2 species (Beninger et al. 2007, this volume) makes this highly probable. Complementary stomach content analyses would help to elucidate this particular aspect.

The $\delta^{13}C$ and $\delta^{15}N$ deviations of oysters from Bourgneuf Bay extended further beyond those of benthic diatom, especially in March, compared with oysters of the previously-studied Marennes-Oléron site (also on the French Atlantic coast) that presented a diet primarily based on microphytobenthos (Fig. 7; Riera & Richard 1996, Riera 1998). In addition, the difference between isotopic deviations of oysters or slipper limpets and benthic diatoms was greater in Bourgneuf Bay than in Oosterschelde (Fig. 7; Riera et al. 2002). Nonetheless, benthic diatoms feasibly contributed a substantial portion to the diets of *Crepidula fornicata*

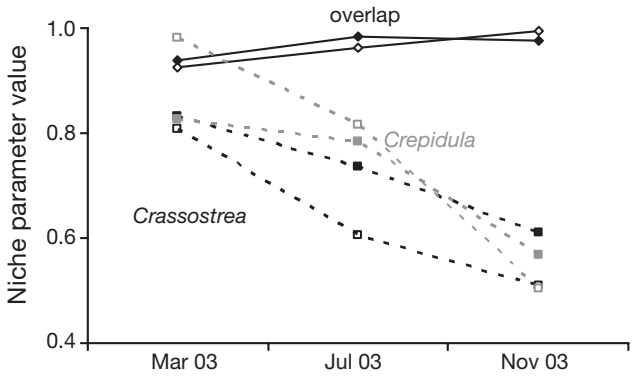

Fig. 6. Levins' standardized niche breadth (dashed lines) of *Crepidula fornicata* (grey) and *Crassostrea gigas* (black), and Pianka's measure of niche overlap between both species (continuous line); obtained from IsoSource (filled symbols) and concentration-dependent model (open symbols) simulations

and *Crassostrea gigas* on all 3 sampling occasions (Figs. 3 & 4). Microphytobenthos, which has an organic composition more readily assimilated than that of angiosperms, may be a major food source to secondary producers (e.g. Currin et al. 1995, Kang et al. 2003).

Phytoplankton was also an important component of the diets of both suspension-feeders. Concomitant grazing on resuspended microphytobenthos and phytoplankton is well documented for intertidal suspension-feeders (e.g. Kang et al. 1999, Rossi et al. 2004). Phytoplankton variability is thus a determinant of individual and population bivalve dynamics in the field (Grant 1996).

Stable isotope compositions of *Crassostrea gigas* and *Crepidula fornicata* in Bourgneuf Bay, together with mixing model analyses, indicated a generally high input by angiosperms and macroalgae. Suspended, variably degraded plant detritus has been shown to be a major trophic substrate for coastal secondary producers, including suspension-feeders, in American saltmarsh food webs (e.g. Currin et al. 1995, Deegan & Garritt 1997). Our *Crepidula fornicata* and *Crassostrea gigas* sampling site, La Couplasse, is close to a total of 9.34 ha of saltmarsh composed of *Halimione portulacoides*, *Salicornia* sp. and *Spartina* sp., and is adjacent to 234.8 ha of macroalgal cover. In addition, infralittoral seaweed beds of unknown extent exist at the entry to the bay (Y. Gruet, Université de Nantes, pers. comm.). Relatively long residence times in Bourgneuf Bay (approx. 2 mo) facilitates physico-chemical and biological modification of detritus particles in the water column.

The estimations of end-member dietary contributions suggested that macroalgae and C_4 angiosperms contributed more than C_3 angiosperms to *Crepidula fornicata* and *Crassostrea gigas* diets, even though C_3 plants predominate in saltmarshes of the French Atlantic coast (European Natura 2000 code 1330; http://natura2000.environnement.gouv.fr/habitats/HAB1330.html). Suspension-feeders obtain maximum benefit from plant detritus in earlier stages of decomposition, before most of the nutrient and energy-rich compounds are used by microheterotrophs (Stuart 1982). Although trophic mediation through bacteria or

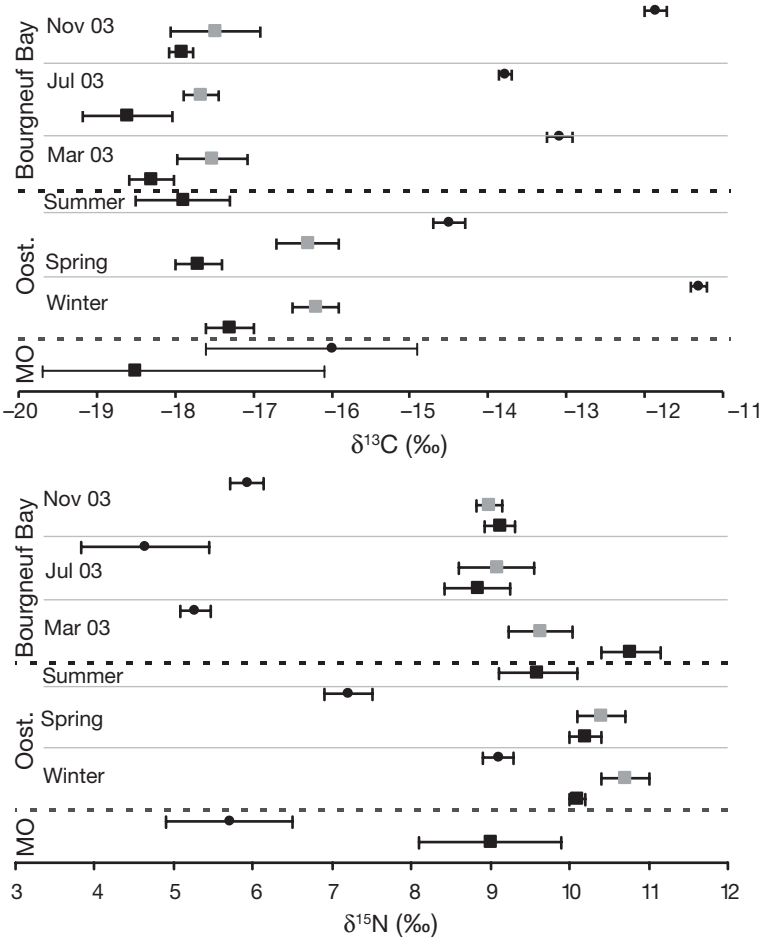

Fig. 7. Mean $\delta^{13}C$ and $\delta^{15}N$ of *Crassostrea gigas* (■), *Crepidula fornicata* (n) and benthic diatoms (●) measured in Bourgneuf Bay (France; present study), the Oosterschelde ('Oost.', The Netherlands; Riera et al. 2002) and Marennes-Oléron Bay ('MO', France; Riera & Richard 1996, Riera 1998). Error bars are either SD or ranges of mean $\delta^{13}C$ from MO. Dashed lines separate locations; grey solid lines separate seasons or months for which data was collected

microzooplankton is often necessary for the digestion of angiosperm tissues (Mann 1988, Langdon & Newell 1990, Riera 1998), no such mediation was observed in the suspension-feeders of the present study: $\delta^{15}N$ values indicated no corresponding trace of enrichment.

In contrast, macroalgal tissues contain relatively large amounts of nitrogen, protein and energy that is readily assimilated by primary consumers (Mann 1988, Bowen et al. 1995); these tissues also constitute a significant source of nutrient-rich detritus, used by suspension-feeders as sources of carbon and nitrogen throughout the year (Bustamante & Branch 1996). In the present study, an increased contribution by plant detritus was observed in March 2003 (i.e. winter assimilation), when microalgal production is minimal. It is thus possible that these suspension-feeders exploit the increased proportion of plant detritus in the winter period, as has been previously reported for other suspension-feeding bivalves (Lucas et al. 1987, Cranford & Grant 1990).

Direct assimilation of plant tissues by oysters and slipper limpets depends on the variety and nature of their digestive enzymes. The extracellular digestive enzymes of bivalves, including oysters, comprise especially cellulases, amylases and laminarinases (Brock et al. 1986). Oysters are thus capable of degrading the major organic components present in detritus (Newell & Langdon 1986). In particular, *Crassostrea virginica* assimilates significant amounts of saltmarsh angiosperm carbon (Newell & Langdon 1986, Langdon & Newell 1990).

Advantages of combined use of IsoSource and concentration-dependent models

Previous stable isotope studies of suspension-feeder diets either did not estimate relative contributions, or quantified source contributions using simple mixing equations in which no more than 2 sources were implicated (Riera et al. 1999, Page & Lastra 2003). We used IsoSource-type linear mixing models to statistically define the feasible relative contributions of end-members to the diets of these 2 suspension-feeders.

The assimilation of food sources was shown to vary according to mollusk species and source type (Tsikhon-Lukanina 1982). The diverse source types implicated in both *Crepidula fornicata* and *Crassostrea gigas* diets could present dissimilar digestible C and N fractions, in addition to different elemental content. Although abundant literature exists on the digestibility of food for humans or bears (Koch & Phillips 2002, Newsome et al. 2004), we are unaware of any such studies on microphage food quality values, and hence the digestibility of the various C and N fractions. Our results, obtained by combined use of IsoSource and concentration-dependent models, described most probable overlaps between the diets of *Crepidula fornicata* and *Crassostrea gigas* despite the lack of literature on digestibility of the different end-members. Based on the incorporation of C and N concentrations, results from the concentration-dependant model indicated a greater similarity in the diets of these 2 species, with a lesser importance of detritus, than did those from the IsoSource model.

Trophic relationships between *Crassostrea gigas* and *Crepidula fornicata*

Given that *Crepidula fornicata* and *Crassostrea gigas* both feed on seston and, that in contrast to the oyster (Cognie et al. 2003), *C. fornicata* does not appear capable of qualitative selection, diet overlap appeared highly probable (Beninger et al. 2007). Isotope comparisons confirmed this assumption: the same food types were ingested on 3 seasonal sampling dates. However, significant differences in the tissue isotope deviations of these 2 species in March and July 2003 revealed that diets differed substantially in winter and spring. Similar conclusions—i.e. same source types but different diets—were reported for these 2 species in the Oosterschelde (The Netherlands; Riera et al. 2002; Fig. 7). In comparison with our study, the dietary differences of specimens from the Oosterschelde were consistently more pronounced, especially with respect to carbon deviations. However, substantial ingestion of macrophyte detritus by these suspension-feeders has not been previously reported from other European Atlantic sites (Oosterschelde and Marennes-Oléron). Interestingly, this corresponds to reduced presence of macrophytes at these sites (Riera & Richard 1996, Riera et al. 2002). These observations bring to attention the need for site-specific diet studies of these invasive species, in order to obtain a realistic understanding of their feeding biology in European coastal ecosystems.

The trophic niche breadth of slipper limpets was either similar to or broader than that of Pacific oysters, indicating that *Crepidula fornicata* is a more generalist suspension-feeder than *Crassostrea gigas.* This may be due, at least in part, to their different capacities for qualitative selective feeding (Beninger et al. 2007). On all 3 sampling dates of the present study, the trophic niches of *Crepidula fornicata* and *Crassostrea gigas* overlapped to variable degrees in Bourgneuf Bay, with greater overlap in November when niche breadths were narrower. This high degree of overlap (consistently > 90%) emphasizes the potential for trophic competition between these 2 invasive species; how-

ever, competition can only be demonstrated when resources are limiting for both species. In the absence of complete source data and functional ecosystem models, demonstration of actual competition must therefore rely on specific physiological and isotopic indices (e.g. shifts in filtration, use of specific diet components). The findings of the present study show that the characteristics of trophic overlap/competition between *Crepidula fornicata* and *Crassostrea gigas* are likely to be highly variable throughout their sympatric range, depending on types and abundances of local food sources.

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