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Intraspecific variation in stable isotope signatures indicates no small-scale feeding interference between a horse mussel and an ascidian

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ABSTRACT: Interspecific feeding interference between neighboring suspension-feeders is an important but understudied structuring process in marine benthic communities. The comparison of $\delta^{13}C$ and $\delta^{15}N$ stable isotope ratios (SIR) between species is widely used to identify the dietary overlap and competition for food. Results from this approach are sometimes substantially biased by the variation of isotope fractionation rates among species and tissues. We suggest that the difference in SIR within a single species in the presence and absence of a potential competitor provides stronger evidence of feeding interference. In the White Sea, shallow subtidal solitary ascidians *Styela rustica* L. frequently develop in clumps on horse mussels *Modiolus modiolus* (L.). Horse mussels without ascidians and ascidian clumps attached to gravel are also common. We analyzed SIR in muscular tissues of neighboring (within 0.25 m^2) *M. modiolus* with and without ascidians attached and in *S. rustica* from nearby (within 0.25 m^2) clumps on gravel and mussels. Species (*M. modiolus* or *S. rustica*), tissue (foot and posterior adductor muscles in *M. modiolus*) and site (8.1 km apart) factors had strong effects on isotope ratios, whilst the presence of a potential competitor had no effect on SIR in either species. We conclude that the diets of *M. modiolus* and *S. rustica* are not affected by co-occurrence and do not overlap much, giving no evidence of competition. Further research should take in account the substantial difference in SIR between the 2 *M. modiolus* muscular tissues studied: −21.582 ± 0.048 ($\delta^{13}C$) and 6.551 ± 0.063 ($\delta^{15}N$) in foot muscle versus −20.970 ± 0.063 ($\delta^{13}C$) and 7.806 ± 0.074 ($\delta^{15}N$) in adductor muscle.

KEY WORDS: Stable isotope ratios · Bivalves · Ascidians · Trophic interactions · Competition

INTRODUCTION

Suspension-feeding organisms often dominate marine benthic assemblages on both hard and soft substrate. Bivalves, ascidians and barnacles may act as foundation species, providing habitats for many other dependent taxa (Fielding et al. 1994, Seed 1996, Castilla et al. 2004, Yakovis et al. 2008). Many suspension-feeders are sessile or nearly so. Their competition for space is an important small-scale structuring process in epibenthic assemblages, and so is their competition for food (Buss 1979, Lohse 2002). However, to date, there is much less direct evidence for the effect of competition for food, possibly because of the complications raised by attempts to manipulate food sources for filter-feeders. The few state-of-art experiments on intraspecific (Pullen & LaBarbera 1991) and interspecific (Okamura 1984, 1985, 1988) small-scale feeding interference left out most potential competitors with relatively large body

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size, likely due to technical limitations of the approach based on direct counting of tagged food particles consumed by each individual.

Stable isotope ratios of carbon and nitrogen are widely used to indirectly assess the relative contribution of different food sources to the diet of animals (see Boecklen et al. 2011 for review). Isotopic signatures are expressed as δ, the ratio of heavy (e.g. ^{13}C and ^{15}N) to light (^{12}C and ^{14}N, respectively) isotopes. Fractionation during the feeding process enriches the tissues of a consumer in relation to the food source by ~1.4 to 3.3‰ for $\delta^{15}N$ and 0.3 to 1.3‰ for $\delta^{13}C$ (McCutchan et al. 2003). Based on the measurements of these ratios in potential food sources (which usually include particulate organic matter of different origin or certain prey species for marine benthic consumers), one may apply a mixing model to estimate their contribution to the diet of a particular species (Phillips et al. 2005). Stable isotope composition of consumer tissues does not reflect the most recent diet. Instead, it is affected by feeding patterns of several weeks to few months preceding sampling, depending on the metabolic rate of a particular organism and tissue (Dalerum & Angerbjörn 2005). Accurate mixing models thus require potential food sources sampled in advance according to previously studied metabolic rates.

Interspecific differences in ^{13}C and ^{15}N signatures have been unhesitatingly interpreted as indicators of feeding segregation between potentially competitive suspension-feeding species (e.g. Dubois et al. 2007). High variation of stable isotope signatures observed among the tissues of a single organism may, however, compromise these conclusions (see Lorrain et al. 2002, Yokoyama & Ishihi 2006, Cabanellas-Reboredo et al. 2009, Deudero et al. 2009, Aya & Kudo 2010 for data on bivalves). In coastal Arctic waters, relatively slow metabolic rates of benthic invertebrates are combined with high seasonal changes in potential food sources for suspension-feeders due to the presence of ice cover and variation in river discharge. Tissue-specific isotope incorporation models (Boecklen et al. 2011) are not yet developed for most species. Therefore, the evidence of interspecific feeding interference or its absence based on mixing models demands multiple repeated sampling of the potential food sources at least several months prior to sampling the consumers. This may not always be technically possible or logistically feasible due to the ice freezing and melting seasons.

Interspecific competition for food alters the diet at least of an inferior competitor in relation to the diet observed in the absence of a superior competitor (Hanson & Leggett 1986, Haken & Batzli 1996). Consequently, intraspecific difference in diet in the presence and absence of a potential competitor would indicate competition for food. Here, we suggest comparing the same tissue of the same species between individuals affected and unaffected by a potential competitor. The individuals compared should be otherwise as similar as possible in size and location. This design avoids the bias caused by variation of stable isotope fractionation rates among species and tissues. It also requires no advance multiple sampling of potential food sources where tissue-specific isotope incorporation rates are unknown. Altered stable isotope signatures in the presence of a potential competitor would prove correlation between this presence and a shift in diet; however the direction of a causal relationship can be only proved by manipulations. Kang et al. (2009) came close to the design we suggest, comparing stable isotope ratios in co- and monocultured ascidians and oysters. However, they sampled co- and monocultured animals from different sites separated by several kilometers and never examined co- and monocultures from the same site. Consequently, their data cannot be used to separate the effects of location and competition for food.

We applied the described approach to 2 large benthic suspension-feeders commonly co-occurring in the White Sea: horse mussels *Modiolus modiolus* (Linnaeus, 1758) and solitary ascidians *Styela rustica* Linnaeus. Horse mussels there are semi-buried in muddy sediment, anchored with byssus threads to submerged gravel, with the siphonal end of the shell pointing upwards (Fig. 1). *S. rustica* develops clumps attached to hard substrates, such as rocks, stones, shells and kelp; at the sites studied, most of them reside on gravel and live *M. modiolus* or their empty shells. According to the size of particles consumed, coexisting species of ascidians and bivalves may share common food sources, like suspended particulate organic matter and phytoplankton (Stuart & Klumpp 1984), or differentiate their diets along the

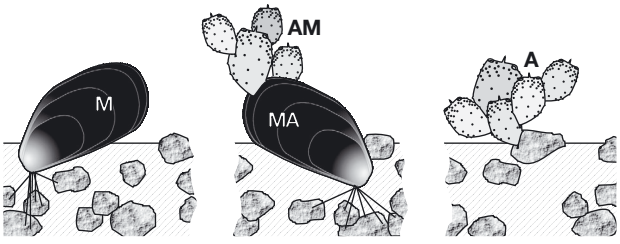

Fig. 1. Sampling on a horse mussel bed. M: *Modiolus modiolus* without *Styela rustica*, MA: *M. modiolus* with *S. rustica*, AM: *S. rustica* on *M. modiolus*, A: *S. rustica* on gravel

size spectra (Lesser et al. 1992). However, no data are available either on the diets of these particular species or on their tissue-specific metabolic rates with respect to isotope incorporation dynamics. Freezing and melting ice prevents sampling of the White Sea shallow subtidal in early winter and, more importantly, in spring, when the seasonal changes in potential food sources may strongly affect the summer isotopic compositions in tissues of the potential competitors. We compared stable isotope ratios in both species from the same sites in the presence and absence of a potential competitor. Significant effect of co-occurrence in *M. modiolus* or *S. rustica* would be evidence for interspecific competition for food. The absence of the effect would not, however, be evidence for absence of competition since the difference in isotopic composition it causes may be beyond the resolution of the method proposed.

MATERIALS AND METHODS

Study sites

The present study was carried out at 2 subtidal sites in the Keret' archipelago at the mouth of Chupa Bay (White Sea, northern Russia). Surface water temperature ranges from −1.2°C in January to 20.1°C in July. Salinity decreases in April to May (18.2‰) and rises in January and July (26.5‰) (Dobretsov & Wahl 2008, Berger 2009). Ice cover lasts from December to May.

Both sites were 15 m deep and sheltered, and the bottom was muddy with gravel. The distance between Site 1 (66° 19′ 50″ N, 33° 49′ 48″ E) and Site 2 (66° 20′ 13″ N, 33° 38′ 58″ E) was 8.1 km (Fig. 2). Both sites had 2 to 5 large horse mussels and 3 to 8 ascidian clumps per m^2 of the bottom. Most ascidians in clumps were *Styela rustica.* Other macrobenthic suspension-feeders, including ascidian species (*Styela coriacea*, *Molgula* spp. and *Boltenia echinata*), in total contributed <5% to the total biomass. Foliose red algae *Phyllophora* spp. and *Phycodrys rubens* co-dominated the assemblage. About one-half of all ascidian clumps were attached to live horse mussels; other clumps were on gravel and empty horse mussel shells.

Field sampling

Within a haphazardly selected 0.25 m^2 plot, SCUBA divers obtained 1 large (71 to 97 mm long)

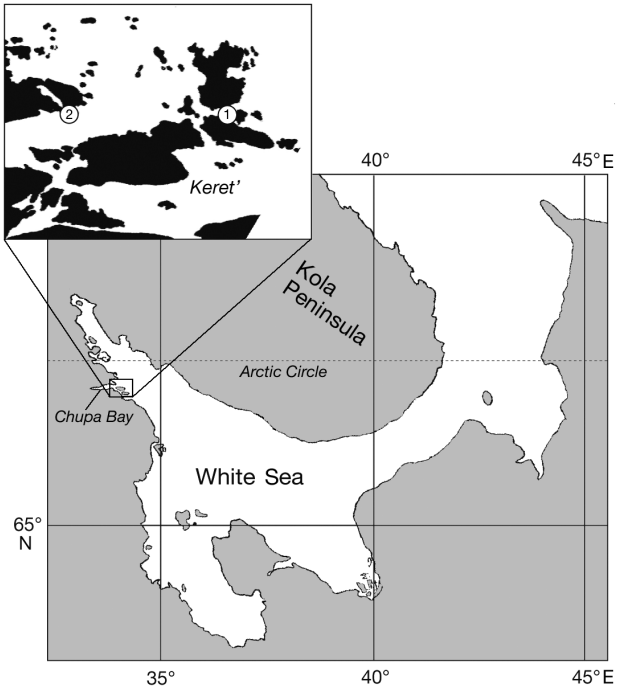

Fig. 2. Sampling area location. O: sampling sites (1 and 2)

Modiolus modiolus individual (MA) with a clump of *Styela rustica* attached (AM), a clump of *S. rustica* on gravel (A) and a large *M. modiolus* without an ascidian clump (M) (Fig. 1). The procedure was repeated 10 times at Site 1 and 3 times at Site 2 on 23 to 28 June 2007. Sampling plots were distributed within 100 m from the base points of the sites; the distance between the plots was not less than 5 m. The disproportion of sample size among sites was due to logistic constraints and had almost no effect on the consistency of the results: stable isotope signatures demonstrated similar patterns of tissue- and species-specific variation at both sites (see Figs. 3 & 4). The approximate area sampled was 250 m^2 at each site. The ascidian clumps collected had 5 to 39 individuals of *S. rustica* with a total wet weight of 14 to 146 g per clump. Site 2 had larger *S. rustica* clumps (75.9 ± 16.8 g; mean ± standard error [SE]) than Site 1 (27.2 ± 3.9 g) (Student *t*-test, $p < 0.001$). Clumps on gravel were insignificantly smaller (31.3 ± 6.3 g) than on *M. modiolus* (52.3 ± 13.1 g).

Preparation and laboratory analysis of samples

Animals were kept alive for up to 24 h in aerated tanks at 5°C prior to processing. We obtained a posterior adductor muscle and a foot muscle from each

MA and M sample. Two different muscle tissues were used to take into account the intraspecific variation of stable isotope ratios between the tissues of similar type. A piece of a body wall muscle was cut from 2 to 3 large (each ~3 to 4 g wet weight) ascidians from each AM and A sample. In total, we prepared 129 samples (see Table 1 for details). Since they included no calcified tissues, no HCl treatment was needed to remove carbonates. Samples were lyophilized and stored at −20°C (Bosley & Wainright 1999). After grinding to powder, ~1 mg of each sample (weighed accurate to 0.001 mg) was used for continuous flow isotope radio mass-spectrometry. A Finnigan Delta Plus Advantage isotope radio mass spectrometer coupled with a Carlo Erba NC 2100 Elemental Analyzer via ConFlo III interface was used to determine $\delta^{13}C$ and $\delta^{15}N$ in samples. Stable isotope ratios were calculated as $\delta X\,(‰) = [(R_{sample} / R_{standard}) - 1] \times 10^3$, where X is ^{13}C or ^{15}N, and R is the corresponding ratio of ^{13}C:^{12}C or ^{15}N:^{14}N. Standards were Pee Dee Belemnite and atmospheric N_2 for carbon and nitrogen, respectively.

As estimated from standards analyzed together with samples (US National Institute of Standards and Technology peach leaves [SRM 1547]), the analytical precision was 0.05‰ for $\delta^{13}C$ and 0.15‰ for $\delta^{15}N$ (SD, n = 32). Calibration standards were International Atomic Energy Agency N1, N2, CH6 and CH7. Samples were processed by the Colorado Plateau Stable Isotope Laboratory (Northern Arizona University, Flagstaff, AZ).

Data analysis

We used Type III sum of squares factorial analysis of variance (ANOVA) followed by Tukey honestly significant difference post-hoc tests to assess the effects of species (fixed) and site (random) on isotope ratios. Type III sum of squares was selected since the design was unbalanced. We performed 2 separate analyses based on horse mussel foot and adductor samples, sharing the same data for ascidians. The $\delta^{13}C$ and $\delta^{15}N$ in foot and adductor muscle samples of *Modiolus modiolus* were compared by paired *t*-test.

We compared stable isotope ratios for *Modiolus modiolus* with and without *Styela rustica* using separate paired *t*-tests for foot and adductor muscle-based data. We also calculated Spearman correlations between $\delta^{13}C$ and $\delta^{15}N$ in foot and adductor muscle samples of *M. modiolus* and the total biomass of ascidians in an attached clump (including zeros in absence of a clump). Since we examined 2 to 3 *S. rustica* individuals from each of the paired A and MA clumps, we applied Type III sum of squares 3-factor nested ANOVA (Sample nested within Site, random; Site, random; *M. modiolus* presence, fixed) followed by Tukey honestly significant difference post-hoc tests to compare isotope ratios in *S. rustica* on mussels and gravel.

All mean values are given ±1 SE, with the exception of Fig. 4, where the error bars are standard deviation.

Table 1. Number of samples analyzed by group

		Site 1	Site 2
Modiolus modiolus			
With *Styela rustica*	Foot	10	3
	Adductor	10	3
Without *S. rustica*	Foot	10	3
	Adductor	10	3
S. rustica			
On *M. modiolus*		30	9
On gravel		29	9

RESULTS

Ascidians and horse mussels had clearly different isotope signatures without any interspecific overlap along the $\delta^{13}C$ axis regardless of the muscular tissue used, adductor or foot (Fig. 3). Species significantly

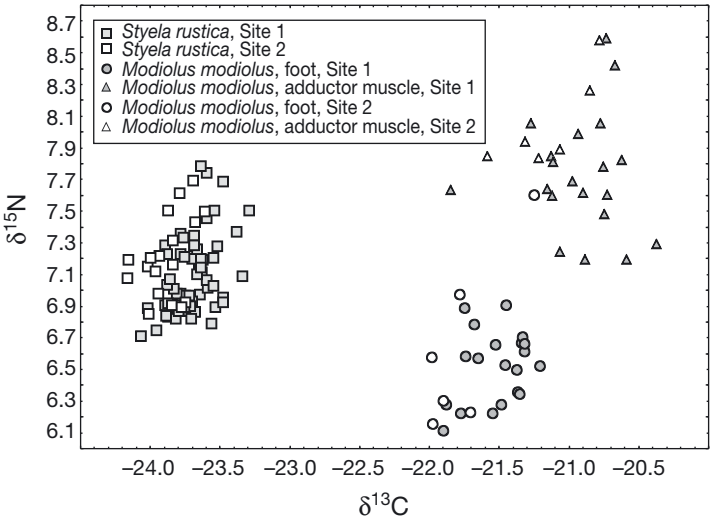

Fig. 3. Scatterplot of $\delta^{13}C$ and $\delta^{15}N$ ratios for *Modiolus modiolus* and *Styela rustica* from the 2 sites

affected both $\delta^{13}C$ and $\delta^{15}N$ ratios (Table 2). *Styela rustica* had average isotopic ratios of −23.746 ± 0.020 for $\delta^{13}C$ and 7.125 ± 0.028 for $\delta^{15}N$, whereas *Modiolus modiolus* had ratios of −21.582 ± 0.048 ($\delta^{13}C$) and 6.551 ± 0.063 ($\delta^{15}N$) in foot muscle and −20.970 ± 0.063 ($\delta^{13}C$) and 7.806 ± 0.074 ($\delta^{15}N$) in adductor muscle. Thus, the difference in $\delta^{15}N$ between the 2 bivalve muscle tissues was nearly twice as much as that between any of them and the ascidian muscle. However, average $\delta^{15}N$ ratio for the *M. modiolus* foot and adductor samples pooled was 7.178 ± 0.100 and was not significantly different from the average $\delta^{15}N$ ratio for *S. rustica*. Pairwise comparisons of isotope ratios for *M. modiolus* foot and adductor muscles revealed the significant difference both in $\delta^{13}C$ and $\delta^{15}N$ ($p < 0.001$, paired *t*-test). The C:N ratio was similar in adductor (3.49 ± 0.03) and foot (3.48 ± 0.01) ($p = 0.567$, *t*-test) samples; both values were significantly lower than the C:N ratio in *S. rustica* muscle (3.86 ± 0.02; $p < 0.001$, *t*-test). On the $\delta^{13}C$ vs. $\delta^{15}N$ scatterplot, *M. modiolus* samples from foot and adductor muscle grouped almost separately, with a small overlap along the $\delta^{15}N$ axis (Fig. 3).

The sampling site similarly affected $\delta^{13}C$ ratio in both species, regardless of the bivalve tissue used for the analysis. The effect of sampling site on $\delta^{15}N$ was significant in the analysis based on adductor muscle samples for *Modiolus modiolus* and insignificant in the one based on foot muscle samples (Tables 2 & 3). The immediate presence of a potential competitor had no effect on isotope ratios in either of the species studied (Fig. 4, Tables 4 & 5). There was no significant correlation of $\delta^{13}C$ or $\delta^{15}N$ either in *M. modiolus* foot or in adductor muscle with the total biomass of *Styela rustica* in an attached clump.

DISCUSSION

Most variation of $\delta^{13}C$ was interspecific. *Styela rustica* had clearly lower $\delta^{13}C$ than *Modiolus modiolus.* The highest variation in $\delta^{15}N$, however, occurred between the adductor and foot muscles of *M. modiolus*. Both the spatial and interspecific variation of $\delta^{15}N$ was markedly lower (Fig. 3).

Muscular tissue is the second most frequently used sample in stable isotope analysis of trophic relationships after the whole body (Boecklen et al. 2011; their Fig. 1c). Of 13 recent isotope-based studies on trophic sources and food partitioning that involved marine benthic bivalves, the following tissues were used: 6 authors examined whole bodies (Kang et al. 1999, 2009, Herman et al. 2000, Riera et al. 2002, Decottignies et al. 2007, Dubois et al. 2007), 3 examined foot muscle (Kasai et al. 2004, Kasai & Nakata 2005, Bucci et al. 2007), 2 studied unspecified (probably adductor) 'muscle' (Iken et al. 2001, Grall et al. 2006), 1 examined the adductor muscle (Page & Lastra 2003) and 1 focused on the mantle (Antonio et al. 2010). At the same time, the variation of stable isotope ratios between bivalve tissues has been assessed in several studies (Lorrain et al. 2002, Yokoyama & Ishihi 2006, Cabanellas-Reboredo et al. 2009, Deudero et al. 2009, Aya & Kudo 2010). Yokoyama & Ishihi (2006) examined both foot and adductor muscles in several bivalve species; 4 other papers did not compare different muscles. None of those studies, however, specifically related the variation of isotope ratios between tissues to their variation between taxonomically different organisms.

The difference we observed in *Modiolus modiolus* between adductor

Table 2. Sum of squares (SS) values from factorial ANOVA comparing isotope ratios by sampling site (1 and 2) and species (*Styela rustica* and *Modiolus modiolus*) based on *M. modiolus* foot and adductor muscle; $^{**}p < 0.01$, $^{***}p < 0.001$, ns: not significant

Source	Type	df	Adductor		Foot	
			$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$
Site	Random	1	0.55^{***}	0.680^{**}	0.63^{***}	0.188^{ns}
Species	Fixed	1	105.98^{***}	7.575^{***}	63.83^{***}	4.521^{***}
Site × Species	Random	1	0.00^{ns}	0.164^{ns}	0.01^{ns}	0.000^{ns}
Error		100	4.23	7.773	3.06	7.214

Table 3. Average $\delta^{13}C$ and $\delta^{15}N$ by species, tissue and sampling site

		$\delta^{13}C$		$\delta^{15}N$	
		Site 1	Site 2	Site 1	Site 2
Modiolus modiolus	Foot	−21.526 ± 0.046	−21.769 ± 0.113	6.523 ± 0.051	6.644 ± 0.228
	Adductor	−20.920 ± 0.071	−21.136 ± 0.124	7.729 ± 0.084	8.060 ± 0.122
Styela rustica		−23.704 ± 0.020	−23.885 ± 0.037	7.099 ± 0.032	7.211 ± 0.060

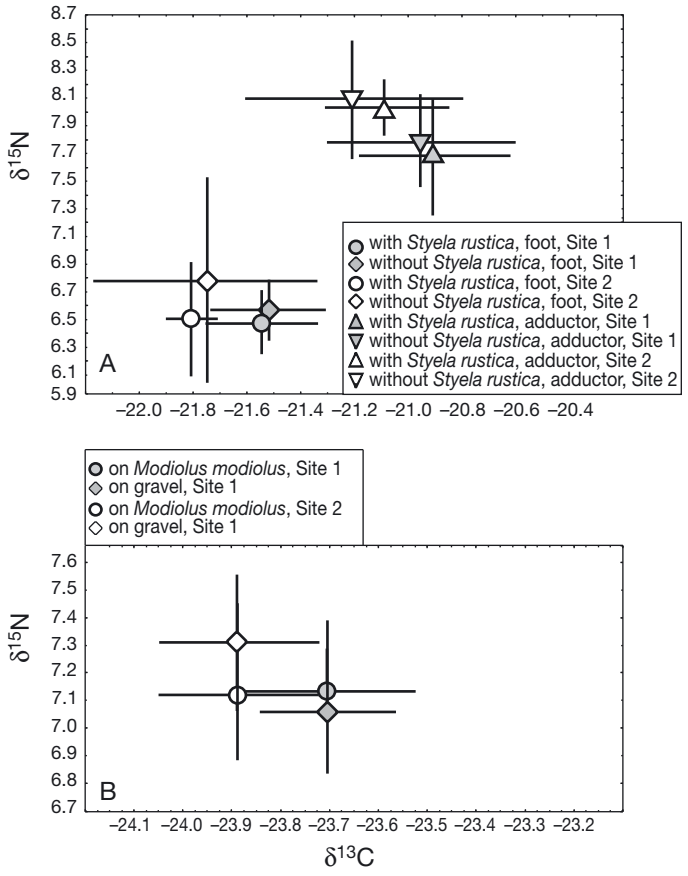

Fig. 4. Mean $\delta^{13}C$ and $\delta^{15}N$ ratios for (A) *Modiolus modiolus* with and without *Styela rustica* and (B) *S. rustica* on *M. modiolus* and on gravel. Error bars are SD

and foot muscles (0.4 to 0.6‰ for $\delta^{13}C$ and 1.2 to 1.4‰ for $\delta^{15}N$; see Table 3) is similar to that reported for *Cyclina sinensis* and *Ruditapes philippinarum* by Yokoyama & Ishihi (2006) for $\delta^{13}C$ but 2-fold higher for $\delta^{15}N$. Adult horse mussels are strongly anchored to gravel by multiple byssal threads and likely do not move much, making their foot muscle relatively idle compared to an adductor; this functional difference may explain the difference in isotope fractionation rates and composition between the 2 tissues. The important implication is that 1.2 to 1.4‰ is quite comparable to the interspecific differences interpreted as evidence of food resource partitioning. For instance, Dubois et al. (2007) examined isotope signatures of the suspension-feeders along the northern French coast. Barnacles *Elminius modestus* and blue mussels *Mytilus edulis* had similar $\delta^{13}C$ and clearly different $\delta^{15}N$, which the authors considered, along with the other results, as an argument against interspecific competition for food. However, the absolute difference they detected in $\delta^{15}N$ was ~1.5‰ (Dubois et al. 2007; their Fig. 2), with both mussels and barnacles examined as whole bodies. Depending on the proportion in which different tissues are mixed in a sample, which, in turn, depends on the body morphology of the organisms compared, interspecific variation in isotope signatures may be substantially biased by tissue-specific inconsistency in isotope fractionation rates. In trophic studies, the use of the same particular tissue to analyze taxonomically distant organisms is commonly considered a sufficient precaution against the bias caused by possible taxonomic variation of fractionation rates (Boecklen at al. 2011). Muscular tissue is preferentially used as it has low lipid content. It appears that our results, similarly to those of Yokoyama & Ishihi (2006), compromise this approach.

The presence of attached ascidians had no effect on the $\delta^{13}C$ and $\delta^{15}N$ of the horse mussels, regardless of the tissue studied. The presence of a live horse mussel as an underlying substrate had no effect on stable isotope ratios of the ascidian muscle tissue. Despite the high variation of stable isotope ratios between the tissues of *Modiolus modiolus*, the ratios were clearly different from those observed in *Styela rustica*. These results are consistent and do not support the hypothesis

Table 4. Comparison of *Modiolus modiolus* isotope signatures with and without *Styela rustica* attached. Mean ratios, paired *t*-test; all results presented were not significant

	$\delta^{13}C$	$\delta^{15}N$
With *S. rustica*		
Foot	−21.599 ± 0.060	6.486 ± 0.073
Adductor	−20.937 ± 0.077	7.752 ± 0.113
Without *S. rustica*		
Foot	−21.566 ± 0.076	6.616 ± 0.103
Adductor	−21.003 ± 0.102	7.860 ± 0.099

Table 5. Sum of squares (SS) values from nested ANOVA comparing isotope ratios in *Styela rustica* on gravel and *Modiolus modiolus* (factors 'Presence of *M. modiolus*' by Site and Sample nested in Site); ***p < 0.001, ns: not significant

Source	Type	df	$\delta^{13}C$	$\delta^{15}N$
Site	Random	1	0.47***	0.186^{ns}
Presence of *M. modiolus*	Fixed	1	0.00^{ns}	0.00^{ns}
Sample (site)	Random	11	0.19^{ns}	0.699^{ns}
Error		63	1.64	3.937
Means				
On gravel			−23.746 ± 0.026	7.112 ± 0.040
On *M. modiolus*			−23.741 ± 0.030	7.125 ± 0.042

that *M. modiolus* and *S. rustica* compete for food. Similar to Dubois et al. (2007), who studied isotope signatures of the ascidian *Ascidiella aspersa* and the mytilid bivalve *Mytilus edulis*, we found ~2‰ less $\delta^{13}C$ in ascidians than in bivalves. Ascidians *Halocynthia roretzi* showed 2.1‰ lower $\delta^{13}C$ and 2.4‰ lower $\delta^{15}N$ than co-cultured oysters *Crassostrea gigas* (Kang et al. 2009). This difference was less (1.39‰, $\delta^{13}C$; 1.29‰, $\delta^{15}N$) between monocultured ascidians and monocultured oysters. Since Kang et al. (2009) collected co-cultured ascidians and oysters at 1 site and monocultured ones at 2 other sites, the lower isotope ratio difference between monocultured filter feeders could result either from feeding interference or site-specific variation in food sources.

To the best of our knowledge, the diets of *Modiolus modiolus* and *Styela rustica* have been never specifically addressed. Both bivalves and ascidians can selectively consume feeding particles by size. According to the data on several species studied, mytilid mussels, to which family *M. modiolus* belongs, best retain particles of 2–3 to 6–7 µm in size. Retention efficiency is much lower at 1 to 2 µm. It also decreases at 7 to 22.5 µm, while most larger particles are rejected within pseudofaeces (Stuart & Klumpp 1984, Lesser et al. 1992, Defossez & Hawkins 1997).

Food capture mechanisms in ascidians ('mucus net trapping') are generally much more effective than mussels' 'cirral trapping' in retaining small (<4 µm) particles (Petersen 2007). *Pyura stolonifera* studied by Stuart & Klumpp (1984) unselectively consume 1–6.35 µm particles with 100% efficiency. Depending on particle concentration, *Styela clava* can switch from preferential ingestion of 5–20 µm particles at lower concentrations to smaller particles (<3.5 µm) at higher concentrations (Jiang et al. 2008). *Halocynthia pyriformis* shows similar switch from 5–15 µm to 2–5 µm as particle concentration rises (Armsworthy et al. 2001). In contrast, *Ciona intestinalis* achieves its highest retention rate at particle sizes >16 µm (Lesser et al. 1992). In addition to size-specific selectivity, mussels (but not ascidians) can also selectively retain organic particles and dismiss inorganic ones (Petersen 2007).

Styela rustica and *Modiolus modiolus* may utilize different size-fractions of available particulate organic matter. Ascidians are likely to use more nanoplankton and fewer larger particles than horse mussels. No significant shift of stable isotope signatures in the presence of a potential competitor shows that the effect of feeding interference (if present at all) is beyond the resolution of the method. Further studies of pairs of suspension-feeders with similar trophic requirements are needed to test the potential of the suggested approach to detect competition for food. Our results also show that interspecific variation in stable isotope ratios should be always related to variation among the tissues of the organisms studied prior to drawing conclusions on food sources partitioning.

Stable isotope ratios provided no evidence of feeding interference between *Modiolus modiolus* and *Styela rustica*, regardless of whether the comparison was made between these 2 species or within 1 species in the presence or absence of the potential competitor. Horse mussels showed an unexpectedly high difference in $\delta^{15}N$ between the foot and adductor muscles, which should be taken in account in further food web studies based on stable isotope signatures.

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LITERATURE CITED

Antonio ES, Kasai A, Ueno M, Kurikawa Y and others (2010) Consumption of terrestrial organic matter by estuarine molluscs determined by analysis of their stable isotopes and cellulase activity. Estuar Coast Shelf Sci 86:401–407

Armsworthy SL, MacDonals BA, Ward JA (2001) Feeding activity, absorption efficiency and suspension feeding processes in the ascidian, *Halocynthia pyriformis* (Stolidobranchia: Ascidiacea): responses to variation in diet-quantity and quality. J Exp Mar Biol Ecol 260:41–69

Aya FA, Kudo I (2010) Isotopic shifts with size, culture habitat, and enrichment between the diet and tissues of the Japanese scallop *Mizuhopecten yessoensis* (Jay, 1857). Mar Biol 157:2157–2167

Berger VY (2009) Production of fucoid brown algae *Ascophyllum nodosum* and *Fucus vesiculosus* in the White Sea. Russ J Mar Biol 35:413–415

Boecklen WJ, Yarnes CT, Cook BA, James AC (2011) On the use of stable isotopes in trophic ecology. Annu Rev Ecol Evol Syst 42:411–440

Bosley KL, Wainright SC (1999) Effects of preservatives and acidification on the stable isotope ratios (^{15}N:^{14}N, ^{13}C:^{12}C) of two species of marine animals. Can J Fish Aquat Sci 56:2181–2185

Bucci JP, Rebach S, DeMaster D, Showers WJ (2007) A comparison of blue crab and bivalve $\delta^{15}N$ tissue enrichment in two North Carolina estuaries. Environ Pollut 145: 299–308

Buss LW (1979) Bryozoan overgrowth interactions — the interdependence of competition for space and food. Nature 281:475–477

Cabanellas-Reboredo M, Deudero S, Blanco A (2009) Stable-isotope signatures ($\delta^{13}C$ and $\delta^{15}N$) of different tissues of *Pinna nobilis* Linnaeus, 1758 (Bivalvia): isotopic variations among tissues and between seasons. J Molluscan Stud 75:343–349

Castilla JC, Lagos NA, Cerda M (2004) Marine ecosystem engineering by the alien ascidian *Pyura praeputialis* on a mid-intertidal rocky shore. Mar Ecol Prog Ser 268: 119–130

Dalerum F, Angerbjörn A (2005) Resolving temporal variation in vertebrate diets using naturally occurring stable isotopes. Oecologia 144:647–658

Decottignies P, Beninger PG, Rincé Y, Riera P (2007) Trophic interactions between two introduced suspension-feeders, *Crepidula fornicata* and *Crassostrea gigas*, are influenced by seasonal effects and qualitative selection capacity. J Exp Mar Biol Ecol 342:231–241

Defossez JM, Hawkins AJS (1997) Selective feeding in shellfish: size-dependent rejection of large particles within pseudofaeces from *Mytilus edulis, Ruditapes philippinarum* and *Tapes decussatus*. Mar Biol 129:139–147

Deudero S, Cabanellas M, Blanco A, Tejada S (2009) Stable isotope fractionation in the digestive gland, muscle and gills tissues of the marine mussel *Mytilus galloprovincialis.* J Exp Mar Biol Ecol 368:181–188

Dobretsov S, Wahl M (2008) Larval recruitment of the blue mussel *Mytilus edulis*: the effect of flow and algae. J Exp Mar Biol Ecol 355:137–144

Dubois S, Orvain F, Marin-Léal JC, 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

Fielding PJ, Weerts KA, Forbes AT (1994) Macroinvertebrate communities associated with intertidal and subtidal beds of *Pyura stolonifera* (Heller) (Tunicata: Ascidiacea) on the Natal coast. S Afr J Zool 29:46–53

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

Haken AE, Batzli GO (1996) Effects of availability of food and interspecific competition on diets of prairie voles (*Microtus ochrogaster*). J Mammal 77:315–324

Hanson JM, Leggett WC (1986) Effect of competition between two freshwater fishes on prey consumption and abundance. Can J Fish Aquat Sci 43:1363–1372

Herman PMJ, Middelburg JJ, Widdows J, Lucas CH, Heip CHR (2000) Stable isotopes as trophic tracers: combining field sampling and manipulative labelling of food resources for macrobenthos. Mar Ecol Prog Ser 204:79–92

Iken K, Brey T, Wand U, Voigt J, Junghans P (2001) Food web structure of the benthic community at the Porcupine Abyssal Plain (NE Atlantic): a stable isotope analysis. Prog Oceanogr 50:383–405

Jiang AL, Yu Z, Cai WG, Wang CH (2008) Feeding selectivity of the marine ascidian *Styela clava.* Aquaculture 39: 1190–1197

Kang CK, Sauriau PG, Richard P, Blanchard GF (1999) Food sources of the infaunal suspension-feeding bivalve *Cerastoderma edule* in a muddy sandflat of Marennes-Oléron Bay, as determined by analyses of carbon and nitrogen stable isotopes. Mar Ecol Prog Ser 187:147–158

Kang CK, Choy EJ, Hur YB, Myeong JI (2009) Isotopic evidence of particle size-dependent food partitioning in co-cultured sea squirt *Halocynthia roretzi* and Pacific oyster *Crassostrea gigas.* Aquat Biol 6:289–302

Kasai A, Nakata A (2005) Utilization of terrestrial organic matter by the bivalve *Corbicula japonica* estimated from stable isotope analysis. Fish Sci 71:151–158

Kasai A, Horie H, Sakamoto W (2004) Selection of food sources by *Ruditapes philippinarum* and *Mactra veneriformis* (Bivalva: Mollusca) determined from stable isotope analysis. Fish Sci 70:11–20

Lesser MP, Shumway SE, Cucci T, Smith J (1992) Impact of fouling organisms on mussel rope culture: interspecific competition for food among suspension-feeding invertebrates. J Exp Mar Biol Ecol 165:91–102

Lohse DP (2002) Relative strengths of competition for space and food in a sessile filter feeder. Biol Bull 203:173–180

Lorrain A, Paulet YM, Chauvaud L, Savoye N, Donval A, Saout C (2002) Differential $\delta^{13}C$ and $\delta^{15}N$ signatures among scallop tissues: implications for ecology and physiology. J Exp Mar Biol Ecol 275:47–61

McCutchan JH, Lewis WM Jr, Kendall C, McGrath CC (2003) Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. Oikos 102:378–390

Okamura B (1984) The effects of ambient flow velocity, colony size, and upstream colonies on the feeding success of Bryozoa. I. *Bugula stolonifera* Ryland, an arborescent species. J Exp Mar Biol Ecol 83:179–193

Okamura B (1985) The effects of ambient flow velocity, colony size, and upstream colonies on the feeding success of Bryozoa. II. *Conopeum reticulum* (Linnaeus), an encrusting species. J Exp Mar Biol Ecol 89:69–80

Okamura B (1988) The influence of neighbors on the feeding of an epifaunal bryozoan. J Exp Mar Biol Ecol 120: 105–123

Page HM, Lastra M (2003) Diet of intertidal bivalves in the Ría de Arosa (NW Spain): evidence from stable C and N isotope analysis. Mar Biol 143:519–532

Petersen JK (2007) Ascidian suspension feeding. J Exp Mar Biol Ecol 342:127–137

Phillips DL, Newsome SD, Gregg JW (2005) Combining sources in stable isotope mixing models: alternative methods. Oecologia 144:520–527

Pullen J, LaBarbera M (1991) Modes of feeding in aggregations of barnacles and the shape of aggregations. Biol Bull 181:442–452

Riera P, Stal LJ, Nieuwenhuize J (2002) $\delta^{13}C$ versus $\delta^{15}N$ of co-occurring molluscs within a community dominated by *Crassostrea gigas* and *Crepidula fornicata* (Oosterschelde, The Netherlands). Mar Ecol Prog Ser 240: 291–295

Seed R (1996) Patterns of biodiversity in the macro-invertebrate fauna associated with mussel patches on rocky shores. J Mar Biol Assoc UK 76:203–210

Stuart V, Klumpp DW (1984) Evidence for food-resource partitioning by kelp-bed filter feeders. Mar Ecol Prog Ser 16:27–37

Yakovis EL, Artemieva AV, Shunatova NN, Varfolomeeva MA (2008) Multiple foundation species shape benthic habitat islands. Oecologia 155:785–795

Yokoyama H, Ishihi Y (2006) Variation in $\delta^{13}C$ and $\delta^{15}N$ among different tissues of three estuarine bivalves: implications for dietary reconstructions. Plankton Benthos Res 1:178–182

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