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 δ13C and δ15N 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 m²) M. modiolus with and without ascidians attached and in S. rustica from nearby (within 0.25 m²) 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 into account the substantial difference in SIR between the 2 M. modiolus muscular tissues studied: −21.582 ± 0.048 (δ13C) and 6.551 ± 0.063 (δ15N) in foot muscle versus −20.970 ± 0.063 (δ13C) and 7.806 ± 0.074 (δ15N) 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 δ$^{15}$N and 0.3 to 1.3‰ for δ$^{13}$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 & Ishii 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
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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 m² 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 m² plot, SCUBA divers obtained 1 large (71 to 97 mm long) *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 m² 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 δ13C and δ15N in samples. Stable isotope ratios were calculated as 

\[ \delta X (\text{‰}) = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 10^3, \]

where \( X \) is 13C or 15N, and \( R \) is the corresponding ratio of 13C:12C or 15N:14N. Standards were Pee Dee Belemnite and atmospheric N2 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 δ13C and 0.15‰ for δ15N (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 δ13C and δ15N 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 δ13C and δ15N 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.

### RESULTS

Ascidians and horse mussels had clearly different isotope signatures without any interspecific overlap along the δ13C axis regardless of the muscular tissue used, adductor or foot (Fig. 3). Species significantly

![Fig. 3. Scatterplot of δ13C and δ15N ratios for *Modiolus modiolus* and *Styela rustica* from the 2 sites](image-url)
affected both δ¹³C and δ¹⁵N ratios (Table 2). Styela rustica had average isotopic ratios of −23.746 ± 0.020 for δ¹³C and 7.125 ± 0.028 for δ¹⁵N, whereas Modiolus modiolus had ratios of −21.582 ± 0.048 (δ¹³C) and 6.551 ± 0.063 (δ¹⁵N) in foot muscle and −20.970 ± 0.063 (δ¹³C) and 7.806 ± 0.074 (δ¹⁵N) in adductor muscle. Thus, the difference in δ¹⁵N between the 2 bivalve muscle tissues was nearly as much as that between any of them and the ascidian muscle. However, average δ¹⁵N ratio for the M. modiolus foot and adductor samples pooled was 7.178 ± 0.100 and was not significantly different from the average δ¹⁵N ratio for S. rustica. Pairwise comparisons of isotopic ratios for M. modiolus foot and adductor muscles revealed the significant difference both in δ¹³C and δ¹⁵N (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 δ¹³C vs. δ¹⁵N scatterplot, M. modiolus samples from foot and adductor muscle grouped almost separately, with a small overlap along the δ¹⁵N axis (Fig. 3).

The sampling site similarly affected δ¹³C ratio in both species, regardless of the bivalve tissue used for the analysis. The effect of sampling site on δ¹⁵N 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 isotopic ratios in either of the species studied (Fig. 4, Tables 4 & 5). There was no significant correlation of δ¹³C or δ¹⁵N either in M. modiolus foot or in adductor muscle with the total biomass of Styela rustica in an attached clump.

### DISCUSSION

Most variation of δ¹³C was interspecific. Styela rustica had clearly lower δ¹³C than Modiolus modiolus. The highest variation in δ¹⁵N, however, occurred between the adductor and foot muscles of M. modiolus. Both the spatial and interspecific variation of δ¹⁵N 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 isotopic ratios between tissues to their variation between taxonomically different organisms. The difference we observed in Modiolus modiolus between adductor

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### 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

<table>
<thead>
<tr>
<th>Source</th>
<th>Type</th>
<th>df</th>
<th>Adductor δ¹³C</th>
<th>Adductor δ¹⁵N</th>
<th>Foot δ¹³C</th>
<th>Foot δ¹⁵N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site</td>
<td>Random</td>
<td>1</td>
<td>0.55***</td>
<td>0.680**</td>
<td>0.63***</td>
<td>0.188ns</td>
</tr>
<tr>
<td>Species</td>
<td>Fixed</td>
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<td>105.98***</td>
<td>7.575***</td>
<td>63.83***</td>
<td>4.521***</td>
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<td>0.00ns</td>
<td>0.164ns</td>
<td>0.01ns</td>
<td>0.000ns</td>
</tr>
<tr>
<td>Error</td>
<td></td>
<td>100</td>
<td>4.23</td>
<td>7.773</td>
<td>3.06</td>
<td>7.214</td>
</tr>
</tbody>
</table>

### Table 3. Average δ¹³C and δ¹⁵N by species, tissue and sampling site

<table>
<thead>
<tr>
<th>Species</th>
<th>Site 1 δ¹³C</th>
<th>Site 2 δ¹³C</th>
<th>Site 1 δ¹⁵N</th>
<th>Site 2 δ¹⁵N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Modiolus modiolus</td>
<td>-21.526 ± 0.046</td>
<td>-21.769 ± 0.113</td>
<td>6.523 ± 0.051</td>
<td>6.644 ± 0.228</td>
</tr>
<tr>
<td>Styela rustica</td>
<td>-20.920 ± 0.071</td>
<td>-21.136 ± 0.124</td>
<td>7.729 ± 0.084</td>
<td>8.060 ± 0.122</td>
</tr>
<tr>
<td></td>
<td>-23.704 ± 0.020</td>
<td>-23.885 ± 0.037</td>
<td>7.099 ± 0.032</td>
<td>7.211 ± 0.060</td>
</tr>
</tbody>
</table>
and foot muscles (0.4 to 0.6‰ for δ\textsuperscript{13}C and 1.2 to 1.4‰ for δ\textsuperscript{15}N; see Table 3) is similar to that reported for 
\textit{Cyclina sinensis} and \textit{Ruditapes philippinarum} by 
Yokoyama & Ishihi (2006) for δ\textsuperscript{13}C but 2-fold higher 
for δ\textsuperscript{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 inter-
preted 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 
\textit{Elminius modestus} and blue mussels 
\textit{Mytilus edulis} had similar δ\textsuperscript{13}C and 
clearly different δ\textsuperscript{15}N, which the au-
thors considered, along with the other 
results, as an argument against interspecific competition for food. However, the absolute difference they 
detected in δ\textsuperscript{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 organ-
isms compared, interspecific variation in isotope sign-
natures may be substantially biased by tissue-specific 
 inconsistency in isotope fractionation rates. In trophic 
studies, the use of the same particular tissue to ana-
lyze taxonomically distant organisms is commonly 
considered a sufficient precaution against the bias 
caused by possible taxonomic variation of fractiona-
tion rates (Boecklen et al. 2011). Muscular tissue is 
preferentially used as it has low lipid content. It ap-
pears that our results, similarly to those of Yokoyama 
& Ishihi (2006), compromise this approach.

The presence of attached ascidians had no effect on 
the δ\textsuperscript{13}C and δ\textsuperscript{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 iso-
toipe ratios of the ascidian muscle tissue. Despite the 
high variation of stable isotope ratios between the tis-
sues of \textit{Modiolus modiolus}, the ratios were clearly dif-
erent from those observed in \textit{Styela rustica}. These re-
sults are consistent and do not support the hypothesis 

![Fig. 4. Mean δ\textsuperscript{13}C and δ\textsuperscript{15}N ratios for (A) \textit{Modiolus modiolus} with and without \textit{Styela rustica} and (B) \textit{S. rustica} on \textit{M. modiolus} and on gravel. Error bars are SD](image)

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

<table>
<thead>
<tr>
<th>Source</th>
<th>Type</th>
<th>df</th>
<th>δ\textsuperscript{13}C</th>
<th>δ\textsuperscript{15}N</th>
</tr>
</thead>
<tbody>
<tr>
<td>With \textit{S. rustica}</td>
<td>Foot</td>
<td>–21.599 ± 0.060</td>
<td>6.486 ± 0.073</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Adductor</td>
<td>–20.937 ± 0.077</td>
<td>7.752 ± 0.113</td>
<td></td>
</tr>
<tr>
<td>Without \textit{S. rustica}</td>
<td>Foot</td>
<td>–21.566 ± 0.076</td>
<td>6.616 ± 0.103</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Adductor</td>
<td>–21.003 ± 0.102</td>
<td>7.860 ± 0.099</td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Source</th>
<th>Type</th>
<th>df</th>
<th>δ\textsuperscript{13}C</th>
<th>δ\textsuperscript{15}N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site</td>
<td>Random</td>
<td>1</td>
<td>0.47***</td>
<td>0.186*</td>
</tr>
<tr>
<td>Presence of \textit{M. modiolus}</td>
<td>Fixed</td>
<td>1</td>
<td>0.00*</td>
<td>0.00*</td>
</tr>
<tr>
<td>Sample (site)</td>
<td>Random</td>
<td>11</td>
<td>0.19*</td>
<td>0.699*</td>
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<tr>
<td>Error</td>
<td>63</td>
<td>1.64</td>
<td>3.937</td>
<td></td>
</tr>
</tbody>
</table>

**Means**

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>δ\textsuperscript{13}C</th>
<th>δ\textsuperscript{15}N</th>
</tr>
</thead>
<tbody>
<tr>
<td>On gravel</td>
<td>63</td>
<td>–23.746 ± 0.026</td>
<td>7.112 ± 0.040</td>
</tr>
<tr>
<td>On \textit{M. modiolus}</td>
<td></td>
<td>–23.741 ± 0.030</td>
<td>7.125 ± 0.042</td>
</tr>
</tbody>
</table>
that *M. modiolus* and *S. rustica* compete for food. Similar to Dubois et al. (2007), who studied isotope signatures of the ascidian *Ascidia aspera* and the mytilid bivalve *Mytilus edulis*, we found ~2% less δ¹³C in ascidians than in bivalves. Ascidians *Halocynthia roretzi* showed 2.1% lower δ¹³C and 2.4% lower δ¹⁵N than co-cultured oysters *Crassostrea gigas* (Kang et al. 2009). This difference was less (1.39%, δ¹³C; 1.29%, δ¹⁵N) 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 δ¹⁵N 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**


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


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