Sedimentary and particulate organic matter: mixed sources for cockle Cerastoderma glaucum in a shallow pond, Western Mediterranean

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Abstract – Seasonal changes in feeding habits and diet of the cockle Cerastoderma glaucum (Mollusca, Bivalvia) were analysed using carbon ($\delta^{13}$C) and nitrogen ($\delta^{15}$N) stable isotopes. I aimed to investigate the role of benthic and pelagic sources in the diet of this dominant infaunal bivalve on a western Mediterranean sandy bottomed pond. Adult C. glaucum and all potential organic sources (particulate and sedimentary organic matter, seagrass, macroalgae, heterotrophic detritus) were collected and analysed for $\delta^{13}$C and $\delta^{15}$N. In total 5 dominant organic sources were found, ranging between $-21.0$ and $-8.0\%_{ee}$ for $\delta^{13}$C and from 3.0 to about $7.0\%_{ee}$ for $\delta^{15}$N. C. glaucum assimilated fraction ranged between $-14.0$ to $-15.0\%_{ee}$ and $6.0$ to $9.0\%_{ee}$ respectively, for carbon and nitrogen, while its estimated fractionation seasonally varied between 1 and 1.5 for carbon and was about 3 for nitrogen. C. glaucum reflected the isotopic composition of its food while maintaining, throughout the year, a fairly constant isotopic composition in its assimilated fraction, despite the high variability in isotopic composition of available sources.

Key words: Infaunal bivalve / Stable isotope / Feeding behaviour / Fractionation / Mediterranean

1 Introduction

Pelagic microalgae (viz. phytoplankton) has been often invoked as the prime component in the diet of suspension feeding bivalves in marine habitats (Newell 1979; Loo and Rosenberg 1989; \textit{sensu} Nadon and Himmelman 2006). However, other forms of detritus can also contribute to the diet of infaunal bivalves. In temperate shallow waters, many bivalve species (e.g. Cerastoderma sp., Tapes sp. etc.) colonise sandy bottoms in detritus-rich and highly wind-stressed environments (Sarà 2006), frequently living in association with seagrasses (Sarà et al. 2003). This typical habitat, together with the general oligotrophy of temperate waters (Margalef 1985) in which filter feeders sort particles, led me to consider the role of other detrital forms of non-pelagic origin (vascular, macroalgal, eutrophic) in infaunal bivalve diet. In such shallow highly dynamic systems, it is not clear how particulate organic matter (POM) and sedimentary organic matter (SOM) can be clearly delimited. POM is probably best described as being continually deposited in the sediments, while the SOM is constantly being re-suspended by wind action (thus, becoming POM; Sarà 2006). Thus, boundaries between POM and SOM are blurred, and suspension consumers seem rely on organic matter originating from both. However, direct
observation of bivalve habitat distribution allows us to hypothe-
size that these organisms assimilate different organic mat-
ter types as a function of their availability (sensu Rosenzweig
1981). This could be the case with Cerastoderma glaucum, an
infaunal suspension-feeding bivalve common in the southern
Mediterranean. It dominates sandy bottoms in sheltered bays
and lagoons and is a key-species of macrobenthic assemblage
at temperate latitudes. It is therefore the perfect model to in-
crease understanding of the role each organic source plays in
the diet of infaunal bivalves living in the above-described en-
vironments, where seagrass dominates, and frequent resuspen-
sion events distribute sedimentary detritus through the water
column. Stomach content analysis is not a reliable tool for
studying the diet of such small invertebrates as infaunal bi-
valves (Nadon and Himmelman 2006). Therefore, stable C and
N isotopes have been used over the last decade as an alter-
native method for investigating diet frames of invertebrates in
coastal environments (Fry and Sherr 1984). Stable isotopes can
be used because the isotopic composition of organisms reflects
that of the food available to them, disregarding a certain degree
of metabolic fractionation (DeNiro and Epstein 1978; Fry and
Sherr 1984; Minagawa and Wada 1984; Vander Zanden and
Rasmussen 2001). However, this commonly accepted scheme is
complicated by recent findings by Nadon and Himmelman (2006),
which reveal a certain degree of uncertainty about weighting the role of pelagic vs. benthic organic matter in sup-
porting diet of benthic invertebrates using stable isotopes ra-
tios. Although some studies have reported on organic assim-
ilation of infaunal species in temperate and shallow intertidal
systems (see Peterson et al. 1985; Dunton and Schell 1987;
Goering et al. 1990; Hobson and Welch 1992; Davenport and
Bax 2002; Kang et al. 2003), I have found no papers to date
that specifically address C. glaucum. A few papers using sta-
ble isotopes deal with the co-generic species C. edule (Kang
et al. 1999; Herman et al. 2000; Sauriau and Kang 2000). In
these studies, C. edule is reported to rely mostly on pelagic
resources, with only occasional dietary input from benthic
sources. The aims of the present study were to identify in
the field, using stable isotopes (δ13C and δ15N): i) the trophic habi-
tat of C. glaucum and, ii) seasonal changes in its diet as a func-
tion of trophic availability.

2 Materials and methods

2.1 Study site

The study was carried out in a pond of a saltworks in
Marsala, western Sicily (37° 52’ N; 12° 28’ E) in 2004. Cer-
astoderma glaucum were collected from a pond (60 000 m2;
48 000 m3; 80 cm average depth) which has a mostly muddy
sand bottom. The pond bottom is subject to diel wind-
induced cycles of resuspension-sedimentation-accumulation
(Sarà 2006), which are repeated daily, making a highly tur-
bid environment (Mannino and Sarà 2006; Sarà 2006). A bal-
ance between evaporation and rainfall controls the hydrody-
namics and maintains the water level. There is wide seasonal
fluctuation in water temperature (min. 9 °C in December, max.
30 °C in August), and salinity values typical of a hyper-saline
environment (about 38%e in winter and 50%e in summer)
(Sarà et al. 1999, 2000). The muddy sand bottoms of the pond
in the study area are covered by the seagrass Cymodocea no-
dosa, while Cystoseira sp., Chaetomorpha linum and Lau-
rencea papillosa are the dominant macroalgae. Phytoplankton
density is quite low (Pusceddu et al. 1999; Sarà et al. 1999;
Sarà et al. 2000; Sarà 2006), with annual average chlorophyll-
a concentrations of 0.7 ± 0.04 µg L−1 (range 0.5–1.7 µg L−1;
Sarà 2006), while the microphytobenthic biomass showed a
higher annual average than those previously measured in other
parts of the western Mediterranean (3.0 ± 0.3 µg g−1 = range
1.6–6.7 µg g−1; Sarà 2006).

2.2 Data collection and analysis

Adult Cerastoderma glaucum (mean length 25.0±2.2 mm)
and all potential organic sources were collected seasonally
between February and December 2004. Samples of seawa-
ter, collected in 5 L Niskin bottles, were filtered through pre-
combusted (450 °C, 4 h) Whatman GF/F filters for the isotopic
analysis of the POM. The first centimetre of sediment was
scraped from the corers to investigate the isotopic composi-
tion of the SOM. Cymodocea nodosa and macroalgae (Chaeto-
morpha linum, Cystoseira sp. and Laurencia papillosa) were
collected seasonally by hand. Epiphytic material was removed
from leaves and stems by scraping. Cerastoderma glaucum
specimens were collected manually, cleaned of epiphytes and
kept alive in filtered pond water for at least 24 h to allow gut
evacuation. Ejection (faeces) and egestion (pseudofaeces) ma-
terials were collected separately using tweezers. Biodeposited
material produced by the dominant bivalve of the pond hard
substrata, the Lessessupian mussel Brachidontes pharaonis (Sarà
et al. 2000), was also collected, and since B. pharaonis isotopic
values were similar to those of C. glaucum, the two sets were
pooled. C. glaucum specimens were killed by freezing and the
flesh dissected from the shell. All samples (POM, SOM, pri-
mary organic matter sources, C. glaucum flesh, C. glau-
cum and B. pharaonis faeces and pseudofaeces) were dried
at 60 °C for several hours (from 24 to 72 h as a function of the
substrate analysed) and ground with a mortar and pes-
tle (Abed-Navandi and Dworschak 2005). The isotopic anal-
yses were performed using a Finnigan Delta-S isotope ratio
mass spectrometer. Isotopic values were expressed in parts
per thousand as deviations from standards (Peedee belemnite lime-
stone for δ13C and nitrogen in air for δ15N; δ13C or δ15N =
\[ \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 10^3 \]
where \( R = ^{13}\text{C}/^{12}\text{C} \) or \( ^{15}\text{N}/^{14}\text{N} \). A one-way ANOVA was performed to test seasonal differ-
ences in isotopic values between each potential organic source.
For the analyses, the heterogeneity of variances was tested
using Cochran’s C test prior to the analysis of variance, and
the Student-Newman-Keuls (SNK) test (Underwood 1997)
allowed the appropriate means comparison. Since it is possible
to determine the contributions of different food sources to the
diet of the cockle from the stable isotope data, but not to dis-
cern whether these are derived from pelagic or benthic de-
tritus, simple mixing models were used in order to identify the
organic sources most important to the isotopic composition
of C. glaucum’s assimilated fraction (Phillips and Gregg
2003). Thus, I focused on the 5 original sources of organic
matter in the pond and ran straight mixing models for each

season (C. glaucum as the target and seagrass, macroalgae, phyttoplankton, diatoms, and bivalve biodeposition material as the potential sources). In the absence of captive experiments specifically designed to calculate the fractionation rate of bi-valves in Mediterranean, mixing models were carried out using 1‰ fractionation of the C isotope and 3.0‰ fractionation of nitrogen (DeNiro and Epstein 1978; DeNiro and Epstein 1981; Fry and Sherr 1984; Minagawa and Wada 1984). However, these chosen values fell well within the range of fractionation used in the current literature regarding bivalves (e.g. Riera et al. 1996; Riera et al. 2000; Sauriau and Kang 2000). To calculate individual contributions from each of the 5 food sources per season, models were run using the IsoSource routine developed by Phillips and Gregg (2003) and the trimmed 1 to 99 percentile range (i.e. Abed-Navandí and Dworschak 2005) and the mean value of source contributions were reported. In the models, all macroalgae (Chaetomorpha linum, Cystoseira sp. and Laurencia papillosa) were pooled together, as their trophic contributions to bivalve diet are very similar as their trophic contributions to bivalve diet are very similar (Phillips and Gregg 2003). Nevertheless, the diet seemed mostly to comprise organic matter, mostly benthic, and the organic matter seemed to derive from bivalve biodeposition material from the two dominant invertebrates, C. glaucum and the mussel B. pharaonis. In the study area, the most enriched organic matter source (ANOVA p < 0.05) was represented by the seagrass C. nodosa, for which δ13C values were mostly depleted for carbon in spring and more enriched in winter (Table 1). As regards nitrogen, values were depleted in autumn and quite variable during the rest of the year (Table 1). The three main dominant macroalgae (Laurencia papillosa, Cystoseira sp. and Chaetomorpha linum) were pooled into a single class (hereafter called macroalgae). Macroalgae organic matter had a more depleted isotopic composition (ANOVA p < 0.05) with respect to the dominant seagrass; carbon ranged between −17.7 and −19.5‰, while nitrogen was more enriched. The biodeposition materials of the two dominant suspension feeders (Cerastoderma glaucum and Brachidontes pharaonis) were the main heterotrophic organic matter sources. Carbon isotopic values were more enriched in summer and autumn (~−15.2‰) and significantly depleted (p < 0.05) in winter and spring (~−20.8‰). In contrast, δ15N was depleted in winter and summer (~−1.5‰) and more enriched in spring and autumn (~4.7‰).

3 Results

3.1 Organic matter sources in the saltworks

The dominant organic sources potentially available for suspension feeders via POM and SOM were (Fig. 1): the seagrass Cymodocea nodosa, dominant macroalgae and biodeposition material from the two dominant invertebrates, C. glaucum and the mussel B. pharaonis. In the study area, the most enriched organic matter source (ANOVA p < 0.05) was represented by the seagrass C. nodosa, for which δ13C values were mostly depleted for carbon in spring and more enriched in winter (Table 1). As regards nitrogen, values were depleted in autumn and quite variable during the rest of the year (Table 1). The three main dominant macroalgae (Laurencia papillosa, Cystoseira sp. and Chaetomorpha linum) were pooled into a single class (hereafter called macroalgae). Macroalgae organic matter had a more depleted isotopic composition (ANOVA p < 0.05) with respect to the dominant seagrass; carbon ranged between −17.7 and −19.5‰, while nitrogen was more enriched. The biodeposition materials of the two dominant suspension feeders (Cerastoderma glaucum and Brachidontes pharaonis) were the main heterotrophic organic matter sources. Carbon isotopic values were more enriched in summer and autumn (~−15.2‰) and significantly depleted (p < 0.05) in winter and spring (~−20.8‰). In contrast, δ15N was depleted in winter and summer (~−1.5‰) and more enriched in spring and autumn (~4.7‰).

Isotopic composition of Cerastoderma glaucum and contribution of organic matter sources to its diet. The annual means of the cockle’s isotopic composition were −14.9 ± 0.6‰ and 8.1 ± 1.2‰ for carbon and nitrogen, respectively (Table 1). δ13C values ranged between the summer and autumn depleted values (~−15.6 ± 0.5‰ and ~−15.0 ± 0.3‰, respectively) and about −14.4 ± 0.2‰ in winter and spring. δ15N values were constantly up at about 8.6 ± 0.5‰ from spring to autumn, and depleted in winter (6.4 ± 0.1‰). The outcome of seasonal mixing models regarding the contribution of each source of dominant organic matter is summarised in Table 2, while the mixing polygon (corrected for fractionation rates; 1.0‰ for carbon and 3.0‰ for nitrogen) calculated on a yearly basis is shown (Fig. 1). In addition, Figs. 2a–e shows the δ13C and δ15N signatures, and the distribution of feasible contributions from each source to the cockle diet. Many of the seasonal contribution ranges presented in Table 2 included the zero value (e.g. in winter, the sand microflora 1–99 percentiles = 0–8%), which means that no clear conclusion can be made about those food sources’ contribution to the C. glaucum diet (Phillips and Gregg 2003). Nevertheless, the diet seemed mostly to comprise organic matter, mostly benthic, and the organic matter seemed to derive from bivalve biodeposition (1–99th percentile: 36–48%) and C. nodosa (1–99th percentile: 6–32%). Phyttoplankton (probabilistically represented only in summer; 1–99th percentile: 6–44%) and macroalgae (probabilistically represented only in spring; 1–99th percentile: 7–48%) played a definite role in the yearly diet of the studied cockle. No conclusions could be made about the ingestion of sand microflora, either.
Fig. 2. Histograms for $\delta^{13}$C and $\delta^{15}$N signatures showing the distribution of feasible contributions from each source to the cockle diet.
Table 1. Statistics of isotopic composition (±se; standard errors for means) of all potential organic matter sources contributing to the seasonal isotopic composition of Cerastoderma glaucum assimilated fraction. Bivalve biodeposits = biodeposition organic matter from bivalves. In the table, isotopic signatures of pure phytoplankton and sand microflora (mostly benthic diatoms) were not reported as extrapolated from current literature.

<table>
<thead>
<tr>
<th>Source</th>
<th>Winter</th>
<th>Spring</th>
<th>Summer</th>
<th>Autumn</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerastoderma glaucum</td>
<td>−14.4</td>
<td>0.2</td>
<td>6.4</td>
<td>0.6</td>
<td>−7.4</td>
</tr>
<tr>
<td>Cymodocea nodosa detritus</td>
<td>−10.6</td>
<td>0.2</td>
<td>6.2</td>
<td>0.4</td>
<td>8.3</td>
</tr>
<tr>
<td>Macroalgae</td>
<td>−15.0</td>
<td>0.2</td>
<td>8.9</td>
<td>0.5</td>
<td>5.1</td>
</tr>
<tr>
<td>Bivalve biodeposits</td>
<td>−14.9</td>
<td>0.6</td>
<td>8.1</td>
<td>1.2</td>
<td>6.4</td>
</tr>
</tbody>
</table>

Table 2. Seasonal contribution (%) and trimmed percentile range (1% and 99%) of organic sources potentially available to Cerastoderma glaucum diet as calculated by means mixing models.

<table>
<thead>
<tr>
<th>Source</th>
<th>Winter</th>
<th>Spring</th>
<th>Summer</th>
<th>Autumn</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phytoplankton</td>
<td>1.8</td>
<td>3.5</td>
<td>26.1</td>
<td>21.4</td>
<td>10.4</td>
</tr>
<tr>
<td>Sand microflora</td>
<td>3.1</td>
<td>43.8</td>
<td>13.1</td>
<td>22.8</td>
<td>16.0</td>
</tr>
<tr>
<td>Cymodocea nodosa detritus</td>
<td>37.0</td>
<td>22.2</td>
<td>6.2</td>
<td>16.9</td>
<td>20.5</td>
</tr>
<tr>
<td>Macroalgae</td>
<td>2.1</td>
<td>27.2</td>
<td>16.5</td>
<td>23.9</td>
<td>9.5</td>
</tr>
<tr>
<td>Biodeposits</td>
<td>56.0</td>
<td>3.3</td>
<td>38.1</td>
<td>15.0</td>
<td>43.6</td>
</tr>
</tbody>
</table>

4 Discussion

The results presented here show that although there was high seasonal biological variability in the available organic sources in the study area, thiscockle incorporates only a narrow isotopic range of its assimilated fraction. It did, in fact, reflect the isotopic composition of its food, but maintained, throughout the year, a fairly constant isotopic composition of its assimilated fraction (−14.8 ± 0.6‰ and 8.1 ± 1.2‰, respectively, for δ13C and δ15N) despite the high variability in isotopic composition of available organic sources. This complies with the hypothesis that bivalves act as long-term integrators of the isotopic composition of their food, levelling short-term intermittent changes (daily, weekly, seasonal) (McKinney et al. 2001). Cerastoderma glaucum in the Sicilian saltworks ponds was generally enriched with respect to pelagic sources, which is consistent with a diet partially supported by benthic primary sources. Nevertheless, the findings of Nadon and Himmelman (2006) on bivalve δ13C values show that these may have been misinterpreted for years as indicators of the importance of benthic primary producers as a food source for the benthos. Despite this, in the case of the present bivalve, C. glaucum in the pond appears to be relying on benthic sources with a possible only negligible contribution by pelagic sources. Indeed, C. glaucum showed carbon isotopic signatures of ~6‰, heavier than phytoplankton. Such an observation would be in line with the particular trophic features of the study area which, from a pelagic point of view, was highly oligotrophic (suspended chlorophyll-a concentration less than 1 µg L−1; Sarà 2006), and consequently, apart from biases due to misinterpretation of stable isotope findings, we can assume that fresh pelagic organic matter plays only a minor role in the diet of cockles. Accordingly, phytoplankton does not seem of great importance in this context, apart from in the summertime (6–44%). The presence of phytoplankton signature in the summer assimilation fraction of cockles corresponds with the phytoplankton spring bloom usually recorded at these temperate latitudes (Margalef 1985) and in the study area (Sarà 2006). On the other hand, a period of 2–3 months (from spring to summer) appears sufficient for a change in the isotopic ratio to occur in bivalves (Riera and Richard 1997). The rest of the year, the cockle diet appears to be dependent on the turbulent environment which it inhabits, i.e. strongly constrained by benthic sources due to wind-induced physical coupling between water column and sediments (sensu Sarà 2006). Thus for most of the year, organic matter contribution to C. glaucum diet appears to derive mainly from seagrass detritus and biodeposition material, as shown by the mixing models. Firstly, since, the pond was a turbid environment (the suspended organic matter reached huge concentrations, up to 230 mg L−1; due to continual cycles of wind-resuspension, Sarà et al. 2000; Sarà 2006), biodeposition material by bivalves could be abundantly elicited.
(Sarà et al. 2000). Biodeposition material is an important source of protenaceous material, mainly consisting of mucus (protein-polysaccharide complexes; Cognie and Barillé 1999). When suspended matter concentrations reach 3–5 mg l⁻¹, bivalves begin to produce pseudofaeces at a mean rate of 5–10 mg h⁻¹ (Navarro et al. 1992; Navarro and Widdows 1997).

Field observations have revealed that the bottom of the pond is completely covered by a fine, brownish organic aggregate layer (limo, G. Sarà, pers. obs.). Such liquid aggregates (Alber and Valiela 1994) were present throughout 2004, forming a layer above the sediment of at least 3–4 cm. It could be an important and readily available source, consisting mainly of labile organic compounds of secondary origin. The cockles seem to rely heavily on this detritus (yearly basis 36–48%).

The second main organic source in the diet of cockles appeared to be seagrass organic matter from the dominant Cymodocea nodosa. This detritus was the most enriched in organic carbon and was the predominant source of primary organic matter in both density and biomass, covering more than 70% (about 42,000 m²) of the pond bottom. Being a perennial vascular plant with main production peaks in spring and summer and a minimum in winter (Pérez and Romero 1994), it is a constant source of refractory detritus (Mann 1988). Degradation of its vascular tissues would proceed as such: loss of labile compounds, microbial colonization and utilization, and finally mechanical fragmentation (Odum et al. 1973; Zieman et al. 1984). Thus, by means of physical (fragmentation induced by wind-resuspension) and biological (action by decomposers) mechanisms, seagrass detritus is continually rearranged and becomes available, over time, to heterotrophic consumers. Its vascular detritus represented the basal isotopic signal of the pond and its C:N ratio, at around 18–20 (G. Sarà unpublished data), fell well within an adequate range for animal nutrition (17:1; Thayer et al. 1977). This explains why the seagrass signature in the diet of C. glauca was consistent throughout the year (6–32%). The other benthic organic sources considered in the present study (sand microflora and macroalgae) appeared not to contribute much to the diet of pond cockles. Although macroalgae organic matter in the pond is the second most dominant primary source (Sarà et al. 2000) and provides nutrient-rich detritus with a very high turnover rate (e.g. Gracilaria tikvahiae and Ulva lactuca; Zieman et al. 1984; Buchsbaum et al. 1991), its contribution to the cockle diet appeared to be negligible for most of the year. This could have been due to the fact that its rapid decaying process would limit its “appearance” over the year in cockle tissues by stable isotopes (sensu Mc Kinney et al. 2001). The same applied to sand microflora, as which is unusually high for a pond at these latitudes (Sarà et al. 2000; Pusceddu et al. 2003). Sand microflora (mostly diatoms; Sarà 2006) should represent an optimal labile organic source for bivalves (Kang et al. 2003), constantly available throughout the year. However, the outcome of mixing models, suggested that sand microflora assumed only a minor role in the diet of cockles throughout 2004. In conclusion, the main result is that stable isotope analysis, in a multi-trophic environment like shallow marine temperate ponds, appears unable to elucidate complicated dietary frames of invertebrates. Indeed, in the absence of captive experiments to investigate the rate of fractionation of organisms, and without other analyses (e.g. stomach contents) supporting the investigation, stable isotopes are inefficient in helping to discern the role of different similar organic sources in the diet of secondary consumers like filter feeders.

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