

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 ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) 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 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. In total 5 dominant organic sources were found, ranging between -21.0 and -8.0‰ for $\delta^{13}\text{C}$ and from 3.0 to about 7.0‰ for $\delta^{15}\text{N}$. *C. glaucum* assimilated fraction ranged between $-14.0/-15.0\text{‰}$ and $6.0/9.0\text{‰}$ 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

Résumé – **Matières organiques particulières et sédimentaires : sources mixtes pour les coques *Cerastoderma glaucum* d'une lagune méditerranéenne.** Les changements saisonniers dans les habitudes et le régime alimentaire de la coque *Cerastoderma glaucum* (Mollusque, Bivalve) ont été analysés en utilisant les isotopes stables du carbone ($\delta^{13}\text{C}$) et de l'azote ($\delta^{15}\text{N}$). Mon objectif est d'étudier le rôle des sources benthiques et pélagiques dans l'alimentation du bivalve dominant d'une lagune méditerranéenne, et qui vit enfoui dans le fond sablo-vaseux. Le *C. glaucum* adulte et toutes les sources organiques potentielles (matière particulaire et sédimentaire, végétaux marins, macroalgues, et détritiques hétérotrophes) ont été collectés et analysés pour leur $\delta^{13}\text{C}$ et $\delta^{15}\text{N}$. Au total 5 sources organiques dominantes ont été trouvées, comprises entre -21 et -8‰ pour $\delta^{13}\text{C}$, et entre 3 à environ 7‰ pour $\delta^{15}\text{N}$. *C. glaucum* assimile des fractions entre $-14/-15 \text{‰}$ et $6/9 \text{‰}$, respectivement, pour le carbone et l'azote, tandis que la matière particulaire varie de façon saisonnière entre 1 et 1,5 pour le carbone et environ 3 pour l'azote. *C. glaucum* reflète la composition isotopique de ses aliments tandis que tout au long de l'année, il maintient une composition isotopique à peu près constante dans la fraction assimilée en dépit d'une grande variabilité dans la composition isotopique des sources disponibles.

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; *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, heterotrophic) 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

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observation of bivalve habitat distribution allows us to hypothesize that these organisms assimilate different organic matter types as a function of their availability (*sensu* Rozensweig 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 increase understanding of the role each organic source plays in the diet of infaunal bivalves living in the above-described environments, where seagrass dominates, and frequent resuspension 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 bivalves (Nadon and Himmelman 2006). Therefore, stable C and N isotopes have been used over the last decade as an alternative 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 supporting diet of benthic invertebrates using stable isotopes ratios. Although some studies have reported on organic assimilation 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 stable 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 ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$): i) the trophic habitat of *C. glaucum* and, ii) seasonal changes in its diet as a function 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. *Cerastoderma 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 turbid environment (Mannino and Sarà 2006; Sarà 2006). A balance between evaporation and rainfall controls the hydrodynamics 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‰ in winter and 50‰ in summer)

(Sarà et al. 1999, 2000). The muddy sand bottoms of the pond in the study area are covered by the seagrass *Cymodocea nodosa*, while *Cystoseira* sp., *Chaetomorpha linum* and *Laurencia 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 $0.7 \pm 0.04 \mu\text{g L}^{-1}$ (range 0.5–1.7 $\mu\text{g L}^{-1}$; Sarà 2006), while the microphytobenthic biomass showed a higher annual average than those previously measured in other parts of the western Mediterranean ($3.0 \pm 0.3 \mu\text{g g}^{-1}$ – range 1.6–6.7 $\mu\text{g g}^{-1}$; Sarà 2006).

2.2 Data collection and analysis

Adult *Cerastoderma glaucum* (mean length 25.0 ± 5.2 mm) and all potential organic sources were collected seasonally between February and December 2004. Samples of seawater, 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 composition of the SOM. *Cymodocea nodosa* and macroalgae (*Chaetomorpha 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) materials were collected separately using tweezers. Biodeposited material produced by the dominant bivalve of the pond hard substrata, the Lessepsian 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, primary organic matter sources, *C. glaucum* flesh, *C. glaucum* 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 pestle (Abed-Navandi and Dworschak 2005). The isotopic analyses 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 limestone for $\delta^{13}\text{C}$ and nitrogen in air for $\delta^{15}\text{N}$): $\delta^{13}\text{C}$ or $\delta^{15}\text{N} = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 10^3$ where $R = {}^{13}\text{C}/{}^{12}\text{C}$ or ${}^{15}\text{N}/{}^{14}\text{N}$. A one-way ANOVA was performed to test seasonal differences 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 discern whether these are derived from pelagic or benthic detritus, 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

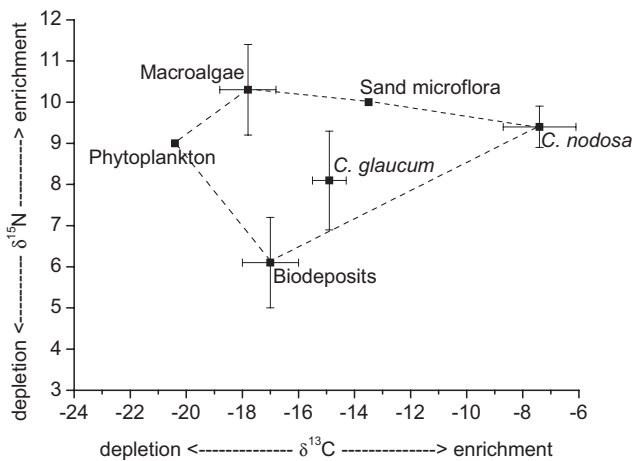


Fig. 1. Biplot ($\delta^{13}\text{C}$ - $\delta^{15}\text{N}$) of isotopic compositions of all the most abundant organic sources potentially available to *Cerastoderma glaucum* throughout the study year, corrected for trophic fractionation effect. Dashed lines encompass the areas where isotopic value for *C. glaucum* (corrected for trophic fractionation) is expected when its diets consist of a mix of the dominant organic matter sources in the study systems. Error bars are means \pm 1 SE.

season (*C. glaucum* as the target and seagrass, macroalgae, phytoplankton, diatoms, and bivalve biodeposition material as the potential sources). In the absence of captive experiments specifically designed to calculate the fractionation rate of bivalves 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-Navandi 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 for carbon and nitrogen (Sarà et al. 2000; Vizzini et al. 2002; Sarà 2006). Furthermore, since the contribution of unicellular autotrophic components (i.e. phytoplankton and sand microflora) to the cockle diets may be virtually indistinguishable due to resuspension (Sarà 2006), pure isotopic signatures of phytoplankton ($\delta^{13}\text{C} = 21.4\text{‰}$; $\delta^{15}\text{N} = 6.0\text{‰}$) and sand microflora (mostly diatoms; $\delta^{13}\text{C} = -14.5\text{‰}$; $\delta^{15}\text{N} = 6.5\text{--}7.0\text{‰}$) were extrapolated from the Mediterranean literature (Dauby 1989; Sarà et al. 2003, 2006; Sarà 2006) and incorporated in the mixing models. However, no seasonal variations for phytoplankton and sand microflora were adopted in mixing models; I therefore considered the average values as those reported in literature.

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 $\delta^{13}\text{C}$ 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 ($\sim -15.2\text{‰}$) and significantly depleted ($p < 0.05$) in winter and spring ($\sim -20.8\text{‰}$). In contrast, $\delta^{15}\text{N}$ was depleted in winter and summer ($\sim 1.5\text{‰}$) and more enriched in spring and autumn ($\sim 4.7\text{‰}$).

Isotopic composition of *Cerastoderma glaucum* and contribution of organic matter sources to its diet. The annual means of the cockle's isotopic composition were $-14.9 \pm 0.6\text{‰}$ and $8.1 \pm 1.2\text{‰}$ for carbon and nitrogen, respectively (Table 1). $\delta^{13}\text{C}$ values ranged between the summer and autumn depleted values ($-15.6 \pm 0.5\text{‰}$ and $-15.0 \pm 0.3\text{‰}$, respectively) and about $-14.4 \pm 0.2\text{‰}$ in winter and spring. $\delta^{15}\text{N}$ values were constantly up at about $8.6 \pm 0.5\text{‰}$ from spring to autumn, and depleted in winter ($6.4 \pm 0.1\text{‰}$). 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 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ 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%). Phytoplankton (probabilistically represented only in summer; 1–99th percentile: 6–44%) and macroalgae (probabilistically represented only in spring; 1–99th percentile: 7–48%) played no definite role in the yearly diet of the studied cockle. No conclusions could be made about the ingestion of sand microflora, either.

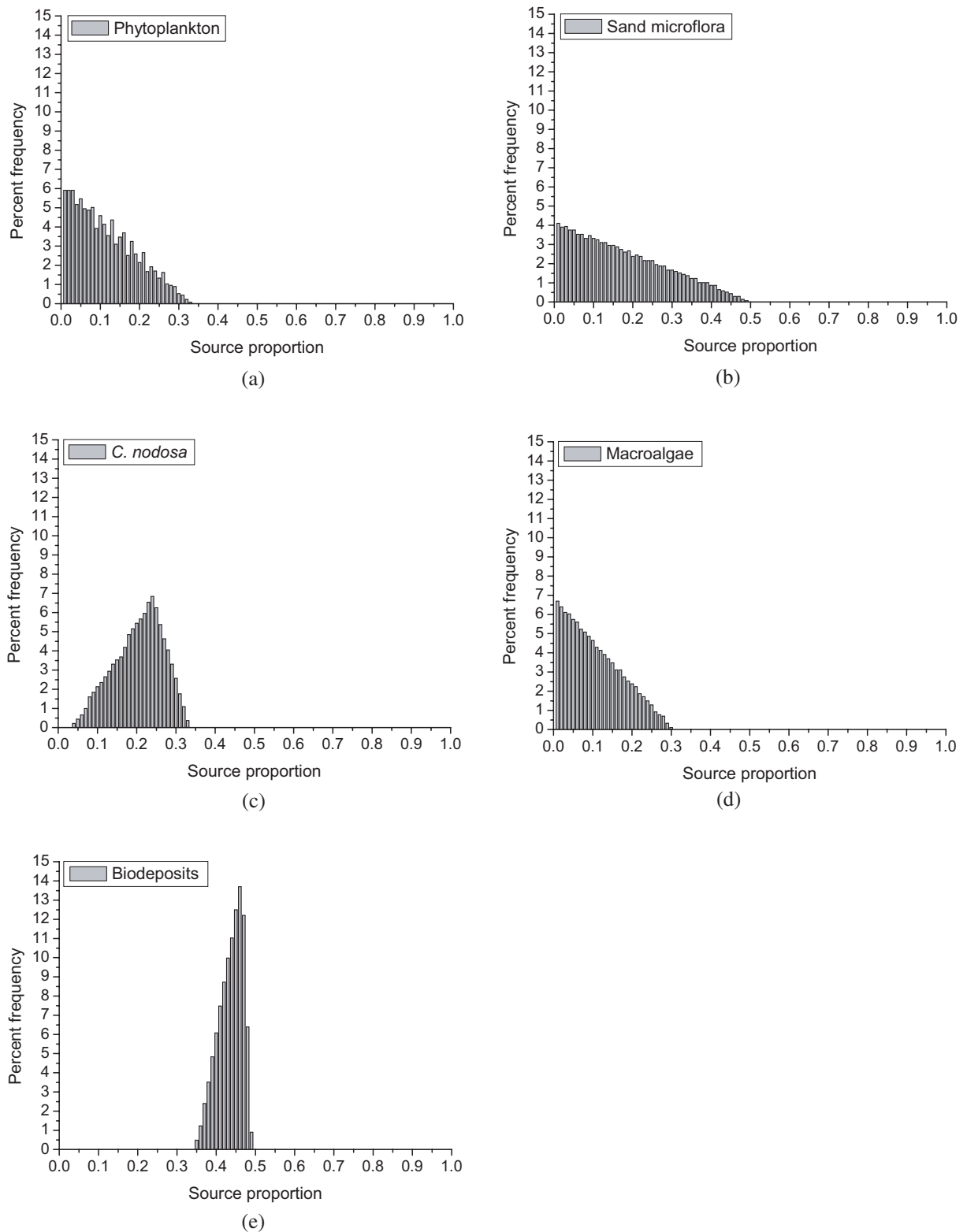


Fig. 2. Histograms for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures showing the distribution of feasible contributions from each source to the cockle diet.

Table 1. Statistics of isotopic composition (\pm 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.

	<i>Cerastoderma glaucum</i>				<i>Cymodocea nodosa</i> detritus			
	$\delta^{13}\text{C}$		$\delta^{15}\text{N}$		$\delta^{13}\text{C}$		$\delta^{15}\text{N}$	
	Mean	\pm se	Mean	\pm se	Mean	\pm se	Mean	\pm se
Winter	-14.4	0.2	6.4	0.6	-7.4	0.3	6.0	0.5
Spring	-14.4	0.3	9.0	1.1	-10.6	0.2	6.2	0.4
Summer	-15.6	0.1	8.0	0.4	-7.7	0.1	8.3	1.1
Autumn	-15.0	0.2	8.9	0.5	-7.8	0.2	5.1	0.6
Year	-14.9	0.6	8.1	1.2	-8.4	1.3	6.4	0.5
	Macroalgae				Bivalve biodeposits			
	$\delta^{13}\text{C}$		$\delta^{15}\text{N}$		$\delta^{13}\text{C}$		$\delta^{15}\text{N}$	
	Mean	\pm sd	Mean	\pm sd	Mean	\pm sd	Mean	\pm sd
Winter	-19.5	1.1	6.2	0.9	-20.4	0.5	1.3	0.1
Spring	-19.3	1.5	7.9	0.8	-21.1	0.9	5.9	0.5
Summer	-17.7	0.8	8.0	1.0	-15.0	0.8	1.8	0.7
Autumn	-18.7	0.9	6.9	1.2	-15.5	0.2	3.4	0.9
Year	-18.8	1.0	7.3	1.1	-18.0	1.0	3.1	1.1

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.

Source	Winter		Spring		Summer		Autumn		Year	
	%	Ile. range	%	Ile. range	%	Ile. range	%	Ile. range	%	Ile. range
Phytoplankton	1.8	0–5	3.5	0–9	26.1	6–44	21.4	0–49	10.4	0–29
Sand microflora	3.1	0–8	43.8	0–84	13.1	0–36	22.8	0–53	16.0	0–44
<i>Cymodocea nodosa</i>	37.0	34–39	22.2	0–46	6.2	0–17	16.9	0–34	20.5	6–32
Macroalgae	2.1	0–6	27.2	7–48	16.5	0–45	23.9	0–57	9.5	0–27
Biodeposits	56.0	55–57	3.3	0–8	38.1	33–46	15.0	0–29	43.6	36–48

4 Discussion

The results presented here show that although there was high seasonal biological variability in the available organic sources in the study area, this cockle 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 ($-14.8 \pm 0.6\text{‰}$ and $8.1 \pm 1.2\text{‰}$, respectively, for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) 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 $\delta^{13}\text{C}$ 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 $\sim 6\text{‰}$, 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 $1 \mu\text{g L}^{-1}$; 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 proteinaceous 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. glaucum* 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 expressed by the sedimentary chlorophyll-a concentration, 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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