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Sources of organic matter in Ria Formosa revealed by stable isotope analysis

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Abstract — The aim of this study is to assess the major sources of organic matter for macroconsumers in the Ria Formosa tidal lagoon. The C, S and N isotopic natural abundances of abundant primary producers of particulate organic matter (POM) and *Mytilus galloprovincialis* muscle and digestive gland were analysed. The chlorophyll a (Chl a), the suspended particulate matter (SPM) and the POM were measured along the Faro-Olhão channel. The range of variation of stable isotope values among primary producers in Ria Formosa was low suggesting difficulties in the assessment of their relative contribution to higher levels of the food web. Chl a values decreased from outer station to inner station, while SPM and POM values increased. The multiple isotope approach illustrates that POM values along the Faro-Olhão channel, may result from a mixture of upland plants, benthic plants and phytoplankton. Mussel values indicate a selective diet of benthic macrophytes and phytoplankton, with the relative proportions of each determined by the location in the channel. During winters, the upland plants may be an important source of organic matter in the inner lagoon while phytoplankton was an important source of organic matter in the outer lagoon. © 1999 Éditions scientifiques et médicales Elsevier SAS

Organic matter / carbon / sulphur / nitrogen / stable isotope natural abundances / coastal lagoon / Ria Formosa

1. INTRODUCTION

The high productivity of coastal ecosystems supports complex food webs which span terrestrial and aquatic environments including consumers with a variety of feeding strategies [11, 24]. Determining the fate of the carbon fixed by primary producers and analysing the trophic structure in salt-marsh estuarine communities is one of the most important considerations in understanding how these ecosystems function. However, our understanding is complicated by the variety of potentially important organic matter sources and the variety of pathways available for nutrient, water, organic matter, and energy exchanges [18, 19, 20].

Stable isotope analysis has more recently been used as an alternative to the standard food web analysis approach (gut content analysis, direct observation both in the field and laboratory, radiotracer techniques and the use of immunological methods) and, in some cases, is a better tool for food web analysis [14, 16]. The strength of this approach is based on the assumption that isotopic ratios are conservative and that physical mixing of end-member sources determines the isotopic distributions of organic matter in natural systems [2, 7]. Early studies attempting to determine sources of organic matter in salt-marsh food webs only used carbon stable isotope. However, this approach may lead to erroneous conclusions because of the difficulty in distinguishing organic matter sources with similar and overlapping $\delta^{13}C$ [11, 18, 21]. Fry and Sherr [8], Peterson et al. [19] and Peterson and Howarth [18] demonstrated that a multiple tracer approach with stable isotopes of carbon, sulphur and nitrogen can distinguish food resources.

The basis for the use of stable isotope analysis in food web studies is that organisms retain the stable isotope signals of the foods they assimilate [4]. Animals are similar in the isotopic compositions of their diets for C and S, but average 3 to 5 ‰ heavier than dietary N [14, 17]. The C isotopic approach is based on the fact that different classes of plants have different sources of CO_2 (air vs. water) and different C isotopic fractionations associated with major photosynthetic pathways (C_3 vs. C_4). The isotopic difference

between seawater sulphate and sulphides makes sulphur useful in distinguishing pelagic vs. benthic producers and phytoplankton vs. salt-marsh plants [7, 9, 18]. Benthic systems and marsh plants tend to be richer in sulphur derived from sulphides and have a lighter $\delta^{34}S$ signal [14]. Because consumers average 3 to 5 ‰ heavier $\delta^{15}N$ than their prey, $\delta^{15}N$ values are useful in estimating the trophic level [7, 17, 18].

The success of these approaches is predicted upon the ability to distinguish the food resources isotopically, including seasonal and spatial variations. Stable isotope analysis can also be applied in conjunction with standard approaches to food web analysis.

This study was aimed at determining the major sources of organic matter for macroconsumers in a shallow mesotidal lagoon, the Ria Formosa, in southern Portugal. Specific goals were: i) to assess the C, S and N isotopic abundances of important primary producers; ii) to assess the amount of chlorophyll a (Chl a), suspended particulate matter (SPM) and particulate organic matter (POM) along the Faro-Olhão channel, where most of the water circulation of the western part of lagoon occurs; and iii) to assess the relative contributions of candidate food sources along the Faro-Olhão channel. Stable isotope values of POM and of the filter feeder *Mytilus galloprovincialis* muscle and digestive gland were analysed. POM isotope values represent the spatial variability of both plant detritus and phytoplankton, suspended in the water column. The digestive gland of mussels contains the POM filtered from water by the animals but not yet assimilated. Isotope values of *M. galloprovincialis* muscle are a time integrated signal of the ingested POM that is assimilated.

2. MATERIALS AND METHODS

2.1. Study site

The Ria Formosa is a system of salt-marshes, mudflats and channels, extending for about 55 km along the coast of southern Portugal, permanently connected to the Atlantic Ocean by six tidal channels (*figure 1*), with no relevant freshwater input. The average depth of the lagoon is 2 m (although the Faro-Olhão channel can reach 30 m). Salinity is in the range of 35.5 to 36.9 ‰ all year round [6]. Benthic macrophytes, such as the salt-marsh species *Spartina maritima*, the seagrass species *Zostera noltii* and *Cymodocea nodosa*, and green mat-forming macroalgae (Ulvales), are reported in the literature to be the main primary producers [22].

The total area of the lagoon is 170 km^2, with an exposed intertidal area of +50 km^2, during spring tides. The intertidal area covered by *S. maritima* is 8 km^2, the area covered by seagrasses is 8.2 km^2 (*Z. noltii* and *C. nodosa*) and the area covered by macroalgae is 2.5 km^2 (Fidalgo, unpubl. data).

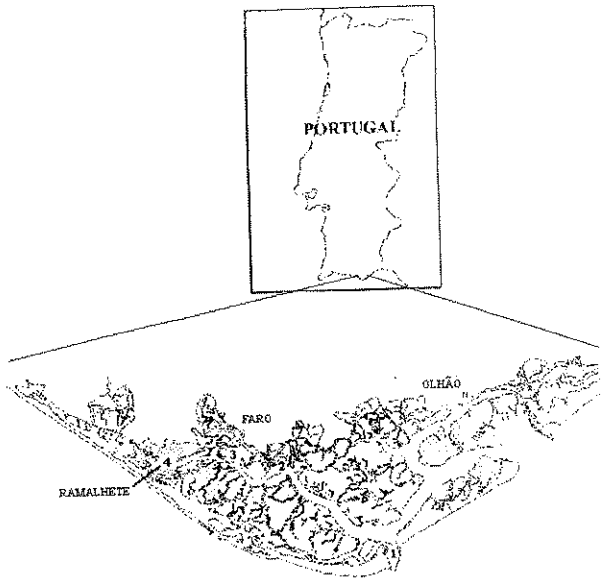

Figure 1. Ria Formosa and sampling sites. Numbers are sampling sites: 1, station 1; 2, station 2; 3, station 3; and 4, station 4.

2.2. Sample collection and processing

The macrophytes *S. maritima*, *Z. noltii*, *C. nodosa*, *Ulva* sp. and the salt-marsh red algae *Bostrychia scorpioides*, were sampled mostly in the summer of 1995 at the 'Esteiro do Ramalhete' (*figure 1*, station 4). Water samples for Chl a, SPM and POM analysis were collected at high tide along the Faro-Olhão channel, from the outlet area, 'Barra Nova' (station 1) to the inside channel area, 'Esteiro do Ramalhete' (station 4), in late winter of 1996 and stored in the dark until filtrations in the laboratory were done. Water samples for POM isotope analyses were vacuum filtered until clogged onto precombusted filters, at 1 m depth, at each station, in the same winter. Juvenile mussels were also collected in the winter of 1996, on navigation marks at the same stations. We chose juvenile life stages because their tissue reflects recently metabolised food sources, rather than adults which would have an integrated isotope signal from a variety of food sources.

Samples for analysis of multiple stable isotopes were processed following the sample preparation guidelines of the Stable Isotope Laboratory, Ecosystem Center, MBL (MA, USA), where analyses were done. Tissues of macrophyte species were cleaned of mud

and debris. When present, epiphytes were removed by scraping with a razor blade. Samples were then dried to constant weight at 60 °C. The dried tissues were ground to a fine powder with a mortar and pestle. Plant tissue samples for ^{13}C were checked for contamination by carbonates. Subsamples of the ground sample were acidified with several drops of 10 % HCl while being observed under a dissecting microscope. If bubbling occurred the whole sample was acidified, redried at 60 °C and stored in glass vials. Plant tissue samples for ^{34}S were ground and rinsed in deionised water to remove seawater sulphate (resuspended in deionised water, centrifuged for 5 min, and supernatant discarded; this procedure was repeated three times), redried at 60 °C and stored in glass vials.

Animal tissue samples for C, N and S stable isotope analysis were dissected to isolate muscle tissue and were dried at 60 °C. The dried tissues were ground to a fine powder with a mortar and pestle. Ground animal ^{13}C tissue samples, suspected of having a carbonate contamination, were acidified with 10 % HCl and redried at 60 °C. Ground animal tissue samples for $\delta^{34}S$ were rinsed in deionised water to remove seawater sulphate and redried at 60 °C.

Each isotope analysis of a species represented a subsample from a pooled sample of several individuals. This was done to minimise the variability associated with analyses of different individual organisms and to gain enough material for S isotope analyses [15].

Chl a was measured by the Lorenzen [13] method while SPM and POM were determined by adapting the Strickland and Parsons [23] method.

2.3. Stable isotope measurements

The abundances of ratios of stable isotopes at natural abundance levels are expressed in the δ notation:

$$\delta\ (‰) = [(R_{sample}/R_{standard}) - 1] \times 10^3$$

where R is $^{13}C:^{12}C$, $^{34}S:^{32}S$, or $^{15}N:^{14}N$; standard is Peedee Belemnite for C, Canyon Diablo triolites for S, and air for N [2].

Samples for ^{13}C and ^{15}N were analysed using an automated elemental analyzer with a cryogenic purification system coupled to a Finnigan Delta S isotope ratio mass spectrometer [10]. After vacuum sealing in Pyrex tubes and combustion, samples for $\delta^{34}S$ were precipitated as $BaSO_4$, converted to SO_2 and analysed on a Finnigan MAT 251 [5].

3. RESULTS AND DISCUSSION

The range of variation of stable isotope values among primary producers of Ria Formosa was low (*figures 2, 3, 4*; *table II*) suggesting difficulties in the assessment of their relative contribution to higher levels of the food web. $\delta^{34}S$ for *S. maritima*, 12.3 ‰,

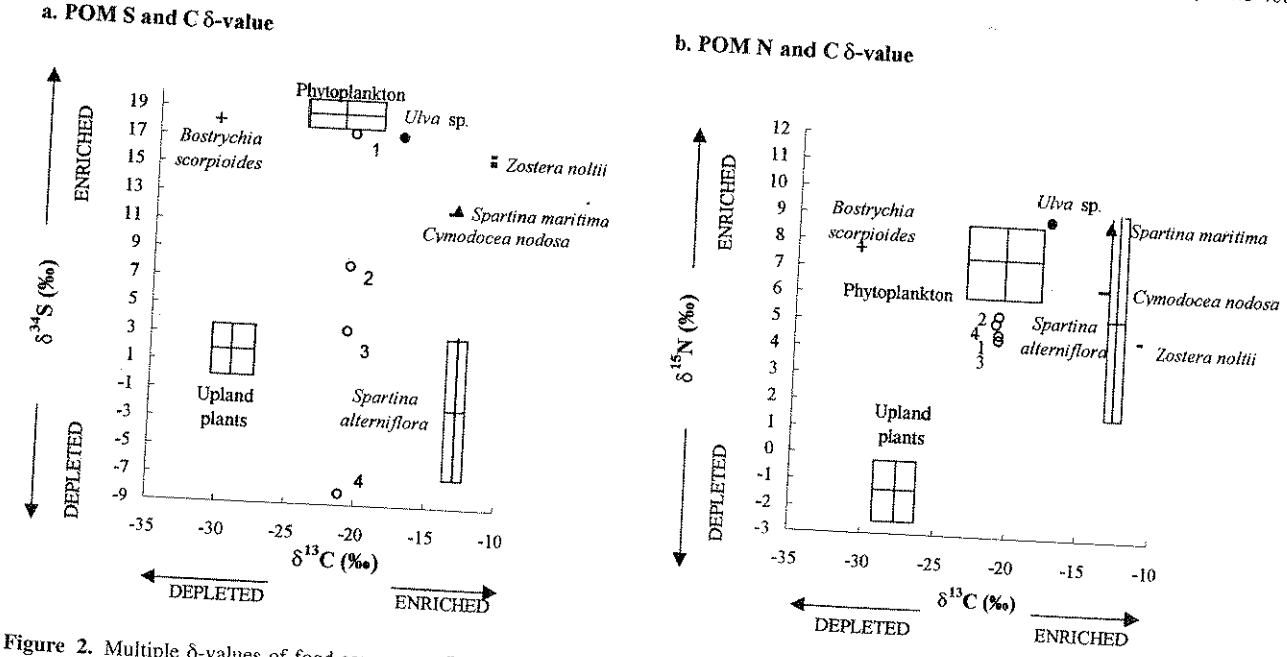

Figure 2. Multiple δ-values of food sources vs. POM of Ria Formosa. Bars are means ± standard deviation of literature derived δ-values of primary producers [4, 19]. Symbols represent δ-values for Ria Formosa primary producers and POM (numbers represent sampling stations): a) carbon and sulphur δ-values; and b) carbon and nitrogen δ-values.

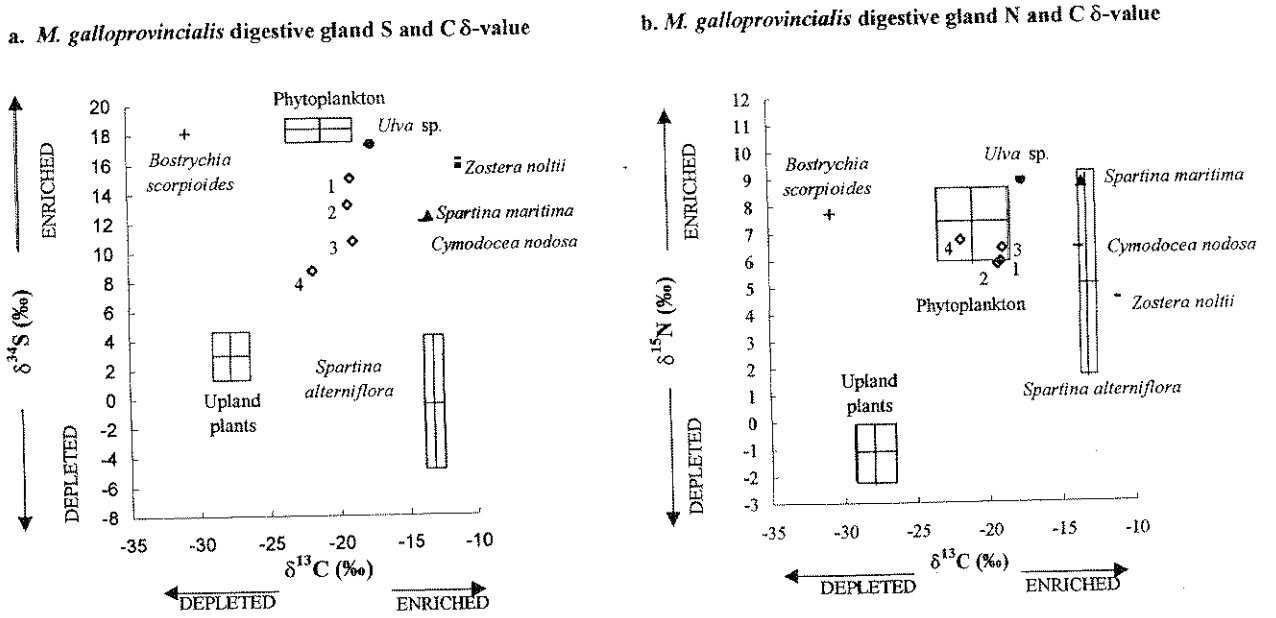

Figure 3. Multiple δ-values of food sources vs. *M. galloprovincialis* digestive gland of Ria Formosa. Bars are means ± standard deviation of literature derived δ-values of primary producers [4, 19]. Symbols represent δ-values for Ria Formosa primary producers and *M. galloprovincialis* digestive gland (numbers represent sampling stations): a) carbon and sulphur δ-values; and b) carbon and nitrogen δ-values.

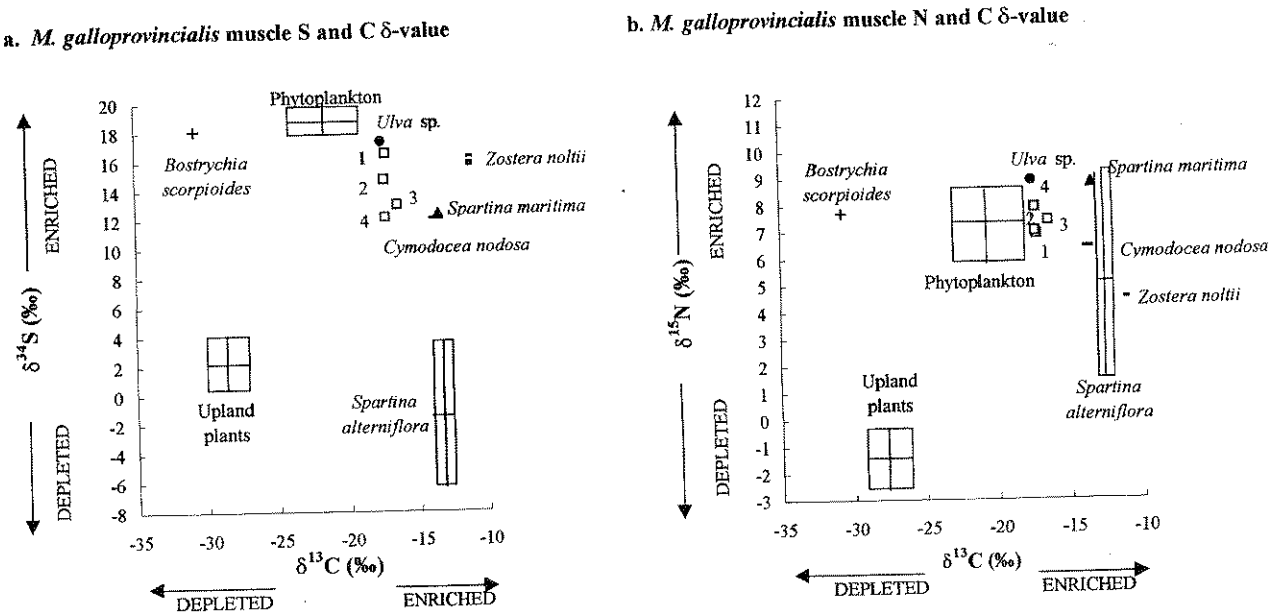

Figure 4. Multiple δ-values of food sources vs. *M. galloprovincialis* muscle of Ria Formosa. Bars are means ± standard deviation of literature derived δ-values of primary producers [4, 19]. Symbols represent δ-values for Ria Formosa primary producers and *M. galloprovincialis* muscle (numbers represent sampling stations): a) carbon and sulphur δ-values; and b) carbon and nitrogen δ-values.

Table I. Spatial distributions of suspended particulate matter (SPM), particulate organic matter (POM), particulate carbon (POC), chlorophyll a (Chl a) and ratio of Chl a to POC, in near-surface waters of Ria Formosa. POC was determined from POM (POC/POM = 0.45).

Station	SPM ($mg \cdot m^{-3}$)	POM ($mg \cdot m^{-3}$)	POC ($mg \cdot m^{-3}$)	Chl a ($mg \cdot m^{-3}$)	Chl a/ POM	Chl a/ POC
1	4394.70	1143.50	514.58	2.67	0.002	0.005
2	5634.15	1355.00	609.75	1.87	0.001	0.003
3	9308.30	1995.00	897.75	1.34	0.001	0.001
4	9081.65	1758.50	791.33	1.87	0.001	0.002

Table II. Isotopic composition (‰) of primary producers, POM and *Mytillus galloprovincialis* digestive gland and muscle in Ria Formosa.

Sample type	Sample station	$\delta^{13}C$	$\delta^{34}S$	$\delta^{15}N$
Bostrychia scorpoides	4	–30.7	18.1	7.7
Ulva sp.	4	–17.5	17.3	8.9
Zostera noltii	4	–11.2	15.6/15.8/16.2	4.5
Cymodocea nodosa	4	–13.7	12.0	6.4
Spartina maritima	4	–13.4	12.3	8.8
POM	1	–20.9	17.4	4.6
	2	–20.9	8.0	5.3
	3	–20.9	3.4	4.4
	4	–21.1	–8.2	5.0
M. galloprovincialis digestive gland	1	–19.0	15.0	5.9
	2	–19.2	13.2	5.8
	3	–18.9	10.7	6.4
	4	–21.8	8.7	6.7
M. galloprovincialis muscle	1	–17.2	16.5	6.9
	2	–17.3	14.7	7.0
	3	–16.4	13.0	7.4
	4	–17.3	12.3	7.9

was heavier than those reported in the literature, –3.9 to 4.9 ‰ [4]. This perhaps reflects good aeration of study site sediment. *Spartina* plants rooted in anoxic sediments apparently use sulphides as a major fraction of their total sulphur uptake, resulting in $\delta^{34}S$ depleted signatures [19]. *B. scorpioides* $\delta^{13}C$ value, –30.7 ‰, was low, close to those of upland plant values, –29.9 to –27.3 ‰ [19], in contrast with *Ulva* sp., –17.5 ‰, and other macroalgae. This suggests a photosynthetic process in *B. scorpioides* which incorporates less ^{13}C than other seaweeds, possibly because its photosynthesis is more efficient in the air than under water (Brotas, pers. comm.).

Suspended particulate matter (SPM) and POM increased from station 1 to station 4 (*table I*). The phytoplankton fraction of POM (Chl a/POM ratio) decreased from outer stations to inner stations (0.002 to 0.001), suggesting a lower contribution of phytoplankton to the total POM at inner stations. Most of the POM was thus associated with components other than living phytoplankton biomass. Chl a/POC ratios in San Francisco Bay [3] ranged from 0.001, when phytoplankton productivity was low, and 0.03 in spring bloom conditions. During winter, Chl a/POC ratio in Ria Formosa ranged from 0.005 in outer stations to 0.001 in inner stations indicating low productivity. Chl a values (*table I*) were similar to values found by Assis et al. [1] for Ria Formosa.

The variation of POM and *M. galloprovincialis* muscle and digestive gland $\delta^{13}C$ values did not reflect any gradient (*figure 5a, table II*) even though the phytoplankton relative abundance decreased from stations 1 to 4 (*table I*). This suggests that the phytoplankton C signal is compensated in the inner stations by a mixture of upland plants, lighter $\delta^{13}C$ signal than phytoplankton, and benthic macrophytes, heavier $\delta^{13}C$ signal than phytoplankton (see *figure 2a*). The analysis of $\delta^{13}C$ values of POM (–21.1 to –20.9 ‰) by itself (*figure 5a, table II*) would erroneously suggest phytoplankton dominance, –24 to –18 ‰ [4]. The POM $\delta^{15}N$ values, 4.4 to 5.3 ‰, also indicate a single dominant source (or balanced mixture) of organic matter at all the stations (*figure 5c, table II*). This highlights the importance of a multiparameter analysis.

Values of $\delta^{34}S$ show a clear gradient, decreasing from station 1 to 4 (*figure 5b, table II*). According to the literature values for phytoplankton and benthic macrophytes (*figures 2a, 3a, 4a*), this would reflect a decreasing effect of phytoplankton vs. an increasing effect of benthic macrophytes in the isotopic signal of POM, mussel digestive gland and muscle. However, $\delta^{34}S$ values of Ria Formosa benthic macrophytes (18.1 to 12.0 ‰) do not explain POM (17.4 to –8.2 ‰) and mussel digestive gland (15.0 to 8.7 ‰) gradients (*figures 2a, 3a, 4a, table II*), suggesting a POM 'contamination' by suspended mineral (pyrite), with low mean S value [20]. Pyrite is the major component of the reduced sulphur pool in marsh sediments and may reflect the long-term mean sulphide $\delta^{34}S$ value [12, 20]. Muscle tissue is not likely to be contaminated by pyrite and its spatial pattern of $\delta^{34}S$ values follow mussel digestive gland values fairly well, but a bit higher (*figure 5b, table II*). This indicates that mussels filter selectively, eliminating the component of POM with very low $\delta^{34}S$ values. The shift of $\delta^{13}C$ values

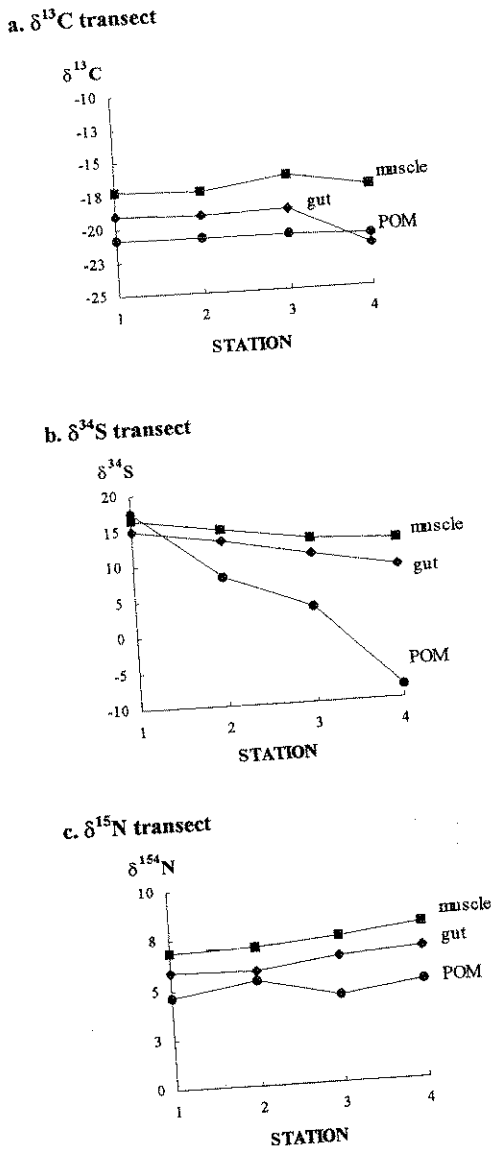

Figure 5. δ-Values of POM, *M. galloprovincialis* digestive gland and muscle along Ria Formosa sampling sites. a) Carbon δ-values; b) sulphur δ-values; and c) nitrogen δ-values.

from POM to *M. galloprovincialis* digestive gland and then to *M. galloprovincialis* muscle also suggests selective exclusion of depleted ^{13}C material plus a bit of trophic enrichment (~1 ‰) from POM to muscle (*figure 5a*, *table II*).

$\delta^{15}N$ values of *M. galloprovincialis* muscle, 6.9 to 7.9 ‰, are approximately 3 ‰ heavier than POM values, 4.4 to 5.3 ‰, showing the expected $\delta^{15}N$ enrichment per trophic level. Mussel digestive gland values were between POM and *M. galloprovincialis* muscle values, 5.8 to 6.7 ‰ (*figure 5c*, *table II*).

The combination of C, S and N isotopes illustrates that POM values along the Faro-Olhão channel may result from a mixture of upland plants, benthic plants (literature values), and phytoplankton (*figure 2*). On the other hand, both mussel digestive gland contents and muscle values (*figures 3, 4*) indicate a selective diet of benthic macrophytes and phytoplankton, excluding upland plant detritus.

4. CONCLUSION

Comparison of S and C isotopic composition of POM and *M. galloprovincialis* muscle and digestive gland provided unique insights into the relative contribution of food sources along the Ria Formosa ocean/inner gradient. It appears from this study that the food sources available in the water column are different at various locations in the channel. However, assessment of seasonal and spatial variations of the isotopic signatures of Ria Formosa benthic macrophytes is needed.

This study indicates that during winters, the upland plants or upper marsh plants may be an important source of organic matter in the inner lagoon. Phytoplankton was an important source of organic matter in the outer lagoon. This approach also suggests that *Bostrychia scorpioides* is not an important source of organic matter in Ria Formosa.

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