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► **To cite this version:**

Ariadna Purroy, Mirjana Najdek, Enrique Isla, Ivan Zupan, Julien Thébault, et al.. Bivalve trophic ecology in the Mediterranean: Spatio-temporal variations and feeding behavior. *Marine Environmental Research*, 2018, 142, pp.234-249. 10.1016/j.marenvres.2018.10.011 . hal-02640334

**HAL Id: hal-02640334**

**<https://hal.science/hal-02640334>**

Submitted on 28 May 2020

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## Bivalve trophic ecology in the Mediterranean: Spatio-temporal variations and feeding behavior

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### Abstract :

The trophic ecology of two bivalves, the clam *Callista chione* and the cockle *Glycymeris bimaculata* was studied using environmental and biochemical variables of the suspended particulate matter and the sediment. Samples were collected from two shallow sites, Pag and Cetina, in the coastal oligotrophic Mediterranean Sea, during a 17 month period.

The temporal variation of the particulate matter reflected a mixture between marine and terrestrial sources throughout the year, with a clear marine influence during summer and fall, and predominance of terrestrial inputs during spring and winter. The digestive gland was a useful rapid turnover tissue, where the carbon isotope signal was species-specific and the nitrogen isotope one was site-specific. FA markers in the digestive gland revealed a mixed diet where *Callista chione* fed more upon fresh material than *G. bimaculata* which relied largely on bacteria-derived detritus.

Overall, little feeding niche overlap was observed between the two species during the year, indicating resource partitioning, expected for a food-limited system. The present trophic ecology study in co-occurring species allowed identifying species-specific feeding adaptations to environmental variability.

### Highlights

► Particulate matter biochemical proxies show terrestrial inputs in the Adriatic Sea. ► POM quality in the shallow oligotrophic Adriatic Sea is best in spring and summer. ► Digestive gland is a useful rapid turnover tissue. ► Bivalve species show feeding adaptations to environmental variation. ► Bacterial diazotroph biomass has major importance in *G. bimaculata* diet. ► Feeding niche overlap between species is low, indicating resource partitioning.

**Keywords** : Feeding ecology, Bivalves, Suspension feeder, Biochemical variables, Stable isotopes, Fatty acids, Adriatic sea, Niche partitioning, Coastal ecosystems

29

30 **1. INTRODUCTION**

31 Coastal systems represent 8.5% of marine biomes and their related physical, chemical and  
32 biological processes are modulated by terrestrial influence (Costanza et al., 1997). In these  
33 systems, a mixture of organic particles from autochthonous and allochthonous origin contribute to  
34 the pool of particulate organic matter (POM) (Bode et al., 2006; Hedges et al., 1997; Savoye et al.,

35 2012). In temperate areas, benthic consumers are able to shift their diets along the year adapting  
36 to the available suspended material, e.g. following the magnitude and seasonality of primary  
37 production (Antonio et al., 2010). Bivalves, as suspension-feeding organisms, participate in the  
38 transfer of POM between the water column and the surface sediment layer (Schubert and Calvert,  
39 2001). In the sediment-water interface, physical factors (e.g. currents, wind) and bioturbation  
40 enhance resuspension enabling a continuous supply of POM to suspension feeding organisms  
41 (Dubois et al., 2014; Orvain et al., 2012). Bivalves mainly feed upon phytoplankton but other  
42 sources such as detritus, bacteria, microphytobenthos and zooplankton can also constitute an  
43 important component of their diet (Davenport et al., 2000; Ezgeta-Balić et al., 2012; Grall et al.,  
44 2006; Kharlamenko et al., 2011; Peharda et al., 2012). The chemical characteristics of POM both in  
45 the pelagic and benthic compartments are tightly coupled to filter feeding activity, e.g. there is a  
46 positive feedback between phytoplankton abundance in the water column and the bivalve grazing  
47 activity (Charles et al., 1999; Page and Lastra, 2003; Prins et al., 1997; Ward and Shumway, 2004).  
48 Thus, investigating the origin and availability of food resources in POM in relation to their  
49 contribution to bivalve's diet allow to distinguish variations in the trophic activity, selective  
50 feeding, inter or intra-specific competition, and biogeochemical functioning of the ecosystem  
51 (Carlier et al., 2007; Dubois et al., 2014; Riera and Richard, 1996).

52

53 In aquatic ecosystems, stable isotope analyses (SI), e.g.  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , and fatty acid (FA)  
54 concentrations have been used to analyze the food web structure and infer trophic relationships  
55 (e.g. Antonio and Richoux, 2016; Giraldo et al., 2016; Kharlamenko et al., 2008, 2011; Pérez et al.,  
56 2013). SI analyses have been widely used to describe diet composition, trophic level and habitat  
57 characteristics in marine organisms (DeNiro and Epstein, 1978; Lopez et al., 2016; Lorrain et al.,  
58 2002; Peterson and Fry, 1987) due to a time-integrated signal of the food sources, assimilated in  
59 animal tissues (Ezgeta-Balić et al., 2014; Martínez del Rio et al., 2009; Peterson and Fry, 1987). The  
60 measurement of  $\delta^{13}\text{C}$  allows the identification of the main primary producers (Post, 2002) and also  
61 discriminates sources from both terrestrial and marine, and benthic and pelagic systems (e.g.  
62 Cloern et al., 2002; Savoye et al., 2003). On the other hand, the  $\delta^{15}\text{N}$  signature is useful to identify  
63 food chain structure, based on the principle that excretion and respiration of lighter N isotopes by  
64 consumers increase the  $\delta^{15}\text{N}$  content in their tissues (Peterson and Fry, 1987). FA are involved in  
65 the organism's energy storage (Parrish et al., 2000) and are effective tracers of food sources, since

66 they remain mostly invariable along trophic pathways, and can be associated to different groups  
67 of organisms, such as primary producers or heterotrophs (e.g. Dalsgaard et al., 2003; Napolitano et  
68 al., 1997). However, the higher the trophic level, the more complex is the interpretation of FA as  
69 dietary indicators (Budge et al., 2002) due to the incorporation of multiple diet sources and  
70 diverse metabolic pathways (Dalsgaard et al., 2003). Still, FA are good discriminators of food  
71 sources in suspended POM and sediment (Bergamino et al., 2014; Connelly et al., 2016; El-Karim et  
72 al., 2016; Ricardo et al., 2015) and also useful describing their seasonal and spatial variations  
73 (Boon and Duineveld, 1996). Lipids play an important role as metabolic energy reserves; thus, FA  
74 analyses in the digestive gland of bivalves have been employed to trace recent food ingestion  
75 (weeks-months) (Deudero et al., 2009; Ezgeta-Balić et al., 2012; Najdek et al., 2013).

76

77 The study of fine-scale spatio-temporal patterns in environmental biochemical variations (e.g. SI,  
78 FA) and in primary producers allow assessing the intra and interspecific variability in the diet of  
79 secondary consumers which is essential for comprehensive ecological studies (Antonio et al., 2012;  
80 Gonçalves et al., 2012; Underwood et al., 2000; Ventrella et al., 2008; Yurkowski et al., 2016).  
81 Likewise, other biomarkers characterizing the composition of suspended particulate matter (SPM)  
82 in coastal ecosystems such as C:N ratios and Chlorophyll *a* are often used as indicators of the  
83 quality of biogenic matter. However, few studies incorporate the analysis of biogenic silica, which  
84 is a major component in diatom biomass (Alomar et al., 2015; Leblanc et al., 2003; Ragueneau et  
85 al., 2002). Therefore, an extended particulate matter characterization including environmental  
86 variables (e.g., temperature, salinity) with the above mentioned biochemical parameters will lead  
87 to more accurate interpretations of benthic trophic ecology.

88

89 The burrowing bivalves *Glycymeris bimaculata* (Poli, 1975) and *Callista chione* (Linnaeus, 1758) are  
90 two common benthic suspension feeders inhabiting Atlantic and Mediterranean waters (Huber,  
91 2010; Poppe and Goto, 1993). *Callista chione* is a commercially important species in many parts of  
92 the Mediterranean Sea (Galimany et al., 2015; Metaxatos, 2004; Tirado et al., 2002), which  
93 demands a thorough knowledge on its general ecology. Both of these species have a relative long  
94 life span of over 40 years (Bušelić et al., 2015; Ezgeta-Balić et al., 2011; Forster, 1981) making them  
95 interesting targets for sclerochronological research that includes use of shell as archives of  
96 environmental change (e.g. Butler and Schöne, 2017). Further on, they co-occur along the eastern

97 Adriatic coast (Peharda et al., 2010) and show spatial variations in growth (Ezgeta-Balić et al.,  
98 2011; Kovačev, 2016) and timing and duration of their reproductive periods (Purroy, 2017).

99 Seawater properties in the shallow eastern coastal Adriatic are exposed to high temporal and  
100 spatial variations mainly characterized by large temperature fluctuations and freshwater inputs  
101 (e.g. Ogrinc et al., 2005; Surić et al., 2015). The intrusion of freshwater is more evident in river  
102 mouth areas, where also the incorporation of nutrients and organic matter from terrestrial origin  
103 stimulates biological productivity and creates a characteristic fluctuating environment. Likewise, in  
104 this karst littoral the common groundwater discharge and coastal springs also influence the  
105 marine environment (Bonacci, 2015; Bonacci et al., 1997; Surić et al., 2015). These conditions have  
106 an ecological impact such as physiological stress, changes in biological responses and shifts in  
107 community structure (Hamer et al., 2010).

108

109 Within this context, the aim of this study was to evaluate the trophic ecology of two co-occurring  
110 suspension feeders in two different coastal settings influenced by freshwater inputs, based on a  
111 multiple tracer approach. To address this, we (1) characterized two coastal settings to identify  
112 relationships between environmental conditions with two main food sources: suspended  
113 particulate matter (SPM) and sediment (Sed); and investigated (2) spatio-temporal variations  
114 (hundreds of Km and months) in feeding patterns in bivalves using SPM and Sed and (3) trophic  
115 interactions to identify whether feeding niche overlap between species occurs. This study  
116 contributes to a better understanding of bivalve feeding mode adaptations to environmental  
117 variability in oligotrophic settings.

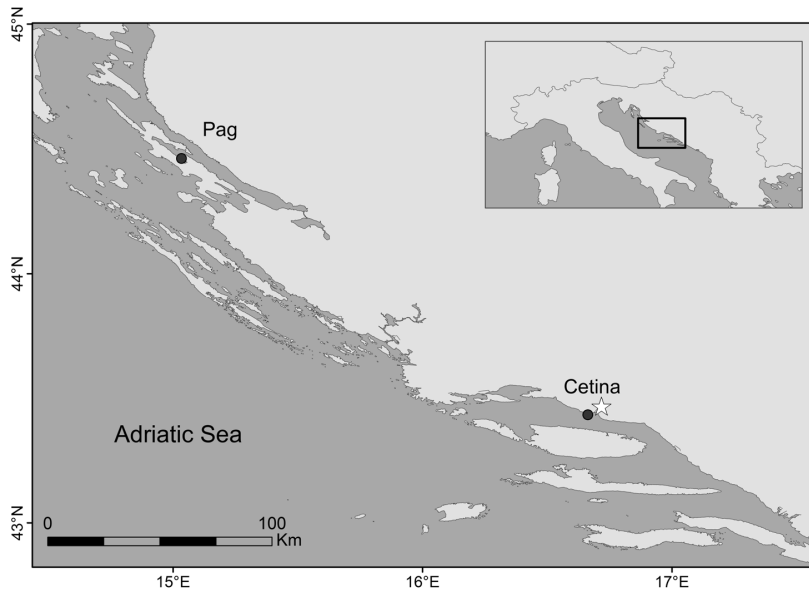
118

## 119 **2. MATERIAL AND METHODS**

### 120 *2.1. Study area and sample collection*

121 The study area is located on the Adriatic coast off central Croatia (Figure 1). Two sites were  
122 selected based on earlier studies, which showed differences in environmental settings and in local  
123 bivalve growth (Ezgeta-Balić et al., 2011). One study site was located in Pag Bay off the island of  
124 Pag (44° 27'42" N, 15°01'36"E) whereas the other site, Cetina (43°26'13" N, 16°41'14"E), was  
125 located close to the mouth of the Cetina River, situated on the main direction of the river plume.  
126 Both study sites have karstic origin and are under the influence of submarine freshwater springs  
127 (Surić, 2005). In these shallow coastal sites, samples were collected by SCUBA and skin diving

128 within a range of 2 to 5 m water depth. Previous studies on the distribution of benthic species  
 129 (Peharda et al., 2010) identified these sites with sufficient population abundances for conducting a  
 130 temporal collection design based on monthly samplings, ensuring collection of specimens  
 131 throughout the study period.



132  
 133 **Fig 1.** Sampling sites in the Croatian Adriatic Sea: Pag and Cetina. A star indicates the location of the Cetina  
 134 river. The black square in the inlet represents the study area.

135  
 136 Study was conducted between May 2014 and October 2015. Seawater temperature was recorded  
 137 hourly with a Tinytag data logger deployed *in situ* and salinity was monthly measured *in situ* with a  
 138 YSI-Pro probe. Twenty adult specimens of both *Glycymeris bimaculata* and *Callista chione*  
 139 (hereafter also referred as Gly and Cal, respectively), were collected monthly at each site,  
 140 measured for biometric analysis and processed within 3 hours by sectioning the muscle and  
 141 dissecting the digestive gland (DG) from each individual. The DG is a rapid turnover tissue enabling  
 142 the detection of isotopic signatures of recent ingestions (days to weeks) (e.g. Ezgeta-Balić et al.,  
 143 2012). Analyses were carried out on three replicates, each of them constituted by a pool of tissues  
 144 from three specimens. Each sample was stored at -20°C for subsequent analysis.

145  
 146 **2.2. Environmental variables and food sources**

147 Seawater samples were collected ~0.5 m above the seafloor using a Niskin bottle (between 10-20  
 148 L) and vacuum filtered in the laboratory. Prior to filtration water containers were gently agitated

149 to homogenize seawater and filters were rinsed with distilled water. Pre-weighed nitrocellulose  
150 filters (Whatman™, 47 mm diameter, 0.45 µm mesh size) were used to measure bulk suspended  
151 particulate matter (SPM) and biogenic silica content (BSi) while pre-combusted (450°C, 6 h) and  
152 pre-weighed glass fiber filters (Whatman™ GF/F, 25 mm diameter, 0.7 µm mesh size) were used  
153 to measure elemental composition and ratios of C (weight %) and N (weight %), and their isotopic  
154 composition ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ; see 2.3), fatty acids (FA) and lipid proportions (see 2.4). The  
155 concentration of SPM was determined on the dry mass of the total suspended particles retained  
156 per unit of volume after drying (60°C, 24 h) (expressed as mg/L). SPM filters were also used for BSi  
157 content analysis following a sequential alkaline digestion (2 to 5 h) with  $\text{NaCO}_3$  to distinguish  
158 between biogenic and lithogenic silica sources (DeMaster, 1991; Mortlock and Froelich, 1989).  
159 Around 1 L of seawater per replicate was filtered for Chlorophyll *a* (Chl *a*) analyses through glass  
160 fiber filters which were folded, wrapped in aluminum foil, frozen and stored at -20°C. Chl *a* was  
161 extracted in 90 % acetone (Strickland and Parsons, 1972) and measured in a Turner Systems  
162 (Sunnyvale, CA) fluorometer (Trilogy). Triplicate samples were conducted for all chemical analysis.  
163 Additionally, satellite-derived Chl *a* data was extracted from the MODIS-Aqua Sensor provided by  
164 the EU Copernicus Marine Service (CMEMS).

165

166 Surface sediment samples (ca. upper 2 cm) (Sed) were collected monthly by SCUBA diving using a  
167 plastic core and preserved at -20°C. Homogenized and ground samples were used for %C, %N and  
168 SI analyses (see 2.3), Chl *a* samples were extracted in 90% acetone (4°C, 12 h) (Lorenzen and  
169 Jeffrey, 1978), BSi content was measured following the procedure for SPM, and total carbon (TC)  
170 was measured in a LECO Truspec CN-2000 analyzer. Inorganic carbon (IC) (expressed in dry weight  
171 %) was calculated by the difference between TC and OC content (see 2.3). To identify the effect of  
172 grain size on the OC content, grain size was determined in a HORIBA LA950V2 laser scattering  
173 particle size distribution analyzer after removal of organic matter in a 20% hydrogen peroxide  
174 solution (Table 1).

175

### 176 *2.3. Stable isotope analysis (SIA)*

177 Particulate organic carbon and nitrogen elemental and isotopic compositions were measured in  
178 filters dried at 50°C for 24 h, 50 mg of Sed and 1 mg of DG. Sed and DG were freeze-dried and  
179 individually homogenized using an agate mortar and pestle. Aiming to remove carbonates, SPM



180 filters and DG samples had a 6 h exposure to HCl fumes (Lorrain et al., 2003) while Sed samples  
181 were exposed to 24 h (due to the high content of carbonates) prior to isotopic analyses. Further,  
182 to avoid effects of acidification in Sed (e.g. Walthert et al., 2010), non-acidified subsamples were  
183 used for the analysis of  $\delta^{15}\text{N}$ . To eliminate residual HCl and water, all samples were ventilated  
184 overnight at room temperature and dried in a hotplate (50°C, 6 h). Filters were folded and placed  
185 into tin cups (12.5 mm x 5 mm) whereas Sed and DG samples were placed into tin cups wrapped  
186 by silver cups to avoid losses due to tin corrosion (8 mm x 5 mm). Stable isotope ratios of carbon  
187 ( $^{13}\text{C}/^{12}\text{C}$ ) and nitrogen ( $^{15}\text{N}/^{14}\text{N}$ ), and elemental %C and %N analyses were conducted on a Carlo  
188 Erba Elemental Analyzer EA1108 coupled to an isotope-ratio mass spectrometer ThermoFinnigan  
189 MAT253 in the Unidade de Técnicas Instrumentais de Investigación, University of A Coruña (Spain).  
190 Results are expressed in standard unit notation (VPDB for the carbon and atmospheric air for the  
191 nitrogen):  $\delta X = ([R \text{ sample} / R \text{ standard}] - 1) \times 1000$ , where X represents  $^{13}\text{C}$  or  $^{15}\text{N}$ , and R is the  
192 ratio of  $^{13}\text{C}/^{12}\text{C}$  or  $^{15}\text{N}/^{14}\text{N}$ , respectively. An acetanilide standard was used for the quantification of  
193 the amounts of %N and %C. Isotopic data are given in the conventional delta notation in units of  
194 parts per thousands (‰) with a resolution of  $\pm 0.15\text{‰}$ . Lipid content in tissues may affect  $\delta^{13}\text{C}$   
195 values, where high lipid concentrations result in depleted  $^{13}\text{C}$  biasing the isotopic signal (DeNiro  
196 and Epstein, 1978; Focken and Becker, 1998). However, previous studies in bivalves did not find  
197 this relationship (Ezgeta-Balić et al., 2014; Lorrain et al., 2002) therefore, lipid extraction was not  
198 conducted in the present study. A two-source isotopic mixing model was used in MixSIAR package,  
199 a graphical user interface (GUI) built on R software (Parnell et al., 2010), that uses an algorithm  
200 based on Bayesian statistics to determine the probability distributions for proportional food  
201 source contribution to the mix diet of consumers (Semmens et al., 2013). This model accounts for  
202 uncertainty in isotopic values when estimating the contribution of sources in the diet due to the  
203 incorporation of diet-tissue discrimination factors. Discrimination factors of  $0.5 \pm 0.13\text{‰}$  for  $\delta^{13}\text{C}$   
204 and  $2.3 \pm 0.18\text{‰}$  for  $\delta^{15}\text{N}$  considered for benthic consumers (Alomar et al., 2015; McCutchan et al.,  
205 2003) were incorporated into the model. Despite the increasing number of studies for prey-  
206 predator fractionation values, there are no species-specific values for our target species;  
207 therefore, conventional fractionation values for benthic consumers can be applied, as supported  
208 by Yokoyama et al., (2005).

209

210 *2.4. Fatty acid analysis (FA)*

211 SPM, Sed and DG samples were collected monthly during one year period, kept frozen and freeze-  
212 dried prior to biochemical analyses. For total lipid analysis all samples were weighted (DG samples  
213 were blended) and after, a dichloromethane-methanol (DCM:MeOH) mixture (2:1) sonicated in a  
214 water bath at 30°C was added. After separation DCM phases were pooled and evaporated to  
215 dryness and weighed. Total lipids of digestive glands were re-dissolved in DCM and neutral lipids  
216 were separated according to Pernet et al., (2012). All extracts (total lipids of SPM and Sed and  
217 neutral lipids of DG were saponified (1.2 M NaOH), acidified (6M HCl) and methylated (14% BF<sub>3</sub> in  
218 methanol) then extracted in DCM. Fatty acid methyl esters (FAMES) were analyzed by Agilent gas-  
219 liquid chromatography (GLC) 6890 N GC System equipped with a 5973 Network Mass Selective  
220 Detector, capillary column (25 m x 0.3 mm x 0.25 µm; cross-linked 5% phenylmethylsiloxane) and  
221 ultra-high purity helium as the carrier gas. The GLC setting was programmed column temperature  
222 rise from 145°C by 4°C min<sup>-1</sup> up to 270°C at a constant column pressure of 2.17 kPa. Retention  
223 times, peak areas and mass spectra were recorded with Chemstation software. Bacterial FAME  
224 standard mix, FAMES mix C18-C20, polyunsaturated fatty acids standards (PUFA1 and PUFA3), cod  
225 liver oil and various individual pure standards of FAME were used (Supelco). FAs were reported as  
226 percentages of the total fatty acids (% TFA, mean ± SD) and grouped as saturated (SFA),  
227 monounsaturated (MUFA), PUFA, and bacterial fatty acids (BACTERIAL;  
228 15:0+15iso+15anteiso+17:0+17iso+17anteiso+18:1(n-7); (Mayzaud et al., 1989; Najdek et al.,  
229 2002). Unsaturation degree (UND) was calculated according to Pirini et al. (2007). The ratio of  
230 C16:1/C16:0 (>1.0) and the relative dominance of C20:5 (n-3) (EPA) and C14:0 are considered as  
231 diatom indicators (Ackman et al., 1968; Kharlamenko et al., 1995; Léveillé et al., 1997) while the  
232 ratio of C22:6 (n-3)/C20:5 (n-3) (DHA/EPA) (>1.0), DHA, 16:0 and C16:1/C16:0 (<1.0) are  
233 predominant in dinoflagellates (Budge and Parrish, 1998; Dalsgaard et al., 2003). DHA, EPA and  
234 ARA (20:4 (n-6)) are considered Essential FA (EFA; Alkanani et al., 2007) and are transferred to  
235 microzooplankton (Ventrella et al., 2008). Herbivorous calanoid copepod markers are C20:1 and  
236 C22:1 (Falk-Petersen et al., 1987). A marker indicator of zooplankton (Sargent and Falk-Petersen,  
237 1988) and detrital matter (Fahl and Kattner, 1993) is C18:1(n-9).

238

## 239 2.5. Statistical analysis

### 240 2.5.1. Environmental variables

241 Two-way ANOVAs were used to test the effect of site (2 levels) and month (17 levels) in SPM, Chl  
242  $\alpha$ , BSi and lipids. A t-test was done for BSi in the sediment to check for site differences. Statistical  
243 assumptions for normality (Shapiro test) and homoscedasticity (Levene's test) were confirmed  
244 prior to all analyses.

245

#### 246 2.5.2. Stable isotopes

247 Two-way ANOVAs were used to assess the effect of site and month on isotopic and C:N molar  
248 ratios in food sources and consumers. The Stable Isotope Bayesian Ellipses method in R (SIBER  
249 package) was used to investigate isotopic niches by examining the dispersion of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$   
250 values together with the MixSIAR package as explained in section 2.3 (Jackson et al., 2011; Parnell  
251 et al., 2010). A Standard Ellipse Area (SEA), which is to bivariate data as SD is to univariate data,  
252 was applied to measure the isotopic niche width using Bayesian inference. SEAs are an alternative  
253 to Convex Hull methods since the ellipses are unbiased with respect to sample size and their  
254 estimation allows robust comparisons between data sets (Jackson et al., 2011). SEA values were  
255 corrected for small sample size ( $\text{SEAC}_c$ ) containing 95% of the data, and Bayesian estimates ( $\text{SEA}_B$ )  
256 were estimated as a measure to trophic width between species-sites. Analyses were performed in  
257 R v3.1.3 (R Core Team, 2015).

258

#### 259 2.5.3. Fatty acids

260 Analyses of similarities (ANOSIM) were performed in all detected FA profiles in SPM and Sed to  
261 determine resemblance among sampling periods and sites, and among consumer groups across  
262 sampling periods (PC-CC, PG-CG, PG-PC and CG-CC, referring to *Callista chione* from Pag (PC) and  
263 Cetina (CC) and to *Glycymeris bimaculata* from Pag (PG) and Cetina (CG). All replicated data were  
264 used and  $\log(x+1)$  transformed to improve normality prior to analysis. Results based on a global R  
265 statistic and p-value were reported. Non-metric multidimensional scaling (nMDS) using a Bray-  
266 Curtis similarity matrix were used to visualize the temporal compositional differences of all  
267 detected monthly mean FA profiles in each compartment with respect to site. Similarity  
268 percentage analysis (SIMPER) and principal component analysis (PCA) were applied to identify the  
269 main contributors of dissimilarities in the FA profiles between sites in each compartment. SIBER  
270 was also used to analyze feeding niche overlap using the x and y coordinates of the nMDS analysis  
271 of FA profiles of consumer groups following Antonio and Richoux (2014). SIBER analyses were

272 performed using R v3.1.3 (Jackson et al., 2011; R Core Team, 2015) and multivariate analysis  
 273 (ANOSIM, nMDS, SIMPER and PCA) using PAST 3.0 (Hammer et al., 2001).

274

275 **Table 1.** Parameters measured in the different compartments at two study sites, Pag and Cetina between  
 276 May 2014 and October 2015.

	SPM	Sed	DG
Chl <i>a</i>	✓	✓	
BSi & total Si	✓	✓	
Granulometry		✓	
%OC, %N, $\delta^{13}\text{C}$ , $\delta^{15}\text{N}$	✓	✓	✓
%IC		✓	
FA	✓	✓	✓
Lipids	✓	✓	✓

277

### 278 3. RESULTS

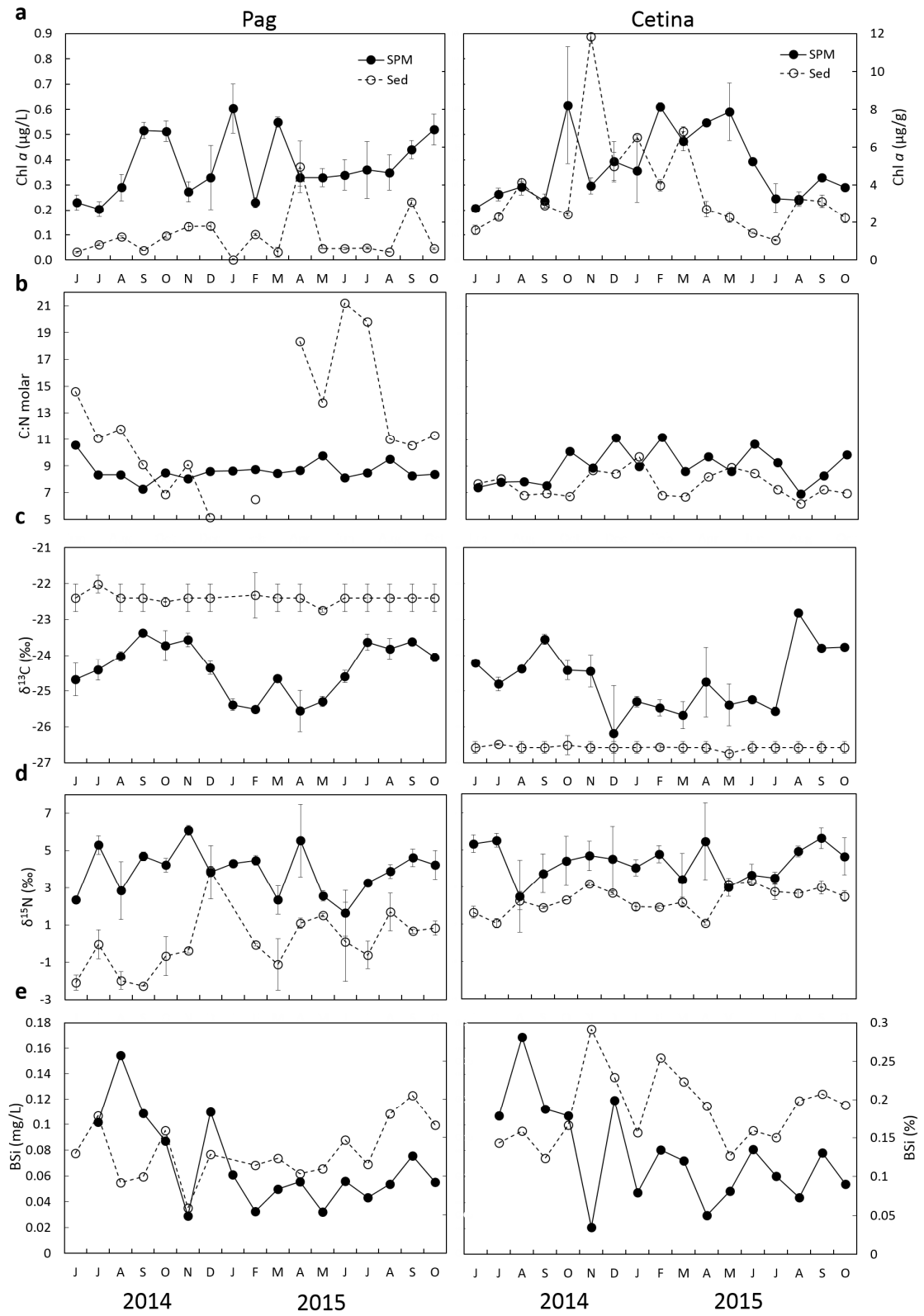
#### 279 3.1. Environmental variables and food sources

280 Temperature at both sites followed a significantly correlated temporal trend ( $r = 0.98$ ,  $P < 0.05$ )  
 281 with monthly means between 8.9°C and 25.1°C in Pag and 12.7°C and 26.4°C in Cetina. Monthly  
 282 salinity values ranged between 34.7 and 38.3 in Pag and 32.2 and 38.5 in Cetina. These values are  
 283 typical in coastal eastern Mediterranean waters (Vilibić et al., 2015). SPM concentrations had  
 284 means of  $1.02 \pm 0.33$  mg/L in Pag and  $0.92 \pm 0.35$  mg/L in Cetina (Table S1) and didn't significantly  
 285 differ between sites ( $P > 0.05$ , Table S2). In Pag, monthly values ranged from 0.43 to 3.98 mg/L and  
 286 highest values were observed in both summers with a peak in March 2015, whereas in Cetina SPM  
 287 ranges varied from 0.53 to 1.99 mg/L with two peaks observed in February and June 2015.  
 288 Temporal variation of Chl  $a_{\text{SPM}}$  were similar between sites ( $P > 0.05$ ) ranging from 0.2 to 0.6  $\mu\text{g/L}$  in  
 289 Pag and 0.2 to 0.7  $\mu\text{g/L}$  in Cetina (Tables S1, S2). In Pag, the maximum Chl  $a_{\text{SPM}}$  values were  
 290 observed in October 2014 and in January, March and October 2015 whereas the lowest  
 291 concentration of Chl  $a_{\text{SPM}}$  was observed in both summers. In Cetina, the highest Chl  $a_{\text{SPM}}$   
 292 concentration was observed during winter and spring. Between June-September 2014 and June-  
 293 August 2015, the low values of both Chl  $a_{\text{SPM}}$  and Chl  $a$  % in SPM, suggested that this period was  
 294 the poorest for Chl  $a$  availability in the water column (Fig 2a, Table S1). A similar annual trend was  
 295 observed from satellite Chl  $a$  extracted from the MODIS-Aqua sensor with overall low Chl  $a_{\text{SPM}}$   
 296 values in summer and fall and high values during spring and winter (Fig S1). Significant differences  
 297 were observed in Chl  $a_{\text{sed}}$  between sites ( $P < 0.001$ ) with ranges from 0.4 to 5.0  $\mu\text{g/g}$  in Pag and 1.1  
 298 to 11.8  $\mu\text{g/g}$  in Cetina; the latter showed high values between November 2014 and March 2015.

299 The annual patterns of Chl  $a_{SPM}$  and Chl  $a_{Sed}$  were significantly correlated in Cetina ( $r = 0.49$ ,  $P <$   
300  $0.05$ ) but not in Pag ( $r = 0.15$ ,  $P > 0.05$ ).

301 The concentration of  $BSi_{SPM}$  showed high temporal variability ranging from 0.03 to 0.20 mg/L in  
302 Pag and from 0.02 to 0.20 mg/L in Cetina and non-significant differences between sites (Tables S1,  
303 S2). Summer 2014 presented the highest  $BSi_{SPM}$  values and no significant correlation was found  
304 between  $BSi_{SPM}$  and Chl  $a_{SPM}$  at any site (all at  $r < 0.28$ ,  $P > 0.05$ ) (Fig 2). The concentrations of  $BSi_{Sed}$   
305 ranged from 0.06 to 0.2% in Pag and 0.1 to 0.3% in Cetina and showed significant differences  
306 between sites (t-test,  $P < 0.05$ ).  $BSi_{SPM}$  and  $BSi_{Sed}$  were coupled during most of the study period (Fig  
307 2e).

308 Sediment grain size was relatively constant with high content of sand and gravel, and low content  
309 of silt and clay (fine sediment), which accounted for  $< 2\%$ . Sand (2 mm – 63  $\mu$ m) represented a  
310 higher portion in Cetina (87%) than in Pag (74%) whereas gravel ( $> 2$ mm) was more abundant in  
311 Pag (26% vs 11%). No significant relationships were found among grain size and OC content ( $r =$   
312  $0.12$ ,  $P > 0.05$ ).



314 **Fig 2.** Temporal variations at Pag and Cetina sampling sites (a) Chlorophyll *a* concentration (Chl *a*) - note  
 315 different units in two vertical axis, (b) C:N molar ratio, (c)  $\delta^{13}\text{C}$ , (d)  $\delta^{15}\text{N}$  and (e) biogenic silica (BSi) in  
 316 suspended particulate matter (SPM) and sediment (Sed) - note different units in two vertical axis. Error bars  
 317 represent standard deviations. Three replicates were used for each data point.

318

### 319 3.2. Stable isotopes

#### 320 3.2.1. Food sources

321 The isotopic temporal variation ranged from -25.6 to -23.4‰ for  $\delta^{13}\text{C}_{\text{SPM}}$  and 1.6 to 6.1‰ for  
 322  $\delta^{15}\text{N}_{\text{SPM}}$  in Pag and from -26.2 and -22.8‰ for  $\delta^{13}\text{C}_{\text{SPM}}$  and 0.03 to 5.6‰ for  $\delta^{15}\text{N}_{\text{SPM}}$  in Cetina (Fig  
 323 2c, 2d). Two-way ANOVAs indicated significant differences between month and the interaction  
 324 with site in  $\delta^{13}\text{C}_{\text{SPM}}$  (at  $P < 0.001$ ) but not between sites ( $P > 0.05$ ), whereas for  $\delta^{15}\text{N}_{\text{SPM}}$  there were  
 325 significant differences between sites, month and their interaction (all at  $P < 0.001$ ; Table S3).  
 326 Greater isotopic values were observed during summer and fall at both sites. In the sediment, the  
 327 isotopic temporal variation ranged from -22.7 to -22.0‰ for  $\delta^{13}\text{C}_{\text{Sed}}$  and -2.3 to 3.9‰ for  $\delta^{15}\text{N}_{\text{Sed}}$  in  
 328 Pag, and from -26.7 to 26.5‰ for  $\delta^{13}\text{C}_{\text{Sed}}$  and 1.1 to 3.3‰ for  $\delta^{15}\text{N}_{\text{Sed}}$  in Cetina. There were  
 329 significant differences in  $\delta^{13}\text{C}_{\text{Sed}}$  between sites (at  $P < 0.001$ ), whereas neither significant temporal  
 330 differences nor in their interaction with site ( $P > 0.05$ ; Table S3). More negative values in  $\delta^{13}\text{C}_{\text{Sed}}$   
 331 than  $\delta^{13}\text{C}_{\text{SPM}}$  were observed in Cetina whereas the contrary was observed in Pag. Significant  
 332 differences in  $\delta^{15}\text{N}_{\text{Sed}}$  (all at  $P < 0.001$ ) made evident the lower and variable values in Pag and  
 333 greater and constant in Cetina (Fig 2d). On the other hand,  $\delta^{15}\text{N}_{\text{SPM}}$  showed more positive values  
 334 than in  $\delta^{15}\text{N}_{\text{Sed}}$  at both sites.

335 The C:N<sub>SPM</sub> molar ratios did not significantly differ between sites ( $P = 0.055$ ), with ranges from 7.3  
 336 to 10.6 in Pag and from 6.9 to 11.2 in Cetina; however, temporal differences within site were  
 337 significant (at  $P < 0.001$ ), and Pag values showed a lower annual mean (Table S3). There was no  
 338 evident annual pattern at any site (Fig 2b). A significant correlation was shown between C:N<sub>SPM</sub>  
 339 and SPM at both sites ( $r = 0.50$ ,  $P < 0.05$ ) and between C:N<sub>SPM</sub> and Chl *a*<sub>SPM</sub> in Cetina ( $r = 0.60$ ,  $P <$   
 340  $0.05$ ), illustrated with the coincidence of peaks in October 2014 and February and April-June 2015  
 341 (Fig 2). BSi<sub>SPM</sub> and C:N<sub>SPM</sub> were not significantly correlated at any site (all at  $r < 0.47$ ,  $P > 0.05$ ). In all  
 342 cases, POC<sub>SPM</sub>/Chl *a*<sub>SPM</sub> values were  $> 200$  (Table S1).

343 C:N<sub>Sed</sub> molar ratios in Pag varied from 5.2 to 21.2 whereas in Cetina from 6.2 to 9.7. In Pag, high  
 344 values were recorded from spring to mid-summer. The range of C:N<sub>Sed</sub> values in Cetina was similar

345 to that observed in  $C:N_{SPM}$  (Fig 2b, Table S3). Inorganic carbon represented 10.7 to 12.1% of  
346 sediment weight in Pag and from 7.6 to 11.6% in Cetina (Table S1). The values in Pag were closer  
347 to the weight % of Carbon in the  $CaCO_3$  molecule, suggesting that  $CaCO_3$  is the main inorganic  
348 carbon constituent in this area. Overall, more than 93% of the TC was inorganic in both sites. Total  
349 Nitrogen values ranged from 0 – 0.05% at both sites (Table S1).

350

### 351 3.2.2. Consumers

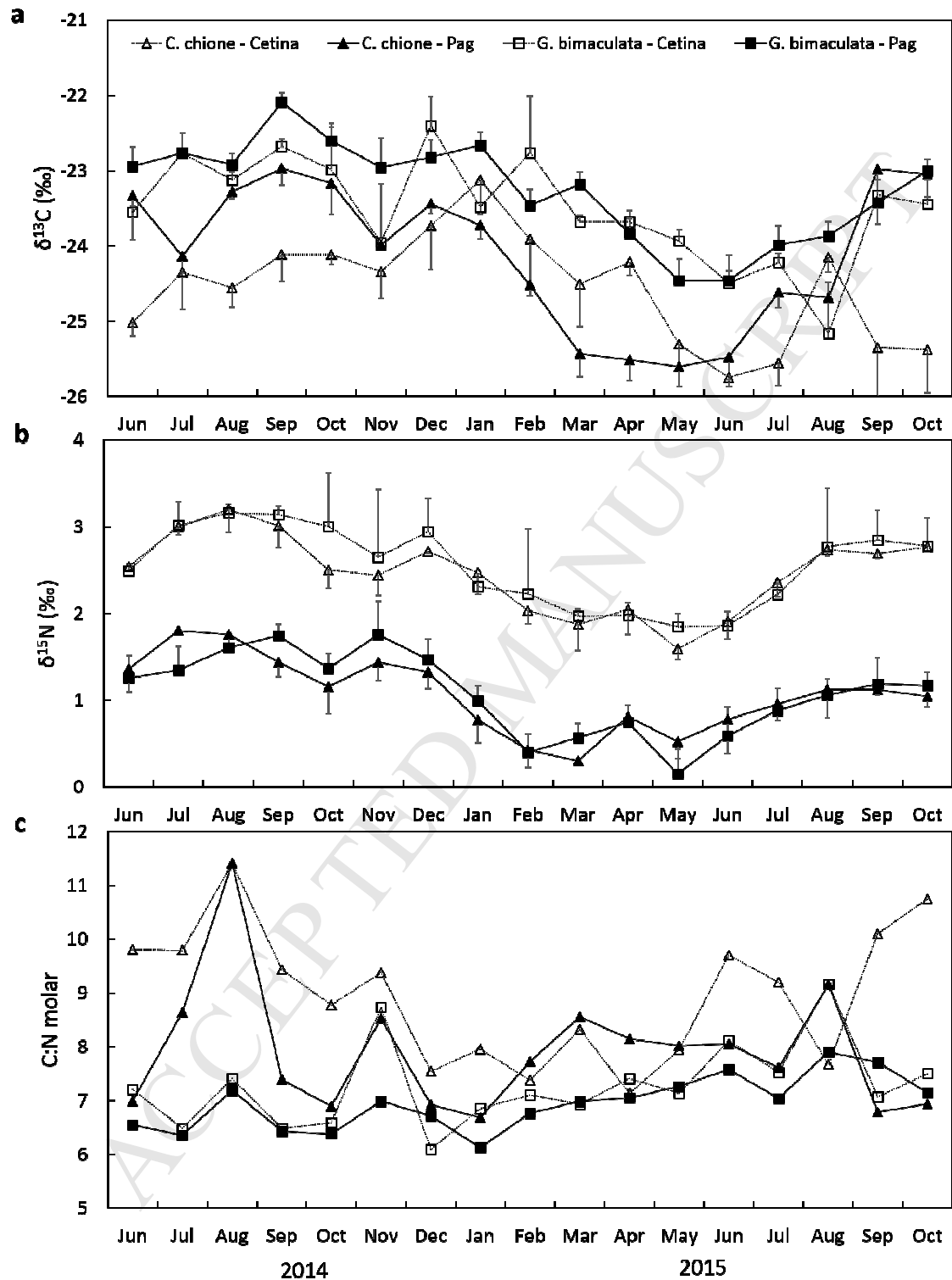
352 The DG isotopic carbon signature showed a temporal variation that ranged from -25.6 to -23.0‰  
353 in Pag and -25.8 to -23.1‰ in Cetina in *C. chione*, and slightly higher  $\delta^{13}C_{DG}$  in *G. bimaculata*, from -  
354 24.5 to -22.1‰ in Pag and -25.2 to -22.4‰ in Cetina (Fig 3a). In *C. chione* the  $\delta^{15}N_{DG}$  varied from  
355 0.3 to 1.8‰ in Pag and 1.6 to 3.2‰ in Cetina and in *G. bimaculata* from 0.2 to 1.8‰ in Pag and 1.9  
356 to 3.2‰ in Cetina (Fig 3b). Two-way ANOVAs revealed significant differences in  $\delta^{13}C_{DG}$  between  
357 sites, month and their interaction for both species (at  $P < 0.001$ ), same for  $\delta^{15}N_{DG}$  values except for  
358 temporal changes between sites for *C. chione* ( $P > 0.05$ ) (Table S3). These results indicated that  
359  $\delta^{13}C_{DG}$  variation was species-dependent with slightly more  $^{13}C$ -depleted values in *C. chione*. In  
360 addition, a temporal offset of weeks in  $\delta^{13}C_{DG}$  values relative to  $\delta^{13}C_{SPM}$  values, observed in both  
361 sites (Figs 2c, 3a). On the other hand,  $\delta^{15}N_{DG}$  values were site-dependent, with more  $^{15}N$ -enriched  
362 values corresponding to Cetina samples. Overall, enriched isotopic values were observed between  
363 June 2014 to January 2015 and July to October 2015, whereas lower values occurred from  
364 February to June 2015. The  $C:N_{DG}$  molar ratio showed significant differences between sites, month  
365 and their interaction for *C. chione* ( $P < 0.001$ ) but they were less evident for *G. bimaculata* ( $P \geq$   
366 0.001) (Fig 3c; Table S3).

367

368 The proportion of each food source on the consumer diets estimated by MixSIAR models revealed  
369 that bivalves used both SPM and Sed; the Gelman-Rubin diagnostic for each model  $< 1.05$ ,  
370 supported the result. However, relative importance of each food source differed between sites.  
371 Sed was more important for Pag populations with a small contribution of SPM during summer,  
372 whereas in Cetina SPM contributed the most to the bivalves diet (Fig 4). The output from SIBER  
373 analysis revealed a large overlap from both convex hull and SEA between species isotopic niche at  
374 each site, indicating an overall proximity in their isotopic niche (Fig 5a). According to the temporal  
375 isotopic distribution results, feeding niche overlap occurred just in November and March,



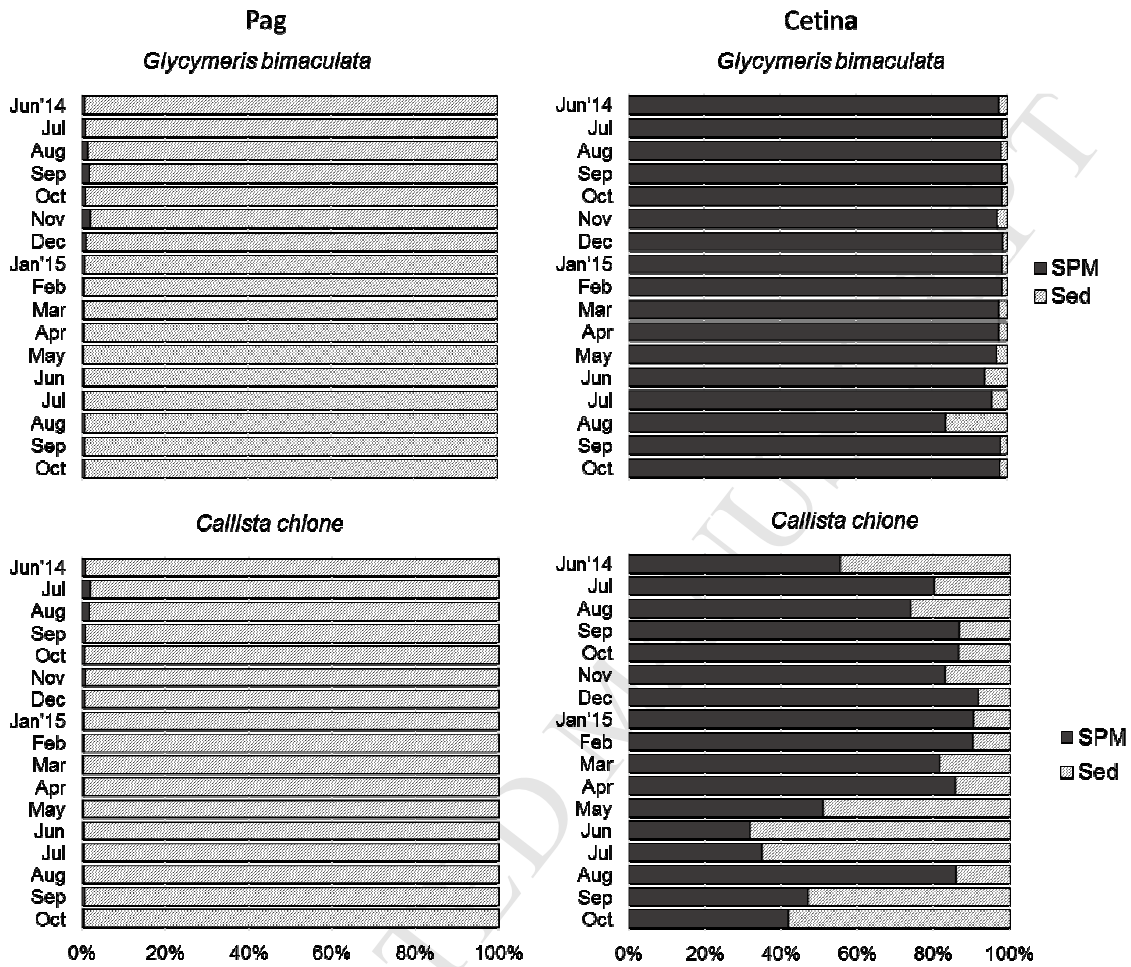
376 revealing different composition of isotopic food sources throughout the year (Fig 5a, Fig S2) and  
377 making the temporal variation observed in Fig 3 clearer. Individuals of Gly<sub>pag</sub> showed the smallest  
378 isotopic feeding niche with a complete SEA overlap and a convex hull overlap with Cal<sub>pag</sub>. Cal<sub>pag</sub> had  
379 a wider niche, where the most enriched values overlapped with those from Gly<sub>pag</sub>. Based on SEA<sub>B</sub>  
380 results, Cal<sub>pag</sub> and Gly<sub>Cetina</sub> have a greater plasticity than the other species at these locations (Fig  
381 5c).



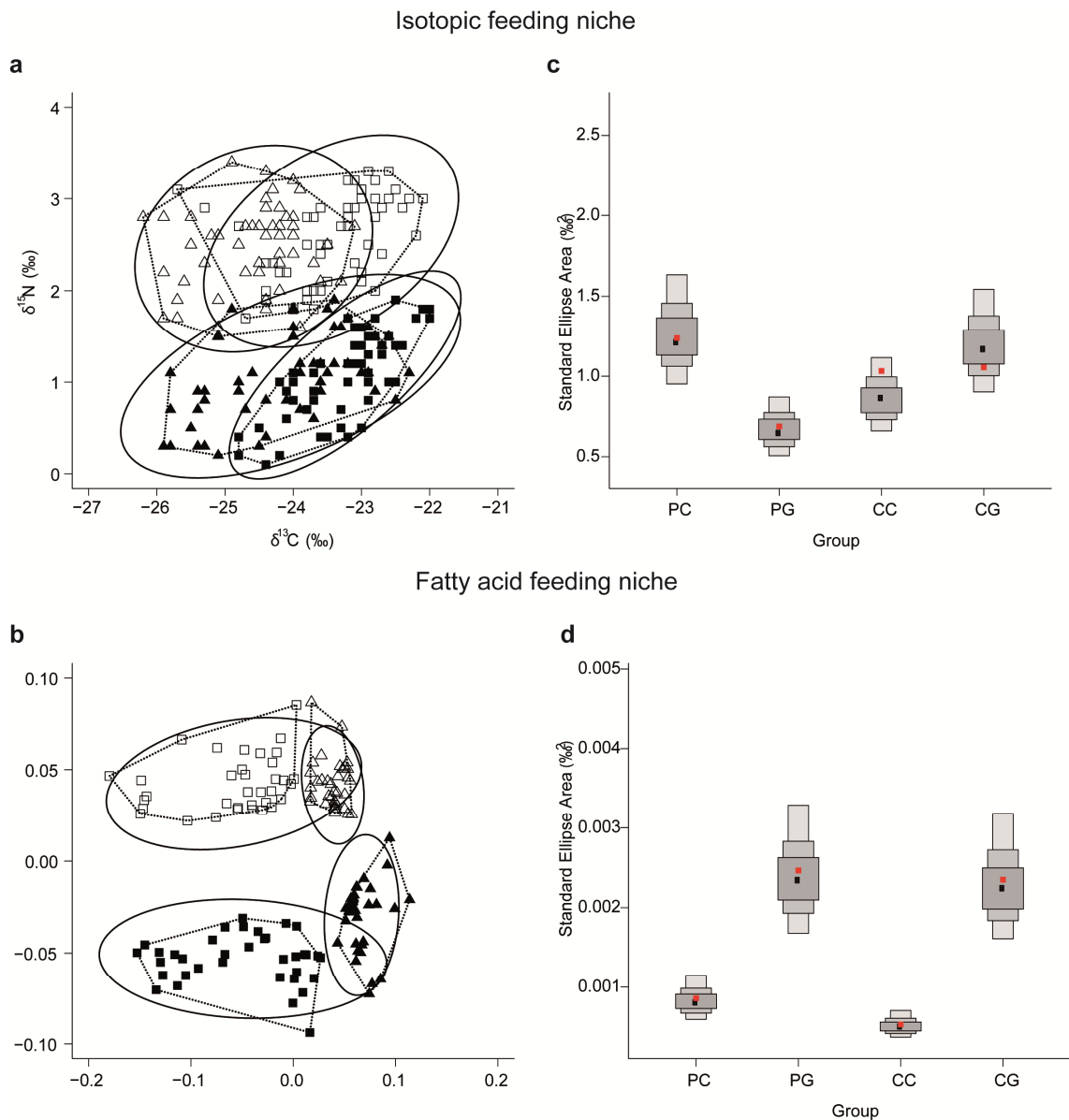
382  
383

Fig 3. Temporal variation in (a)  $\delta^{13}\text{C}$  (‰), (b)  $\delta^{15}\text{N}$  (‰) and (c) C:N molar ratio in the consumer's digestive

384 gland from both sites. *Callista chione* (*C. chione*) and *Glycymeris bimaculata* (*G. bimaculata*) from Pag and  
 385 Cetina.



386  
 387 **Fig 4.** Contribution of food sources: suspended particulate organic matter (SPM) and sediment (Sed) to the  
 388 diet of *Glycymeris bimaculata* and *Callista chione* at two sites: Pag and Cetina. Output from a two-source  
 389 MixSIAR model for each population.



390

391 **Fig 5.** Isotopic (SI) and fatty acid (FA) feeding niche of two benthic suspension feeders at two sampling sites  
 392 during 17 months. SIBER output using (a) pooled stable isotope bi-plot and (b) nMDS x-y ordinates of pooled  
 393 FA profiles. *Glycymeris bimaculata* (squares) and *Callista chione* (triangles) in Pag (filled) and Cetina (empty).  
 394 The continuous line represents the small sample size corrected standard ellipse areas ( $SEA_c$ ) and the convex  
 395 hulls areas are the dotted lines of calculated feeding niche widths for each species. Mean standard ellipse  
 396 area (SEA) estimates for each group (PC= *Callista chione* from Pag; CC= *C. chione* from Cetina; PG=  
 397 *Glycymeris bimaculata* from Pag and CG= *G. bimaculata* from Cetina) for SI (c) and FA (d). Boxed areas  
 398 indicate the  $SEA_B$  with Bayesian 50, 75 and 95% credible interval with the mode indicated by black squares.

399 The maximum likelihood estimate for the corresponding SEAc as depicted in Fig 5 is indicated by red  
400 squares.

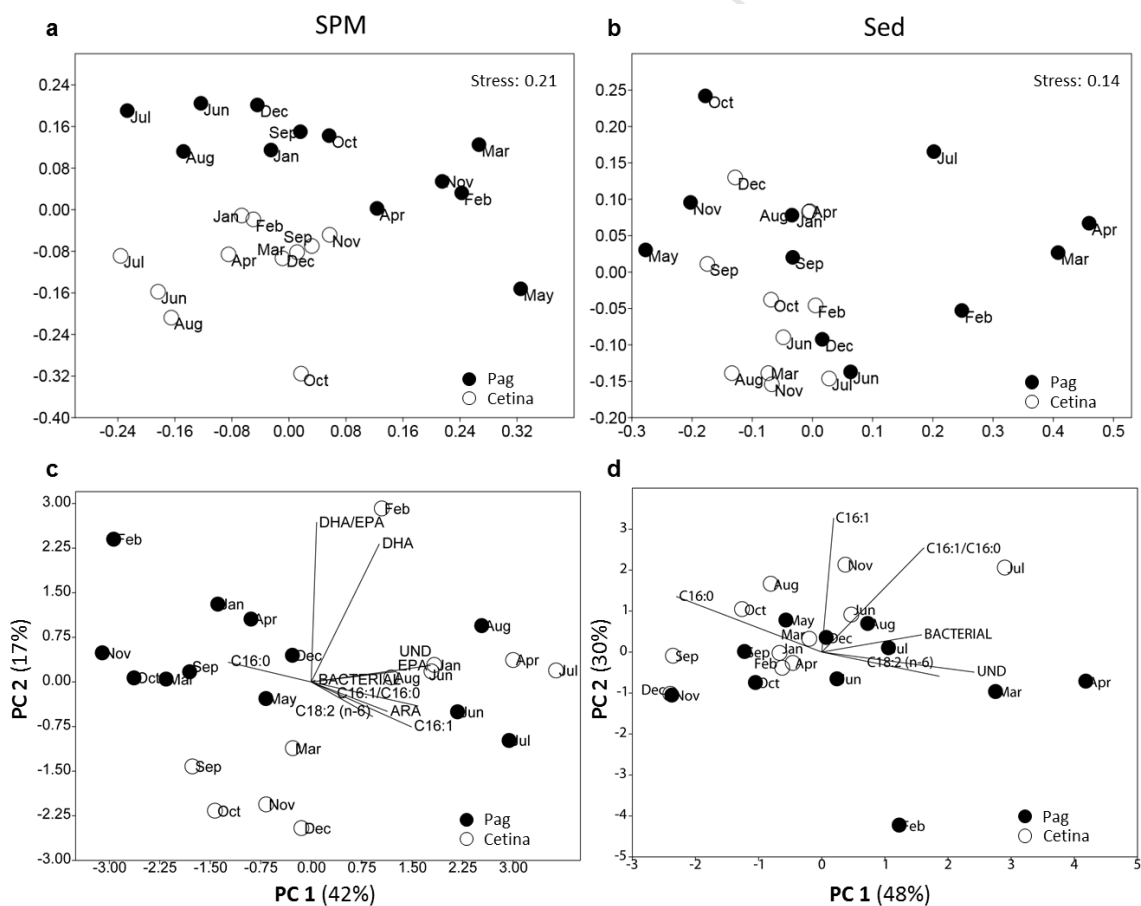
401

### 402 3.3. Fatty acids

403 FA with values  $> 0.01$  (1% of total FA) are represented in Table S5 for SPM and Sed and in Table S6  
404 for DG. All compartments showed the higher proportions on saturated fatty acids (SFA), compared  
405 to MUFA and PUFA, with higher TFA% during fall and in DG also during winter. The lowest SFA  
406 values were observed during the summer, when PUFA was more abundant.

#### 407 3.3.1. Food sources

408 Considering all  $FA_{SPM}$  ( $n = 33$ ) and  $FA_{Sed}$  ( $n = 24$ ), a two-way ANOSIM and nMDS were performed  
409 showing significant differences between sites and month ( $r > 0.99$ ,  $P < 0.001$ , and  $r > 0.84$ ,  $P <$   
410  $0.001$ , respectively) and nMDS revealed the large temporal spread of the data between sites,  
411 which was more evident in SPM than in Sed (Fig 6a,b).



412

413 **Fig 6.** The upper panels show the non-metric multidimensional scaling (nMDS) of (a) suspended particulate  
414 organic matter (SPM) and (b) sediment (Sed), based on all FA profile averages. The bottom panels show the  
415 Principal component analysis (PCA) output using most influential FA profiles (see text) in (c) SPM and (d)  
416 Sed. Sampling sites are represented as filled dots for Pag and blank dots for Cetina.

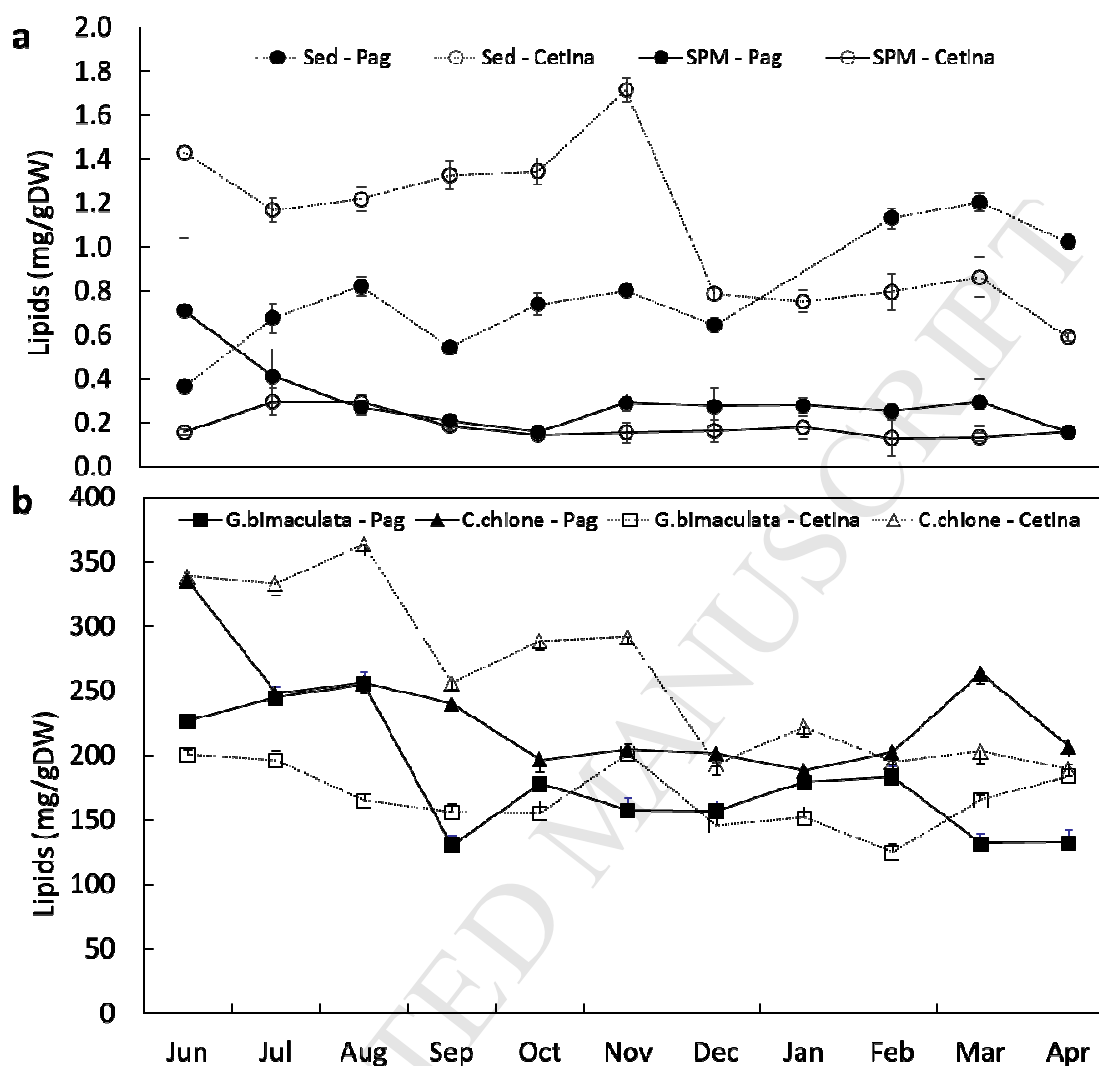
417

418 SIMPER analyses revealed that the FA C16:1, C18:2 (n-6) and PUFA were the profiles which better  
419 explained dissimilarities between sites for both SPM and Sed, with a cumulative contribution of  
420 nearly 50% for both compartments (Table S7).

421 To observe the spatio-temporal variations of the main FA profiles for each food source (SPM and  
422 Sed) identified with SIMPER, PCAs were performed (Fig 6c,d). In the case of  $FA_{SPM}$ , January and  
423 April (only at Cetina) and summer months (at both sites) were positively related to PC1 (explaining  
424 42% of the variance) due to the contribution of EPA, UND, 16:1, 16:1/16:0, 18:2, BACTERIAL and  
425 ARA. This statistical relationsuggested thatphytoplankton material was combined with bacterial  
426 and terrestrial components in the SPM in those months. In PC2, the contribution of DHA and  
427 DHA/EPA showed that SPM sampled in February at Cetina had organic matter with good quality as  
428 well. In contrast, the negative relation of  $FA_{SPM}$  in PC1 indicated by C16:0, showed that the organic  
429 matter available in SPM in Pag during fall, winter and spring had relatively poorer quality.  
430 However,  $FA_{SPM}$  from Pag were more enriched with DHA than  $FA_{SPM}$  from Cetina during the same  
431 period (Fig 6c). The spatio-temporal separation of  $FA_{Sed}$  samples was less evident than the one  
432 observed for  $FA_{SPM}$ , suggested that most of the samples represent a food mixture without  
433 predominant components. However, a small prevalence of the terrestrial component could be  
434 detected in March and April at Pag, whereas a phytoplankton component was prevalent during  
435 summer at Cetina (Fig 6d).

436

437 The concentration of  $lipids_{SPM}$  differed significantly between sites; however, it followed a similar  
438 temporal pattern ( $P < 0.001$ ; Table S2) ranging from 0.16 to 0.71 mg/L in Pag and from 0.13 to 0.30  
439 mg/L in Cetina (Fig. 7). At both sites, the lowest concentration of  $lipids_{SPM}$  took place between  
440 August and October (Fig 7a). In  $lipids_{Sed}$ , values ranged from 0.36 to 1.20 mg/g in Pag and 0.59 to  
441 1.72 mg/g in Cetina with different temporal patterns between sites; the concentration was  
442 significantly higher in Cetina ( $P < 0.001$ ; Table S2).



443  
 444 **Fig 7.** Temporal variation of lipid concentration in (a) sediment (Sed; dashed line) and suspended particulate  
 445 organic matter (SPM; continuous line) and (b) *Glycymeris bimaculata* and *Callista chione* at Pag (fill) and  
 446 Cetina (blank).

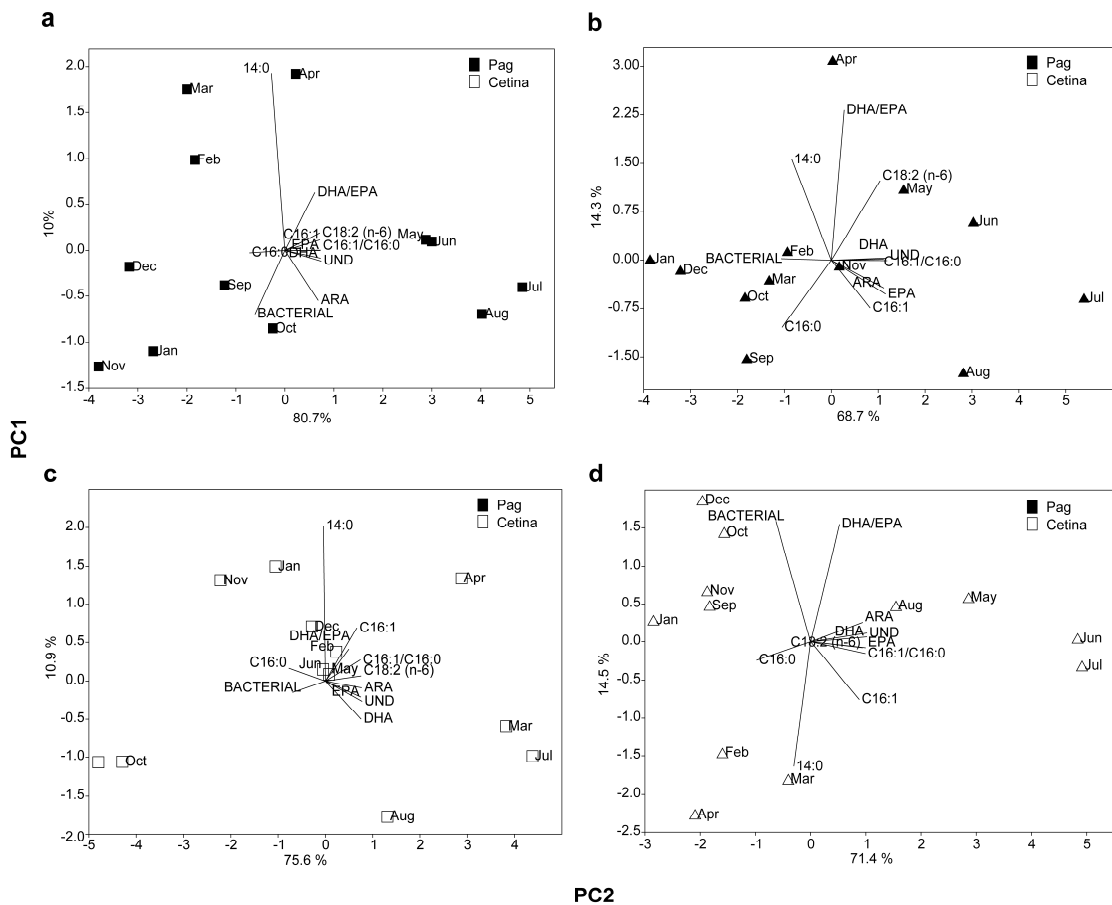
447

### 448 3.3.2. Consumers

449 Results for FA<sub>DG</sub> were derived from the same pooled individuals than for SI. Most predominant FA  
 450 group were SFA (> 58 TFA%), followed by MUFA (> 23 TFA%) and PUFA (> 5 TFA%) (Table S6).  
 451 ANOSIM revealed significant differences on all FA profiles (n = 38) among each group formed by  
 452 species-site (r = 0.83, P < 0.001) and these were more evident between species than sites in the  
 453 pairwise comparison (r = 1, P < 0.001 between species; r < 0.53, P < 0.001 between sites). SIMPER  
 454 analyses and PCA revealed the FA profiles that most contributed to temporal differences for each

455 consumer group, with PC1 explaining more than 68% and PC2 up to 14.5% (Table S8; Fig 8). PUFA,  
456 C20:5 and C22:6 contributed to nearly 50% of the differences between each group.  
457 Generally, in both sites and for both species, PC1 axis clearly separated spring and summer  
458 months from fall and winter, according to the digestion of high quality fresh material or bacterial  
459 material enriched with saturated components, respectively (Fig 8; Table S6).  
460 In late spring and summer, Gly<sub>pag</sub>'s digestive glands were strongly positively related to PC1 (EPA,  
461 DHA, 16:1, 16:1/16:0, UND) and separated from the fall, winter and early spring according to  
462 negative relations to PC1 (16:0). The latter samples were additionally separated according to the  
463 influence of BACTERIAL FA (between Sep–Jan) and C14:0 (between Feb–Apr) positively and  
464 negatively related to PC2, respectively (Fig 8a). In Cal<sub>pag</sub>'s digestive glands, a positive relationship  
465 of fresh material markers to PC1 was found between May and August, with a more pronounced  
466 terrestrial component (C18:2) in May and June, and phytoplankton (16:1, EPA) in July and August.  
467 A BACTERIAL component (negative PC1) was more digested during fall, winter and early spring,  
468 being more enriched with C16:0 or C14:0 (Apr) (Fig 8b). The digestive glands of Gly<sub>Cetina</sub> from  
469 March, April, June and August were positively loaded to PC1, according to enrichment with all  
470 unsaturated FA components and separated from September, October, November and January,  
471 due to enrichment with the BACTERIAL component negatively related to PC1. Samples from  
472 February, May, June and December were indifferent to both axis, which suggested the digestion of  
473 mixture without prevalence of any food component (Fig 8c). In Cal<sub>Cetina</sub>'s digestive glands, samples  
474 were positively related to PC1 (EPA, DHA, ARA, 16:1, 16:1/16:0, UND) between May and August,  
475 and separated from the other months according to negative relation of 16:0 to PC1. Additionally,  
476 the influence of the BACTERIAL component separated fall and early winter samples (between  
477 Sep–Jan) from the late winter and early spring (Feb–Apr) which were enriched with C14:0 (Fig 8d).  
478





479

480 **Fig 8.** Principal component analysis (PCA) output using most influential fatty acid profiles in Pag for (a)  
 481 *Glycymeris bimaculata* (b) *Callista chione* and Cetina for (c) *G. bimaculata* (d) *C. chione*.

482

483 Overall, spring and summer were the seasons with the fresher material found in the digestive  
 484 gland of both specimens, in contrast to fall and winter, where the BACTERIAL component was  
 485 dominant. The herbivorous calanoid copepod markers (C20:1 and C22:1) were more abundant in  
 486 spring and summer; oleic acid (C18:1) characteristic of zooplankton was more abundant in *C.*  
 487 *chione*, and a diatom marker (C14:0) was more abundant in *G. bimaculata*. In addition, within the  
 488 SFA pool, the bacteria marker associated to detrital matter (C20:0) was slightly higher in *G.*  
 489 *bimaculata*, especially at Cetina (Table S6). SIBER analysis of FA revealed a substantial overlap  
 490 between SEA but not convex hulls from each consumer group (Fig 5b). The FA feeding niche plot  
 491 also showed that consumers were grouped by site and the highest niche plasticity corresponded  
 492 to *G. bimaculata* at both sites (Fig 5d).

493 The concentration of lipids<sub>DG</sub> ranged from 188.34 to 335.39 mg/g in Cal<sub>Pag</sub>, 189.59 to 363.90 mg/g  
494 in Cal<sub>Cetina</sub>, 130.53 to 255.36 mg/g in Gly<sub>Pag</sub> and from 125.07 to 201.78 mg/g in Gly<sub>Cetina</sub>. Overall,  
495 there was a decreasing trend from summer to spring (Fig 7b).

496

#### 497 **4. DISCUSSION**

498 In coastal zones, marine particulate organic matter is composed by a mixture of terrestrial and  
499 marine particles and living and detrital material mainly derived from plankton, bacteria, fecal  
500 pellets or zooplankton remains such as exoskeletons (Berto et al., 2013; Savoye et al., 2012). The  
501 coastal fringe in the eastern Mediterranean is especially important due to its oligotrophic  
502 conditions, where environmental variation (e.g. wind, river discharges) may exert stronger  
503 influence on the nutrient and biota dynamics in its shallow water column. Within this frame, it is  
504 important for ecological studies, particularly for trophic ecology approaches, to disentangle food  
505 sources and their variability to gain insights into the relationships between biotic and abiotic  
506 factors that shape the ecosystem.

507

##### 508 *4.1. Characterization of food sources*

509 The  $\delta^{13}\text{C}$  pattern in the SPM showed isotopically lighter carbon during winter and spring (down to -  
510 26‰). This was presumably due to high precipitation events associated to river discharge and  
511 more positive  $\delta^{13}\text{C}$  values during summer and fall (up to -22.8‰), closer to those related to marine  
512 environments (e.g.  $\sim$ -22‰ in marine phytoplankton) (Fry and Sherr, 1984; Harmelin-Vivien et al.,  
513 2008), suggesting a progressive mixing of terrestrial and marine materials (e.g. Berto et al., 2013;  
514 Savoye et al., 2003).

515 Unlike SPM, large spatial rather than temporal variations were observed in  $\delta^{13}\text{C}$  values in  
516 Sed, which averaged  $-22\text{‰}\pm 0.4$  at Pag and  $-26\text{‰}\pm 0.2$  at Cetina. These values were within those  
517 reported for locations in the Adriatic Sea (Faganeli et al., 2009; Ogrinc et al., 2005; Žvab Rožič et  
518 al., 2015) receiving marine and terrigenous inputs and suggested that the isotopic signature in Sed  
519 may reflect a longer time-integrated signal than that in SPM. The upper sediment layer in Pag  
520 showed more positive  $\delta^{13}\text{C}$  values which could be attributed to microphytobenthic production,  
521 which is most likely contributing to primary production in other areas in the Adriatic Sea  
522 (Šestanović et al., 2009). In contrast, the proximity to the river mouth suggests that the influence

523 of terrestrial matter in Cetina should be responsible of the  $^{13}\text{C}$ -depleted signal as shown in other  
524 estuarine areas (Hedges et al., 1997; Sarma et al., 2014).

525

526 The interpretation of the nitrogen signal requires considering the potential contribution of several  
527 sources, namely  $\text{N}_2$ ,  $\text{NO}_3^-$ ,  $\text{NH}_4^+$  or DON (e.g. Berto et al., 2013; Currin et al., 1995; Peterson, 1999).  
528 On the one hand, the phytoplankton and bacterioplankton  $\delta^{15}\text{N}$  signature ranges in the order of -2  
529 to 5‰ (Rees et al., 2006), whereas on the other, the Eastern Mediterranean basin is  $^{15}\text{N}$ -depleted  
530 due to its oligotrophic nature (Pantoja et al., 2002; Sachs and Repeta, 1999). Given that our  
531  $\delta^{15}\text{N}_{\text{SPM}}$  data ranged from 1.6 to 6.1‰ in Pag and from 2.5 to 5.5‰ in Cetina, it is hard to  
532 distinguish the drivers of the  $\delta^{15}\text{N}_{\text{SPM}}$  signal; however, a combination of factors modulating the  
533 observed results seem more feasible. The  $\sim 4\text{‰}$  temporal variation observed along the study  
534 period suggests that a mixture of freshwater and marine water influenced both study sites (Fig  
535 2d). The higher values coincided in months with high precipitation events (typically fall) that  
536 increased river discharges (Cetina area) and pulses of underwater springs (characteristic from Pag  
537 area) (Surić et al., 2015) which potentially enhanced the enrichment in the nitrogen isotopic  
538 composition signal (Xue et al., 2009).

539 In the sediment, more negative and variable values (ranging from -2.3 to 3.9‰) were  
540 found in Pag; negative values are likely associated to benthic diazotroph community. For example,  
541 in oligotrophic water bodies, diazotroph biomass is responsible of atmospheric nitrogen fixation  
542 (Rees et al. 2006, Pantoja et al. 2002, Wada et al. 2012), particularly by blue-green algae (Currin et  
543 al., 1995; Minagawa and Wada, 1986). Studies on cultured cyanobacteria revealed that nitrogen  
544 isotope ratios ranging from -1.5 to -3.0‰ during  $\text{N}_2$  fixation were associated to high  $\delta^{13}\text{C}$  in  
545 cyanobacteria cells (Wada et al., 2012). Alike, studies on cyanobacteria in sedimentary organic  
546 matter in the NW and Eastern Mediterranean showed  $\delta^{15}\text{N}$  ranges from -2 to 2‰, suggesting that  
547 the observed values were the result of bacterial organic matter remineralization (Kerhervé et al.,  
548 2001; Möbius, 2013). This association would imply that bacterial nitrogen fixation is a main  
549 contributor to the  $\delta^{15}\text{N}$  signal in PON in the coastal system off Pag island. On the other hand,  
550 comparatively more constant but also relatively low values in Cetina (averaging  $2.3\text{‰} \pm 0.2$ ), were  
551 similar to those in Mediterranean estuarine areas (Carlier et al., 2007).

552

553 Based on the live phytoplankton C:N molar ratio of 6, the Redfield ratio is a traditional indicator to  
554 discriminate fresh relative to degraded marine and to distinguish marine from terrestrial organic  
555 matter (Redfield, 1958). Bacterial cells have lower C:N molar ratio than phytoplankton due to  
556 preferential mineralization of N (Newton et al., 1994), therefore, in periods of food scarcity C:N  
557 ratios may decrease due to bacterial presence. The C:N<sub>SPM</sub> values (between 7 and 11, Fig. 2b) in  
558 Pagsuggest a high proportion of phytoplanktonic material (e.g. Schubert and Calvert, 2001)  
559 throughout the year and a particular contribution of bacterial material in December and February,  
560 what seems to be in concordance with the isotopic signals previously discussed. At Cetina, C:N<sub>SPM</sub>  
561 ranged from 7 to 11 indicating a mixture of phytoplankton with marine degraded and/or  
562 terrestrial OM in agreement with other estuarine systems, probably enhanced by riverine inputs  
563 (Dias et al., 2016; Hedges et al., 1997; Savoye et al., 2003). The presence of several C:N ratio peaks  
564 (e.g. October 2014, December, February and June 2015; Fig 2b) in Cetina site was most likely  
565 related to resuspension events that incorporated degraded material into the water column. The  
566 coincidence with high suspended particulate matter values (Table S1) provides further support to  
567 this idea. The low relationship between C:N and biogenic silica suggest that the non-siliceous  
568 phytoplankton community could be more important than diatoms, radiolarians and  
569 silicoflagellates in the carbon pool in this shallow water column.

570 In the sediment at Pag, the overall higher C:N<sub>sed</sub> ratios (between 5 and 31) suggested  
571 higher abundance of bacteria during fall and winter, in agreement with the observed N isotopic  
572 signature (Fig 2b,d). The C:N<sub>sed</sub> ratios in Cetina were more constant (averaging 7.7) and coexisted  
573 with comparatively higher percentage of biogenic silica, implying a higher concentration of fresh  
574 OM with diatom abundance. However, the FA marker for diatom was quite low (as discussed next)  
575 suggesting that the presence of empty frustules explains the high BSi values and providing further  
576 support to the presence of bacteria in the OM pool.

577 In general, the low %C and %N in the sediment were within the range of other reports  
578 from the Adriatic, e.g. 0.5-1.3 %C and 0.1-0.17 %N (Ogrinc et al., 2005) and 0.02-0.15 %N  
579 (Matijević et al., 2009) corroborating the poor nutritional quality of the sediment in this  
580 oligotrophic basin. In addition, the POC/Chl *a* values were > 200 indicating high chlorophyll *a*  
581 degradation most likely due to the strong presence of heterotrophic/mixotrophic organisms  
582 (Bentaleb et al., 1998; Galois et al., 1996) as reported in estuarine areas in the Adriatic Sea (Šolić et

583 al., 2015), which have also been found to be key bacterial grazers in coastal oligotrophic waters  
584 (Unrein et al., 2013).

585

586 Seasonal FA patterns in the SPM also indicated different sources of OM at two sites. The most  
587 important components of SPM and Sed lipids were 14:0, 18:0, 18-1(n-9), 20:1, 20:0 and BACTERIAL  
588 FA (Fig 6, Table S6). Within SFA, C20:0, which could be linked to bacteria associated to detrital  
589 matter collected from the sediment (Galap et al., 1999) or suspended particulate matter was  
590 higher during winter and it was more abundant in Pag, coincident with SI interpretations. The high  
591 proportion of MUFA, 18-2(n-6) and BACTERIAL FA in Cetina suggested that terrestrial inputs  
592 predominated in the OM in SPM, also supporting the observations of SI results. Usually, the FA  
593 marker C18:2(n-6) is associated with terrestrial sources (Budge and Parrish, 1998; Fischer et al.,  
594 2014; Parrish et al., 1996), seagrass (Kharlamenko et al., 2011) or protozoa (Zhukova and  
595 Kharlamenko, 1999). The FA 18:1(n-9) was particularly abundant during spring and summer, and it  
596 has been associated with zooplankton (Sargent and Falk-Petersen, 1988) and cyanobacteria  
597 (Antonio and Richoux, 2016) Relatively high PUFA concentrations (particularly EFA's; Fig 6)  
598 suggested that the best OM quality in SPM took place during summer at both sites. However, in  
599 Pag, slightly higher values coincident with relatively low C:N and high BSi values, indicated a larger  
600 contribution of fresh siliceous phytoplankton biomass in that site. Further, herbivorous calanoid  
601 copepod markers were more abundant in Cetina during fall and winter, presumably following  
602 phytoplankton biomass peaks. In the Sed, high PUFA concentrations were also observed in  
603 summer, suggesting an effective pelagic-benthic coupling along the study area.

604 Phytoplankton FA markers,  $\delta^{13}\text{C}$ , C:N ratios and  $\text{BSi}_{\text{SPM}}$  values suggested that SPM is of  
605 better quality and has more phytoplankton influence during spring and summer. Often,  $\delta^{13}\text{C}$  is  
606 positively correlated to Chl *a* concentrations (e.g. Miller and Page, 2012) and probably this  
607 relationship is more evident in shallow coastal areas, such as the present study area. However, the  
608 temporal mismatch between Chl *a* and the rest of measured parameters in the present study was  
609 most likely influenced by the high amount of resuspended material different to phytoplankton  
610 (e.g. bacteria and empty diatom frustules) that may obscure the signal of Chl *a*, which is related  
611 solely to phytoplankton biomass.

612

613 The combined SI and FA temporal variation profiles in the SPM and Sed described an alternation  
614 between a freshwater dominated system in winter to a marine-water dominated one in summer.  
615 Indeed, the particulate matter in Pag appeared of better quality due to abundance of EFA within  
616 PUFA in both compartments and by the lighter values of  $\delta^{13}\text{C}$  and less SFA observed in the  
617 sediment. However, the nitrogen isotopic signature better distinguished the sources contributing  
618 to the OM pool at each site resulting in a better tracer of environmental changes than the carbon  
619 isotope (Fig 3a). Overall, results suggested that bacteria seemed to play a major role in the organic  
620 carbon and nitrogen budgets in the particulate matter in the coastal system off the island of Pag.  
621 BACTERIAL FA showed that bacteria were abundant in both compartments at both sites, making  
622 evident their importance as food source (Galois et al., 1996; Šestanović et al., 2009).

623

#### 624 4.2. Spatial and temporal variation in bivalve diet

625 The isotopic composition of the digestive gland of *Callista chione* and *Glycymeris bimaculata*  
626 revealed a pronounced temporal variation at two sampling sites. The carbon isotopic variation  
627 indicated to be species-specific, with slightly higher values in *G. bimaculata* while large differences  
628 in the nitrogen isotope were site-specific, with higher values shown in Cetina (Fig 3a,b). The  
629 temporal variation in  $\delta^{13}\text{C}_{\text{DG}}$  was coupled to that in  $\delta^{13}\text{C}_{\text{SPM}}$ , indicating that bivalves adjusted their  
630 dietary shifts depending on the availability of food mainly in SPM (Figs 2,3). This coupling  
631 suggested that SPM is an important food source what is in agreement with other studies in coastal  
632 systems (Antonio and Richoux, 2014; DeNiro and Epstein, 1978; Kang et al., 1999; Kasai et al.,  
633 2004; Riera and Richard, 1996). Bivalves increase their  $\delta^{13}\text{C}$  values due to the preferential release  
634 of  $^{12}\text{C}$  during respiration (Kang et al., 1999; Kharlamenko et al., 2001; Page and Lastra, 2003), and  
635 our results showed a slight  $^{13}\text{C}$ -enrichment in DG with respect to SPM, presumably reflecting this  
636 process. In our study, *G. bimaculata* presented slightly heavier  $\delta^{13}\text{C}$  values than *C. chione*  
637 indicating that they feed more upon autochthonous particles (i.e. phytoplankton derived organic  
638 matter) showing a stronger dependence on primary producers, as seen in other studies (France,  
639 1995; Kang et al., 2003; Kharlamenko et al., 2008; Nadon and Himmelman, 2006). Another stable  
640 isotope study conducted on *C. chione* from the NW Mediterranean showed a more enriched  
641 isotopic signature ( $\delta^{13}\text{C}$ :  $-19.4\text{‰} \pm 0.5$ ,  $\delta^{15}\text{N}$ :  $4.7\text{‰} \pm 0.4$ ,  $n=5$  in Carlier et al., 2007), suggesting that  
642 same species may reflect local environmental differences along the Mediterranean Sea.

643

644 The  $\delta^{15}\text{N}$  in Cetina populations was ca. 1‰ higher than in Pag, allowing a clear distinction between  
645 sites (Fig 3b, Table S3). The ranges of  $\delta^{15}\text{N}$  in specimens at Cetina fell within those from other  
646 filter-feeders in the Adriatic Sea (Ezgeta-Balić et al., 2014), whereas the more negative  $\delta^{15}\text{N}$   
647 signature from Pag populations has not been previously reported for bivalves in the Adriatic Sea.  
648 These results together with the low isotopic signal observed in the Sed in Pag suggested that there  
649 are particular environmental conditions in that site. The temporal  $\delta^{15}\text{N}$  signal variation showed  
650 more negative values during winter and spring, coincident with the carbon isotope signal. In Pag  
651 populations, these values were closer to those of Sed, suggesting an important contribution of the  
652 Sed pool to their diet (further discussed in the next paragraph). These results emphasize the  
653 importance of studies on temporal variation due to the rapid imprint that environmental changes  
654 have on animal tissues. The observed spatio-temporal intraspecific variation from SI data support  
655 the use of the digestive gland as a rapid OM turnover tissue (Deudero et al., 2009; Ezgeta-Balić et  
656 al., 2014).

657

658 FA profiles complemented the information on the spatio-temporal variation of the diet  
659 composition provided by isotopic signatures. For all populations, temporal differences on FA  
660 profiles were more evident than the spatial variation. In *G. bimaculata*, SFA values were similar to  
661 those reported in *Glycymeris nummaria* (up to 82% TFA) in the south Adriatic (Najdek et al., 2016)  
662 suggesting these FA greatly contribute to the diet of *Glycymeris sp.* in the eastern Adriatic, perhaps  
663 reflecting specific selection patterns, in contrast to *C. chione* and other bivalves (Ezgeta-Balić et al.,  
664 2012; Najdek et al., 2013). Within the SFA pool, the bacteria marker associated to detrital matter  
665 (C20:0) was slightly higher in *G. bimaculata*, especially at Cetina. PUFA were higher in *C. chione*  
666 especially during summer, particularly in Pag (Table S6). PUFA, especially EFA, are essential for  
667 growth but also a high degree of unsaturation (UND) is characteristic of healthy mollusks (Dupčić  
668 Radić et al., 2014). Overall, diatom, dinoflagellate and herbivorous calanoid copepod markers  
669 contributed most to the bivalves' diet during spring and summer at both sites, particularly in *C.*  
670 *chione*. Further, since oleic acid, a marker of zooplankton, was more abundant in *C. chione*, it  
671 seems that zooplankton is preferential food for this bivalve. All these observations suggested that  
672 *C. chione* preferentially uptakes food with better quality than *G. bimaculata*.

673



674 Overall detritus, diatom, dinoflagellate, zooplankton and EFA markers were the most abundant in  
675 studied bivalve's diet in agreement with other studies in the south-eastern Adriatic Sea. In these  
676 studies, other filter-feeders such as *Mytilus galloprovincialis*, *Ostrea edulis* and *Modiolus barbatus*  
677 (Ezgeta-Balić et al., 2012), *Pinna nobilis* (Najdek et al., 2013), *Arca noae* (Dupčić Radić et al., 2014;  
678 Ezgeta-Balić et al., 2012) and *G. nummaria* (Najdek et al., 2016) revealed ingestion of a mixed diet.  
679 The higher concentration of lipids in bivalves coincided with those months with the best indicators  
680 of food quality in SPM (e.g. spring and summer). *Callista chione* fed more upon fresh material than  
681 *G. bimaclulata* which relied largely on bacteria-derived detritus, as observed in other species of the  
682 same genus, such as *G. glycymeris* (Galap et al., 1999) and *G. nummaria* (Najdek et al., 2016). The  
683 importance of bacteria in bivalves' diet has also been reported in other shallow coastal areas  
684 (Antonio et al., 2012; Kang et al., 2003). Altogether, our results corroborated the importance of  
685 heterotrophic bacteria in the carbon cycle and in the contribution to the maintenance of benthic  
686 communities in oligotrophic waters such as the Adriatic Sea (Šantić et al., 2013; Unrein et al.,  
687 2013).

688

#### 689 4.3. Contribution of food sources to bivalve diet and feeding niche

690 A two source mixing model was used to elucidate the contribution of each source to bivalves' diet  
691 through time (Fig 4). Based on our results, the contribution of the Sed pool dominated in the  
692 populations from Pag with a slight contribution of SPM during summer, when a major  
693 concentration of fresh material was available in the water column. In contrast, the SPM pool was  
694 the main contributor to the diet composition at Cetina especially for *G. bimaclulata* (> 97%). Dual  
695 isotope and fatty acid analyses characterized each site as different feeding niches (Fig 5). Within  
696 sites, the isotopic feeding niches showed an overlap only in November and March at Cetina,  
697 revealing different food sources composition during the rest of the year, whereas at Pag, the  
698 overlap was evident during fall and winter (Fig S2). A larger contribution of Sed was clear in Pag  
699 populations supported by the <sup>15</sup>N-depleted values found in the DG. Isotopic niches alone should be  
700 carefully addressed as remarked in other studies (Albó-Puigserver et al., 2015; Fry, 2013; Karlson  
701 et al., 2015), especially when analyzed in bulk matter, where the detailed isotopic signature of  
702 specific components (e.g. dinoflagellates, diatoms, copepods, bacteria, etc.) could be obscured,  
703 particularly in a poorly investigated area (i.e. lacking specific enrichment factors and baseline  
704 studies).



705

706 At Pag, the highest niche plasticity was observed for *C. chione* suggesting its ability to  
707 consume more isotopically diverse food sources than *G. bimaculata*. Niche plasticity allows to  
708 reduce intraspecific competition when food availability is scarce (Gutt, 2006) by consuming  
709 different fractions of POM (Riera, 2007). The narrower isotopic range in *G. bimaculata* would  
710 suggest that both species compete for food in a period of food scarcity, however, the fatty acid  
711 feeding niche revealed that despite the overlap, *G. bimaculata* is more opportunistic. This  
712 behavior matches with the trophic activity of *C. chione* which appeared to be more selective  
713 within the available organic pool. Morphological differences could also play a role in the selection  
714 of food particles. The presence of siphon in *C. chione* could restrict its ability to pick particles from  
715 the environment. On the contrary, little is known about the feeding habitats of *G. bimaculata*  
716 which has no inhalant siphon and presumably has the ability to feed upon particles in the  
717 interstitial water.

718 Our results suggest that both species have the capacity to ingest particles from both SPM  
719 and Sed pools. This type of intraspecific variation was also observed in the Pacific oyster  
720 *Crassostrea gigas* in France (Riera and Richard, 1996). SPM is a dynamic pool of autochthonous  
721 and allochthonous material thus, multiproxy approaches facilitate distinction in the origin of SPM.  
722 We can conclude that each species obtained their carbon from different sources and that the  
723 coupling of nitrogen isotopic composition between Sed and DG was useful to distinguish  
724 populations (Fig 5b). A larger number of bacterial FA markers in *G. bimaculata* indicated that this  
725 species fed more upon bacteria than *C. chione*. In agreement with this observation, bacteria just  
726 constituted a small proportion of the diet in *C. chione* in the western Mediterranean (Charles et  
727 al., 1999). *G. bimaculata* might act as an opportunistic feeder in this respect, incorporating  
728 resuspended particles with more detrital-derived material such as seen in *P. magellanicus*  
729 (Shumway et al., 1987). Bacteria seems to contribute significantly in periods of food scarcity to  
730 satisfy nitrogen bivalve requirements (Langdon and Newell, 1990), which would be the case in the  
731 present study, especially for Pag.

732

### 733 CONCLUSIONS

734 The temporal variation of particulate matter in the coastal central Adriatic Sea reflected a mixture  
735 between marine and terrestrial sources throughout the year, with a clear marine influence during

736 summer and fall, and terrestrial inputs during spring and winter. Detrital material associated to  
737 bacterial FA was more abundant in SPM from Pag, whereas in Cetina, both detrital and terrestrial  
738 FA had the highest concentrations. In addition, EFA and zooplankton markers indicated that during  
739 spring and summer OM had better quality at both sites. The more negative carbon isotope  
740 signature from sediment in Cetina suggested a greater influence of terrestrial matter from the  
741 river discharges. It is difficult to draw conclusions on the temporal variation of  $\delta^{15}\text{N}$  signal;  
742 however, this signal is most likely related to the activity of diazotroph biomass, particularly in Pag.

743

744 The  $\delta^{13}\text{C}$  was species-specific, with more positive values in *G. bimaculata* than *C. chione* whereas  
745  $\delta^{15}\text{N}$  was site-specific, with more positive values in Cetina than in Pag. A small temporal offset was  
746 observed in  $\delta^{13}\text{C}_{\text{DG}}$  with respect to  $\delta^{13}\text{C}_{\text{SPM}}$ , indicating the dietary shift adjustment (weeks) of  
747 bivalves to available SPM. The observed spatio-temporal intraspecific variation supported the use  
748 of digestive gland as a rapid turnover tissue. FA in the digestive gland revealed a mixed diet where  
749 *C. chione* fed more upon fresh material than *G. bimaculata*, which relied largely on bacteria-  
750 derived material.

751

752 Overall, our results suggested that there was little feeding niche overlap between *C. chione* and *G.*  
753 *bimaculata* during the year, strongly indicating resource partitioning between species, typical in a  
754 food-limited system such as the central eastern Adriatic. The pronounced temporal variation of  
755  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  within populations highlighted interspecific isotopic variation in bivalve populations.  
756 In Cetina, SPM greatly contributed to the diet of bivalves whereas in Pag, Sed appeared to have  
757 major importance. The study of trophic ecology in co-occurring species allowed identifying  
758 species-specific feeding adaptations to environmental variability providing valuable information  
759 for other ecological studies and complementing challenging research opportunities, i.e.  
760 sclerochronology.

761

## 762 **ACKNOWLEDGMENTS**

763 This research was supported by the EU within the framework (FP7) of the Marie Curie  
764 International Training Network ARAMACC (604802), and AP by a European Community Marie-  
765 Curie Fellowship (Call: FP7-PEOPLE-2013-ITN). We are grateful to Filip Bukša and Mario Zokić for  
766 the collection of specimens and to master students for help in the laboratory.

767

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