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# Tracing Groundwater Sources in Coastal Food Webs: Nitrogen and Carbon Isotope Values in Mussels in a Mediterranean Lagoon

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1           **Tracing groundwater sources in coastal food webs:**  
2 **nitrogen and carbon isotope values in mussels in a**  
3 **Mediterranean lagoon**

4  
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33 **ABSTRACT**

34

35 Whilst the role that groundwater discharge plays in supporting primary  
36 production in coastal ecosystems is well recognized, there is still limited  
37 evidence on its impacts higher up the food chain. In this study, we investigate the  
38 role of groundwater discharge in supplying nitrogen and carbon to the  
39 Mediterranean mussels (*Mytilus galloprovincialis*) in Salses-Leucate lagoon  
40 (France). Nitrogen ( $\delta^{15}\text{N}$ ) and carbon ( $\delta^{13}\text{C}$ ) isotope values in mantle and gill  
41 tissues of Mediterranean mussels are used to trace nitrogen and carbon sources  
42 at groundwater-exposed sites and a control site. Our results show similar isotope  
43 values of mantle and gill tissues, likely due to relatively stable environmental  
44 conditions in the study sites and to biochemical composition of tissues. Diet shift,  
45 fractionation and the physiological function of tissues could also explain this  
46 observation.  $\delta^{15}\text{N}$  values in mussel tissues increase overall in winter at both the  
47 groundwater-influenced sites (4.9 to 7.8‰) and the control site (3.4 to 8.5‰) in  
48 comparison with those in autumn (3.5 to 7.4‰ at the groundwater-influenced  
49 sites and 4.5 to 6.9‰ at the control site), likely due to reduced food supply and  
50 slower metabolism in winter, resulting in  $^{15}\text{N}$  enrichment. In contrast, mussel  
51  $\delta^{13}\text{C}$  values decrease in winter at the groundwater-influenced sites (-23.4 to -  
52 21.6‰) and the control site (-22.0 to -19.0‰), suggesting input of  $^{13}\text{C}$ -depleted  
53 sources originating from groundwater input. The comparison of the isotopic  
54 values of mussel tissues and potential food sources indicates that the main diet  
55 of *M. galloprovincialis* in Salses-Leucate lagoon is a mixture of groundwater  
56 particulate organic matter (POM), lagoon POM and marine POM (POM as proxy  
57 of phytoplankton). Depleted  $\delta^{13}\text{C}$  values in mussel tissues, particularly at  
58 groundwater-exposed sites, provide evidence of uptake of groundwater-derived  
59 nutrients. The ecological benefit of groundwater input on filter feeders results  
60 thus from local increases in food availability (mainly phytoplankton), supported  
61 by groundwater-derived nitrogen and carbon. In summary, this study shows the  
62 combined (a) indirect effect of groundwater discharge on mussel growth via  
63 uptake of their food, phytoplankton and (b) the likely direct feeding of  
64 groundwater POM by *M. galloprovincialis*. It shows thus the important ecological  
65 implication of groundwater inputs in coastal lagoon food webs.

66

67 **Keywords:** submarine groundwater discharge, nitrogen and carbon stable  
68 isotopes, phytoplankton, *Mytilus galloprovincialis*, coastal lagoon

## 69 **1 Introduction**

70 Groundwater discharge has been recognized as an important pathway of  
71 dissolved inorganic nitrogen and carbon in coastal ecosystems (Valiela et al.  
72 1992; Rutkowski et al. 1999; Burnett and Dulaiova 2003; Rodellas et al. 2018).  
73 The inputs of dissolved inorganic nitrogen and carbon associated with  
74 groundwater discharge can sometimes rival surface water inputs (Dorsett et al.  
75 2011; Rodellas et al. 2015; Sugimoto et al. 2016). It plays an important ecological  
76 role in coastal ecosystems (*e.g.* eutrophication and algal blooms) (Van Cappellen  
77 et al. 1993; Null et al. 2012; Kwon et al. 2017). Groundwater discharge may also  
78 be a direct source of particulate organic nitrogen and carbon to filter-feeding  
79 organisms such as mussels (Hopkinson et al. 1998; Dhillon and Inamdar 2014). A  
80 growing number of studies show the effects of groundwater input on nutrient  
81 budgets, and more recently on phytoplankton growth in coastal ecosystems  
82 (Valiela et al. 1992; Null et al. 2012; Kwon et al. 2017; Andrisoa et al. 2019b).  
83 However, there are still limited studies that document the indirect effects of  
84 groundwater discharge higher up the food chain, in particular on filter feeders,  
85 which depend on phytoplankton as primary food source.

86  
87 Coastal lagoons are highly dynamic transitional (brackish) water bodies, which  
88 are controlled by physical processes of both marine and terrestrial origin. They  
89 support a wide range of ecosystem services that are relied upon by coastal  
90 communities, including aquaculture, fishery and tourism (Kjerfve 1994; Anthony  
91 et al. 2009; Kennish and Paerl 2010). These semi-enclosed ecosystems are  
92 characterized by a restricted exchange with the sea, and they are heavily  
93 dependent on watershed inputs. In the Mediterranean Sea, which is an  
94 oligotrophic basin characterized by limited surface water inputs due to low  
95 rainfall in the region, groundwater discharge is a major source of dissolved  
96 nitrogen and carbon in coastal lagoons (Malta et al. 2017; Rodellas et al. 2018).  
97

98 Stable nitrogen ( $\delta^{15}\text{N}$ ) and carbon ( $\delta^{13}\text{C}$ ) isotopes have been widely used to trace  
99 sources of nitrogen and carbon in coastal ecosystems (Chanton and Lewis 1999;  
100 Fry 2002; Kendall et al. 2007). They provide time-integrated information of  
101 feeding relationships and energy flow through food webs (Moore and Suthers  
102 2005; Fry 2007; Perkins et al. 2014). Typically, the  $\delta^{15}\text{N}$  value provides  
103 information on trophic levels, nitrogen sources and residence time while  $\delta^{13}\text{C}$   
104 value allows to identify primary food sources and to discriminate between  
105 terrestrial and marine origin (O'Reilly et al. 2002; Piola et al. 2006). Trophic  
106 enrichment occurs along the food webs for both  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  and the average  
107 enrichment factors are 3-4 ‰ and 1‰ for  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ , respectively (Post  
108 2002; Caut et al. 2009; Kilham et al. 2009). Nitrogen and carbon stable isotope  
109 techniques are commonly used in coastal ecology, but rarely applied to trace  
110 groundwater sources and fate. In addition, most studies on nitrogen and carbon  
111 stable isotopes in coastal food webs focus on the impacts of wastewater sources  
112 and aquaculture activities (McClelland and Valiela 1998; Carlier et al. 2009;  
113 Martínez-Durazo et al. 2019), but rarely on groundwater inputs. A recent study  
114 conducted in Salses-Leucate lagoon (France) by Andrisoa et al. (2019b)  
115 investigated the  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values of phytoplankton and showed that  
116 groundwater discharge is the major source of dissolved inorganic nitrogen and  
117 carbon for phytoplankton growth (60 – 94% of N). Here we investigate whether  
118 this groundwater-derived nitrogen and carbon is transferred to filter feeders  
119 through phytoplankton feeding.

120

121 The Mediterranean mussel *Mytilus galloprovincialis* is among the commercially  
122 most important filter-feeders along the Mediterranean coastline (Ladagnous and  
123 Le Bec 1997). They are naturally present and widely distributed in many  
124 lagoons. *M. galloprovincialis* is a filter-feeding bivalve that acquires its food from  
125 particulate organic material (POM) by filtering large volumes of water over  
126 their ciliated gills (Inoue et al. 2021). POM is usually comprised of  
127 phytoplankton, detritus, and bacteria (Liénart et al. 2017, 2020). This water  
128 filtration allows them to efficiently accumulate nitrogen and carbon from the  
129 water and primary producers, thereby providing a time-integrative measure of  
130 nitrogen and carbon inputs in their tissues. Mussels have relatively long lifespan

131 and slow tissues turnover rate (turnover rates of 80 to 350 days) (Raikow and  
132 Hamilton 2001; Hill and McQuaid 2009) in comparison with their diet  
133 phytoplankton, which have a lifespan of few days to a week (Cloern et al. 2002).  
134 The isotope values of a consumer are often understood to represent its diet, but  
135 it should be noted that this isotopic composition is time-specific and represents  
136 an average ratio related to tissue turnover rate and the life of the organism.  
137 Previous studies in mussels have suggested that isotopic enrichment depends on  
138 tissue turnover rates and, consequently, it will vary according to tissue type  
139 (Deudero et al. 2009; Cabanellas-Reboredo et al. 2009; LaFrancois et al. 2018). In  
140 mussel, gill tissue has a higher turnover rate, followed by mantle and then  
141 adductor tissue (i.e. gill > mantle > adductor) (Deudero et al. 2009; Cabanellas-  
142 Reboredo et al. 2009; Hill and McQuaid 2009). There is some evidences in  
143 *Mytilus edulis* that gill tissue is more active metabolically than mantle tissue  
144 (Livingstone and Farrar 1984) and bivalves commonly have higher lipid  
145 contents in gill than mantle tissues (Wenne and Polak 1989; Ahn et al. 2000).  
146 Therefore, in order to better understand the isotope values of mussels, different  
147 type of tissue must be considered. Moreover, the C/N ratio measured in  
148 biological tissues may inform on the nutritional quality of food sources, the lipid  
149 content and/or the physiological state of the organism (Smaal and Vonck 1997;  
150 Elser et al. 2003; Post et al. 2007; Bracken 2017).

151

152 In this study, we examine the variations of  $\delta^{15}\text{N}$ ,  $\delta^{13}\text{C}$  and C/N ratios in two types  
153 of tissues (gill and mantle) of the Mediterranean mussel *M. galloprovincialis* in  
154 the groundwater-fed Salses-Leucate lagoon (France) in autumn and winter. Here  
155 we investigate the indirect role of groundwater processes in supplying nitrogen  
156 and carbon to filter-feeding organisms through phytoplankton feeding as well as  
157 the direct feeding of groundwater POM by *M. galloprovincialis*. We study both gill  
158 and mantle tissues because gill tissues have higher turnover rates and are likely  
159 not impacted by physiological cycles while mantle tissues reflect long-term  
160 assimilation of food (Hill and McQuaid 2009).

161

## 162 2 Materials and Methods

### 163 2.1 Study sites

164 Salses-Leucate lagoon is located in the southwestern Mediterranean region (Fig.  
165 1). The lagoon has a surface area of 5600 ha and a mean water depth of 1.5m.  
166 The catchment area is 160 km<sup>2</sup>, which is mainly covered by agriculture (32%)  
167 and natural pastures (28%) (Ladagnous and Le Bec 1997). The region  
168 experiences rainfall during autumn and spring (500 mm per year) with little rain  
169 during summer (Fig. 2), and is characterized by strong northwesterly winds,  
170 regularly exceeding 10 m s<sup>-1</sup>, particularly in winter months. The lagoon is  
171 connected with the Mediterranean Sea by three permanent artificial openings in  
172 the eastern part of the lagoon (Fig. 1). Direct continental surface water inputs are  
173 limited (e.g. rivers...); temporary streams are present in the southern part of the  
174 lagoon, but they are dry most of the time and freshwater input from these  
175 sources are considered negligible (Carlier et al. 2009). Throughout the year, the  
176 lagoon receives freshwater from two karstic groundwater springs (Font Dame  
177 and Font Estramar) connected with the lagoon via short streams. The mean  
178 water flow are  $3.0 \times 10^5 \text{ m}^3 \text{ d}^{-1}$  and  $2.0 \times 10^5 \text{ m}^3 \text{ d}^{-1}$ , respectively (Arnaud 1968;  
179 Fleury et al. 2007). Salinity at the groundwater-influenced sites (Font Dame)  
180 ranges from 15.5 to 34.1 throughout the year, whilst at the control site salinity  
181 ranges between 27.2 and 39.4 (Fig. 2). Average lagoon water temperature ranges  
182 between 6.2 °C in winter and 25.6 °C in summer. Water level in the lagoon is  
183 highly variable and is mainly influenced by precipitation and wind speed (Fig. 2).  
184 A sewage treatment plant (primary and secondary stages) intermittently  
185 discharges treated wastewater (ca. 500 m<sup>3</sup> d<sup>-1</sup>) to the western part of the lagoon  
186 from Salses-le-Chateau village (3500 inhabitants).

187

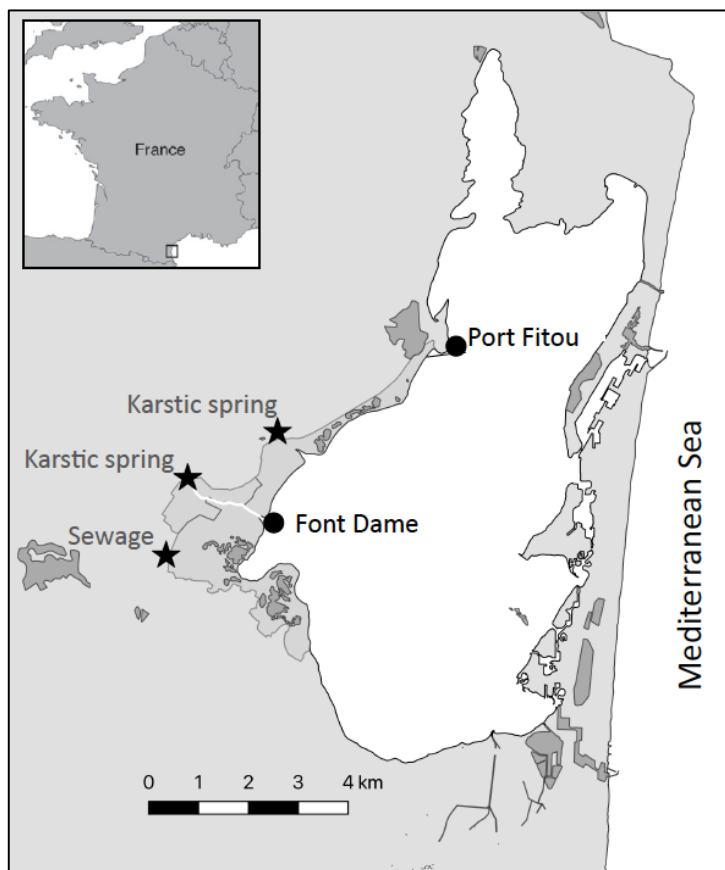
188 To evaluate the influence of groundwater inflow on the isotope values of the  
189 Mediterranean mussel, two different locations in the lagoon were selected: a  
190 groundwater-influenced site (Font Dame) and a control site (PF: Port Fitou) far  
191 from groundwater influence and representative of lagoon water conditions (Fig.  
192 1). At Font Dame, the water column is highly stratified due to fresh groundwater

193 inflow and two depths were thus studied: Font Dame Surface (FDS) and Font  
194 Dame Deep (FDD).

195

196 A recent study conducted in Salses-Leucate lagoon indicated that nitrogen and  
197 carbon originating from groundwater springs are a major source of nitrogen and  
198 carbon for primary producers (Andrisoa et al. 2019b). Groundwater springs and  
199 porewater fluxes supply 60 - 94% of the nitrogen used to support primary  
200 producer growth in the lagoon. The remaining percentage is accounted for by  
201 nitrogen input from sewage effluents. Moreover, groundwater springs in Salses-  
202 Leucate have been shown to provide favourable environmental conditions for  
203 fast growth of Mediterranean mussels by investigating shell growth (Andrisoa et  
204 al. 2019a).

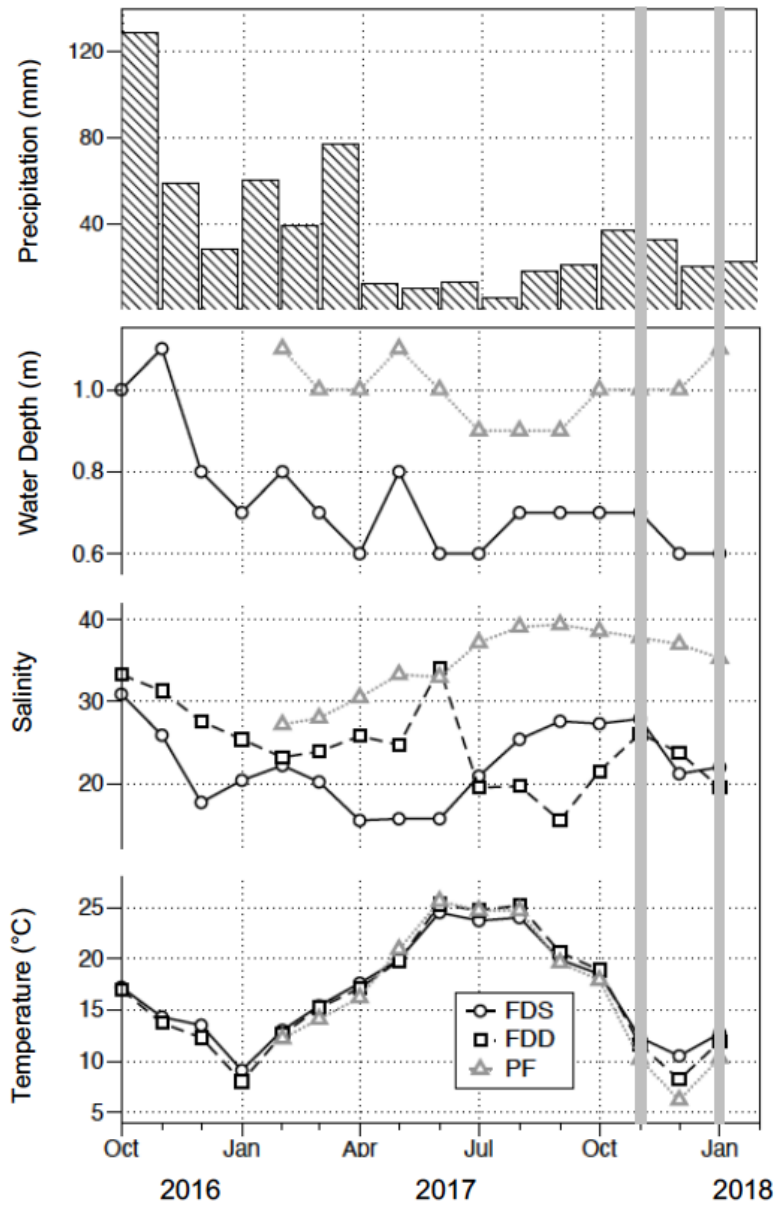
205



206

207 **Fig. 1:** Location of Salses-Leucate lagoon on the French Mediterranean coastline.  
208 The groundwater-influenced sites (FDS: Font Dame Surface and FDD: Font Dame  
209 Deep), the control site (PF: Port Fitou) and the sewage treatment plant are  
210 shown. Karstic springs are groundwater sources.





212

213 **Fig. 2:** Monthly variations in precipitation, water level, salinity and temperature  
 214 from October 2016 to January 2018 in groundwater influenced-sites (FDS and  
 215 FDD) and a site far from groundwater influences (PF). Precipitation data were  
 216 provided by Météo France. Periods of the sampling surveys are indicated by  
 217 vertical grey lines.

218

## 219 2.2 Sample collection and analysis

### 220 2.2.1 Mussel samples

221 Mediterranean mussels *M. galloprovincialis* naturally present in the lagoon were  
222 collected on 10 October 2016 at Font Dame and 17 February 2017 at Port Fitou  
223 for a catch-recovery experiment. At Font Dame, the water column is highly  
224 stratified due to fresh groundwater inflow (Fig. 2) and mussels collected in the  
225 lagoon were hence placed in two cages at 50 cm (surface cage, FDS) and 20 cm  
226 (bottom cage, FDD) above the bottom (mean water depth ca. 0.7 m). At Port  
227 Fitou (PF), only one cage was installed at 50 cm above the bottom because the  
228 water column was well mixed (mean water depth ca. 1 m). Mussels from the  
229 cages installed at FDS (n = 30, shell length: 37 – 82 mm), FDD (n = 24, shell  
230 length: 39 – 81 mm) and PF (n = 42, shell length: 46 – 79 mm) were collected in  
231 November 2017 (n = 40) and January 2018 (n = 56) for nitrogen and carbon  
232 stable isotope analyses. Mussels from the same size group were collected during  
233 each campaign to avoid a potential effect of size on the isotopic values. The two  
234 sampling periods allow to understand seasonal variations (autumn and winter)  
235 in mussel isotopic compositions. In the laboratory, mussel samples were cleaned  
236 to remove all epibionts and other attached animals. Mussels were dissected and  
237 samples from the mantle and gill were collected. The mantle and gill samples  
238 were subsequently oven-dried overnight at 60°C, ground and homogenized with  
239 a mortar and pestle. Approximately 1-2 mg of the ground samples was loaded  
240 into tin capsules and analysed for  $\delta^{15}\text{N}$ ,  $\delta^{13}\text{C}$  and C/N ratios. Samples were  
241 acidified with 50  $\mu\text{L}$  of 0.25N  $\text{H}_2\text{SO}_4$  to remove carbonates, which may interfere  
242 with  $\delta^{13}\text{C}$  measurements (Kennedy et al. 2005).

243

### 244 2.2.2 Food source samples

245 To characterize the nitrogen and carbon isotope values in mussel food sources,  
246 POM samples, a proxy for phytoplankton (Carlier et al. 2007; Liénart et al. 2017),  
247 were collected in June 2016, November 2016 and November 2017 at the  
248 groundwater influenced sites (Font Dame Surface and Font Dame Deep), the  
249 control site (Port Fitou) and from the potential nutrient and POM sources,  
250 including the karstic groundwater springs (n = 6), the sewage effluents (n = 2)

251 and the sea (n = 4). For POM sampling, water samples of 0.4 to 1 L were filtered  
252 through GF/F filters (0.7 µm) under vacuum until clogging. Filters were kept  
253 frozen (-20°C) until analyses. Seagrass may also be a source of POM to mussels,  
254 and seagrass leaves were hence collected from the dominant species *Zostera*  
255 *noltii* in the lagoon (n = 12). Seagrass samples were rinsed with deionized water,  
256 bagged and transported on ice to the laboratory. In the laboratory, samples were  
257 oven-dried at 60°C, ground and homogenized with a mortar and pestle.  
258 Approximately 1 mg of the ground samples was used for isotope analyses. Like  
259 mussel samples, seagrass samples were acidified with 50 µL of 0.25N H<sub>2</sub>SO<sub>4</sub> to  
260 remove carbonates to avoid interference with δ<sup>13</sup>C measurements.

261

### 262 **2.2.3 Stable isotope and elemental analyses**

263 Stable isotope and elemental analyses were conducted using an elemental  
264 analyzer mass spectrometer Integra CN Sercon at the Mediterranean Institute of  
265 Oceanography (Marseille) (Raimbault et al. 2008; Lacoste et al. 2016). Organic  
266 standard glycine was used to calibrate the particulate nitrogen measurements  
267 and to track the consistency of the combustion. The measured isotopic  
268 compositions of nitrogen (δ<sup>15</sup>N) and carbon (δ<sup>13</sup>C) were expressed as the  
269 relative difference between isotopic ratios in sample and in conventional  
270 standards (atmospheric N<sub>2</sub> for nitrogen and Vienna Pee Dee Belemnite for  
271 carbon):

$$272 \quad \delta^{15}\text{N or } \delta^{13}\text{C (‰)} = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000$$

273 where R is the ratio <sup>15</sup>N/<sup>14</sup>N or <sup>13</sup>C/<sup>12</sup>C. The precision of replicate analyses was  
274 0.5‰ and 0.3‰ for δ<sup>15</sup>N and δ<sup>13</sup>C, respectively.

275

### 276 **2.3 Statistical analysis**

277 Data normality and homogeneity of variances were tested with Shapiro-Wilk and  
278 Levene's tests, respectively. We used paired *t*-test to compare the isotope values  
279 in mussel tissues (mantle and gill). Two-way ANOVA was applied to assess the  
280 spatial and seasonal variability in isotopic and elemental ratios in mussel tissues.

281 The *t*-test statistics was used to compare the isotope values ( $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ ) and  
282 C/N ratios between groundwater-influenced sites (Font Dame Surface, Font  
283 Dame Deep), which were pooled together for this comparison only, and the  
284 control site (Port Fitou) during each campaign. Differences between the two  
285 sampling periods were also tested using *t*-test statistics. For spatial and temporal  
286 comparisons, both mantle and gill tissues were used. All statistical analyses were  
287 performed using R software.

288

## 289 **3 Results**

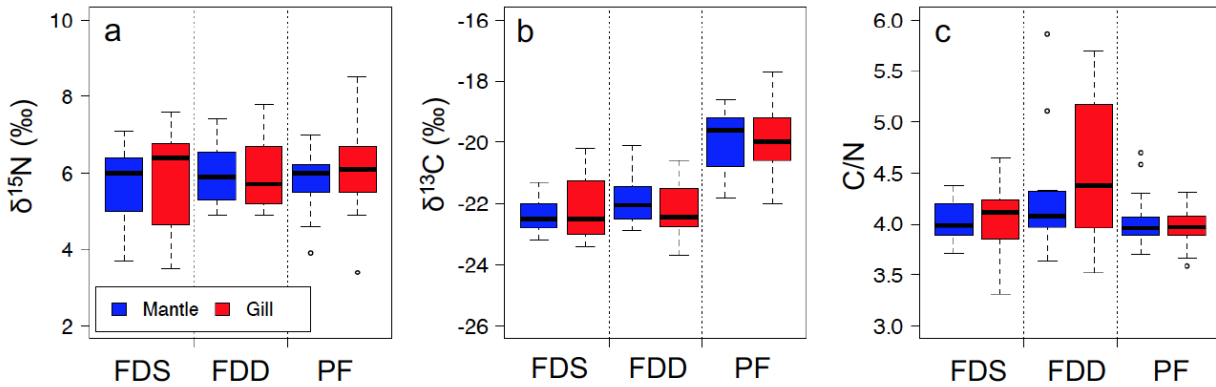
### 290 **3.1 Variation in mussels' tissue isotopic values and elemental ratios**

#### 291 **3.1.1 Variation between tissues**

292 The  $\delta^{15}\text{N}$ ,  $\delta^{13}\text{C}$  and C/N values in mantle and gill tissues were compared at the  
293 three study sites (Fig. 3). For all sites and all seasons, the  $\delta^{15}\text{N}$  values ranged  
294 from 3.7 to 7.4‰ for mantle tissues and from 3.4 to 8.5‰ for gill tissues. The  
295  $\delta^{13}\text{C}$  values ranged between -23.2 and -18.6‰ for mantle tissues and between -  
296 23.7 and -17.7‰ for gill tissues. The C/N ratios ranged from 3.6 to 5.9 and from  
297 3.3 to 5.7 for mantle and gill tissues, respectively. The paired *t*-tests showed that  
298 the  $\delta^{15}\text{N}$  values did not differ between tissues neither at FDS ( $t = -0.42$ ,  $p =$   
299  $0.675$ ), FDD ( $t = 0.04$ ,  $p = 0.966$ ) nor at PF ( $t = -1.09$ ,  $p = 0.280$ ). Likewise, the  
300  $\delta^{13}\text{C}$  values were not different neither at FDS ( $t = -0.83$ ,  $p = 0.416$ ), FDD ( $t = 0.66$ ,  
301  $p = 0.516$ ) nor at PF ( $t = 0.15$ ,  $p = 0.878$ ). For C/N ratio, no difference was  
302 observed between tissues neither at FDS ( $t = 0.32$ ,  $p = 0.752$ ), FDD ( $t = -0.82$ ,  $p =$   
303  $0.422$ ), nor at PF ( $t=0.77$ ,  $p = 0.446$ ). The overall difference between mantle and  
304 gill tissues was 0.01 – 0.31‰ for both isotopes, which is below detection limit at  
305 an analytical precision of the mass spectrometer (0.5‰ for  $\delta^{15}\text{N}$  and 0.3‰ for  
306  $\delta^{13}\text{C}$ ). Therefore, we consider all gill and mantle samples indiscriminately  
307 throughout the paper.

308

309



311

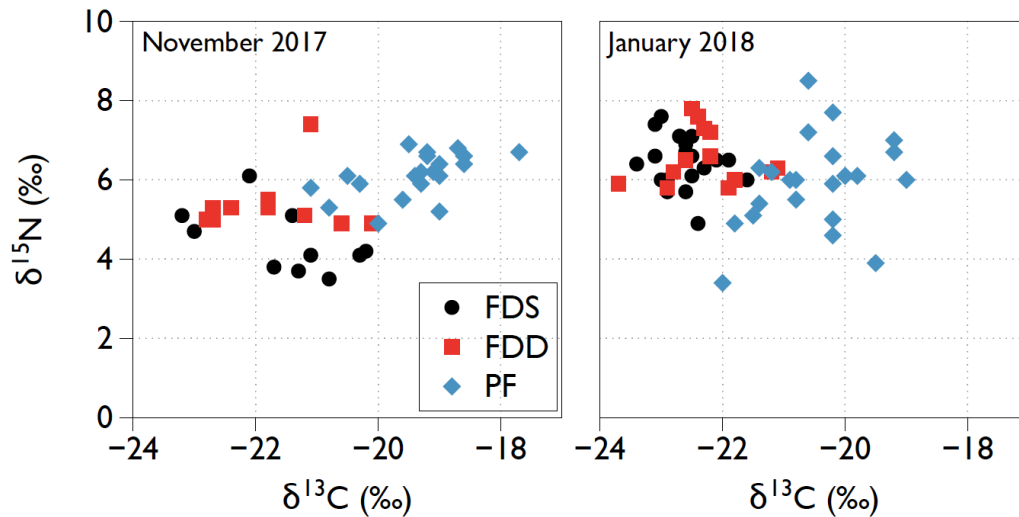
312 **Fig. 3:**  $\delta^{15}\text{N}$  (a),  $\delta^{13}\text{C}$  (b) and C/N ratios (c) in mussel mantle and gill at the three  
 313 study sites (FDS, FDD and PF)

314

### 315 3.1.2 Spatial variability

316 At the groundwater-influenced sites, the  $\delta^{15}\text{N}$  values in mussel tissues ranged  
 317 from 3.5 to 7.6‰ (n = 30) at Font Dame Surface and from 4.9 to 7.8‰ (n = 24)  
 318 at Font Dame Deep. At the control site Port Fitou, the  $\delta^{15}\text{N}$  values ranged  
 319 between 3.4 and 8.5 (n = 42) (Fig. 4). The two-way ANOVA showed that the  
 320 spatial variations had no effect on the  $\delta^{15}\text{N}$  values of mussels ( $F_{2,90} = 0.75$ , p =  
 321 0.476). The  $\delta^{15}\text{N}$  values differed between the groundwater-influenced and the  
 322 control site in November (*t-test*:  $t = -5.14$ ,  $p < 0.0001$ ) but not in January (*t-test*:  $t$   
 323 = 1.98,  $p = 0.057$ ). The mussel  $\delta^{13}\text{C}$  values ranged between -23.4 and -20.2‰ (n  
 324 = 30) at Font Dame Surface, between -23.7 and -20.1‰ (n = 24) at Font Dame  
 325 Deep and between -22.0 and -17.7‰ (n = 42) at Port Fitou (Table 1). The  $\delta^{13}\text{C}$   
 326 values varied between sites (two-way ANOVA:  $F_{2,90} = 93.22$ ,  $p < 0.0001$ ) and  
 327 increased at Port Fitou in comparison with those from the groundwater-  
 328 influenced sites for both November (*t-test*:  $t = -5.14$ ,  $p < 0.0001$ ) and January (*t-*  
 329 *test*:  $t = 1.98$ ,  $p < 0.0001$ ). The elemental C/N ratios ranged between 3.3 to 4.7  
 330 (n=30) at Font Dame Surface, between 3.5 to 5.9 (n=24) at Font Dame Deep and  
 331 between 3.6 and 4.7 (n = 42) at Port Fitou. The C/N ratios varied between study  
 332 sites (two-way ANOVA:  $F_{2,90} = 11.11$ ,  $p < 0.0001$ ). In addition, the ratios between  
 333 the groundwater-influenced sites (FDS and FDD) and the control site (PF) were  
 334 different for November (*t-test*:  $t = 2.52$ ,  $p = 0.019$ ) but not for January (*t-test*:  $t =$   
 335 0.73,  $p = 0.467$ ). Overall, the  $\delta^{15}\text{N}$ ,  $\delta^{13}\text{C}$  and C/N ratio values from this study were

336 in good agreement with values from the literature for the target species *M.*  
 337 *galloprovincialis* in the Mediterranean region (Table 1). However, the  $\delta^{13}\text{C}$  values  
 338 from the groundwater-influenced sites were relatively lower compared to values  
 339 previously reported from other coastal sites in the Mediterranean region (Table  
 340 1).  
 341



342  
 343 **Fig. 4:** Nitrogen and carbon isotopic values at groundwater-influenced sites  
 344 (FDS: Font Dame Surface and FDD: Font Dame Deep) and a control site (PF: Port  
 345 Fitou) during the two sampling periods in November 2017 and January 2018.  
 346

347 **Table 1:** The  $\delta^{15}\text{N}$ ,  $\delta^{13}\text{C}$  and C/N ratio values of food sources and tissues of *M.*  
 348 *galloprovincialis* in Salses-Leucate lagoon from this study and from different  
 349 coastal sites in the Mediterranean region. *n* corresponds to number of samples.

References	Type of sample	Sites / samples	<i>n</i>	$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰)	C/N
This study	Food source	Lagoon POM	10	3.4 to 7.8	-25.1 to -20.5	6.4 to 8.5
		Sewage POM	2	9.8 to 12.8	-28.2 to -25.6	6.0 to 6.3
		groundwater POM	6	0.1 to 5.3	-36.5 to -27.0	14.4 to 57.9
		Marine POM	4	2.9 to 8.7	-23.6 to -19.5	8.4 to 9.2
		Seagrass	12	3.6 to 7.0	-15.0 to -7.5	15.1 to 25.6
	Mussel tissue	Font Dame Surface	30	3.5 to 7.6	-23.4 to -20.2	3.3 to 4.7
		Font Dame Deep	24	4.9 to 7.8	-23.7 to -20.1	3.5 to 5.9
		Control site	42	3.4 to 8.5	-22.0 to -17.7	3.6 to 4.7
		Coastal lagoon	6	6.3 to 7.8	-20.0 to -19.7	-
Carrier et al. (2009)		Coastal lagoon	6	6.3 to 7.8	-20.0 to -19.7	-

Deudero et al. (2009)		Coastal sites	45	3.3 to 8.9	-22.8 to -18.4	-
Carabel et al. (2009)		Coastal sites	5	6.3 to 7.4	-18.3 to -17.3	3.5 to 3.8
Rumolo et al. (2017)		Coastal sites	1247	1.9 to 7.9	-20.1 to -17.3	4.3 to 6.0
Briant et al. (2018)		Coastal sites	19	3.2 to 4.9	-20.3 to -18.1	4.5 to 6.0
Özdilek et al. (2019)		Coastal sites	35	5.5 to 7.5	-22.5 to -19.5	-

### 350 3.1.3 Temporal variability

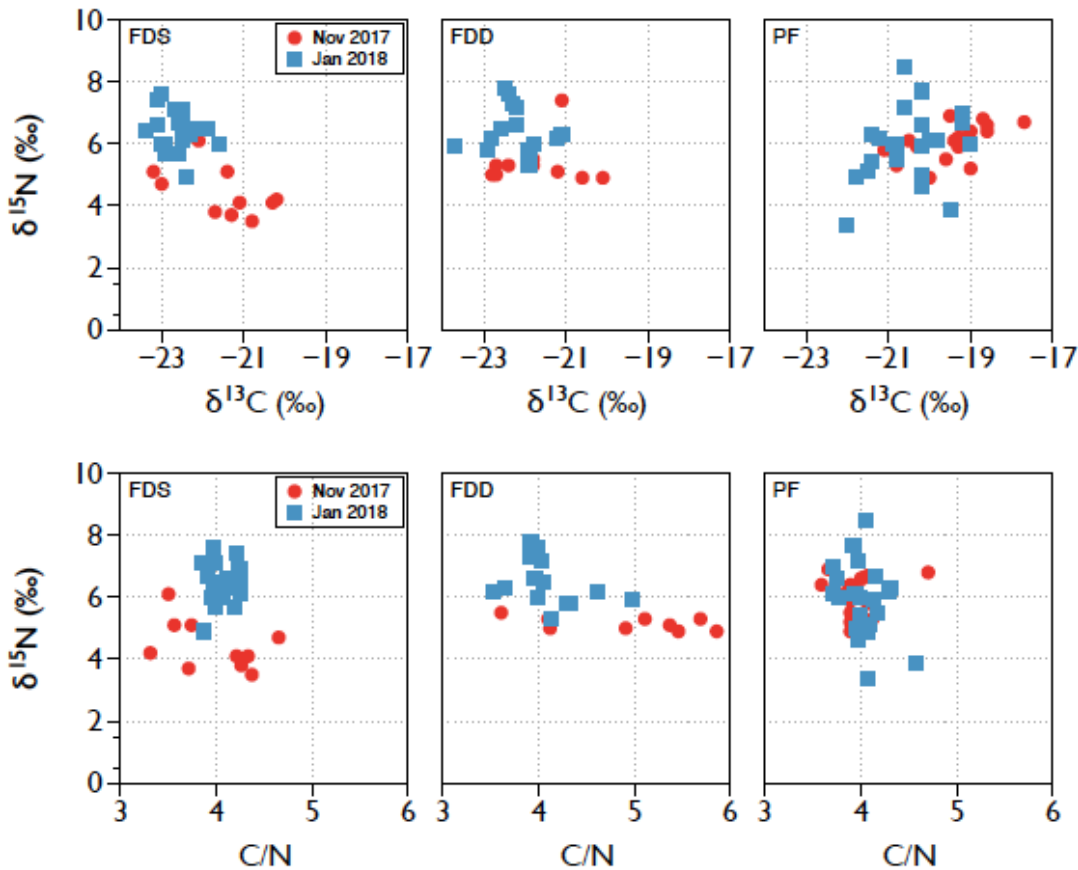
351 In November 2017, mussel  $\delta^{15}\text{N}$  values ranged between 3.5 and 6.1‰, between  
352 4.9 and 7.4‰ and between 4.4 and 6.9‰ at Font Dame Surface, Font Dame  
353 Deep and at Port Fitou, respectively (Fig. 5). In January 2018, the  $\delta^{15}\text{N}$  values  
354 were between 4.9 and 7.6‰ at Font Dame Surface, between 5.3 and 7.8‰ at  
355 Font Dame Deep and between 3.4 and 8.5‰ at Port Fitou. The  $\delta^{15}\text{N}$  values  
356 showed a decrease in November 2017 compared to those of January 2018 at  
357 Font Dame Surface (*t-test*:  $t = -6.89$ ,  $p < 0.0001$ ) and Font Dame Deep (*t-test*:  $t = -$   
358  $3.55$ ,  $p = 0.002$ ) while no change was observed at Port Fitou (*t-test*:  $t = 0.72$ ,  $p =$   
359  $0.479$ ) (Fig. 5).

360

361 In November 2017, the  $\delta^{13}\text{C}$  values ranged between -23.2 and -20.2‰, between -  
362 22.8 and -20.1‰ and between -21.1 and -17.7‰ at Font Dame Surface, Font  
363 Dame Deep and Port Fitou, respectively (Fig. 5). In January 2018, the  $\delta^{13}\text{C}$  values  
364 ranged between -23.4 and -21.6‰, between -23.7 and -21.1‰ and between -  
365 22.0 and -19.0‰ at Font Dame Surface, Font Dame Deep and Port Fitou,  
366 respectively. The  $\delta^{13}\text{C}$  values were higher in November 2017 than those from  
367 January 2018 at Font Dame Surface (*t-test*:  $t = 3.29$ ,  $p = 0.007$ ) and Port Fitou (*t-*  
368 *test*:  $t = 4.24$ ,  $p = 0.0001$ ). In contrast, no difference was observed between  
369 November 2017 and January 2018 samples at Font Dame Deep (*t-test*:  $t = 1.51$ ,  $p$   
370  $= 0.150$ ) (Fig. 5).

371

372 For C/N ratios, no differences were found between seasons neither at Font Dame  
373 Surface (*t-test*:  $t = -0.62$ ,  $p = 0.550$ ) nor at Port Fitou (*t-test*:  $t = -1.34$ ,  $p = 0.188$ ).  
374 However, the ratios were slightly higher in January 2018 than those in  
375 November 2017 at Font Dame Deep (*t-test*:  $t = 2.63$ ,  $p = 0.022$ ). At Font Dame  
376 Deep, C/N ratios ranged between 3.61 and 5.86 in November 2017 and between  
377 3.52 and 4.97 in January 2018 (Fig. 5).



378

379 **Fig. 5:** Temporal variability of mussel isotopic compositions ( $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$   
 380 values) and C/N ratios in November 2017 and January 2018 at Font Dame  
 381 Surface (FDS), Font Dame Deep (FDD) and Port Fitou (PF).

382

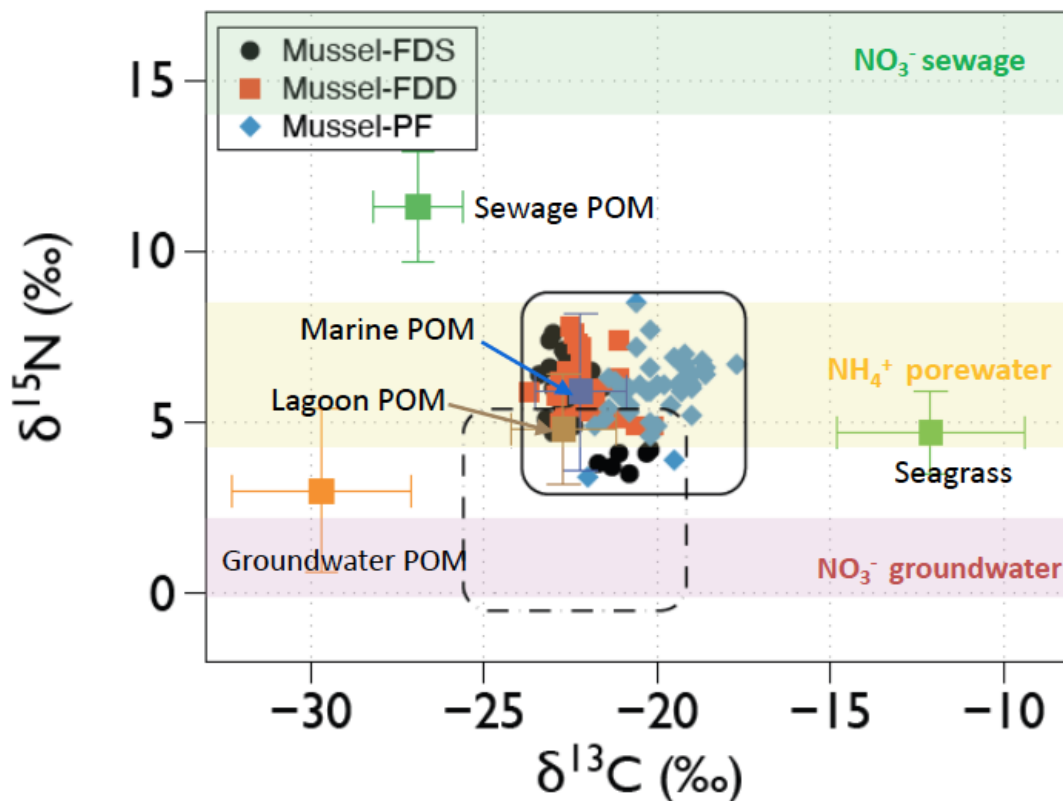
### 383 3.2 Isotope values of potential food sources

384 The  $\delta^{15}\text{N}$  values of particulate organic matter collected in the lagoon ranged  
 385 between 3.4 and 7.8 ‰, with a mean of  $4.8 \pm 1.6$ ‰ while the  $\delta^{13}\text{C}$  values ranged  
 386 between -25.1 to -20.5‰ with a mean value of  $-22.7 \pm 1.5$ ‰ (Table 1; Fig. 6).

387 Sewage POM is the most enriched in  $^{15}\text{N}$ , as the  $\delta^{15}\text{N}$  values ranged from 9.8 to  
 388 12.8‰ (mean  $\pm$  SD =  $11.3 \pm 1.5$ ‰) (Table 1; Fig. 6). The  $\delta^{13}\text{C}$  values of sewage  
 389 POM ranged from -28.2 to -25.6‰ (mean  $\pm$  SD =  $-26.7 \pm 1.3$ ‰). The POM of  
 390 groundwater springs is depleted in  $^{15}\text{N}$ , with the  $\delta^{15}\text{N}$  values ranging between  
 391 0.1 and 5.3‰ and a mean value of  $1.7 \pm 2.8$ ‰. The  $\delta^{13}\text{C}$  from groundwater POM  
 392 showed the lowest values ranging between -36.5 to -27.0‰ (mean  $\pm$  SD =  $-29.7$   
 393  $\pm 2.6$ ‰). The isotopic values of marine POM ranged between 2.9 and 8.7‰  
 394 (mean  $\pm$  SD =  $5.9 \pm 2.3$ ‰) for  $\delta^{15}\text{N}$  and between -24.1 and -19.5‰ (mean  $\pm$  SD =



395  $-22.2 \pm 2.1\text{‰}$ ) for  $\delta^{13}\text{C}$ . Seagrasses are the most enriched in  $^{13}\text{C}$  with values of  
 396  $\delta^{13}\text{C}$  values ranging between  $-15.0$  to  $-7.5\text{‰}$  and a mean value of  $-12.1 \pm 2.7\text{‰}$ .  
 397 The  $\delta^{15}\text{N}$  values of seagrass ranged between  $3.6$  and  $7.0\text{‰}$ .  
 398  
 399 The C/N ratios of POM from the lagoon ( $6.4$  to  $8.5$ ), sewage ( $6.0$  to  $6.3$ ) and the  
 400 Mediterranean Sea ( $8.4$  to  $9.2$ ) were lower compared to those from groundwater  
 401 POM and seagrass, with values ranging from  $14.1$  to  $57.9$  and from  $15.1$  to  $25.6$ ,  
 402 respectively (Table 1).



403  
 404 **Fig. 6:** Nitrogen and carbon isotope values for mussel tissues at Font Dame  
 405 Surface (FDS), Font Dame Deep (FDD) and at Port Fitou (PF) and potential food  
 406 sources for Mediterranean mussels. The green-, yellow-, and pink-shaded  
 407 rectangle indicate the  $\delta^{15}\text{N}$  range of  $\text{NO}_3^-$  in sewage ( $14.0 - 21.5\text{‰}$ ), of  $\text{NH}_4^+$  in  
 408 sediment porewater ( $4.5 - 8.5\text{‰}$ ) and of  $\text{NO}_3^-$  in groundwater spring ( $0.1 -$   
 409  $2.2\text{‰}$ ), respectively (Andrisoa et al. 2019b). The  $\delta^{15}\text{N}$  values reach up to  $21.5\text{‰}$   
 410 for  $\text{NO}_3^-$  sewage. Mean  $\pm$  SD values for food sources are shown ( $n = 10$  for lagoon  
 411 POM,  $n = 5$  for groundwater POM,  $n = 2$  sewage POM,  $n = 4$  for marine POM and  $n$   
 412  $= 8$  for seagrass). The dashed square indicates the area corresponding to the  
 413 expected  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  of the potential food source for mussels, taking into

414 account the trophic enrichment factor of 3.4‰ for  $\delta^{15}\text{N}$  and 1.7‰ for  $\delta^{13}\text{C}$  (Post  
415 2002; Kilham et al. 2009; Carlier et al. 2009).

416

## 417 **4 Discussion**

### 418 **4.1 Mussel tissue types**

419 This study is among the few that compare the  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  isotopic values and  
420 C/N ratios in mantle and gill tissues in the Mediterranean mussel *M.*  
421 *galloprovincialis*. Such comparison is of importance in applications of isotope  
422 analyses of bio-indicators as they can mask abiotic effects if present (Piola et al.  
423 2006). This study shows an absence of difference in isotopic and elemental ratios  
424 between mantle and gill tissues (Table 2), similar to results reported from  
425 bivalves *Pinctada imbricata* and *Isognomon alatus* in other coastal ecosystems  
426 (Graniero et al. 2016). This indicates that mantle and gill tissues are effectively  
427 interchangeable for nitrogen and carbon isotope analyses for the Mediterranean  
428 mussels in the studied lagoon. However, several studies have shown that isotopic  
429 enrichment processes are tissue-specific and depends on tissue turnover rates  
430 (Logan et al. 2006; Deudero et al. 2009). Tissues with low turnover rates, such as  
431 the muscle and mantle, integrate dietary isotopic values over longer period while  
432 tissues that have higher turnover rates, such as gill and digestive gland, reflect  
433 the diet during a short period. Deudero et al. (2009) reported, through isotopic  
434 fractionation of *M. galloprovincialis*, depleted values in digestive gland, followed  
435 by gills and then muscle tissue. Difference in isotopic values among different  
436 tissues has also been found in other marine organisms such as crustacean *Maja*  
437 *brachydactyla* (Bodin et al. 2007) and bivalve *Pecten maximus* (Lorrain et al.  
438 2002). We hypothesise that the relatively stable environmental conditions in the  
439 study sites (partially driven by the constant supply of water and nutrients by  
440 groundwater inputs) might contribute explaining the minor variability between  
441 mantle (low turnover rate) and gills (high turnover rate) observed in this study.  
442

443 The similar nitrogen and carbon isotopic values between the mantle and gill  
444 tissues in this study seems surprising as it is overall believed that gill tissue is  
445 more active metabolically than mantle tissue (Livingstone and Farrar 1984;

446 Deudero et al. 2009; Cabanellas-Reboredo et al. 2009). The differences of  
447 isotopic values between animal tissues are often associated with differences in  
448 the biochemical composition of those tissues (DeNiro and Epstein 1978;  
449 Sweeting et al. 2005; Hill and McQuaid 2009). For instance, a tissue containing a  
450 high proportion of lipids would probably have a more negative  $\delta^{13}\text{C}$  value than a  
451 tissue with a lower lipid content (Ezgeta-Balić et al. 2014). Conversely, the  
452 absence of difference of isotopic values between gill and mantle tissues in this  
453 study are likely associated with similarities in their biochemical composition,  
454 particularly in lipid content. Indeed, the C/N ratios, used as proxy of lipid content  
455 (Elser et al. 2003; Post et al. 2007), did not show difference (Fig. 3), suggesting  
456 similar proportion of lipids in gill and mantle tissues. Diet shift, fractionation and  
457 the physiological function of tissues (mantle) could also explain this similar  
458 isotope values between mantle and gill tissues (Lorrain 2002; Sweeting et al.  
459 2005; Ezgeta-Balić et al. 2014).

460

#### 461 **4.2 Seasonal variability**

462 Due to the absence of difference in isotope and C/N ratios between gill and  
463 mantle tissues, gill and mantle data are pooled together for the study of seasonal  
464 variability and food sources. Our results show seasonal differences in the  
465 isotopic values at the groundwater-influenced sites (Fig. 5). In winter (January),  
466 mussels exhibit increased  $\delta^{15}\text{N}$  values but decreased  $\delta^{13}\text{C}$  values compared to the  
467 values from autumn (November). In winter, food supply is low and mussel  
468 metabolism is slower and it might be that body condition has deteriorated  
469 (Smaal and Vonck 1997), resulting in an increase of their tissue  $\delta^{15}\text{N}$  values (Hill  
470 and McQuaid 2009). Indeed, the condition indices, representing the physiological  
471 condition of mussels, were lower in winter ( $7.4 \pm 0.6\%$  for FDS and  $5.9 \pm 1.6\%$   
472 for FDD) in comparison with those in autumn ( $8.9 \pm 5.1\%$  for FDS and  $6.3 \pm 1.2\%$   
473 for FDD) for *M. galloprovincialis* in Salses-Leucate lagoon (Andrisoa et al. 2019a).  
474 In addition, lagoon salinity slightly decreases in winter (Fig. 2) and at low  
475 salinity, filter feeders reduce filtration of water, tightly closing their shell valves,  
476 isolating themselves from the environment (Shurova 2001). Prolonged isolation  
477 can suppress the vital processes of bivalves and thereby adversely affect their

478 physiological conditions. Considering the mussels' tissue turnover rates, the  
479 isotopic values observed in mussels from the present study reflect ca. the past 3  
480 – 4 months of mussel's food (Raikow and Hamilton 2001; Hill and McQuaid  
481 2009). In this study, the  $\delta^{15}\text{N}$  enrichment in winter is probably in relation to the  
482 limited amount of food and possible stress from low salinity (Adams and Sterner  
483 2000; Olive et al. 2003; Haubert et al. 2005). A potential starvation-induced  $\delta^{15}\text{N}$   
484 enrichment in mussels suggests that during starvation, the uptake of nitrogen is  
485 close to zero, but nitrogen loss continues, even if at low rate (Hill and McQuaid  
486 2009). This  $\delta^{15}\text{N}$  enrichment is often coupled with high C/N ratios due to  
487 decreased N content, indicating nutritional stress. The C/N ratios in this study  
488 show no seasonal variation (except for Font Dame Deep), probably due to  
489 permanent supply of nitrogen and carbon from groundwater sources. This  
490 seasonal change of both  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values in mussels may also indicate a  
491 change in the isotope values of food sources (Riera 2007; Cabanellas-Reboredo  
492 et al. 2009). During winter, higher rainfall and runoff results in higher terrestrial  
493 material inputs (surface and/or groundwater inputs), as well as increased net  
494 production with more N inputs associated with rainfall (Carlier et al. 2007).  
495 Terrestrial materials (dissolved or particulate) have generally low  $\delta^{13}\text{C}$  values  
496 (e.g., Carlier et al. 2007; Berto et al. 2013). Low  $\delta^{13}\text{C}$  values in food webs thus  
497 generally indicate food sources of terrestrial origin (Kasai and Nakata 2005;  
498 Piola et al. 2006). However, surface water inflow to Salses-Leucate lagoon is  
499 negligible and thus can be ruled out (Carlier et al. 2007, Andrisoa et al. 2019b).  
500 Thus, the low  $\delta^{13}\text{C}$  values in food webs at this site, particularly in winter, are  
501 most probably influenced by groundwater inputs. The role of groundwater as a  
502 major conveyor of nutrients in this region has already been reported in previous  
503 studies (Rodellas et al. 2018; Andrisoa et al. 2019b). The seasonal variation of  
504  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values in mussels is not related to that of their C/N ratios,  
505 suggesting that lipid content cannot explain the seasonal variability in mussel  
506  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values.

507

508 At Font Dame Surface, the increased  $\delta^{15}\text{N}$  values and decreased  $\delta^{13}\text{C}$  values  
509 during winter suggest seasonal change in food source and no change in lipid  
510 content (no change in C/N ratio). Font Dame Surface is permanently impacted by

511 groundwater sources and thus this change suggests seasonal change in food  
512 supply from groundwater sources or change in their isotope values. At Font  
513 Dame Deep, the increased  $\delta^{15}\text{N}$  values and C/N values, but not  $\delta^{13}\text{C}$  values,  
514 suggests potential occurrence of starvation or/ and stress from low salinity  
515 during winter. At Port Fitou, no seasonal change is observed (except for the  $\delta^{13}\text{C}$   
516 values) consistent with the stable environmental conditions at this location (Fig.  
517 2).

518

### 519 **4.3 Potential nitrogen and carbon sources for mussels**

520 Most of the studies on nitrogen and carbon stable isotope composition  
521 of mussels in coastal lagoon systems focus on the impacts of anthropogenic  
522 activities and natural functioning of coastal food webs (Carlier et al. 2007, 2009;  
523 Lefebvre et al. 2009; Graniero et al. 2016; Briant et al. 2018), but rarely on  
524 groundwater inputs. This study was specifically designed to further investigate  
525 the indirect role that groundwater dissolved inorganic nitrogen and carbon play  
526 (via phytoplankton feeding) in coastal food webs and the direct use of  
527 groundwater POM by *M. galloprovincialis* as main food source in coastal lagoon  
528 ecosystems. Considering trophic enrichment factors, the  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values of  
529 the main diet of *M. galloprovincialis* in Salses-Leucate lagoon can be estimated, as  
530 indicated in Fig. 6 (dashed square). Thus, the isotopic values of the main diet of  
531 mussels are supposed to be closer to those of expected potential food source (the  
532 dashed square in Fig. 6). Our results show that the  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values of  
533 groundwater POM, lagoon POM and marine POM sources are closer to those of  
534 expected potential food sources (Fig. 6). It is therefore reasonable to consider  
535 the main diet of mussel in Salses-Leucate lagoon as a mixture of groundwater  
536 POM, lagoon POM and marine POM (discussed below). The  $\delta^{15}\text{N}$  values in sewage  
537 POM are significantly higher (9.8 – 12.8‰) compared to those of expected  
538 potential food source of mussels (-0.5 to 5.1‰; Fig. 6), as commonly reported in  
539 the literature (Aravena et al. 1993; McClelland et al. 1997; Cole et al. 2005;  
540 Kendall et al. 2007). This indicates little impact of sewage effluents on caged  
541 mussels in this study. It should be noted that Salses-Leucate lagoon is an  
542 oligotrophic lagoon and it is perhaps not surprising that the impact of the  
543 sewage treatment plant to the lagoon is minor (IFREMER 2014). The lowest  $\delta^{15}\text{N}$

544 values of expected potential food source (dashed rectangle in Fig. 6) suggest the  
545 existence of a food source with depleted  $^{15}\text{N}$ . In the oligotrophic Mediterranean  
546 region, dissolved atmospheric dinitrogen ( $\text{N}_2$ ) could be used as nitrogen source  
547 for primary producers, resulting in low  $\delta^{15}\text{N}$  values in diazotrophs (ca.  $-1\text{‰}$ ;  
548 Liénart et al. 2017). This indicates that diazotrophs are also a potential food  
549 source for *M. galloprovincialis* in Salses-Leucate lagoon. The  $\delta^{13}\text{C}$  values of  
550 seagrass ( $-15.0$  to  $-7.5\text{‰}$ ) are significantly higher compared to those of mussel  
551 tissues ( $-23.7$  to  $-17.7\text{‰}$ ), suggesting that contribution of live seagrass to the  
552 diet of *M. galloprovincialis* in Salses-Leucate lagoon is negligible. This unlikely  
553 contribution of seagrass to the diet of bivalve has also been reported for  
554 *Spondylus crassisquama* in the Ojo de Liebre lagoon, Mexico (Mathieu-Resuge et  
555 al. 2019) and for *Brachidontes pharaonic* in the western Mediterranean Sea (Sarà  
556 et al. 2007). Bivalves do not prefer seagrass due to high indigestible lignin and  
557 inhibitory polyphenols (Harrison 1982; Cebrian 1999). Salses-Leucate lagoon is  
558 a shallow lagoon, allowing proliferous seagrass to develop with the euphotic  
559 zone with ca. 60% of seagrass cover dominated by *Zostera noltii* (IFREMER  
560 2014). It has been shown that less than 10% of seagrass enters the food webs via  
561 grazing and the main transfer route of seagrass organic matter is the detrital  
562 pathway (Benner and K'nees 1988; Cebrián et al. 1997). Indeed, degraded  
563 seagrass particles are likely to have different isotope and elemental ratios than  
564 fresh particles due to degradation process occurring (Berto et al. 2013b; Liénart  
565 et al. 2017). The  $\delta^{13}\text{C}$  values of sedimentary organic matter ( $-19.5.8$  to  $-17.8\text{‰}$ )  
566 measured in Salses-Leucate lagoon by Carlier et al. (2009) (not measured in this  
567 study) are comparable to those of mussels' tissue ( $-23.7$  to  $-17.7\text{‰}$ ), suggesting  
568 that seagrass detritus may contribute to the diet of *M. galloprovincialis*.

569

570 The stable isotope values reveal that groundwater POM, lagoon POM and marine  
571 POM are the main food sources for mussels at the investigated sites. Previous  
572 stable isotope studies have shown the significant role that lagoon POM (Carlier et  
573 al. 2007) and marine POM (Kang et al. 1999) play in supplying food for filter  
574 feeders in coastal ecosystems. Our results show that lagoon POM and marine  
575 POM are dominated by phytoplankton (C/N ratios between 6.4 and 9.2; Table 1),  
576 which are the preferred food source for mussels (Ogilvie et al. 2000; Penney et

577 al. 2001). Liénart et al. (2017) reported that marine POM is composed of ca. 84%  
578 of phytoplankton in coastal systems. The  $\delta^{13}\text{C}$  values in groundwater POM are  
579 slightly lower compared to those of expected potential food sources (Fig. 6). This  
580 discrepancy could be explained by a selective assimilation of one component of  
581 groundwater POM with high isotopic values by the filter feeders. A further  
582 investigation on stable isotopic composition of different biochemical fractions of  
583 POM may be of interest. Dietary proteins, for instance could have an enriched  
584  $\delta^{13}\text{C}$  values relative to bulk material (Lorrain et al. 2002). Moreover, mussels  
585 from groundwater-influenced sites are depleted in  $^{13}\text{C}$  compared to those from  
586 control site and their  $\delta^{13}\text{C}$  values are closer to those of groundwater POM.  
587 Several processes may contribute to the low  $\delta^{13}\text{C}$  values in food webs, including  
588 incorporation of carbon from  $\text{CH}_4$ -oxidizing bacteria, which leads to  $\delta^{13}\text{C}$  values  
589 in consumers below  $-40\text{‰}$  (Grey 2016). However, no mussels have values this  
590 low in this study, so it is unlikely that  $\text{CH}_4$ -oxidizing bacteria are responsible for  
591 the low food web  $\delta^{13}\text{C}$  values. The  $^{13}\text{C}$ -depleted tissues, particularly at the  
592 groundwater-influenced sites, likely reflect the assimilation of  $^{13}\text{C}$ -depleted soil-  
593 respired  $\text{CO}_2$  in groundwater by primary producers, suggesting the indirect  
594 effect of groundwater discharge on mussel growth via uptake of their food,  
595 phytoplankton. In addition, the  $\delta^{15}\text{N}$  values of POM in this study suggest  
596 important N inputs from groundwater sources (Fig. 6). The beneficial effects of  
597 groundwater input on filter feeders results thus from the local increases in food  
598 availability, supported by groundwater-derived nitrogen and carbon. Very few  
599 studies have reported the role of groundwater input to the ecological and  
600 physiological condition of *M. galloprovincialis* in coastal ecosystems (Piló et al.  
601 2018; Andrisoa et al. 2019a). Whilst the ecological implication of groundwater in  
602 food webs has often been overlooked in coastal ecosystems, it has been widely  
603 studied in freshwater ecosystems (McClelland et al. 1997; Vander Zanden et al.  
604 2005; Pettit et al. 2016; Burrows et al. 2018).

605

606 A previous study by [Andrisoa et al. \(2019b\)](#) in Salses-Leucate lagoon suggested  
607 that groundwater discharge is an important source of inorganic nitrogen and  
608 carbon supporting phytoplankton growth. The present study shows that this  
609 groundwater-derived inorganic nitrogen and carbon is indirectly transferred to

610 mussels *M. galloprovincialis* present in the lagoon via their food source  
611 phytoplankton and groundwater POM is directly used by mussels *M.*  
612 *galloprovincialis* as main food source. In coastal ecosystems, phytoplankton form  
613 the base of food webs and are consumed by primary consumers like mussels,  
614 zooplankton, small fishes and crustaceans. Primary consumers are in turn  
615 consumed by larger carnivorous fishes and invertebrates. Since groundwater  
616 fuels phytoplankton growth and primary production in this coastal lagoon, it  
617 thus likely supports the whole lagoon food webs. Indeed, Encarnação et al.  
618 (2015) reported an increased meiofauna diversity associated with groundwater  
619 inputs in the south coast of Portugal. Previous studies in coastal systems also  
620 suggested the effect of groundwater input to increase the growth rate (Andrisoa  
621 et al. 2019a) and the abundance (Piló et al. 2018) of *M. galloprovincialis*. In  
622 addition, groundwater discharge has been reported to enhance species richness,  
623 abundance and biomass of fishes and invertebrates in Japanese coastal waters  
624 (Hata et al. 2016; Utsunomiya et al. 2017). Overall, the results of this study  
625 combined with those of Andrisoa et al. (2019b) suggest that groundwater input  
626 may fuel primary production and phytoplankton growth in coastal systems and  
627 thus likely enhance nitrogen and carbon transfer to lagoon food webs.

## 628 **5 Conclusion**

629 This study shows seasonal and spatial variations of the isotopic values of mussel  
630 mantle and gill tissues in a groundwater-fed coastal lagoon. The  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$   
631 values in mantle and gill tissues of *M. galloprovincialis* are similar, likely related  
632 to relatively stable environmental conditions in the study sites (partially driven  
633 by the constant supply of water and nutrients by groundwater inputs) and to the  
634 physiological status of the filter feeders. The  $\delta^{15}\text{N}$  values in mussel tissues are  
635 significantly higher in winter than in autumn likely due to reduced food supply in  
636 winter, and thereby adversely affect mussel physiological conditions. However,  
637 mussel  $\delta^{13}\text{C}$  shows overall lower values in winter, suggesting input of  $^{13}\text{C}$ -  
638 depleted sources from groundwater, as surface water input in the region is  
639 known to be negligible. The seasonal variation of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values in mussels  
640 is not related to that of their C/N ratios, suggesting that lipid content cannot  
641 explain the seasonal variability in mussel  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values. Further



642 investigation on the ecophysiology of mussels is needed. In winter, the increased  
643  $\delta^{15}\text{N}$  values and decreased  $\delta^{13}\text{C}$  values at Font Dame Surface suggest a change of  
644 food source while the increased  $\delta^{15}\text{N}$  values and C/N ratio at Font Dame Deep  
645 suggests the occurrence of starvation and environmental stress. Our results  
646 show that the main diet of *M. galloprovincialis* in Salses-Leucate lagoon is a  
647 mixture of groundwater POM, lagoon POM and marine POM, with negligible  
648 contributions from sewage input. Lower  $\delta^{13}\text{C}$  values in tissues, particularly at  
649 groundwater-influenced sites, provide further evidence of the relevance of  
650 groundwater inputs on mussel diet. Local increases in food availability (mainly  
651 phytoplankton), supported by groundwater-derived nitrogen and carbon result  
652 in overall beneficial effects of groundwater input to filter feeders. In conclusion,  
653 this study elucidates the indirect role of groundwater in supplying nitrogen and  
654 carbon to filter feeders via their food source phytoplankton and suggests the  
655 additional likely direct feeding of groundwater POM by mussels. It shows  
656 therefore the important ecological implications of groundwater input in coastal  
657 lagoon food webs.

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