



HAL
open science

Different particle sources in a bivalve species of a coastal lagoon: evidence from stable isotopes, fatty acids, and compound-specific stable isotopes

Margaux Mathieu-Resuge, Gauthier Schaal, Edouard Kraffe, Rudolph Corvaisier, Oanez Lebeau, Salvador Lluch-Cota, Rosa Salgado García, Martin Kainz, Fabienne Le Grand

► To cite this version:

Margaux Mathieu-Resuge, Gauthier Schaal, Edouard Kraffe, Rudolph Corvaisier, Oanez Lebeau, et al.. Different particle sources in a bivalve species of a coastal lagoon: evidence from stable isotopes, fatty acids, and compound-specific stable isotopes. *Marine Biology*, 2019, 166 (7), pp.89. 10.1007/s00227-019-3535-z . hal-02406541

HAL Id: hal-02406541

<https://hal.science/hal-02406541>

Submitted on 17 Jun 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Different particle sources in a bivalve species of a coastal lagoon: evidence from stable isotopes, fatty acids, and compound-specific stable isotopes

Mathieu-Resuge Margaux ^{1,4,*}, Schaal Gauthier ¹, Kraffe Edouard ¹, Corvaisier Rudolph ¹,
Lebeau Oanez ², Lluch-Cota Salvador E. ³, Salgado García Rosa L. ³, Kainz Martin J. ⁴,
Le Grand Fabienne ¹

¹ Univ Brest, CNRS, IRD, Ifremer, LEMAR, IUEMPLouzané, France

² Univ Brest, CNRS, UMS3113, IUEMPLouzané, France

³ Centro de Investigaciones Biológicas del Noroeste (CIBNOR) La Paz, Mexico

⁴ WasserCluster Lunz-Inter-University Centre for Aquatic Ecosystem Research Lunz am See, Austria

* Corresponding author : Margaux Mathieu-Resuge, email address : m.mathieuresuge@gmail.com

Abstract :

The trophic fate of various food sources is of central interest for ecologists, yet not well understood in coastal lagoon food webs. In this field study, fatty acids (FA), stable isotopes (SI), and compound-specific isotopic analysis (CSIA) on FA were used to investigate how diets from oceanic and local sources are retained in a bivalve species (*Spondylus crassisquama*; Lamarck 1819) along a transect in the Ojo de Liebre lagoon (BCS, Mexico). Results from SI and FA indicated the contribution of oceanic diatoms at the entrance of the lagoon, through ¹⁵N enrichment, and higher proportions of 16:1n-7 and 20:5n-3 in digestive glands. In the inner bay, higher abundance of 18-carbon FA (18:1n-9, 18:3n-3, 18:4n-3) suggested a higher contribution of microheterotrophs, including (dino)flagellates and ciliates, to the diet of this bivalve derived from local production. Significant spatial differences for the $\delta^{13}\text{C}$ of FA highlighted changes in the origin of food sources. Indeed, a ¹³C depletion was observed in the $\delta^{13}\text{C}$ of heterotrophic flagellates biomarkers in individuals from the innermost station, revealing that their origin in the diet of bivalves differs within the lagoon, highlighting the importance of local processes (sediment resuspension, remineralization) in the trophic functioning of the lagoon. The $\delta^{13}\text{C}$ values of FA considered as diatoms biomarkers (16:1n-7 and 20:5n-3) were consistent, which suggests that diatoms assimilated have very similar origins throughout the lagoon. The complementary of the tracers used here allowed for a better understanding of the trophic functioning of this coastal lagoon submitted to oceanic influences.

45 **Introduction**

46 Coastal lagoons are complex and productive ecosystems, and their primary production is
47 supported by a highly diverse origin of nutrients (Nixon 1982). At the interface between land
48 and ocean, lagoons are characterized by shallow water and a large diversity of potential food
49 sources, including continental inputs, salt marsh, seagrass, macroalgae, and phytoplankton,
50 and resulting in marked spatial patterns in food web interactions (Carlier et al. 2008; Clavier
51 et al. 2014). In coastal lagoons, benthic assemblages are largely dominated by suspension-
52 feeding bivalves (Bachelet et al. 2000). Because these organisms are sedentary primary
53 consumers, they are good indicators of processes occurring at the base of the food web
54 (Marchais et al. 2013). Therefore, studying the nature and the origin of food sources
55 involved in their diet provides a spatially and temporally integrated overview of organic
56 matter transfer between benthic and pelagic systems, and across ecosystem boundaries
57 (i.e., between lagoons and the nearby coastal ocean).

58 The trophic ecology of suspension-feeding invertebrates inhabiting coastal environments has
59 been extensively investigated over the last 20 years using indirect biomarker-based
60 approaches. Among the most common, the analysis of bulk carbon and nitrogen stable
61 isotopes (SI) has provided valuable insights into the origin and nature of bivalve food sources
62 (Riera 2007; Marchais et al. 2013). Because primary producers typically display contrasted SI
63 composition according to their benthic vs pelagic (France 1995) or freshwater vs marine
64 (Riera and Richard 1996) origin, and because the SI composition of consumers are indicative
65 of their source, this approach is currently widely used to address questions in trophic
66 ecology (Fry 1988; Layman et al. 2012). However, to be efficient, this method requires
67 primary producers displaying contrasted SI composition. Although different species of
68 planktonic microalgae can vary in their SI values (Falkowski 1991; Vuorio et al. 2006), it is

69 generally difficult to differentiate between potential diet sources that share the same
70 habitat (Kharlamenko et al. 2001). Therefore, it remains a challenge for bulk SI ecology to
71 assess the dietary contribution of different primary producers to consumers.

72 The analysis of fatty acids (FA) is a powerful and complementary approach, which is based
73 on the assumption that most animals have limited ability to biosynthesize polyunsaturated
74 FA (PUFA) or FA of bacterial origin *de novo* (Budge et al. 2006), and thus the FA composition
75 of a consumer resembles that of its food source (Dalsgaard et al. 2003). Among the different
76 FA synthesized by primary producers, some of them are considered as trophic biomarkers
77 because they characterize specific food sources; for example, palmitoleic acid (16:1n-7) and
78 eicosapentaenoic acid (20:5n-3) are used as biomarkers of diatoms, and stearidonic acid
79 (18:4n-3) and docosahexaenoic acid (22:6n-3) are abundant in (dino)flagellates (Parrish et al.
80 2000; Kharlamenko et al. 2001; Alfaro et al. 2006). Therefore, it is possible to assess the
81 contribution of specific taxonomic groups (e.g. diatoms, dinoflagellates, marine plants) in the
82 diet of primary consumers (Kelly and Scheibling 2012); by paying attention when analyzing
83 bivalve tissues presenting different turnover rates resulting in diverse integrating dietary
84 information over time (e.g. the digestive gland (DG), has faster turnover rates than muscle
85 tissues) (Lorrain et al. 2002; Dalsgaard et al. 2003; Nerot et al. 2015). Because SI can be
86 useful in assessing food sources, in particular when using stable C and N isotopes in
87 combination (Lorrain et al. 2002; Schaal et al. 2016) and FA can differentiate between algal,
88 bacterial or even terrestrial sources (Kainz et al. 2002), these two approaches have been
89 successfully used in combination to unravel trophodynamic trajectories in a variety of
90 coastal environments (Alfaro et al. 2006; Jaschinski et al. 2008; Allan et al. 2010). The
91 combined use of SI and FA is based on the assumption that specific SI values or FA molecules
92 are attributed to distinct sources, which is unlikely in complex ecosystems (Kharlamenko et

93 al. 2001). For instance, it would be impossible to characterize the precise origin (i.e. oceanic
94 or coastal) of the diet of a suspension feeder assimilating planktonic dinoflagellates and
95 benthic diatoms using only bulk SI and FA approaches.

96 Compound-specific isotope analysis (CSIA) represents an ecologically complementary
97 approach, through which the identification of the SI composition of source-specific
98 compounds can characterise their origin. For example, CSIA has been successfully used in
99 trophic ecology to highlight the contribution of ice algae to the diet of bivalves (Gaillard et al.
100 2017), or dietary shifts in benthic invertebrates at the vicinity of finfish farming (Colombo et
101 al. 2016).

102 Lagoon, such as the Ojo de Liebre lagoon (Baja California Sur, Mexico), are of particular
103 interest for trophic ecology as they rely on a wide diversity of both local sources
104 (autochthonous) such as local phytoplankton or resuspended benthic sources, including
105 eelgrass detritus (*Zostera marina*; Linnaeus, 1753), and allochthonous sources, such as
106 oceanic phytoplankton supplied to the lagoon by tide current, especially during important
107 oceanic events occurring from April to June in this region (Du et al. 2015)..

108 In this context, the aim of this study was to apply three trophic biomarker-based approaches
109 to examine spatial patterns of organic matter sources and their biochemical composition
110 assimilated by *S. crassisquama*. It was hypothesized that lower oceanic phytoplankton
111 availability in the inner part of the lagoon, which is less connected to the ocean, would result
112 in higher reliance on local food sources. Stable isotope, FA, and CSIA values in consumers
113 located close the ocean were thus expected to resembled those of the ocean, testing the
114 effect of spatial diet proximity to the ocean and how far its impact is perceived inside the
115 lagoon; by contrast, consumers furthest away from the ocean were isotopically and in their

116 FA composition expected to be closer to the autochthonous diet sources highlighting the
117 effect of local diet incorporation.

118

119 **Material and methods**

120 *Sampling*

121 The Ojo de Liebre lagoon is a 446 km² lagoon situated on the Pacific coast of Baja California
122 hosting one of the most important bivalve fishery (in particular *Spondylus crassiquama*;
123 Lamarck 1819) of western Mexico, was sampled in June 2017 at three stations along a
124 transect (Fig. 1); Station 1: located 3.5 km outside the bay; Station 2: at 6.1 km from the
125 mouth, inside the lagoon; and Station 3: 15.5 km inside the lagoon. Sampling depths ranged
126 from 7 to 10 m. Stations 1 and 3 corresponded to the most outer and inner sites,
127 respectively, where the target species, *Spondylus crassisquama*, could be found. At each
128 station, 10 individuals of *S. crassisquama* of similar size (~12 cm of length), and potential
129 food sources (particulate organic matter; POM, and, sediment-water interface organic
130 matter; SOM) were collected by scuba divers. The *Zostera marina* fragments, at two
131 different degradation stages (later called fresh and detrital), were also collected from the
132 shore at the eastern part of the sampling area. Bivalves were placed in cooler boxes with
133 water from sampling sites at ambient temperature, while collected surface water and
134 sediment cores and eelgrass detritus were protected from light using black plastic bags until
135 transportation (within 3 hours) to the lab facilities at the Northwestern Center for Biological
136 Research (CIBNOR, Guerrero Negro, BCS). Once in the laboratory, bivalve DG and adductor
137 muscles were dissected, packed and stored in liquid nitrogen.

138 Food sources (POM, SOM, and eelgrass fragments) were prepared for analysis by the
139 following methods; a) sediment-water interface were soaked in filtered sea water (GF/F 0.7
140 μm), and allowed to settle. The supernatant containing fine organic particles (SOM),
141 potentially affected by resuspension events and therefore available to benthic suspension-

142 feeders, was filtered on pre-combusted (6 h, 450°C) GF/F filters. Four subsamples of SOM
143 (two for SI, and two for FA analyses) were prepared for each sampling station, packed and
144 frozen in liquid nitrogen; b) particles from surface water were filtered on pre-combusted (6
145 h, 450°C) GF/F filters until clogging. Immediately after filtration, filters subsamples of POM (n
146 = 2 for SI, and n = 2 for FA analyses) were packed and frozen in liquid nitrogen immediately
147 after filtration, and; c) large epiphytes were first removed from eelgrass fragments, which
148 were then packed and stored in liquid nitrogen. Once at the main laboratory (CIBNOR, La
149 Paz), all samples were stored at -80°C until further analysis.

150 *Stable isotopes analysis*

151 Muscles and eelgrass samples were freeze-dried (24 h) and homogenized using a ball mill.
152 Because bivalve muscles are generally poor in lipids, lipids were not removed from muscle
153 tissues prior to SI analysis (Lorrain et al. 2002). Indeed, the measured C:N ratios did not
154 exceed 3.5, rendering lipid removal unnecessary for SI analysis (Post et al. 2007). Muscle
155 tissues (~25 mg dry weight) and particles of SOM and POM (scraped off from the freeze-
156 dried filters; ~1.5 mg dry weight) were packed in tin capsules (8x5 mm) for subsequent bulk
157 SI analysis.

158 All samples were analyzed by continuous flow on a Flash EA 2000 IRMS elemental analyzer
159 coupled to a isotope ratio mass spectrometer (IRMS; Thermo Fisher Scientific Delta V Plus).
160 Isotopic calibration was done using international standards (IAEA-600 Caffeine, IAEA-CH-6
161 Sucrose and IAEA-N-2 Ammonium Sulphate). The standard deviation calculated with the
162 acetanilide working standard (Thermo Scientific), repeatedly measured after 7 samples, was
163 ± 0.1 ‰ (n = 19) for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. Results were expressed in delta (δ) units with
164 respect to international standards (Vienna Pee Dee Belemnite for $\delta^{13}\text{C}$ and atmospheric

165 nitrogen for $\delta^{15}\text{N}$), following the equation: $\delta^{13}\text{C}$ or $\delta^{15}\text{N} = [(R_{\text{sample}}/R_{\text{standard}})-1] \times 10^3$
166 (expressed in ‰), where R is $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$.

167 *Fatty acid analysis*

168 *Lipid extraction*

169 Total lipids of freeze-dried and homogenized bivalve DG and eelgrass fragments (between
170 0.2 and 0.4 g), and POM and SOM filter samples were extracted using chloroform/methanol
171 (6mL; 2:1; v/v) applying a modified method of Folch et al. (1957). Samples were then
172 sonicated at 4°C during 5 min, to ensure complete extraction of lipids. Until further analysis,
173 all lipid extracts were stored at -20°C under nitrogen atmosphere to avoid oxidation.

174 *Separation of neutral lipids*

175 DG lipids were separated into neutral and polar lipids (the latter was not analyzed in the
176 present study) following the method described elsewhere (Le Grand et al. 2014). In brief, an
177 aliquot (1/6) of the total lipid extract was evaporated under nitrogen, recovered with 3
178 washes using chloroform/methanol (0.5 mL; 98:2; v/v) and spotted at the top of a silica gel
179 column (40 mm x 4 mm, silica gel 60A 63-200 μm rehydrated with 6% H_2O ; 70-230 mesh).
180 Neutral lipids (NL) were eluted using chloroform/methanol (10 mL; 98:2; v/v) and collected
181 in glass vials containing an internal standard (2.3 μg of tricosanoic acid; C23:0). Lipid
182 fractions were then stored at -20°C until further analyses.

183 *Fatty acid analysis by gas chromatography*

184 The NL fraction of DG as well as aliquots of POM, SOM and eelgrass total lipid extracts were
185 evaporated to dryness under nitrogen. Fatty acids methyl esters (FAME) were obtained by
186 an acidic transesterification following a modified method by (Le Grand et al. 2014). In brief,

187 transesterification was performed by the addition of 0.8 mL of a H₂SO₄/methanol mixture
188 (3.4 %; v/v) to the dried extracts containing NL and heated at 100°C for 10 min. After cooling,
189 hexane (0.8 mL) and distilled water saturated in hexane (1.5 mL) were added. Vials were
190 then homogenized and centrifuged (at 738 g for 1 min at 20°C). Only the organic phase–
191 containing FAME was kept and washed two more times with distilled water saturated in
192 hexane (1.5 mL). At each step, the aqueous phase was discarded.

193 Analyses of FAME were conducted on a Varian CP8400 gas chromatograph (GC), by
194 simultaneous separation on two columns with two different phase polarities: one polar
195 (ZBWAX: 30 m x 0.25 mm ID x 0.2 µm, Phenomenex) and another apolar column (ZB5HT: 30
196 m x 0.25 mm ID x 0.2 µm, Phenomenex). The GC runs followed these temperature programs:
197 the GC oven temperature was raised to 150°C at 50°C min⁻¹, then to 170°C at 3.5°C min⁻¹, to
198 185°C at 1.5°C min⁻¹, to 225°C at 2.4°C min⁻¹, and finally to 250°C at 5.5°C min⁻¹ and
199 maintained for 15 min) and equipped of two splitless injectors regulated at 220°C, and two
200 flame-ionization detectors (280°C), using hydrogen as vector gas. Identification of FAME was
201 realized by comparison of their retention times with those of commercial standards from
202 Sigma (Supelco 37 Component FAME Mix, the PUFA No.1 and No.3, and the Bacterial Acid
203 Methyl Ester Mix) and an in-house standard mix from marine bivalves and microalgae. The
204 FA content (%) was expressed as the mass percentage of the total FA content.

205 *Compound-specific isotopic analysis on fatty acids*

206 Five bivalve samples previously analyzed for FA composition were randomly selected for
207 compound-specific isotopic analysis. The utilization of GC (Thermo Fisher Scientific GC
208 ISOLINK TRACE ULTRA) allowed the separation of FAME using the same apolar phase (Agilent
209 DB5) and the same analytical program as for GC-FID analysis (see above). Each of the FA was

210 converted into CO₂ by combustion in the ISOLINK furnace and transferred to the CONFLO IV
211 interface and then introduced to the IRMS (Delta V Plus; Thermo Fisher Scientific). As above,
212 FAME were identified by comparison of their retention time with those of commercial
213 standards and in-house standard mixtures as described above. 18:1n-9 and 18:3n-3 did
214 coelute on the apolar column used in GC-C-IRMS analysis, and were therefore analyzed
215 simultaneously. Fatty acids kept for δ¹³C analyses were selected based on their abundance
216 and detection in CSIA (i.e., with amplitudes > 800 mV).

217 *Calibration of δ¹³C values*

218 The δ¹³C values were calibrated using the F8-3 standard mixture of eight acid ethyl and
219 methyl esters (14:0, 16:0, 18:0, and 20:0, with δ¹³C values ranging from -26.98±0.02‰ to -
220 30.38±0.02‰) supplied by Indiana University Stable Isotope Reference Materials, inserted
221 before and after every 3 analytical replicates of each sample.

222 *Correction of the δ¹³C contribution of the carbon added by trans-esterification*

223 Because of the addition of a methyl group derived from the methanol used in the
224 transesterification reaction, δ¹³C of FAME analyzed here differed from the original δ¹³C of FA
225 present in source material. To correct the contribution of the carbon from this additional
226 methyl group, several free FA (14:0, 16:0, 16:1n-7, 17:0, 18:0, 18:1n-9, 18:2n-6, 18:3n-3,
227 18:3n-6, 20:0, 20:4n-6, 20:5n-3, 22:0, 23:0, and 24:0; Sigma), were trans-esterified with the
228 same methanol as the one used for samples. Prior to trans-esterification, the δ¹³C values of
229 each of those free FA were determined by using continuous flow on a Flash EA2000
230 elemental analyzer coupled to a Delta V Plus mass spectrometer (Thermo Fisher scientific).
231 After trans-esterification of free FA, the δ¹³C values of the resulting FAME were measured
232 using the GC-C-IRMS system. Then, an average correction was calculated using the following

233 equation: $\delta^{13}C_{Methanol} = -n * \delta^{13}C_{FA} + (n + 1) * \delta^{13}C_{FAME}$, where n is the number of
234 carbon atoms in the free FA (Abrajano et al. 1994). The average $\delta^{13}C$ value (-39.6‰) was
235 calculated for the methyl-derived carbon based on the difference between the $\delta^{13}C$ values of
236 the corresponding free FA and their respective FAME and this value was used to correct the
237 FAME $\delta^{13}C$ by rearranging the above equation. Further studies found no kinetic isotope
238 effect associated to trans-esterification (Budge et al. 2008; Graham et al. 2014), thus this
239 aspect was not considered for $\delta^{13}C$ analysis.

240 *Analytical error*

241 The analytical error (consisting of the SD of 10 analyses of the C16:0 FA laboratory standard
242 interspersed during the samples run) was 0.2 ‰. Therefore the analytical precision was
243 estimated to be $\leq 0.2\%$.

244 *Statistical Analysis*

245 Normality (Shapiro-Wilks test) and homoscedasticity (Bartlett-test) were tested prior to
246 parametric or non-parametric tests. Parametric tests were applied when conditions of
247 normally distributed data and homoscedasticity were satisfied, otherwise non-parametric
248 tests with Bonferroni's adjustment method were performed. The isotopic variation in bivalve
249 muscles was compared among stations by Kruskal-Wallis (KW) tests followed by Conover-
250 Iman multiple comparisons. Only the FA $>0.5\%$ of total FA were considered to compare the
251 dissimilarity among stations, by a permutational variance analyses (PERMANOVA).
252 Subsequently, a test of similarity percentages analyses (SIMPER) was carried out to assess
253 the most discriminant FA responsible of the difference among stations ($>80\%$). Each FA
254 accounting for $>80\%$ of dissimilarity among stations, and presenting an abundance

255 permitting its detection in CSIA in all DG samples, was accounted. For that, one-way analysis
256 of variance (ANOVA) followed by Tukey's HSD, or KW tests followed by Conover-Iman
257 multiple comparison were carried out to compare the difference of their percentage of
258 neutral lipids, and their respective $\delta^{13}\text{C}$ values, between the individuals from the three
259 stations. All statistical analyses and graphics were performed with the free software R (Core
260 Team 2017), with R Version 3.4.1 (2017 06 30).

261

262 Results

263 *Stable isotopes analyses*

264 No significant differences in bulk $\delta^{13}\text{C}$ values were observed in bivalve muscles between the
265 different stations (KW test, $H_{25} = 2.12$, $P = 0.35$). In contrast, a significant $\delta^{15}\text{N}$ difference
266 was observed (KW test, $H_{25} = 17.71$, $P < 0.001$) (Fig. 2). Individuals from stations 1 and 2
267 displayed similar $\delta^{15}\text{N}$ (8.3‰ and 8.5‰, respectively) (Fig. 2), while station 3 was ^{15}N
268 depleted (7.2‰). For logistical reasons, only two replicates of POM and SOM could be
269 sampled per site, which could not allow to statistically compare the results for these
270 potential food sources. The pattern observed in POM was the same as observed for bivalve
271 muscles with stations 1 and 2 being ^{15}N enriched compared to station 3 (from 6.5‰ and
272 6.9‰ for stations 1 and 2 respectively, to 4‰ at station 3) (Fig. 2). The POM $\delta^{13}\text{C}$ value
273 sampled outside the lagoon (-22.8‰) was slightly lower than at stations 2 and 3 (-21.1‰
274 and -20.9‰, respectively). SOM displayed quite a different spatial pattern than the one
275 observed for POM, with station 1 being ^{15}N enriched and ^{13}C depleted ($\delta^{15}\text{N} = 7.3$ ‰, $\delta^{13}\text{C} = -$
276 14.5‰) compared to the two inner stations 2 and 3 ($\delta^{15}\text{N} = 5.2$ ‰ and 4.6‰, $\delta^{13}\text{C} = -10.2$ ‰
277 and -13‰, respectively). Both samples of *Zostera marina* were ^{13}C enriched (-10.0‰ for
278 fresh eelgrass and -11.2‰ for detrital eelgrass) compared to other samples. Fresh eelgrass
279 displayed higher $\delta^{15}\text{N}$ than detrital eelgrass (5.6‰ and 1.8‰, respectively) (Fig. 2).

280

281 *Fatty acids composition*

282 The FA composition of neutral lipids of DG varied significantly among stations (PERMANOVA,
283 $F = 48.76$, $P < 0.001$). Individuals from stations 1 and 2, the closest to the mouth of the bay,
284 were characterized by higher contents of 14:0, 20:4n-6, and the diatom biomarkers 16:1n-7
285 and 20:5n-3 than individuals from station 3 (ANOVA or KW tests followed by post-hoc; Table

286 1). The PUFA 22:6n-3 was slightly more abundant in individuals from station 1 (12.1%) than
 287 in those from station 2 (11.4%) and station 3 (10.5%) (Table 1). The DG of individuals from
 288 the most inner station in the lagoon (station 3) had significantly higher contents of C18-FA
 289 (18:0, 18:1n-9, 18:3n-3, and 18:4n-3) and C16-FA (ANOVA or KW tests followed by post-hoc;
 290 Table1).

291 Some FA in POM samples presented the same spatial variations than in bivalve DG, in
 292 particular 16:1n-7 that presented higher proportions at the external station (Table 2), and
 293 18:4n-3 that showed higher values at the inner station (Table 2). When compared to bivalve
 294 DG, the PUFA 18:4n-3 and 20:5n-3 in POM were similar along the sampled transect (Table 2),
 295 and the 18:1n-9 content was lower at station 2 and 3 (3% and 2.8%, respectively) compared
 296 to station 1 (5.2%) (Table 2).

297

298 *Compound-specific isotopic analyses*

299 The $\delta^{13}\text{C}$ values of almost all selected FA of bivalve DG differed significantly among
 300 stations (ANOVA or KW tests followed by post-hoc; Table 1), with station 3 being different
 301 from stations 1 and 2, except for 20:4n-6, 20:5n-3 and 18:1n-7 (Table 1, Fig. 3). The
 302 differences among stations were significant for most FA, however, the range of variation did
 303 not exceed 1‰ for some FA, such as 16:1n-7 (from -27‰ at the external station, to -25.9‰
 304 at the inner station; Table 1), and 20:5n-3 at station 2 (-26.9‰) differed significantly from
 305 station 3 (-27.6‰; Table 1) (Fig. 3). The variability of $\delta^{13}\text{C}$ values of 18:1n-9+18:3n-3 was
 306 1.9‰ (Fig. 3), ranging from -29.7‰ (station 1) to -29.1‰ (station 2), and finally to -31.0‰
 307 (station 3; Table 1). Because these FA coeluted on the apolar column used for GC-C-IRMS
 308 analysis, they were reported jointly. The $\delta^{13}\text{C}$ values of 18:4n-3 displayed the highest range
 309 of variability (2.3‰) (Fig. 3), ranging from -29.2‰ at stations 1 and 2 to -31.5‰ at station 3

310 (Table 1). Finally, 22:6n-3 varied in its $\delta^{13}\text{C}$ values among the stations (1.8‰; Fig. 3) with
311 higher $\delta^{13}\text{C}$ values at the external stations 1 and 2 (-26.1‰ and -26.9‰, respectively)
312 compared to lower $\delta^{13}\text{C}$ values at the inner station 3 (-27.5‰) (Table 1). For C18 FA (18:1n-9,
313 18:3n-3, 18:4n-3) and 22:6n-3 of bivalve DG, samples from station 3 were always more
314 depleted in ^{13}C compared to other stations (Table 1 and Fig. 3).

315 Similar trends in isotopic variability of FA were found in POM samples than in bivalve DG,
316 with 18C and 22:6n-3 always more depleted in ^{13}C with increasing distance from the mouth
317 of the lagoon (Table 2). For example, the $\delta^{13}\text{C}$ values of 18:4n-3 were similar at station 1 (-
318 31.6‰) and 2 (-31.4‰) and more depleted at station 3 (-32.3‰; Table 2), and the 22:6n-3
319 showed $\delta^{13}\text{C}$ depleted values along the transect (from -25.6‰ and -27.5‰ at stations 1 and
320 2 to -34.9‰ at station 3; Table 2). Like in DG samples, both FA, 16:1n-7 and 20:5n-3 were ^{13}C
321 enriched at the internal station (Table 2) in POM samples. Indeed, 16:1n-7 and 20:5n-3
322 showed respectively $\delta^{13}\text{C}$ from -26.7‰ and -29.5‰ at the external station, to -25.8‰ and -
323 28.7‰ at the station near to the mouth, and to -25.7‰ and -27.5‰ at the inner station
324 (Table 2).

325

326 **Discussion**

327 Results from this study suggest that organic matter from the ocean is transported into the
328 lagoon, contributing to the primary production and to the diet of benthic filter feeders. The
329 CSIA on FA data indicate that diatoms (indicated by 16:1n-7 and 20:5n-3) assimilated by
330 bivalves have a very similar marine origin throughout the lagoon, according to their
331 comparable $\delta^{13}\text{C}$ values among stations. Moreover, results also demonstrate that a diversity
332 of heterotrophic flagellates, characterized by higher contents of 18 carbon FA, and 22:6n-3
333 may also enter the diet of bivalves. The variable $\delta^{13}\text{C}$ values of these FA among stations
334 suggest that their origin varies according to the location within the lagoon, and depleted
335 values at the inner station indicate an influence of local processes in the diet of these
336 consumers.

337 The most striking result from bulk SI analysis of bivalve muscles and POM was the significant
338 ^{15}N enrichment observed near the entrance of the lagoon, with individuals from stations 1
339 and 2 displaying similar $\delta^{15}\text{N}$ values that were higher than in the inner bay. This reveals an
340 oceanic influence, and particularly the contribution of ^{15}N oceanic inputs to the diet of
341 bivalves near the entrance of the lagoon, but also that such dietary influence of the ocean
342 did not extend to the innermost part of the lagoon. This oceanic influence may be explained
343 by active wind-driven seasonal upwelling occurring outside the lagoon from April to June
344 (Ibarra-Obando et al. 2001; Du et al. 2015). It has been suggested that water masses
345 containing upwelled biological material could be transported into coastal lagoons of the
346 Pacific coast of Baja California (Zaytsev 2003), promoting primary production and potentially
347 affecting their trophic functioning. The hypothesis that upwelled waters affect the diet of
348 bivalves inside the lagoon is based on the fact that during upwelling conditions, the biomass
349 of large phytoplankton increases (Taylor and Landry 2018), and as upwelled nitrate is rapidly

350 consumed by phytoplankton (O'Reilly et al. 2002), its $\delta^{15}\text{N}$ increases. Although coastal
351 upwelling influence on the studied lagoon was not supported by chlorophyll a or
352 temperature data, such high $\delta^{15}\text{N}$ with no changes in $\delta^{13}\text{C}$ have been reported in various
353 systems under upwelling influence worldwide (O'Reilly et al. 2002; Hill and McQuaid 2008;
354 Reddin et al. 2015).

355 The spatial differences found in the FA composition of neutral lipids in the DG of bivalves
356 indicates a change in the proportion and possibly in the nature of food sources with
357 increasing distance from the mouth of the lagoon. The DG at the entrance of the lagoon
358 (stations 1 and 2) had higher 16:1n-7 and 20:5n-3 contents when compared to individuals
359 from the inner station (station 3), indicating a higher contribution of diatoms in their diet
360 (Parrish et al. 2000; Alfaro et al. 2006). A previous study revealed that during the late spring,
361 diatoms are the dominant phytoplankton taxa found in the Northern California current (Du
362 et al. 2015). The higher importance of diatoms in the diet of bivalves near the entrance of
363 the lagoon therefore supports the influence of oceanic primary production. Individuals from
364 the inner station had lower contents of these two FA, suggesting a dilution of oceanic inputs
365 further into the lagoon. The importance of 22:6n-3 in DG of individuals at the external
366 station suggests that dinoflagellates also represent an important dietary contribution of
367 *Spondylus crassisquama* (Parrish et al. 2000). This contribution decreases slightly with
368 increasing distance from the mouth of the lagoon, being the lowest at the internal station,
369 suggesting a decreasing contribution of dinoflagellates to this station. However, the high
370 abundance of 18:4n-3, another dinoflagellate marker (Kharlamenko et al. 2001; Bachok et al.
371 2003; Nerot et al. 2015), at the inner station (station 3) suggests the opposite. Such
372 conflicting information by these two dinoflagellate markers may indicate different
373 assemblages being assimilated by bivalves in the lagoon, but such change in dietary

374 biomarkers does not necessarily suggest any shift in contribution of dinoflagellates to the
375 diets of these consumers. Bivalves from the inner bay were also characterized by higher C18-
376 FA contents (18:1n-9, 18:3n-3). These FA can be characteristic of different dietary sources,
377 such as seagrass (Kharlamenko et al. 2001) or heterotrophic flagellates constituting microbial
378 food webs (Zhukova 1991). Although seagrass beds are known to be a source of dissolved
379 organic matter (DOM; Fenton and Ritz 1988), bivalve muscles were too ^{13}C depleted as
380 compared to seagrass $\delta^{13}\text{C}$ to be in accordance with a significant trophic contribution of
381 *Zostera marina*. Consequently, the abundance of these FA likely reflects the contribution of
382 heterotrophic microorganisms (*i.e.* bacteria, ciliates, flagellates, dinoflagellates), relying on
383 locally produced DOM, to the diet of bivalves. This highlights a higher diversity of food
384 sources for bivalves at the internal station than for those submitted to oceanic influences
385 and shows that diet source variation within this and possibly other lagoons is based on
386 allochthonous and autochthonous contribution. Such various diet sources could also present
387 differences in terms of quality and quantity, which consequently impact bivalve stocks and
388 consequently fisheries. More detailed understanding of spatio-temporal dynamics of these
389 food resources would lead to a better management of bivalve stocks in the Ojo de Liebre
390 lagoon and elsewhere.

391 Inferring diet composition based on CSIA requires FA-specific fractionation coefficients,
392 which are not available for our species, and would require extensive laboratory experiments
393 (Ruess and Chamberlain 2010; Bec et al. 2011). Consequently, our approach did not aim to
394 characterize the accuracy of food sources retention by bivalves, but to scrutinize the trophic
395 spatial variability in an effort to provide evidence of a different spatial origin of the diets for
396 this consumer (Ramos et al. 2003; Van den Meersche et al. 2004). Although no difference in
397 bulk $\delta^{13}\text{C}$ values was observed in muscles of individuals from the different stations, CSIA

398 detect the isotopic composition of FA that may indicate source-specific differences of FA
399 (Budge et al. 2008; Colombo et al. 2016). Some differences were observed in the range of
400 variation in FA $\delta^{13}\text{C}$ along the transect depending on biomarkers considered (diatoms vs
401 heterotrophic flagellates). In DG neutral lipids, diatom biomarkers (16:1n-7 and 20:5n-3)
402 displayed the smallest range of variation among the three stations ($\leq 1\text{‰}$), which is of little
403 ecological value. The spatial consistency in the $\delta^{13}\text{C}$ values of these diatom biomarkers
404 suggests that diatoms assimilated by bivalves have all oceanic origin throughout the bay.
405 Therefore, it seems that phytoplankton of local origin (produced within the lagoon) is of
406 relatively little dietary importance for *S. crassisquama*. The C18-FA 18:1n-9 and 18:3n-3 were
407 both considered to reveal the contribution of microheterotrophs (flagellates, ciliates, see
408 above). Even if the three stations presented significant differences in their $\delta^{13}\text{C}$ for 18:1n-9
409 and 18:3n-3, stations 1 and 2 displayed very similar $\delta^{13}\text{C}$ values (only differed by 0.6‰),
410 while station 3 was depleted in ^{13}C by 1.9‰ compare to the other stations. This difference
411 suggests that the origin of these microheterotrophs differed within the lagoon.
412 Microheterotrophs are central elements of microbial food webs, feeding on bacteria and
413 other small unicellular organisms, and ultimately relying on DOM (Azam et al. 1983). The ^{13}C
414 depletion observed at the inner station suggests a local source of DOM, which is of primary
415 importance for bivalves in the lagoon. Although the lagoon of Ojo de Liebre hosts one of the
416 largest eelgrass bed of the Northern Pacific (Cabello-Pasini et al. 2003), a seagrass-derived
417 DOM seems unlikely because seagrass is usually ^{13}C enriched compared to other marine
418 primary producers (Hemminga and Mateo 1996), which was also observed in the present
419 study. The possible DOM release from the sediment is also not in agreement with $\delta^{13}\text{C}$
420 measured for these FA (around -24.5‰ in SOM). In fact, such ^{13}C -depleted values in coastal
421 environments are usually associated with red algae, which can be extremely low in $\delta^{13}\text{C}$

422 (Raven et al. 2002). Although no extensive population of such algae was observed during
423 sampling, it is possible that such populations exist in the inner bay, supplying a pool of
424 dissolved organic carbon that is subsequently trophically transferred to bivalves.
425 Dinoflagellate FA biomarkers (18:4n-3 and 22:6n-3) had a very similar spatial distribution
426 pattern as observed for biomarkers of other microheterotrophs (18:1n-9 and 18:3n-3), with
427 a marked ^{13}C depletion at the inner station. Therefore, in contrast to diatoms, dinoflagellates
428 entering the diet of bivalves do not seem to be of marine origin throughout the lagoon. The
429 similarity observed between the CSIA in microheterotrophs and dinoflagellates might
430 suggest that the same factors affect the SI composition of these two possible food sources.
431 In fact, although dinoflagellates are usually described as primary producers, heterotrophy is
432 a widespread pattern in this group (Jeong et al. 2010). Based on these results, it is possible
433 that dinoflagellates assimilated by *S. crassisquama* in the inner part of the lagoon may rely
434 on heterotrophy, emphasizing a potentially important role of the microbial food web in this
435 enclosed ecosystem.

436

437 **Conclusion**

438 The use of CSIA provided a new scale of complexity in the diet of *Spondylus crassisquama* in
439 the Ojo de Liebre lagoon. Although dietary diatoms retained in bivalves appeared to have
440 the same oceanic origin throughout the lagoon, this study indicates that the heterotrophic
441 component of bivalve diet varies spatially in the lagoon, highlighting an influence of local
442 processes in the diet of these consumers. The combination of the three methods used here
443 showed to be complementary, resulting in a more informative analysis of spatial feeding
444 ecology of this and likely also other lagoons. The lower $\delta^{13}\text{C}$ observed at the most inner

445 station could not be explained in this study, although any potential role of seagrass in
446 providing DOM for the associated microbial food web could be discarded based on different
447 SI data. Further research is warranted to identify how the origin of basal resources vary and
448 support food webs in lagoons and to better understand the quantitative importance of
449 dietary sources for bivalves.

450 **Acknowledgements**

451 Authors would like to thank the contribution of the Guerrero Negro unit of CIBNOR for
452 hosting the first steps of sample preparation after fieldwork, the LIPIDOCEAN analytical
453 facilities for hosting all fatty acid analyses, and the Pôle Spectrométrie Océan (Clément
454 Tanvet) for running bulk SI and CSIA analyses.

455 Authors thank the fishermen crew for the fieldwork, but also Laura Guzman for their
456 precious help during sampling and dissections, and Dr. Elena Palacios and Dr. Kitty
457 Arredondo Vega, for the laboratory facilities provided for the preparation of samples at the
458 CIBNOR La Paz. Authors address their thanks to the Reserva de la biosfera desierto de “el
459 Vizcaino” for their help and support in carrying out sampling for this study. Authors would
460 like to thank Aurelien Boye for his precious help and advices about statistical analyses.

461 Authors also thank Pr. Sandra Shumway and an anonymous reviewer, for their help in
462 improving this paper.

463

464 **Funding**

465 This study was supported by the ECOS-ANUIES program (PROPHYMUS project), the
466 "Laboratoire d'Excellence" LabexMER (ANR-10-LABX-19) and co-funded by a grant from the
467 French government under the program "Investissements d'Avenir". MMR's PhD fellowship
468 was provided by the French Research Ministry and Region Bretagne.

469

470 **Compliance with Ethical Standards**

471 **Conflict of interest**

472 The authors declare that they have no conflict of interest.

473 **Ethical approval**

474 All applicable international, national and/or institutional guidelines for the care and use of
475 bivalves *Spondylus crassisquama* were followed. The individuals of *S. crassisquama* have
476 been sampled under the permit PRMN/DGOPA-011/2017, delivered by the Mexican
477 government.

478 **References**

- 479 Abrajano TA, Murphy DE, Fang J, Comet P, Brooks JM (1994) $^{13}\text{C}^{12}\text{C}$ ratios in individual fatty
480 acids of marine mytilids with and without bacterial symbionts. *Org Geochem* 21:611–617.
481 doi: 10.1016/0146-6380(94)90007-8
- 482 Alfaro AC, Thomas F, Sergent L, Duxbury M (2006) Identification of trophic interactions
483 within an estuarine food web (northern New Zealand) using fatty acid biomarkers and stable
484 isotopes. *Estuar Coast Shelf Sci* 70:271–286. doi: 10.1016/j.ecss.2006.06.017
- 485 Allan EL, Ambrose ST, Richoux NB, Froneman PW (2010) Determining spatial changes in the
486 diet of nearshore suspension-feeders along the South African coastline: Stable isotope and
487 fatty acid signatures. *Estuar Coast Shelf Sci* 87:463–471. doi: 10.1016/j.ecss.2010.02.004
- 488 Azam F, Fenchel T, Field JG, Gray JS, Meyer-Reil LA, Thingstad F (1983) The ecological role of
489 water-column microbes in the sea. *Mar Ecol Prog Ser* 257–263.
- 490 Bachelet G, De Montaudouin X, Auby I, Labourg P-J (2000) Seasonal changes in macrophyte
491 and macrozoobenthos assemblages in three coastal lagoons under varying degrees of
492 eutrophication. *ICES J Mar Sci* 57:1495–1506.
- 493 Bachok Z, Mfilinge PL, Tsuchiya M (2003) The diet of the mud clam *Geloina coaxans*
494 (Mollusca, Bivalvia) as indicated by fatty acid markers in a subtropical mangrove forest of
495 Okinawa, Japan. *J Exp Mar Biol Ecol* 292:187–197. doi: 10.1016/S0022-0981(03)00160-6
- 496 Bec A, Perga M-E, Koussoroplis A, Bardoux G, Desvillettes C, Bourdier G, Mariotti A (2011)
497 Assessing the reliability of fatty acid-specific stable isotope analysis for trophic studies.
498 *Methods Ecol Evol* 2:651–659. doi: 10.1111/j.2041-210X.2011.00111.x

- 499 Budge SM, Iverson SJ, Koopman HN (2006) Studying trophic ecology in marine ecosystems
500 using fatty acids: a primer on analysis and interpretation. Mar Mammal Sci 22:759–801. doi:
501 10.1111/j.1748-7692.2006.00079.x
- 502 Budge SM, Wooller MJ, Springer AM, Iverson SJ, McRoy CP, Divoky GJ (2008) Tracing carbon
503 flow in an arctic marine food web using fatty acid-stable isotope analysis. Oecologia
504 157:117–129.
- 505 Cabello-Pasini A, Muñiz-Salazar R, Ward DH (2003) Annual variations of biomass and
506 photosynthesis in *Zostera marina* at its southern end of distribution in the North Pacific.
507 Aquat Bot 76:31–47. doi: 10.1016/S0304-3770(03)00012-3
- 508 Carlier A, Riera P, Amouroux J-M, Bodiou J-Y, Desmalades M, Grémare A (2008) Food web
509 structure of two Mediterranean lagoons under varying degree of eutrophication. J Sea Res
510 60:264–275. doi: 10.1016/j.seares.2008.10.006
- 511 Clavier J, Chauvaud L, Amice E, Lazure P, Van Der Geest M, Labrosse P, Diagne A, Carlier A,
512 Chauvaud S (2014) Benthic metabolism in shallow coastal ecosystems of the Banc d’Arguin,
513 Mauritania. Mar Ecol Prog Ser 501:11–23.
- 514 Colombo SM, Parrish CC, Whiticar MJ (2016) Fatty acid stable isotope signatures of molluscs
515 exposed to finfish farming outputs. Aquac Environ Interact 8:611–617.
- 516 Core Team R (2017) R: A language and environment for statistical computing. R Foundation
517 for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>.
- 518 Dalsgaard J, St. John M, Kattner G, Müller-Navarra D, Hagen W (2003) Fatty acid trophic
519 markers in the pelagic marine environment. Adv Mar Biol 46:225–340. doi: 10.1016/S0065-
520 2881(03)46005-7

- 521 Du X, Peterson W, O'Higgins L (2015) Interannual variations in phytoplankton community
522 structure in the northern California Current during the upwelling seasons of 2001-2010. Mar
523 Ecol Prog Ser 519:75–87.
- 524 Falkowski PG (1991) Species variability in the fractionation of ^{13}C and ^{12}C by marine
525 phytoplankton. J Plankton Res 13:21–28. doi: 10.1093/oxfordjournals.plankt.a042367
- 526 Fenton GE, Ritz DA (1988) Changes in carbon and hydrogen stable isotope ratios of
527 macroalgae and seagrass during decomposition. Estuar Coast Shelf Sci 26:429–436.
- 528 Folch J, Lees M, Sloane-Stanley GH (1957) A simple method for the isolation and purification
529 of total lipids from animal tissues. J Biol Chem 226:497–509.
- 530 France RL (1995) Carbon-13 enrichment in benthic compared to planktonic algae: foodweb
531 implications. Mar Ecol Prog Ser 124:307–312.
- 532 Fry B (1988) Food web structure on Georges Bank from stable C, N, and S isotopic
533 compositions. Limnol Oceanogr 33:1182–1190. doi: 10.4319/lo.1988.33.5.1182
- 534 Gaillard B, Meziane T, Tremblay R, Archambault P, Blicher ME, Chauvaud L, Rysgaard S,
535 Olivier F (2017) Food resources of the bivalve *Astarte elliptica* in a sub-Arctic fjord: a multi-
536 biomarker approach. Mar Ecol Prog Ser 567:139–156.
- 537 Graham C, Oxtoby L, Wang SW, Budge SM, Wooller MJ (2014) Sourcing fatty acids to juvenile
538 polar cod (*Boreogadus saida*) in the Beaufort Sea using compound-specific stable carbon
539 isotope analyses. Polar Biol 37:697–705.
- 540 Hemminga MA, Mateo MA (1996) Stable carbon isotopes in seagrasses: variability in ratios
541 and use in ecological studies. Mar Ecol Prog Ser 140:285–298.

- 542 Hill JM, McQuaid CD (2008) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ biogeographic trends in rocky intertidal
543 communities along the coast of South Africa: Evidence of strong environmental signatures.
544 Estuar Coast Shelf Sci 80:261–268. doi: 10.1016/j.ecss.2008.08.005
- 545 Ibarra-Obando SE, Camacho-Ibar VF, Carriquiry JD, Smith SV (2001) Upwelling and lagoonal
546 ecosystems of the dry Pacific coast of Baja California. In: Coastal Marine Ecosystems of Latin
547 America. Springer, pp 315–330
- 548 Jaschinski S, Brepohl DC, Sommer U (2008) Carbon sources and trophic structure in an
549 eelgrass *Zostera marina* bed, based on stable isotope and fatty acid analyses. Mar Ecol Prog
550 Ser 358:103–114.
- 551 Jeong HJ, Du Yoo Y, Kim JS, Seong KA, Kang NS, Kim TH (2010) Growth, feeding and ecological
552 roles of the mixotrophic and heterotrophic dinoflagellates in marine planktonic food webs.
553 Ocean Sci J 45:65–91.
- 554 Kainz M, Lucotte M, Parrish CC (2002) Methyl mercury in zooplankton the role of size,
555 habitat, and food quality. Can J Fish Aquat Sci 59:1606–1615.
- 556 Kelly JR, Scheibling RE (2012) Fatty acids as dietary tracers in benthic food webs. Mar Ecol
557 Prog Ser 446:1–22.
- 558 Kharlamenko VI, Kiyashko SI, Imbs AB, Vyshkvartzev DI (2001) Identification of food sources
559 of invertebrates from the seagrass *Zostera marina* community using carbon and sulfur stable
560 isotope ratio and fatty acid analyses. Mar Ecol Prog Ser 220:103–117.
- 561 Layman CA, Araujo MS, Boucek R, Hammerschlag-Peyer CM, Harrison E, Jud ZR, Matich P,
562 Rosenblatt AE, Vaudo JJ, Yeager LA (2012) Applying stable isotopes to examine food-web
563 structure: an overview of analytical tools. Biol Rev 87:545–562.

- 564 Le Grand FL, Soudant P, Siah A, Tremblay R, Marty Y, Kraffe E (2014) Disseminated Neoplasia
565 in the soft-shell Clam *Mya arenaria*: membrane lipid composition and functional parameters
566 of circulating cells. Lipids 49:807–818. doi: 10.1007/s11745-014-3917-4
- 567 Lorrain A, Paulet Y-M, Chauvaud L, Savoye N, Donval A, Saout C (2002) Differential $\delta^{13}\text{C}$ and
568 $\delta^{15}\text{N}$ signatures among scallop tissues: implications for ecology and physiology. J Exp Mar
569 Biol Ecol 275:47–61. doi: 10.1016/S0022-0981(02)00220-4
- 570 Marchais V, Schaal G, Grall J, Lorrain A, Nerot C, Richard P, Chauvaud L (2013) Spatial
571 variability of stable isotope Ratios in Oysters (*Crassostrea gigas*) and primary producers
572 along an estuarine gradient (Bay of Brest, France). Estuaries Coasts 36:808–819. doi:
573 10.1007/s12237-012-9584-x
- 574 Nerot C, Meziane T, Schaal G, Grall J, Lorrain A, Paulet Y-M, Kraffe E (2015) Spatial changes in
575 fatty acids signatures of the great scallop *Pecten maximus* across the Bay of Biscay
576 continental shelf. [http://ac.els-cdn.com.scd-proxy.univ-brest.fr/S0278434315300522/1-s2.0-S0278434315300522-main.pdf?_tid=cc01133c-2f39-11e7-8950-](http://ac.els-cdn.com.scd-proxy.univ-brest.fr/S0278434315300522/1-s2.0-S0278434315300522-main.pdf?_tid=cc01133c-2f39-11e7-8950-00000aab0f02&acdnat=1493731274_d207b602cfd94a22be277a4effaa33b7)
577 [S0278434315300522-main.pdf?_tid=cc01133c-2f39-11e7-8950-](http://ac.els-cdn.com.scd-proxy.univ-brest.fr/S0278434315300522/1-s2.0-S0278434315300522-main.pdf?_tid=cc01133c-2f39-11e7-8950-00000aab0f02&acdnat=1493731274_d207b602cfd94a22be277a4effaa33b7)
578 [00000aab0f02&acdnat=1493731274_d207b602cfd94a22be277a4effaa33b7](http://ac.els-cdn.com.scd-proxy.univ-brest.fr/S0278434315300522/1-s2.0-S0278434315300522-main.pdf?_tid=cc01133c-2f39-11e7-8950-00000aab0f02&acdnat=1493731274_d207b602cfd94a22be277a4effaa33b7). Accessed 2
579 May 2017
- 580 Nixon SW (1982) Nutrient dynamics, primary production and fisheries yields of lagoons.
581 Oceanol Acta Spec Issue 357–371.
- 582 O'Reilly CM, Hecky RE, Cohen AS, Plisnier P-D (2002) Interpreting stable isotopes in food
583 webs: Recognizing the role of time averaging at different trophic levels. Limnol Oceanogr
584 47:306–309. doi: 10.4319/lo.2002.47.1.0306

- 585 Parrish CC, Abrajano TA, Budge SM, Helleur RJ, Hudson ED, Pulchan K, Ramos C (2000) Lipid
586 and phenolic biomarkers in marine ecosystems: analysis and applications. In: Marine
587 chemistry. Springer, pp 193–223
- 588 Post DM, Layman CA, Arrington DA, Takimoto G, Quattrochi J, Montaña CG (2007) Getting to
589 the fat of the matter: models, methods and assumptions for dealing with lipids in stable
590 isotope analyses. *Oecologia* 152:179–189. doi: 10.1007/s00442-006-0630-x
- 591 Ramos CS, Parrish CC, Quibuyen TAO, Abrajano TA (2003) Molecular and carbon isotopic
592 variations in lipids in rapidly settling particles during a spring phytoplankton bloom. *Org*
593 *Geochem* 34:195–207.
- 594 Raven JA, Johnston AM, Kübler JE, Korb R, McInroy SG, Handley LL, Scrimgeour CM, Walker
595 DI, Beardall J, Vanderklift M, Fredriksen S, Dunton KH (2002) Mechanistic interpretation of
596 carbon isotope discrimination by marine macroalgae and seagrasses. *Funct Plant Biol*
597 29:355–378. doi: 10.1071/pp01201
- 598 Reddin CJ, Docmac F, O'Connor NE, Bothwell JH, Harrod C (2015) Coastal upwelling drives
599 intertidal assemblage structure and trophic ecology. *PloS One* 10:e0130789.
- 600 Riera P (2007) Trophic subsidies of *Crassostrea gigas*, *Mytilus edulis* and *Crepidula fornicata*
601 in the Bay of Mont Saint Michel (France): a $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ investigation. *Estuar Coast Shelf Sci*
602 72:33–41. doi: 10.1016/j.ecss.2006.10.002
- 603 Riera P, Richard P (1996) Isotopic determination of food sources of *Crassostrea gigas* along a
604 trophic gradient in the estuarine bay of Marennes-Oléron. *Estuar Coast Shelf Sci* 42:347–360.
- 605 Ruess L, Chamberlain PM (2010) The fat that matters: soil food web analysis using fatty acids
606 and their carbon stable isotope signature. *Soil Biol Biochem* 42:1898–1910.

- 607 Schaal G, Nerot C, Grall J, Chouvelon T, Lorrain A, Mortillaro J-M, Savoye N, Brind'Amour A,
608 Paulet Y-M, Le Bris H (2016) Stable isotope ratios in benthic-demersal biota along a depth
609 gradient in the Bay of Biscay: a multitrophic study. *Estuar Coast Shelf Sci* 179:201–206. doi:
610 10.1016/j.ecss.2015.10.023
- 611 Taylor AG, Landry MR (2018) Phytoplankton biomass and size structure across trophic
612 gradients in the southern California Current and adjacent ocean ecosystems. *Mar Ecol Prog*
613 *Ser* 592:1–17.
- 614 Van den Meersche K, Middelburg JJ, Soetaert K, Van Rijswijk P, Boschker HT, Heip CH (2004)
615 Carbon-nitrogen coupling and algal-bacterial interactions during an experimental bloom:
616 modeling a ^{13}C tracer experiment. *Limnol Oceanogr* 49:862–878.
- 617 Vuorio K, Meili M, Sarvala J (2006) Taxon-specific variation in the stable isotopic signatures
618 ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of lake phytoplankton. *Freshw Biol* 51:807–822.
- 619 Zaytsev (2003) Coastal upwelling activity on the Pacific shelf of the Baja California peninsula.
620 *J Oceanogr* 59:489–502.
- 621 Zhukova NV (1991) The pathway of the biosynthesis of non-methylene-interrupted dienoic
622 fatty acids in molluscs. *Comp Biochem Physiol Part B Comp Biochem* 100:801–804.
- 623

624 **Table captions**

625 Table 1 – Neutral lipid fatty acid (FA) composition (mean \pm SD; mass % of total FA; n = 10)
626 and Compound Specific Isotopic analysis (CSIA) of neutral lipid FA (mean \pm SD; $\delta^{13}\text{C}$ ‰; n = 5)
627 of *Spondylus crassisquama* digestive gland in June 2017, for the 3 stations (1, 2, and 3). Only
628 the FA accounting for more than 80 % of dissimilarity between stations (SIMPER test) and
629 presenting the greatest abundance permitting their good detection in CSIA are shown.
630 Different letters indicate significant difference between the stations (ANOVA or KW tests
631 followed by multiple comparison tests, at the significant level $\alpha < 0.05$). *In CSIA, 18:1n-9
632 and 18:1n-3 are coeluted and $\delta^{13}\text{C}$ value is presented in the same line.

633

634 Table 2 – Total fatty acid (FA) composition (mean; mass % of total FA; n = 2) and Compound
635 Specific Isotopic analysis (CSIA) of FA (mean; $\delta^{13}\text{C}$ ‰; n = 2) of potential food sources, for the
636 3 stations (1, 2, and 3). Only the FA accounting for more than 80 % of dissimilarity between
637 stations (SIMPER test) and presenting the greatest abundance permitting their good
638 detection in CSIA are shown. NA: Not analyzed. *In CSIA, 18:1n-9 and 18:1n-3 are coeluted
639 and $\delta^{13}\text{C}$ value is presented in the same line.

640

641

642 **Figure captions**

643 **Fig. 1** Location of the 3 sampling stations in the Ojo de Liebre lagoon (Baja California Sur,
644 Mexico). Station 1 is located at 3.5 km outside the bay. Station 2 and 3 are located inside the

645 lagoon, respectively at 6.1 km and 15.5 km from the mouth of the lagoon. (Map modified
646 from Google earth).

647

648 **Fig. 2** Stable Isotope (SI) bi-plot ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, mean values \pm SD when $n > 2$) of the adductor
649 muscles of *Spondylus crassisquama* and of its potential food sources, in June 2017, at the 3
650 sampled stations. Samples from station 1 are shown in dark blue, 2 in light blue, and 3 in
651 green. Bivalves are represented by squares, POM by circles, SOM by triangles, and eelgrass
652 by crosses (* fresh; x detrital).

653

654 **Fig. 3** Box plot of $\delta^{13}\text{C}$ values (‰; $n = 5$) of some selected neutral lipid fatty acid (FA) of
655 *Spondylus crassisquama* digestive gland in June 2017 at each stations (1, 2, and 3). Only the
656 FA accounting for more than 80 % of dissimilarity between stations, presented the greatest
657 abundance permitting their good detection in CSIA, and present in all samples are shown.
658 Different letters indicate significant difference between the stations (ANOVA or KW tests
659 followed by multiple comparison tests, at the significant level $\alpha < 0.05$). Samples from
660 station 1 are shown in dark blue, 2 in light blue, and 3 in green.

661

662

663

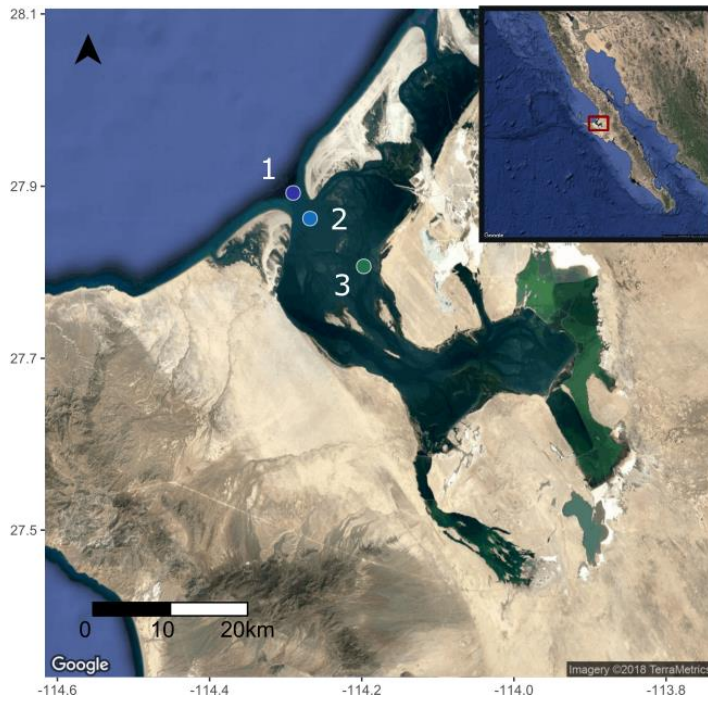
Table 1

	<i>Fatty Acids (mass % of neutral lipid FA)</i>			<i>Compound Specific Isotopic Analysis ($\delta^{13}\text{C}$ ‰)</i>		
	Station 1	Station 2	Station 3	Station 1	Station 2	Station 3
14:0	5.8 ± 0.6 ^A	6.1 ± 0.5 ^A	4.2 ± 0.3 ^B	-28.2 ± 0.2 ^a	-28.3 ± 0.2 ^a	-29.7 ± 0.3 ^b
16:0	21.6 ± 0.4 ^B	21.9 ± 0.9 ^B	23.9 ± 0.9 ^A	-27.5 ± 0.3 ^a	-27.1 ± 0.1 ^a	-29.9 ± 0.6 ^b
18:0	4.1 ± 0.5 ^B	3.9 ± 0.4 ^B	5.6 ± 0.5 ^A	-26.8 ± 0.3 ^a	-26.4 ± 0.2 ^a	-28.3 ± 0.4 ^b
16:1n-7	10.1 ± 0.8 ^A	10.5 ± 0.8 ^A	5.0 ± 0.2 ^B	-27.0 ± 0.2 ^b	-26.6 ± 0.1 ^b	-25.9 ± 0.4 ^a
18:1n-7	2.8 ± 0.1	2.8 ± 0.2	2.8 ± 0.1	-27.5 ± 0.5	-27.2 ± 0.6	-27.8 ± 0.4
18:1n-9*	3.4 ± 0.1 ^B	3.3 ± 0.3 ^B	4.8 ± 0.4 ^A	-29.7 ± 0.3 ^a	-29.1 ± 0.2 ^b	-31.0 ± 0.2 ^c
18:3n-3*	3.4 ± 0.1 ^B	3.3 ± 0.1 ^B	5.3 ± 0.2 ^A			
18:4n-3	5.1 ± 0.4 ^B	5.4 ± 0.5 ^B	6.6 ± 0.4 ^A	-29.2 ± 0.9 ^a	-29.2 ± 0.6 ^a	-31.5 ± 1.1 ^b
20:4n-6	1.7 ± 0.1 ^A	1.6 ± 0.1 ^A	1.0 ± 0.1 ^B	-26.7 ± 0.4	-26.8 ± 0.6	-26.3 ± 1.5
20:5n-3	12.0 ± 0.3 ^A	11.4 ± 0.8 ^A	8.6 ± 1.2 ^B	-27.1 ± 0.3 ^{ab}	-26.9 ± 0.4 ^a	-27.6 ± 0.5 ^b
22:6n-3	12.1 ± 1.0 ^A	11.4 ± 0.8 ^{AB}	10.5 ± 0.9 ^B	-26.1 ± 0.2 ^a	-25.7 ± 0.4 ^a	-27.5 ± 0.8 ^b

Table 2

	<i>Fatty Acids (mass% of total lipid FA)</i>						<i>Compound Specific Isotopic Analysis ($\delta^{13}\text{C}$ ‰)</i>									
	POM			SOM			Eelgrass		POM			SOM		Eelgrass		
	Station 1	Station 2	Station 3	Station 1	Station 2	Station 3	Fresh	Detrital	Station 1	Station 2	Station 3	Station 1	Station 2	Station 3	Fresh	Detrital
14:0	9.7	11.4	7.2	2.6	3.8	3.2	0.8	1.9	-26.7	-25.9	-27.7	NA	NA	NA	-18.9	-26.4
16:0	21.3	25.0	26.0	16.3	22.2	20.6	21.8	25.6	-30.5	-31.1	-34.5	-27.2	-23.6	-23.3	-17.5	-22.3
18:0	6.4	6.1	4.5	6.4	6.1	6.3	1.9	2.6	-29.0	-29.8	-30.8	-30.1	-27.7	-26.6	-16.4	-25.3
16:1n-7	6.1	5.4	5.1	7.3	12.0	8.3	0.4	1.0	-26.7	-25.8	-25.7	-23.5	-23.4	-22.2	-15.8	-17.2
18:1n-7	3.1	2.6	3.0	6.7	3.9	5.2	0.4	1.4	-23.5	-23.1	-20.9	-21.1	-20.1	-19.4	NA	NA
18:1n-9*	5.2	3.0	2.8	4.1	3.0	4.3	1.4	1.9	-28.4	-29.9	-30.8	-25.8	-24.6	-24.5	NA	NA
18:3n-3*	2.2	2.5	3.3	1.8	0.9	1.2	38.3	29.7								
18:4n-3	3.6	3.8	3.9	0.9	1.3	1.5	0.1	0.3	-31.6	-31.4	-32.3	NA	NA	NA	NA	NA
20:4n-6	0.2	0.1	0.1	0.4	0.2	0.2	0.1	0.5	NA	NA	NA	-26.0	-23.2	-23.3	NA	NA
20:5n-3	5.5	5.3	5.3	7.4	7.2	8.8	0.2	1.5	-29.5	-28.7	-27.5	-25.4	-21.2	-22.8	NA	NA
22:6n-3	7.9	7.1	4.3	5.8	2.7	3.4	0.3	0.7	-25.6	-27.5	-34.9	-25.1	-21.7	-24.4	NA	NA

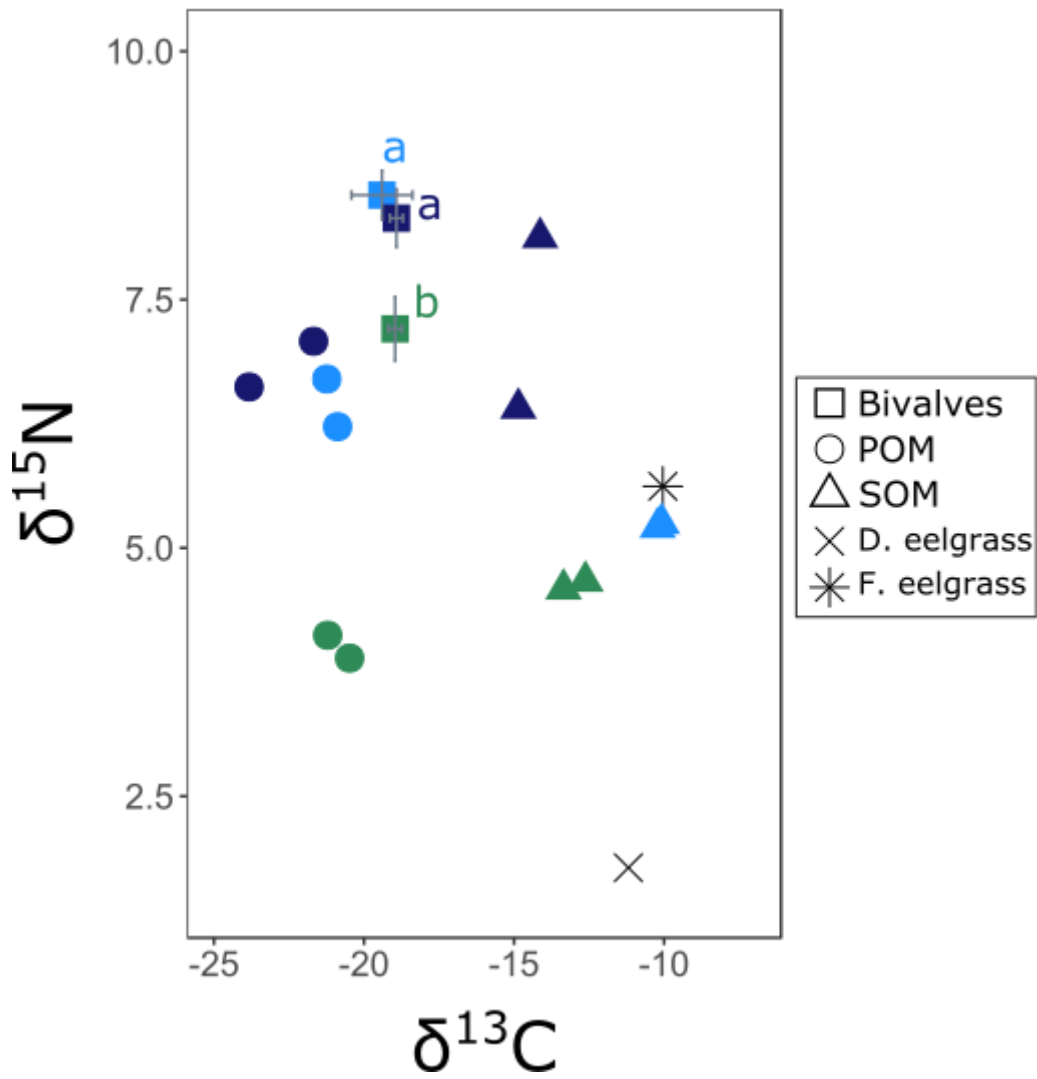
1 **Fig. 1**



2

3

4 **Fig. 2**

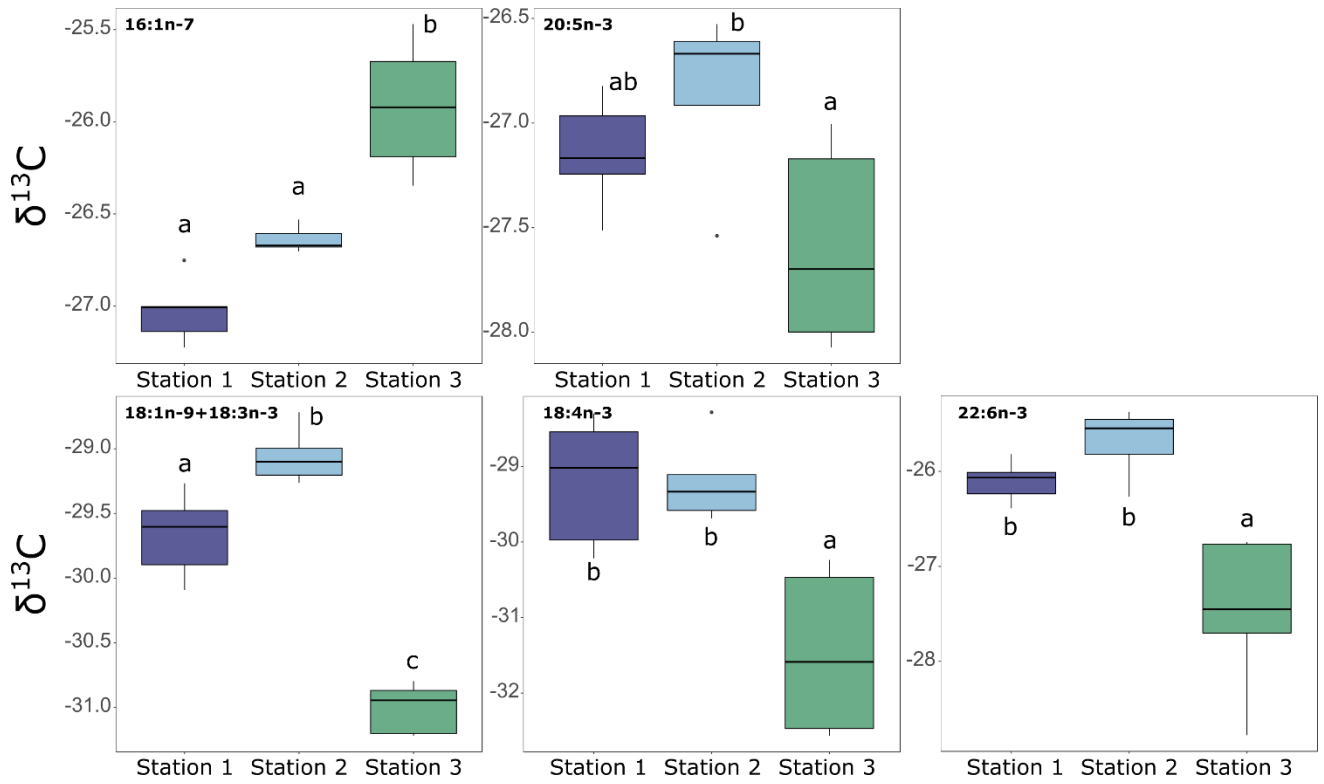


5

6

7

8 **Fig. 3**



9