1 **A wolf in sheep's clothing: carnivory in dominant sea urchins**

2 **in the Mediterranean**

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13 **Running head:** Trophic level of Mediterranean sea urchins

14   ABSTRACT: *Arbacia lixula* and *Paracentrotus lividus* are the dominant sea urchins in

15   the Mediterranean sublittoral, where they are key structuring species due to their

16   grazing activity. It has been commonly accepted that competition between both species

17   is minimized by specializing in different algal foods: *A. lixula* is considered to feed

18   mainly on encrusting coralline algae, while *P. lividus* prefers fleshy macroalgae. We

19   used stable isotope analysis to test if these species occupy different trophic positions at

20   three western Mediterranean and one Macaronesian locations. Our results

21   unambiguously show that *A. lixula* always occupies a higher trophic level than *P.*

22   *lividus*, with a δ^{15}N comparable in some locations to strict carnivores such as *Actinia*

23   *schmidti* or *Marthasterias glacialis*. A temporal monitoring at one locality showed that

24   this signature of a higher trophic level is consistent throughout the year. These results

25   are incompatible with the current belief of an herbivorous diet for *A. lixula* and suggest

26   that it must be considered an omnivore tending to carnivory in Mediterranean

27   ecosystems, feeding at least partially on sessile animals such as Cirripedia, Hydrozoa or

28   Bryozoa. A parallel analysis of gut contents showed a predominance of vegetal items in

29   both species, although *A. lixula* consistently had a higher abundance of animal

30   components than *P. lividus*. Our results challenge the validity of using gut content

31   observations alone for characterizing the trophic behaviour of omnivorous marine

32   invertebrates that feed on a variety of food sources with different digestibility.

33   KEY-WORDS: *Arbacia lixula* · *Paracentrotus lividus* · trophic relationships · benthic

34   community · stable isotope analysis.

INTRODUCTION

The edible common sea urchin *Paracentrotus lividus* (Lamarck, 1816) and the black sea urchin *Arbacia lixula* (Linnaeus, 1758) are the two dominant echinoid species in shallow rocky bottoms in the Mediterranean, where they coexist (Palacín et al. 1998b, Benedetti-Cecchi et al. 1998). Their grazing activity is commonly considered to greatly influence benthic communities (Sala et al. 1998, Palacín et al. 1998a, Bulleri et al. 1999). Their coexistence has raised questions regarding how these two abundant species interact and, specifically, whether and how they partition resources (Bulleri et al. 1999, Chiantore et al. 2008, Privitera et al. 2008). The currently prevalent view is that they are competitors for algal foods, although such putative competition seems alleviated by a selective preference of *P. lividus* for erect seaweeds, while *A. lixula* tends to feed more on encrusting coralline algae (Kempf 1962, Régis 1978, Verlaque & Nedelec 1983, Frantzis et al. 1988, Bulleri et al. 1999, Boudouresque & Verlaque 2001, Privitera et al. 2008).

This herbivorous behaviour described in *A. lixula* is, however, in sharp contrast with other species in the genus *Arbacia,* where omnivorous or unambiguously carnivorous diets have been reported. North American *A. punctulata* feeds on sessile animals, sand dollars and other *Arbacia* individuals, besides some algae (Harvey 1956, Karlson 1978, Cobb & Lawrence 2005). The diet of South Atlantic *A. dufresnei* is mainly carnivorous (Penchaszadeh 1979, Penchaszadeh & Lawrence 1999). The Pacific *A. spatuligera* showed preference for animal food over common species of algae from its habitat (Silva et al. 2004). Moreover, some observations indicate omnivorous or carnivorous behaviour of *A. lixula* outside the Mediterranean (Marques 1984, Cabral de Oliveira 1991, Tavares & Borzone 2005).

59   The crucial importance of sea urchins in shaping benthic ecosystems (Lawrence

60   1975) has been demonstrated by many ecological experiments along the Mediterranean

61   coasts (e.g. Benedetti-Cecchi & Cinelli 1995, Sala & Zabala 1996, Benedetti-Cecchi et

62   al. 1998, Palacín et al. 1998a, Bulleri et al. 1999, Guidetti et al. 2004, Bonaviri et al.

63   2011). The underlying premise in these experiments is that sea urchins are

64   predominantly herbivorous and that their effects are mainly due to their grazing on

65   benthic algae. In particular, population outbreaks of both *A. lixula* and *P. lividus* are

66   able to create barrens in rocky substrates (Verlaque 1987, Hereu 2004), affecting both

67   productivity and diversity of benthic assemblages (Bulleri et al. 2002, Privitera et al.

68   2008). The feeding behaviour and the herbivorous nature of *P. lividus* have been

69   repeatedly assessed; however, much less information is available about the ecological

70   role played by *A. lixula* in Mediterranean ecosystems. In fact, Privitera et al. (2008)

71   demonstrated that both species occupy different trophic niches in resource-limited

72   (barren) areas, again in the sense that *A. lixula* fed mainly on encrusting corallines while

73   *P. lividus* fed on non-encrusting macrophytes. A knowledge gap about the effective diet

74   of *A. lixula,* essential for designing and interpreting ecological studies, still persists.

75   Filling this gap seems necessary not only for basic research, but also for management

76   purposes (e.g. marine reserves or local fisheries).

77   We used a combination of stable isotope analysis and gut content examination for

78   assessing the diet and establishing the trophic position of *A. lixula* and *P. lividus*

79   coexisting in western Mediterranean rocky bottoms.

80

81   **MATERIALS AND METHODS**

82   **Study sites and sampling procedures.** Gut contents and isotopic signatures of

83   both sea urchin species were explored both temporally, performing a year-round follow-

84   up at a single site, and spatially, sampling at two additional western Mediterranean sites

85   at a single time point. We sampled also a non-Mediterranean site for reference

86   information. This design aimed at establishing the robustness of the patterns found.

87   The temporal sampling was performed at Tossa de Mar (NE Spain, 41º 43.2' N, 2º

88   56.4' E, Fig. 1) from December 2008 to December 2009. This location is fully described

89   elsewhere (Ballesteros 1988, 1989, 1992, 1993) and is characterized by gently sloping

90   rocks extending from the surface to 12 m depth, which show a rich algal cover, being

91   almost devoid of barren zones. We sampled between 2 and 6 m depth, where the

92   dominant communities are the *Corallina elongata* community (Ballesteros 1988) at

93   zones with high hydrodynamism and the *Stypocaulon scoparium* community

94   (Ballesteros 1993) at zones with a moderate to low hydrodynamism. Sea urchin

95   densities during the sampling period were 0.6 ± 0.8 and 5.7 ± 4.7 adult individuals

96   ($\pm$SD) m^{-2}, for *A. lixula* and *P. lividus* respectively, measured at a depth of 3 m

97   following the transect method as in Turon et al. (1995).

98   Ten *A. lixula* and ten *P. lividus* individuals were collected bimonthly by scuba

99   diving. Only adults with test diameter >35 mm in *A. lixula* and >40 mm in *P. lividus*

100   were sampled. The bimonthly sampling periodicity seems adequate to detect possible

101   diet shifts (Tieszen et al. 1983, Hobson & Clark 1992). Samples of the dominant taxa

102   from the three macroalgal divisions (*Stypocaulon scoparium, Dictyota dichotoma* and

103   *Padina pavonica:* Phaeophyta; *Codium vermilara* and *Flabellia petiolata*: Chorophyta

104   and *Corallina elongata, Sphaerococcus coronopifolius, Peyssonnelia* sp. and

105   *Lithophyllum incrustans*: Rhodophyta) were collected at the same times. In addition,

106   other invertebrates were also sampled throughout the year, including herbivores (*Patella*

107   sp., *Amphitoe* sp.), detritivores (*Ophiothrix fragilis, Echinaster sepositus*), suspension

108   feeders (*Balanus* spp.) and carnivores (*Actinia schmidti, Marthasterias glacialis,*

109   *Ophioderma longicauda*), in order to characterize the different levels of the local

110   trophic web. All samples were frozen (-20 ºC) shortly after collection for later analysis.

111   Additional sampling was carried out at two different locations (distant ca. 200 and

112   900 km from the previous one) in December 2009, in order to examine the consistency

113   of the results. Although densities were not quantified, both sea urchin species were

114   present at these localities (again with dominance of *P. lividus*) with largely overlapping

115   depth distributions. These sampling points were Torredembarra, (NE Spain, 41º 7.9' N,

116   1º 23.7' E) and Carboneras (SE Spain, 36º 59.6' N, 1º 53.4' W) (Fig. 1). The location at

117   Torredembarra is characterized by a shallow rocky habitat (0 - 3 m), surrounded by a

118   sandy bottom. The macroalgal assemblages are poorly developed, and the main primary

119   producer is *Jania rubens,* with scarce presence of other algae such as *Corallina*

120   *elongata* or *Dictyota dichotoma*. The Carboneras site is a shallow rocky habitat (0 - 4

121   m) with a denser algal cover, where the dominant producers were *Jania rubens*,

122   *Stypocaulon scoparium* and *Peyssonnelia* sp., with a well-developed *Posidonia*

123   *oceanica* meadow located nearby. At these two sites, samples were obtained only of the

124   two echinoids and of representative algal species, following the same procedures as

125   above. Thus, three communities with quite different characteristics were sampled in this

126   study, accounting for some of the diversity of Mediterranean shallow habitats were *A.*

127   *lixula* and *P. lividus* can coexist.

128   Finally, samples of sea urchins (of the same sizes detailed above) for stable

129   isotope analysis were collected in November 2009 at one Atlantic site: Caleta (Arico,

130   SE Tenerife, Canary Islands, 28º 6.1' N, 16º 28.7' W, Fig. 1), between 0 and 3 m depth.

131   In this location rock boulders dominate at shallow depths, with a poorly developed algal

132   community including sparse patches of *Caulerpa webbiana* and *Lobophora variegata.*

133   While *A. lixula* and *P. lividus* are known to broadly share spatial niches at the Canary

134   Islands (Tuya et al. 2007), in this locality, however, the former was only found in

135   vertical walls, while the second was located under the stones at the bottom.

136   **Stable isotope analyses.** Muscles of the Aristotle's lantern of all collected sea

137   urchins were used to perform isotopic analyses, and some of the same individuals were

138   used for gut analyses (see below). Algae were sampled by slicing several pieces of

139   different parts of the thalli after carefully scraping epibionts off their surface. For faunal

140   specimens, we sliced a small portion of a specified part of the body: the foot for *Patella*,

141   an arm for Ophiuroidea and Asteroidea and the body column for *Actinia*, while the

142   whole body of amphipods and cirripeds (excluding the shell) was used.

143   Before isotopic analyses, samples were rinsed in distilled water, freeze-dried and

144   ground to a fine powder. Isotopically lighter lipids may influence carbon isotope ratios

145   in animal tissues (Attwood & Peterson 1989, Hobson & Welch 1992), so five samples

146   of each species were reanalysed after lipid removal by chloroform-methanol 2:1

147   extraction (Folch et al. 1957). Passing & Bablok (1983) regression did not show any

148   significant differences in the $\delta^{13}C$ for any species (data not shown), probably due to low

149   lipid content in the sampled tissues. Thus, values of untreated samples were used

150   thereinafter. Carbonate rich samples (Corallinaceae, *Padina pavonica*, Ophiuroidea,

151   Asteroidea) were rinsed several times with HCl 0.1 M to remove inorganic carbonates

152   (Tomas et al. 2006). As HCl treatment has been reported to alter the $\delta^{15}N$ values (Bunn

153   et al. 1995), samples including calcareous elements were split into two subsamples, one

154   analysed after acid treatment for $\delta^{13}C$ and the other, untreated, for $\delta^{15}N$.

155   Aliquots of 0.3 or 1 mg of dried powder from faunal or algal samples,

156   respectively, were placed into tin capsules and crimped for combustion. Samples were

157   oxidised in a Flash EA1112 furnace coupled to a Delta C stable isotope mass

158   spectrometer through a Conflo III interface (Thermo-Finnigan). Isotope ratios are

159   expressed as δ values in parts per thousand (‰) according to the equation: $\delta X =$

160    $[(R_{sample}/R_{standard}) - 1] \times 1000$, where X (‰) is ^{13}C or ^{15}N, and R is the ratio of

161   corresponding element ($^{13}C/^{12}C$ or $^{15}N/^{14}N$), in sample or standard. The standard values

162   were Pee Dee Belemnite for ^{13}C and atmospheric nitrogen for ^{15}N. IAEA standards were

163   inserted every 12 samples for calibration. Replicate assays of standards indicated

164   measurement errors of ± 0.1 ‰ and ± 0.2 ‰ for carbon and nitrogen, respectively.

165   Trophic levels were calculated according to the equation of Hobson & Welch

166   (1992): $TL = 1 + (N_m - N_b) / TE$. Where TL is the trophic level of the species, N_m is the

167   mean $\delta^{15}N$ value of the species, N_b is the average basis $\delta^{15}N$ value of producers

168   (baseline) and TE is the trophic enrichment factor in the ecosystem. A constant TE

169   factor of 3 ‰, commonly accepted for aquatic benthic ecosystems involving

170   invertebrates (Vander Zanden & Rasmussen 2001, Jaschinski et al. 2008, Wan et al.

171   2010), was used. The baseline for $\delta^{15}N$ was estimated averaging the values obtained for

172   the different algal species analysed, except in the Atlantic site, where we did not collect

173   algae. In this case, we assigned *P. lividus* a value of TL = 2 (strict herbivore) and used it

174   as a baseline for calculating the TL of *A. lixula*.

175   **Gut content analyses.** The gut contents of sea urchins of both species collected at

176   Tossa de Mar in June and December, or at the other two Mediterranean locations in

177   December, were analyzed. Sea urchins (from 5 to 10 individuals per species and month

178   or locality) were dissected and the total gut contents of each specimen were examined

179   under a binocular microscope after disaggregation of the pellets. Some small calcareous

180   remnants were collected and examined under a scanning electron microscope. Algal

181   fragments were identified to genus level, while faunal items were classified into the

182   following taxonomic groups: Foraminifera, Porifera, Hydrozoa, Polychaeta,

183   Gastropoda, Bivalvia, Bryozoa, Cirripedia, Ostracoda, Copepoda, Amphipoda/Isopoda,

184   Decapoda and Other. Echinoid fragments, which were present in the gut of some

185   specimens, were not included in the analysis, since we cannot assure that they were not

186   an artefact resulting from sample manipulation.

187   The frequency of occurrence of each food item in a species (FO_i) was calculated

188   as the fraction of individuals having ingested this item (Pillay 1952, Hyslop 1980). The

189   volumetric occupation of ingested items was assessed by quantifying 25 squares of a

190   Petri dish with a 5 mm-grid. The surface occupied by the items present in each square

191   was semiquantitatively estimated using a scale from 1 to 5, and the occupation indices

192   of all items were calculated for every sea urchin individual, dividing the sum of the

193   semiquantitative scores assigned to a given food item by the total sum of the scores for

194   all measured squares. The volumetric index of each food item in a species (V_i) was then

195   obtained as the mean value of all individuals. A feeding index (FI_i) reflecting the

196   relative importance of each food item in the diet of each species at a given location, was

197   calculated following Lauzane (1975), as $FI_i = FO_i \times V_i$ and then standardized as a

198   percentage of the sum of the feeding indices for all items.

199   The relative contribution of animal and vegetal matter in the gut contents was

200   quantified by addition of the standardized feeding indices of all items of either animal or

201   vegetal origin (cumulative feeding indices). These indices summarize the carnivorous or

202   herbivorous character of the diet, as inferred from gut contents.

203   **Statistical analyses.** Two-way ANOVA with species as fixed factor and month as

204   random factor was performed to assess temporal variation of isotopic signatures of both

205   sea urchin species at Tossa de Mar throughout the year. Two-way ANOVA with species

206   as fixed factor and locality as random factor was used to compare the signatures and the

207   calculated trophic levels of both sea urchin species at the sampled localities. We also

208   used two-way ANOVA with species (fixed) and locality (random) as factors to formally

209   analyse differences in the cumulative animal feeding indices. As in mixed models the

210   expected mean square for the fixed factor (species in our case) includes the variance

211   component for the interaction term, the fixed factor tests for the effect of species over

212   and above the variation due to the interaction and the residual. It is therefore

213   interpretable even in the presence of significant interaction (Quinn & Keough 2002).

214   Notwithstanding, when interaction was significant we performed separate *t*-tests with

215   Bonferroni correction (unbalanced data prevented us from using other multiple

216   comparison tests) at each locality to check that the effect was coherent across sites.

217   The assumptions of normality and homoscedasticity of the variables were checked

218   with the Kolmogorov-Smirnov and Cochran tests, respectively. In some cases the data

219   did not comply with these assumptions, and rank transformed data were used instead

220   (detailed in Results). In two instances this transformation did not solve the lack of

221   homoscedasticity (detailed in Results), but we performed the analysis anyway as the

222   rank transformation is robust to deviations from assumptions of parametric procedures

223   (Conover & Iman 1981, Potvin & Roff 1993). All analyses were performed with

224   STATISTICA 6.1 software.

225

226   **RESULTS**

227   **Stable isotope analyses.** At Tossa de Mar, the annual average of δ^{15}N values found for

228   *A. lixula* (8.2 ‰) was comparable to those of typical carnivores such as *Actinia schmidti*

229   or *Marthasterias glacialis* (Table 1, Fig. 2). In contrast, herbivorous grazers and

230   detritivores had lower δ^{15}N values comprised between 4.6 ‰ for the amphipod

231   *Amphitoe* sp. and 5.3 ‰ for *Echinaster sepositus*, while *P. lividus* showed a slightly

232   higher value of 5.9 ‰, possibly indicating a higher intake of animal items than its more

233   strictly herbivorous counterparts. Seaweeds, as expected, showed lower δ^{15}N values,

234 ranging from 1.9 ‰ for *Flabellia petiolata* to 4.2 ‰ for *Sphaerococcus coronopifolius*,

235 while the most abundant species were within the range of 3 to 3.5 ‰. The mean value

236 for all algae, used as the baseline for calculating consumers' trophic levels at Tossa de

237 Mar, was 3.13 ‰.

238 When analysed on a temporal basis (Fig. 3), δ^{15}N values in *A. lixula* were

239 significantly higher than in *P. lividus* (species factor, $p < 0.001$, Table 2), while time

240 and the interaction were not significant (Table 2), indicating that the difference in

241 trophic levels is not subject to temporal variation. The mean difference was 2.3 ‰.

242 Likewise, δ^{13}C values showed a high degree of individual variability (Fig. 3), but

243 overall they were also significantly higher (by ca. 0.7 ‰) for *A. lixula* than for *P. lividus*

244 (species factor, $p < 0.001$, Table 2, Fig. 3), suggesting again a higher trophic level for *A.*

245 *lixula* (the trophic enrichment factor for carbon in marine coastal trophic webs is ca. 0.8

246 ‰ according to France & Peters 1997). No clear temporal trend was apparent for δ^{13}C

247 values (time and interaction not significant, Table 2).

248 In the two additional Mediterranean locations, as well as in the Atlantic one, δ^{15}N

249 values obtained from *A. lixula*'s muscle exceeded those from *P. lividus*, as did the

250 estimated trophic levels (Table 3). This suggests that the tendency to a more

251 omnivorous/carnivorous diet of *A. lixula* is probably widespread through all its

252 distribution range. A two-way ANOVA of δ^{15}N values revealed a significant effect of

253 species (fixed) and locality (random), as well as a significant interaction term (Table 4),

254 suggesting different adaptations of sea urchin species in different conditions (see

255 below). Separate analyses (*t*-tests with Bonferroni correction) at fixed levels of the

256 locality factor revealed that the differences in δ^{15}N values between sea urchin species

257 were significant (all $p < 0.01$) at all sites. Likewise, the analysis of trophic level

258 between species and localities revealed a significant effect of species and a significant

259   interaction between species and locality (Table 4); the inter-specific differences being

260   again significant in all localities (*t*-tests with Bonferroni correction, all $p < 0.01$). The

261   significant interaction term is probably due to the plasticity that can be observed in the

262   derived trophic levels of both species among the different localities and ecosystems.

263   Thus, where algal cover was dense, as happened in Tossa de Mar and Carboneras, *P.*

264   *lividus* showed a trophic level of around 2, compatible with a mainly herbivorous diet,

265   whereas *A. lixula* showed trophic levels of around 2.7, corresponding to a

266   predominantly carnivorous omnivore. On the contrary, where algal resources were

267   scarce (as in Torredembarra) both sea urchins tended to increase their animal intake,

268   rising their $\delta^{15}N$ values and trophic levels. Our results showed a trophic level of 2.7 for

269   *P. lividus* at Torredembarra, whereas *A. lixula* had a level of 3.0 (which would

270   correspond to a strict carnivore) in this location. In Tenerife, as algal samples were not

271   available for isotopic analysis, a baseline value for producers cannot be used, but the

272   difference between $\delta^{15}N$ values of both sea urchins was the biggest of all locations

273   sampled, and corresponded exactly to one trophic level.

274   The $\delta^{13}C$ signatures at the additional localities revealed, as in Tossa de Mar, a

275   higher enrichment in *A. lixula* (Table 3). For this variable, no significant interaction

276   between species and locality was found, while the main factors were highly significant

277   (Table 4), highlighting the higher $\delta^{13}C$ value in *A. lixula* as well as a noticeable spatial

278   heterogeneity in isotopic signature. The increase in $\delta^{13}C$ in *A. lixula* relative to *P.*

279   *lividus* in Torredembarra was similar to that in Tossa, but it was much higher at the

280   other two localities (Carboneras and Tenerife, Table 3), suggesting a different carbon

281   source for both sea urchins in these localities.

282   **Gut content analyses.** Gut content analyses in Tossa de Mar (Table 5) revealed a

283   higher abundance of animal items in *A. lixula* than in *P. lividus*. In addition, the ingested

284   material of *P. lividus* showed remarkable temporal differences. Thus, *Dictyota* and

285   *Dasycladus*, the most frequent algal items found in June, did not appear in the gut

286   contents of samples collected in December, when *Corallina* abundance increased. On

287   the contrary, the gut contents of *A. lixula* showed very scarce seasonal changes, being

288   dominated by small filamentous algae such as *Cladophora* and *Polysiphonia*, and

289   crushed fragments of encrusting corallines (*Lithophyllum*). Sessile animals such as

290   hydrozoans, cirripeds and polychaetes were also commonly found throughout the year.

291   These six items, with the addition of bryozoans in June, constituted the main

292   components of *A. lixula* gut contents, with little variation between seasons.

293   At the two other Mediterranean localities analysed, the results of the gut content

294   analysis confirmed the higher prevalence of animal items in the diet of *A. lixula* relative

295   to *P. lividus*, although with strong variability, probably associated to changes in benthic

296   algal cover. Thus, at the Torredembarra site, where algae were less abundant, some

297   animal items appeared frequently in *P. lividus* guts, such as the bryozoan *Schizoporella*

298   *errata*, which was common in this habitat. Conversely, in Carboneras, a location with a

299   well developed algal cover, the relative amount of animal remnants in the gut of both

300   sea urchins was the least of all localities sampled. Remarkably, cirripeds were absent of

301   this location, and *Jania* appeared as the main food source for both sea urchin species.

302   The cumulative feeding indices in the three localities showed that the diet of *A.*

303   *lixula* has a significantly higher animal component than that of *P. lividus* (Fig. 4, Table

304   6). The locality factor was also highly significant, reflecting the marked spatial

305   heterogeneity, but no significant interaction was detected. Whereas for *P. lividus* gut

306   contents were always dominated by the algal fraction, that of *A. lixula* displayed a much

307   higher variability in the relative contribution of animal and vegetal matter among the

308   different localities, ranging from a predominantly animal component in Torredembarra

309   to a dominance of vegetal diet in Carboneras (Fig. 4).

310

311   **DISCUSSION**

312   Our results show that *A. lixula* occupies a higher trophic level than *P. lividus*, as

313   shown by its $\delta^{15}N$, consistently higher across the sampled localities in the former than in

314   the latter. Their estimated trophic levels indicate that *A. lixula* is an omnivore tending to

315   carnivory, while *P. lividus* is a predominantly herbivore that can turn into an omnivore

316   in some instances. The (at least partial) carnivory in *A. lixula* is further supported by the

317   analyses of gut contents, which reveal a consistently higher proportion of animal food

318   items ingested in *A. lixula* as compared to *P. lividus*. However, gut content analysis

319   alone do not reveal the full extent of the trophic gap between the two species, since

320   vegetal components are the dominant ones in most situations analysed (except for *A.*

321   *lixula* in Torredembarra). Finally, the results for $\delta^{13}C$ are coherent with those of $\delta^{15}N$,

322   indicating an overall enrichment of the signature of *A. lixula* with respect to *P. lividus*.

323   The results for carbon, however, should be taken with caution as this isotope is best

324   suited to detect differences in sources of food rather than trophic levels (Cardona et al.

325   2007). This implies that the role of *A. lixula* in the shallow subtidal in the Mediterranean

326   should be, at least in part, re-evaluated. Specifically, the notion of a putative strong

327   competition for food should be carefully re-examined.

328   The suitability of stable isotope analysis, and specifically $\delta^{15}N$, for identifying

329   trophic levels in marine ecosystems has been clearly established (e.g. Cherel et al.

330   2008). Much closer to the scope of the present study, this tool has revealed differences

331   in the trophic levels of sympatric sea urchins (Vanderklift et al. 2006). These authors

332   found that two littoral Australian echinoids previously thought to be herbivorous

333   (*Phyllacanthus irregularis* and *Centrostephanus tenuispinus*) had actually an

334   omnivorous behaviour tending to carnivory. The differences in $\delta^{15}N$ between *A. lixula*

335   and *P. lividus* that we report here, based on a wide temporal and geographical scale, are

336   comparable to those found between both Australian purportedly herbivorous species

337   and *Heliocidaris erythrogramma*, which proved to be a true strict herbivore.

338   In previous studies, animal items had been reported in the gut contents of both

339   Mediterranean sea urchins (Maggiore et al. 1987, Privitera et al. 2008, Chiantore et al.

340   2008), but were mostly disregarded as anecdotal or accidental captures, which may be

341   true for *P. lividus* but certainly not for *A. lixula*. The long-held misconception about the

342   herbivory of *A. lixula* may stem from several causes, but mainly from the fact that most

343   primary information on this issue came from studies of gut contents, which target

344   ingested, rather than assimilated, food. While it is true that the ecological impact of an

345   organism (in this case, *A. lixula*) feeding activity may depend mostly on what is

346   ingested, rather than on what is assimilated, gut content analysis can introduce some

347   biases on our perception of an animal diet if used alone. Gut content analyses cannot be

348   dismissed, though, as they provide the only direct taxonomical information about what

349   the sea urchins ingest and, in combination with stable isotope analyses, can shed light

350   on important aspects of their feeding strategy.

351   In addition, if diverse kinds of foodstuff have differential digestibility, results

352   can be biased towards less digestible material. It is remarkable in this sense that most

353   faunal items found in the gut of *P. lividus* are nearly intact and easily identifiable,

354   probably reflecting the little ability of this species for assimilating animal material. The

355   opposite is true for *A. lixula*, which seems to perform complete digestions of animal

356   tissues. Conversely, undigested filamentous algae, even the most delicate ones, are

357   regularly found intact in the guts of *A. lixula*. In a study on *A. lixula* from Brazil, Cabral

358   de Oliveira (1991) found that 50% of the algae present in its faecal pellets survived

359   digestion and were able to grow when cultured, in contrast to algae egested by

360   herbivorous sea urchins such as *Lytechinus variegatus* or *Echinometra lucunter*.

361   Another cause that can contribute to the misconception about *A. lixula* herbivory

362   is the fact that the gut contents of *A. lixula* that we examined consisted largely of small

363   crushed pieces of pinkish-greyish carbonates, which can be easily interpreted as

364   fragments of calcareous algae. However, using scanning electron microscopy, we have

365   unambiguously identified many of these pieces as fragments of shells of the common

366   western Mediterranean barnacle *Balanus perforatus*. Thus, we must consider the

367   possibility that cirriped shell remnants may have been mistaken for encrusting corallines

368   in some studies which were carried out under the undisputed paradigm of an

369   herbivorous *A. lixula*.

370   The finding that *A. lixula* is an omnivore tending to carnivory may shed light on

371   unexpected results of some ecological experiments. For example, the removal of *P.*

372   *lividus* had no effect and did not trigger an increase of the population of *A. lixula*

373   (Gianguzza et al. 2006), as would be expected if inter-specific competition occurred

374   between both species. Artificially reducing or increasing the density of *A. lixula* in

375   selected patches had no effect on the percent cover of encrusting corallines (Benedetti-

376   Cecchi et al. 1998, Bulleri et al. 1999), but the removal of *A. lixula* produced an

377   increase in the density of *B. perforatus* and a decrease in the density of limpets (Bulleri

378   et al. 1999), opposite to what would be expected if *A. lixula* was an herbivorous

379   consumer of filamentous algae and trophic competitor of *Patella* sp.

380   Finally, our results add some information about the putative competition between

381   the two main Mediterranean sea urchins. Densities of *P. lividus* in the NW

382   Mediterranean are usually higher (an order of magnitude on average, Palacín et al.

383   1998b) than those of *A. lixula*. This fact challenges the idea that both species engage in

384   strong competitive interactions or, at least, it suggests that *P. lividus* is able to

385   outcompete *A. lixula*, whose shift to a different diet may help to avoid exclusion..

386   However, both species can locally coexist at high densities (Guidetti et al. 2004, Tuya et

387   al. 2007), and *A. lixula* can be the dominant sea urchin in some communities (Benedetti-

388   Cecchi et al. 1998). Furthermore, both species segregate spatially in some cases (Kempf

389   1962, Chelazzi et al. 1997, Bulleri et al. 1999), as happens in our Atlantic location,

390   where *A. lixula* is restricted to vertical walls. Thus, interference competition between

391   these species is likely to happen in many places. Agonistic interactions (Shulman 1990)

392   have never been observed between them, so exploitative competition seems more likely,

393   and feeding flexibility can be an important mechanism to alleviate its effects. On the

394   other hand, it has to be emphasized that factors other than direct trophic competition,

395   such as resistance to hydrodynamism (Tuya et al. 2007), resistance to predation

396   (Guidetti 2006) or presence of predators which could modulate sea urchin behaviour

397   (Freeman 2006) could also be involved in shaping the distribution and abundance of

398   these two sea urchin species.

399   Few studies have addressed the possibly different foraging behaviour of these sea

400   urchins species. Apparently, *A. lixula* shows a higher mobility than *P. lividus* in barren

401   zones (Bonaviri et al. 2011), so that a wider area can be impacted by its grazing activity.

402   The strong Aristotle's lantern that allows *A. lixula* to scrape the substrate for searching

403   its prey, and the fact that this species tends to be more abundant than *P. lividus* in barren

404   zones which offer relatively few algal food in comparison to animal prey (Guidetti &

405   Dulcic 2007), could be better explained in the light of its tendency to carnivory.

406   Studies comparing feeding strategies between these species in communities with

407   dominance of *A. lixula* are necessary to ascertain the trophic position of both species

408 under competitive pressures different from those found in the present study.

409 Interestingly, at high densities, *A. lixula* may be expected to limit the abundance of its

410 prey populations, and so it can compete with carnivores such as asteroids. Thus, the

411 omnivory and feeding plasticity of *A. lixula* adds complexity to models of community

412 structure, including possible trophic loops and increased connectivity between species

413 (Camus et al. 2008).

414   In conclusion, the finding that *A. lixula* is an omnivore tending to carnivory has

415 important implications for the dynamics of shallow water communities in the

416 Mediterranean, as it suggests not only a reduced competition for food with the

417 coexisting echinoid *P. lividus*, but also opens new views to understand biotic

418 interactions in these communities. Given the important functional role of these echinoid

419 species in shaping sublittoral assemblages, and the fact that one of them (*P. lividus*)

420 sustains heavy fisheries in some areas, the results presented here should be taken into

421 consideration both in basic studies of ecosystem functioning and in applied issues of

422 environmental and fisheries management.

423

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612 Table 1. Mean isotopic signatures (SD in parentheses) and derived trophic levels (TL=1

613 for primary producers) of animal species at the site where sampling was performed

614 bimonthly (Tossa de Mar). n: number of individuals analysed.

	n	Trophic Level	δ¹⁵N ‰	δ¹³C ‰
Actinia schmidti	5	2.7	8.3 (0.6)	-19.8 (0.7)
Arbacia lixula	**72**	**2.7**	**8.2 (0.5)**	**-18.3 (1.1)**
Ophioderma longicauda	5	2.5	7.6 (0.9)	-17.5 (0.9)
Marthasterias glacialis	5	2.4	7.5 (0.4)	-15.8 (1.0)
Balanus spp.	5	2.2	6.8 (0.3)	-19.4 (0.3)
Paracentrotus lividus	**71**	**1.9**	**5.9 (0.4)**	**-19.0 (0.9)**
Echinaster sepositus	5	1.7	5.3 (0.4)	-15.6 (0.3)
Ophiothrix fragilis	4	1.7	5.2 (0.7)	-20.4 (1.0)
Patella sp.	5	1.6	5.1 (0.4)	-17.0 (0.6)
Amphitoe sp.	4	1.5	4.6 (0.7)	-22.1 (1.2)

615   Table 2. Summary of factorial 2-way ANOVA for assessing significant differences in

616   the isotopic signatures at Tossa de Mar between species (*A. lixula* and *P. lividus*, fixed

617   factor) and sampling times (random factor).

Variable	Effect	Df	MS	*F*	*p*-level
$\delta^{15}N$	Species (S)	1	187.663	493.66	**<0.001**
	Time (T)	6	0.632	1.66	0.28
	S x T	6	0.381	2.00	0.07
	Error	129	0.190		
$\delta^{13}C$ [a]	Species (S)	1	30481.07	15.82	**<0.001**
	Time (T)	6	6005.13	3.11	0.10
	S x T	6	1929.10	1.50	0.18
	Error	129	1282.21		

[a] Rank-transformed. No homogeneity of variance achieved.

618 Table 3. Calculated trophic levels and ^{15}N signatures for *A. lixula*, *P. lividus* and the

619 mean value for seaweeds (baseline) at the four sampled locations (SD in parentheses).

620 ^{13}C signatures are also shown for both sea urchin species. N/A: Data not available.

	n	Trophic level	δ^{15}N ‰	δ^{13}C ‰
Tossa				
A. lixula	72	2.7 (0.17)	8.2 (0.5)	-18.3 (1.1)
P. lividus	71	1.9 (0.13)	5.9 (0.4)	-19.0 (0.9)
Seaweeds average	37	1.0	3.1 (0.7)	
Torredembarra				
A. lixula	10	3.0 (0.17)	10.6 (0.5)	-17.2 (0.6)
P. lividus	9	2.7 (0.12)	9.6 (0.4)	-17.9 (0.5)
Seaweeds average	12	1.0	4.6 (0.7)	
Carboneras				
A. lixula	9	2.7 (0.16)	10.4 (0.5)	-11.8 (0.6)
P. lividus	10	2.1 (0.19)	8.5 (0.6)	-16.7 (0.7)
Seaweeds average	18	1.0	5.3 (0.5)	
Tenerife				
A. lixula	10	3.0 (0.12)	10.7 (0.4)	-11.2 (0.7)
P. lividus	7	2.0 (0.14)	7.5 (0.4)	-17.8 (0.4)
Seaweeds average		N/A	N/A	

621   Table 4. Summary of factorial 2-way ANOVA for assessing significant differences in

622   the isotopic signatures and calculated trophic levels between species (*A. lixula* and *P.*

623   *lividus*, fixed factor) at the four sampled locations (random factor).

Variable	Effect	Df	MS	*F*	*p*-level
$\delta^{15}N$	Species (S)	1	98.355	31.27	**0.01**
	Location (L)	3	87.491	22.04	**0.01**
	S x L	3	3.970	18.42	**<0.001**
	Error	190	0.216		
$\delta^{13}C$ [a]	Species (S)	1	35884.07	20.27	**0.007**
	Location (L)	3	84754.14	46.79	**0.005**
	S x L	3	1811.29	1.11	0.34
	Error	190	1624.53		
Trophic	Species (S)	1	10.928	31.268	**0.01**
Level	Location (L)	3	1.667	3.779	0.15
	S x L	3	0.441	18.418	**<0.001**
	Error	190	0.024		

[a] Rank-transformed. No homogeneity of variance achieved.

624 Table 5. Summary of standardized feeding indices (only the 12 highest values) for

625 major food items (SD in parentheses) as derived from gut content analysis of *A. lixula*

626 and *P. lividus* at three Mediterranean locations. Given the prominent seasonal changes

627 in the algal assemblages, feeding indices have been calculated separately for June and

628 December at the site where sampling was performed over time (Tossa de Mar). Animal

629 items are shown in bold.

Tossa de Mar - June

A. lixula n = 6		*P. lividus* n = 6	
Cladophora	19.4 (9.4)	*Dictyota*	40.0 (21.6)
Polysiphonia	19.1 (9.4)	*Dasycladus*	22.3 (10.0)
Lithophyllum	17.3 (8.3)	*Stypocaulon*	11.9 (9.8)
Bryozoa	8.1 (12.7)	*Polysiphonia*	7.1 (6.1)
Hydrozoa	7.6 (4.9)	*Ceramium*	5.3 (4.7)
Cirripedia	5.6 (6.2)	**Bryozoa**	3.0 (3.1)
Polychaeta	5.2 (2.4)	*Corallina*	3.0 (3.6)
Stypocaulon	4.5 (4.5)	*Cladophora*	1.7 (2.5)
Foraminifera	3.6 (2.6)	*Jania*	1.4 (1.9)
Dictyota	2.5 (3.3)	*Colpomenia*	1.3 (1.8)
Ostracoda	2.3 (2.7)	Other seaweed	1.2 (0.9)
Porifera	1.1 (1.1)	*Sphaerococcus*	0.8 (1.4)

Tossa de Mar - December

A. lixula n = 9		*P. lividus* n = 5	
Lithophyllum	19.6 (13.6)	*Corallina*	52.2 (7.1)
Polysiphonia	17.1 (10.6)	*Stypocaulon*	21.2 (5.3)
Hydrozoa	14.9 (10..9)	*Peyssonnelia*	9.6 (6.1)
Cirripedia	11.6 (6.1)	*Jania*	7.3 (5.4)
Cladophora	9.3 (7.9)	*Cladophora*	3.0 (2.2)
Polychaeta	8.3 (6.8)	*Polysiphonia*	1.7 (2.2)
Foraminifera	3.7 (1.4)	*Cystoseira*	1.3 (1.6)
Jania	3.2 (2.4)	**Porifera**	1.0 (1.0)
Corallina	2.8 (3.6)	**Polychaeta**	0.8 (0.8)
Porifera	2.0 (2.5)	*Lithophyllum*	0.8 (0.9)
Bryozoa	2.0 (2.0)	**Hydrozoa**	0.3 (0.3)
Stypocaulon	1.6 (3.8)	*Halimeda*	0.2 (0.4)

Torredembarra - December

A. lixula n = 5		*P. lividus* n = 5	
Cirripedia	55.5 (12.8)	*Jania*	64.9 (5.5)
Hydrozoa	23.3 (6.9)	*Corallina*	8.8 (3.0)
Polysiphonia	8.9 (4.7)	*Posidonia*	8.4 (3.9)
Porifera	8.5 (4.7)	**Bryozoa**	5.7 (4.3)
Cladophora	1.3 (1.9)	*Polysiphonia*	3.0 (3.0)
Bivalvia	0.8 (1.1)	*Codium.*	2.1 (1.4)
Ceramium	0.7 (0.6)	*Stypocaulon*	1.9 (1.5)
Stypocaulon	0.3 (0.4)	*Cladophora*	1.6 (1.2)
Gastropoda	0.2 (0.4)	**Hydrozoa**	1.6 (1.3)
Polychaeta	0.2 (0.4)	Other seaweed	0.6 (1.1)
Bryozoa	0.2 (0.3)	*Peyssonnelia*	0.6 (0.6)
Other metazoa	0.1 (0.2)	*Padina*	0.2 (0.3)

Carboneras - December

A. lixula n = 7		*P. lividus* n = 10	
Jania	34.1 (26.3)	*Jania*	59.9 (10.6)
Lithophyllum	21.2 (23.6)	*Posidonia*	11.6 (5.8)
Cladophora	17.2 (14.0)	*Peyssonnelia*	11.0 (11.0)
Porifera	8.3 (8.8)	*Cladophora*	5.0 (4.3)
Stypocaulon	5.8 (7.0)	*Stypocaulon*	4.1 (6.0)
Ceramium	4.0 (5.9)	*Udotea*	3.3 (2.2)
Polysiphonia	2.2 (3.4)	Other seaweed	2.6 (5.7)
Peyssonnelia	1.9 (3.2)	*Lithophyllum*	1.6 (2.4)
Other seaweed	1.6 (3.2)	*Padina*	0.7 (0.8)
Foraminifera	1.4 (1.9)	*Corallina*	0.1 (0.2)
Corallina	0.9 (2.0)	**Polychaeta**	0.1 (0.1)
Hydrozoa	0.5 (1.1)	**Porifera**	0.0 (0.1)

630 Table 6. Summary of factorial 2-way ANOVA to assess significant differences in the

631 cumulative animal feeding index (sum of standardized feeding indices of animal items),

632 between both sea urchin species (fixed factor) and location (random factor).

Variable	Effect	Df	MS	*F*	*p*-level
Cumulative animal feeding index [a]	Species (S)	1	2799.113	450.15	**0.002**
	Location (L)	2	917.827	149.62	**0.007**
	S x L	2	6.135	0.60	0.55
	Error	35	10.220		

[a] Rank-transformed.

633   Fig. 1. Sampling locations.
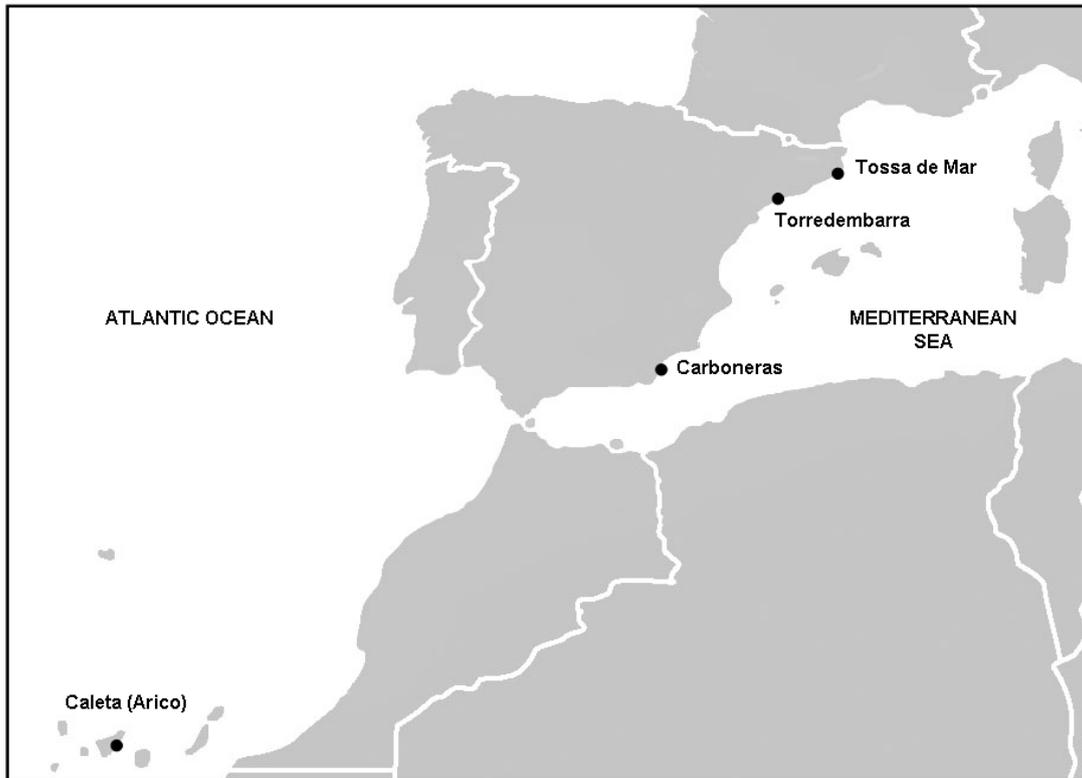
634   Fig. 2. Plot of δ^{15}N and δ^{13}C isotopic signatures (mean $\pm$ SD) of species examined

635   during the bimonthly sampling (Tossa de Mar). Producers are represented by open

636   squares, whereas consumers are represented by open circles. Both sea urchin species are

637   represented by solid circles.

638   Metazoa: Act: *Actinia schmidti*, ARB: *Arbacia lixula*, Olo: *Ophioderma longicauda*,

639   Mar: *Marthasterias glacialis*, Bal: *Balanus* spp., PAR: *Paracentrotus lividus*, Ech:

640   *Echinaster sepositus*, Ofr: *Ophiothrix fragilis*, Pat: *Patella* sp., Amp: *Amphitoe* sp.

641   Algae: 1: *Sphaerococcus coronopifolius*, 2: *Flabellia petiolata*, 3: *Stypocaulon*

642   *scoparius*, 4: *Corallina elongata*, 5: *Dictyota dichotoma*, 6: *Codium vermilara*, 7:

643   *Peyssonnelia* spp. 8: *Lithophyllum incrustans*, 9: *Padina pavonica*.
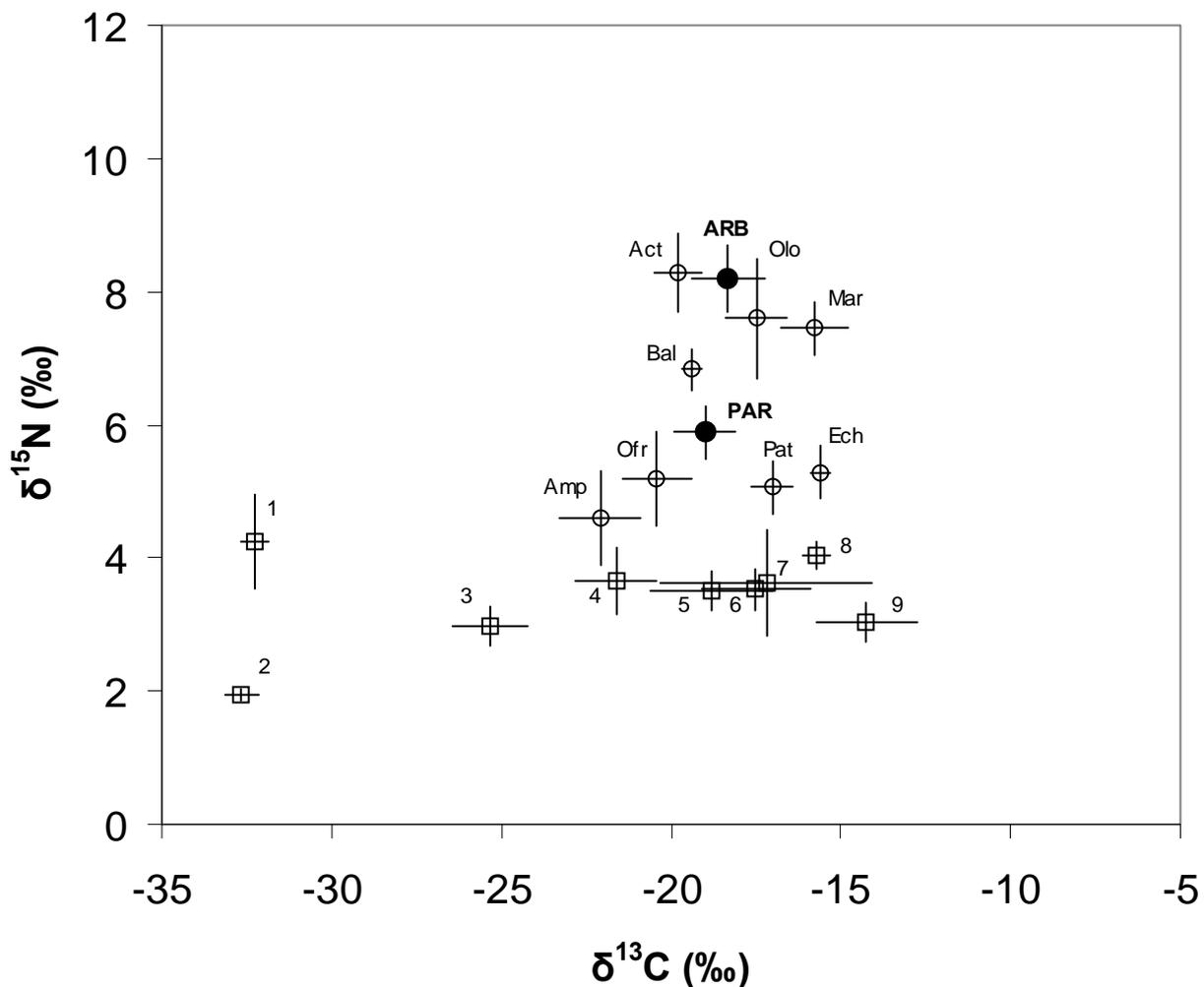
644 Fig. 3. Isotopic signatures of *A. lixula* and *P. lividus* at Tossa de Mar as a function of

645 time. Mean values ± SD are displayed for every sampled month. The number of

646 individuals analysed for each observation was from 8 to 12.
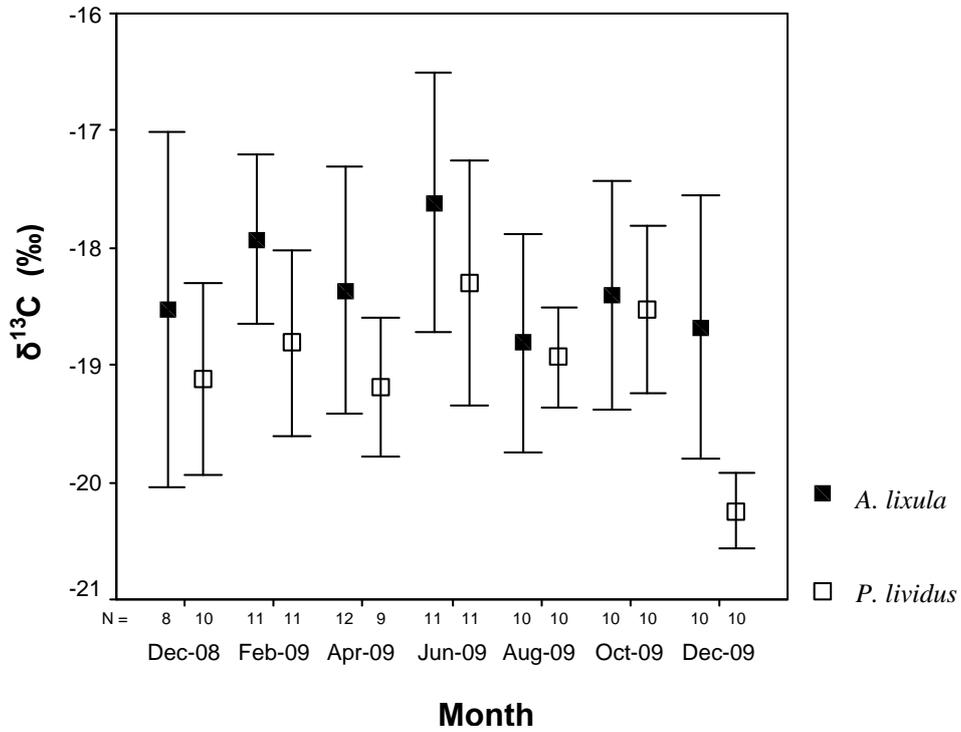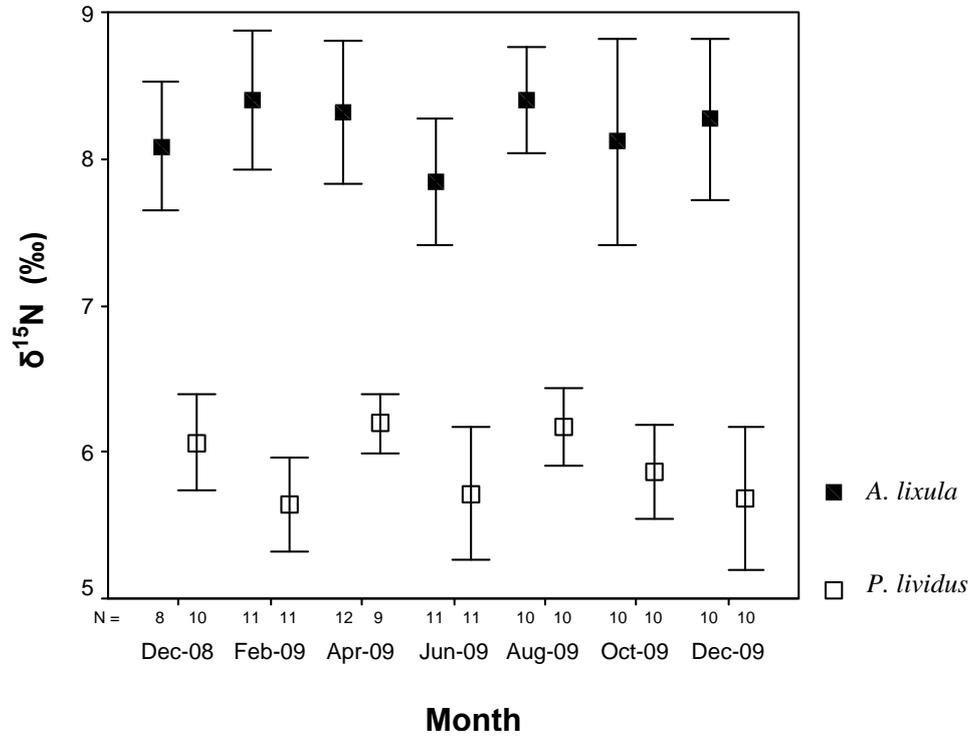
647   Fig. 4. Cumulative feeding indices (mean + SD) for animal and vegetal food items,

648   derived from gut content analyses of *A. lixula* and *P. lividus* collected in December at

649   three Mediterranean locations.