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1 **Determination of fish trophic levels in an estuarine system**

2

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15 **Abstract**

16

17           The concept of trophic level is particularly relevant in order to improve knowledge of  
18 the structure and the functioning of an ecosystem. A precise estimation of fish trophic levels  
19 based on nitrogen isotopic signatures in environments as complex and fluctuant as estuaries  
20 requires a good description of the pelagic and benthic trophic chains and a knowledge of  
21 organic matter sources at the bottom. In this study these points are considered in the case of  
22 the Gironde estuary (south west France, Europe). To obtain a good picture of the food web,  
23 fish stomach content analyses and a bibliographic synthesis of the prey feeding ecology were  
24 carried out. Fish trophic levels were calculated from these results and  $\delta^{15}\text{N}$  data. The feeding  
25 link investigation enabled us to identify qualitatively and quantitatively the different preys  
26 consumed by each fish group studied, to distinguish the prey feeding on benthos from those  
27 feeding on pelagos and to characterize the different nutritive pools at the base of the system.  
28 Among the species studied, only *Liza ramada* and the flatfish (*Platichthys flesus* and *Solea*  
29 *solea*) depend mainly on benthic trophic compartments. All the other fish groups depend on  
30 several trophic (benthic and/or pelagic) sources. These results enabled us to correct the  
31 calculation of fish trophic levels which are coherent with their feeding ecology data obtained  
32 from the nitrogen isotopic integrative period. The present work shows that trophic positions  
33 are linked with the feeding ecology of fish species and vary according to individual size.  
34 Ecological data also allow the correction of the isotopic data by eliminating absurd results and  
35 showing the complementarity of the two methods. This work is the first to consider source  
36 variability in the fish food web. This is an indispensable step for trophic studies in a dynamic  
37 environment. The investigation of matter fluxes and recycling processes at the food web base  
38 would provide an useful improvement in future estuarine food web research.

39 *Keywords:* stomach contents;  $\delta^{15}\text{N}$ ; predator-prey relationships; organic matter sources; fish

40 trophic levels; estuarine ecosystem

41 *Regional index terms:* Europe; France; Gironde estuary; Lat. 45°20'N; Long. 0°45'W

## 42 **1. Introduction**

43

44 In recent years, there has been increased interest in use of carbon and nitrogen stable  
45 isotopes to characterize the trophic web structure and the energy flows of aquatic ecosystems  
46 (e.g. Kwak and Zedler, 1997). For a living being, the carbon isotope ratio  $^{13}\text{C}/^{12}\text{C}$  provides an  
47 estimate of the origin of the assimilated organic matter (De Niro and Epstein, 1978; Fry and  
48 Sherr, 1984; Post, 2002) and the nitrogen carbon ratio  $^{15}\text{N}/^{14}\text{N}$  gives its trophic level (De Niro  
49 and Epstein, 1981; Minagawa and Wada, 1984; Hesslein et al., 1991; Wada et al., 1991).

50 For fish, stable isotope analysis represents a complementary approach to traditional  
51 feeding studies: stomach contents reflect the qualitative and quantitative ingestion of species  
52 at a given time whereas stable isotope analyses represent an integrative record of the food that  
53 has really been assimilated by the fish during a period prior to the sampling (e.g. Persson and  
54 Hansoon, 1999; Davenport and Bax, 2002; West et al., 2003; Winemiller et al., 2007). The  
55 isotopic integrative time varies from days to years according to the ecosystem, the species,  
56 their growth rate and the tissue considered (Tieszen et al., 1983; Hesslein et al., 1993;  
57 Guelinckx et al., 2007; Church et al., 2009; Suring and Wing, 2009). For example, Perga &  
58 Gerdeaux (2003) have estimated that the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of muscle tissue only reflected the  
59 food consumed during the spring and summer growth period.

60 The combined use of these two methods can provide a detailed picture of the structure  
61 of an estuarine fish food web by i) describing trophic relationships between different  
62 biological compartments (stomach contents analyses) and ii) estimating the trophic position of  
63 species one alongside the other (nitrogen stable isotope analyses) (e.g. West et al., 2003; Akin  
64 and Winemiller, 2008).

65 In the Gironde estuary,  $\delta^{15}\text{N}$  seemed to be a good marker to improve knowledge of the  
66 food web structure, even if anomalies in fish trophic positions have been identified (Pasquaud

67 et al., 2008). For example, *Platichthys flesus*, a predator of small benthic crustaceans has a  
68 higher average  $\delta^{15}\text{N}$  level than *Argyrosomus regius* which tends to be ichthyophagous. These  
69 observations can be explained by an enrichment of the  $\delta^{15}\text{N}$  signatures in benthic species  
70 compared to pelagic species for an equivalent trophic position (e.g. Sherwood and Rose,  
71 2005). Moreover, a precise estimation of the fish trophic positions requires knowledge of  
72 sources at the bottom of the food chains (Vander Zanden et al., 1997; Vander Zanden and  
73 Rasmussen, 1999; Sherwood and Rose, 2005; Pasquaud et al., 2008).

74 The aim of the present study was to characterize fish trophic levels precisely according  
75 to their size class from  $\delta^{15}\text{N}$  values, taking account of variability. Hence, the objectives were  
76 (1) to determine the food chains during the nitrogen isotopic integrative period in the Gironde  
77 estuary from fish stomach content analyses and from literature data for the prey; (2) to  
78 attribute to each fish species (or size class) studied a baseline  $\delta^{15}\text{N}$  value taking into account  
79 the proportion of benthic/pelagic prey in their feeding and the  $\delta^{15}\text{N}$  signatures of the nutritive  
80 pool identified, and (3) use this information to calculate fish/size class trophic levels.

81

## 82 **2. Materials and methods**

83

### 84 *2.1. Study area*

85

86 The Gironde estuary is located in South West France (45°20'N, 0°45'W; Fig. 1). It is  
87 12 km wide at the mouth and 76 km long between the ocean and the Bec d'Ambès, where the  
88 Garonne and Dordogne rivers meet and which also constitutes the upstream salinity limit. The  
89 watershed of this system represents an area of 81 000 km<sup>2</sup>. The hydrodynamic conditions are  
90 highly variable due to the interactions of marine and fluvial flows, leading to temperature and  
91 salinity gradients. There are also considerable seasonal variations. During the period under

92 consideration (April to September 2004), discharge values varied widely with a flood event in  
93 spring (up to 3,600 m.s<sup>-3</sup> in May) and an important drought period in summer (about 250 m.s<sup>-3</sup>  
94 from July to September; unpublished data). In consequence, salinity values varied and were  
95 especially low in June 2004. Temperatures stayed relatively high in summer, fluctuating  
96 between 13.1 and 24.7°C. The dynamic conditions involved high turbidity values reaching  
97 500 mg.L<sup>-1</sup> (Sottolichio and Castaing, 1999; David et al., 2007) and a restricted light  
98 penetration. In consequence, primary production was reduced and the food web seems based  
99 on the detritus pool (Lobry et al., 2008). In spite of these constraining conditions, the Gironde  
100 estuary shows a high biodiversity and seems to be a particularly well-preserved ecosystem.

101

## 102 2.2. *Sampling surveys*

103

104 Fish were sampled approximately bimonthly from April 2004 to September 2004,  
105 across ten stations located in the upstream area of the saline Gironde estuary (Fig. 1). An otter  
106 trawl (4 m opening and a cod-end with a mesh size of 8 mm) was used. Fish sampling was  
107 carried out during daylight hours at high tide and was restricted to 15-minute episodes in  
108 order to limit regurgitation or abnormal feeding. All the sampled fish were identified,  
109 counted, weighed, measured and finally frozen until laboratory analysis could be carried out.  
110 Some of the most abundant fish species or genus, separated in some cases into size classes,  
111 are presented here (Table 1). Only cohorts spending the totality of their spring and summer  
112 growth period (i.e. period considered in that study, see paragraph 2.3.) inside the system were  
113 selected.

114

## 115 2.3. *Fish stomach content analyses*

116

117 The fish stomach contents were analyzed in order to describe their trophic  
118 relationships during the nitrogen isotopic integrative time in the Gironde estuary (Table 1).  
119 Crossing literature information (Tieszen et al., 1983; Perga and Gerdeaux, 2005; Miller, 2006;  
120 Guelinckx et al., 2007), we have estimated that the  $\delta^{15}\text{N}$  of the muscle tissue (used in these  
121 isotopic analyses, see Pasquaud et al., 2008 for more details) reflected the food consumed  
122 during the warm growth period, i.e. from April to September. It is important to note that we  
123 only selected specimens belonging to the same cohorts during the period considered, i.e. we  
124 took account for the individual growth along the integrative time.

125 The different items were examined under binocular microscope and identified to the  
126 highest possible taxonomic separation. The volume of each prey was estimated using the  
127 point method described by Pillay (1952) and Hyslop (1980).

128 For *Liza ramada*, only the stomach contents collected in September 2004 were  
129 analyzed. Even if these data do not provide this species food variability over the isotopic  
130 integrative period, they were nevertheless considered to give data elements on its feeding  
131 ecology and thus to estimate the origin of the organic matter at the base of its trophic chain in  
132 the Gironde estuary.

133 Due to this species' specific diet, a particular methodology was used, adapted from  
134 Almeida et al. (1993) and Laffaille et al. (2002): a 200 mg sample was removed from the  
135 stomach and suspended in 5 ml of formaldehyde (5%). First, the samples were analyzed under  
136 binocular microscope to identify zooplanktonic organisms. Next, a known volume (0.03 ml)  
137 was pipetted onto a slide for microscopic observation ( $\times 500$ ). Items were identified using  
138 diatoms and phytoplankton determination keys (Cupp, 1943; Prygiel and Coste, 2000).  
139 Diatom volumes were estimated from literature data (Padisak and Adrian, 1999) and  
140 zooplankton volumes were calculated from their length. The volumetric percentage was then  
141 estimated for each item identified.

142

#### 143 2.4. Prey feeding ecology

144

145 A bibliographic review was carried out to identify general feeding ecology of benthic  
146 prey (molluscs, annelid polychaetes and crabs), hyperbenthic prey (amphipods, isopods and  
147 shrimps) and pelagic prey (mysids, copepods and diverse metazooplankton) in order to  
148 characterize the main source of organic matter on which they eat in the Gironde estuary. The  
149 fish feeding ecology was estimated from the stomach content analysis results obtained in the  
150 present study and a published work (Pasquaud et al., 2008).

151

#### 152 2.5. Available data

153

154 The  $\delta^{15}\text{N}$  signatures of fish species (or size classes) measured by Pasquaud et al.  
155 (2008) and of their prey (unpublished data) were used in this study (Table 1).

156 Since the benthic trophic chain is generally richer in  $\delta^{15}\text{N}$  than the pelagic trophic  
157 chain (Sherwood and Rose, 2005), a distinction was made between isotopic values from the  
158 benthic organic matter and the pelagic matter. Due to sampling constraints, our isotope data  
159 set did not include sufficient  $\delta^{15}\text{N}$  values to characterize suspended matter, i.e. pelagic source.  
160 Consequently, averaged  $\delta^{15}\text{N}$  signatures of two primary consumers characterizing two  
161 different pelagic nutritive pools during the considered period were chosen, one detritivorous  
162 *Eurytemora affinis* and the other phytoplanktivorous *Acartia* spp. The averaged  $\delta^{15}\text{N}$   
163 signature of a benthic primary consumer, *Cerastoderma edule*, was used to characterize the  
164 benthic baseline.

165

## 166 2.6. Fish trophic level

167

168 Fish trophic level (TL) was estimated as:  $TL = [(\delta^{15}N_{pred} - \delta^{15}N_{base}) / \Delta\delta^{15}N] + TL_{base}$ ,

169 where  $\delta^{15}N_{pred}$  is the  $\delta^{15}N$  signature of the predator in question,  $\delta^{15}N_{base}$  is the  $\delta^{15}N$  signature

170 of a representative baseline for the predator and  $TL_{base}$  is the trophic level of that baseline.

171 Primary consumers (*Eurytemora affinis*, *Acartia* spp. and *Cerastoderma edule*) were used for

172 that baseline. So,  $TL_{base}$  is equal to 2.  $\Delta\delta^{15}N$  represents the trophic fractionation of  $\delta^{15}N$ ,

173 estimated at 3.4‰ (Post, 2002).

174 The fish preys feed on benthic and/or pelagic food chains (phytoplanktonic and/or

175 detritic) and/or intermediate food chains (mixing of the two pelagic sources), determined from

176 their feeding ecology. The fish species or size class studied present different diets. Thus from

177 the knowledge of the food chains, we estimated for each fish predator group the  $\delta^{15}N_{base}$ ,

178 calculated as:  $\delta^{15}N_{base} = (\%V_B \times \delta^{15}N_B + \%V_H \times \delta^{15}N_H + \%V_D \times \delta^{15}N_D + \%V_I \times \delta^{15}N_I) / 100$ ,

179 where %V is the volumetric percentage of the prey B, H, D or I in the stomachs of a predator;

180 B groups together all the prey feeding almost exclusively on the benthic food chain, H the

181 prey feeding almost exclusively on the phytoplanktonic food chain, D the prey feeding almost

182 exclusively on the detritic (pelagic) food chain, and I the prey feeding both on detritic and

183 phytoplanktonic food chains.

184

## 185 3. Results

186

### 187 3.1. Fish trophic ecology

188

189 The diet of each fish species was identified during their growth period (warm period)

190 from stomach content analyses and expressed as a volumetric percentage (%V; Table 2).

191 A large proportion of *Liza ramada* stomach contents was composed of organic debris  
192 (%V = 86.53%). The rest was predominantly micro-algae in volume (essentially diatoms).

193 The flatfish, *Solea solea* and *Platichthys flesus*, fed on the benthic and suprabenthic  
194 compartments. The diet of *P. flesus* consisted mainly of the bivalve *Cerastoderma edule*  
195 (72.62%) and the isopod *Synidotea laticauda* (11.68%). *S. solea* fed more on the polychaetes  
196 *Neanthes succinea* (13.69%) and *Pectinaria koreni* (12.02%), the amphipods *Gammarus* spp.  
197 (19.46%) and the shrimp *Crangon crangon* (10.35%). Some vegetal debris (11.59%) was  
198 found in its diet.

199 *Pomatoschistus* spp. fed mainly on the suprabenthic compartment. The majority of  
200 their stomach contents consisted of *Gammarus* spp. (66.67%) and the mysid *Neomysis integer*  
201 (26.19%).

202 All other species (*Dicentrarchus labrax*, *Argyrosomus regius*, *Anguilla anguilla* and  
203 *Dicentrarchus punctatus*) had a more diversified diet, feeding from the supra-, epibenthic and  
204 pelagic compartments on organisms such as amphipods, isopods, shrimps and copepods. They  
205 also are ichthyophagous. Large and small *A. regius* fed especially on *Gammarus* spp.  
206 (respectively 14.92 and 11.87%), *C. crangon* (35.76 and 17.96%) and *N. integer* (12.15 and  
207 17.16%). Moreover, small *A. regius* consumed the mysids *M. slabberi* (23.52%) and fish  
208 larvae (20.78%), whereas large *A. regius* fed on the shrimp *Palaemon* spp. (13.02%). *S.*  
209 *laticauda* was the preferential prey for large *D. labrax* (11.90%), *A. anguilla* (25.59%) and *D.*  
210 *punctatus* (44.30%). The other preferential prey of *D. punctatus* was *M. slabberi* (18.37%).  
211 The diet of *A. anguilla* was also composed of *Gammarus* spp. (33.71%) and *Palaemon* spp.  
212 (12.75%). Large *D. labrax* had the most diversified diet, eating many preys such as  
213 *Gammarus* spp. (38.89%), *C. crangon* (11.72%), *N. integer* (12.89%), copepods (12.50%)  
214 and fish (12.10%). Finally, small *D. labrax* did not have the same diet as large individuals.

215 Their stomach contents were composed of a large proportion of *C. crangon* (25%), *Palaemon*  
216 spp. (28.30%), *Acartia* spp. (16.67%) and fish (16.67%).

217 Results represented ingested biomasses and from these the most important prey was  
218 determined (% V>10%; Table 2; Fig. 2).

219

### 220 3.2. Prey trophic ecology

221

222 A bibliographic review provides information on prey trophic ecology in the Gironde  
223 estuary or in similar estuarine systems (Table 3).

224 Organisms living on or in the sediment have diverse types of food habits: bivalves are  
225 suspension feeders (*C. edule*), deposit feeders (*Macoma balthica*) or grazers (*Littorina*  
226 *saxatilis*); polychaeta are carnivorous (*Nephtys* spp. or *Neanthes* spp.) or deposit feeders  
227 (*Polydora* spp., *Notomastus latericeus*, *Heteromastus filiformis*, *Pectinaria koreni*) and crabs  
228 are carnivorous. Some isopods (*Sphaeroma serratum* and *Cyathura carinata*), amphipods  
229 (*Bathyporeia* spp and *Corophium volutator*), shrimps (*C. crangon*) and copepods  
230 (*Acanthocyclops trajani*) also live on the sediment. However, all these species also use the  
231 sediment as a source of organic matter. In the pelagic compartment, organisms feed on  
232 phytoplankton, detritus or both. A few organisms, such as *M. slabberi*, *Schistomysis* spp.,  
233 *Gastrosaccus* spp. and *Acartia* spp., are exclusively herbivorous and depend on  
234 phytoplankton as a source of organic matter. Others, such as *Eurytemora affinis*, depend  
235 exclusively on detritus at the food web baseline. Most of the pelagic organisms consume both  
236 phytoplankton and detritus (e.g. *Euterpina acutifons*, the crustacean larvae).

237 From this trophic knowledge, the base of the fish food chains could be characterized  
238 (sources of organic matter; Fig. 2; Table 4).

239

240 3.3. Fish trophic levels

241

242 The characterization of fish and prey diets gives a good picture of the Gironde food  
243 web (Fig. 2). It highlights the fact that the fish species studied use diverse sources of organic  
244 matter by eating prey with different food habits. Moreover, from the feeding ecology  
245 information of preys and their  $\delta^{15}\text{N}$  values, we observed an enrichment of the  $\delta^{15}\text{N}$  signatures  
246 for the benthic species compared to the pelagic ones for an equivalent trophic position. In  
247 addition, an organism feeding on a detritic pelagic source got a higher  $\delta^{15}\text{N}$  signatures  
248 compared to the ones using a phytoplanktonic source.

249 To identify a  $\delta^{15}\text{N}_{\text{base}}$  for each fish group (genus, species or size class), the relative  
250 proportion of each category of prey (B, H, D, I; cf. definitions part 2.6) in the diet was  
251 estimated (Table 4). All the fish fed on both pelagic and benthic food chains, but in different  
252 proportions. For example, *Pomatoschistus* spp. ate only 3.57% of benthic prey whereas small  
253 *D. labrax* ate 37.53%. *P. flesus*, *S. solea* and *L. ramada* ate mostly from the benthic  
254 compartment, with 79.96, 59.41 and 94.68% of their diet respectively depending on this food  
255 chain.

256 The  $\delta^{15}\text{N}$  value of *C. edule*, used to characterize the benthic organic matter, is  $7.97 \pm$   
257  $0.39\text{‰}$  and for *E. affinis* (pelagic/detritic food chain) and *Acartia* spp.  
258 (pelagic/phytoplanktonic food chain) the values are  $7.13 \pm 2.67\text{‰}$  and  $6.12 \pm 1.51 \text{‰}$   
259 respectively. We assume that these three representative baseline organisms are primary  
260 consumers and have a position of 2. Fish  $\delta^{15}\text{N}$  signatures have higher values, varying between  
261  $10.70 \pm 0.50\text{‰}$  for *Pomatoschistus* spp. and  $13.60 \pm 0.12\text{‰}$  for large *D. labrax*.

262 The  $\delta^{15}\text{N}_{\text{base}}$  signature for each fish species (or size class) were estimated and from this  
263 their trophic levels were calculated (TL; Table 4). Large predators, such as large *D. labrax*  
264 and *A. anguilla*, were positioned at the higher TL (respectively 3.98 and 3.89), followed by *D.*

265 *punctatus* and large *A. regius* (3.58). *L. ramada* (2.87), and *Pomatoschistus* spp. (3.14) were  
266 found at the lower TL. Small *A. regius* and *D. labrax* and benthic fish *S. solea* and *P. flesus*  
267 were at the intermediate position, at TL = 3.15, TL = 3.43; TL = 3.30 and TL = 3.36  
268 respectively.

269

## 270 **4. Discussion**

271

### 272 *4.1. Trophic web of the Gironde estuary*

273

274 A precise estimation of fish trophic levels based on  $\delta^{15}\text{N}$  values requires a good  
275 knowledge of the trophic web in order to characterize the different nutritive pools at the base  
276 of the system and to describe food linkages allow us to distinguish pelagic food chains from  
277 benthic ones (Vander Zanden and Rasmussen, 1999; Mc Cuthan et al., 2003).

278 In environment as complex and fluctuant as estuaries, it appears necessary to consider  
279 the temporal feeding ecology of the nitrogen isotopic integrative period. Crossing  
280 bibliographic data, this time was estimated for fish to their warm growth period. Nevertheless,  
281 this time is species and life-stage specific (e.g. Miller, 2006; Guelinckx et al., 2007). It will be  
282 interesting to complete this study by an experimental approach to define precisely the  
283 different turnover times.

284 Fish stomach content analyses were carried out the warm period and enabled us to  
285 identify qualitatively and quantitatively the different preys consumed by each fish group  
286 studied. For both small and large groups of *D. labrax*, *D. punctatus* and *L. ramada*, the  
287 number of samples was reduced and/or limited to a restricted part of the period studied  
288 compared to other species, which led to less accurate results. Nevertheless, they were  
289 considered because they give information on these species diets in the Gironde estuary. In

290 addition, the trophic positions of these species obtained in that study are coherent with the  
291 description of their feeding ecology (Cf. following paragraph).

292         The general diet trends in their preys have been characterized from bibliographic data,  
293 mainly coming from Gironde estuary, to estimate the origin and the importance of each  
294 source for fish groups. Due to available isotopic data, one benthic baseline and two pelagic  
295 ones were distinguished. The knowledge of food chains and the  $\delta^{15}\text{N}$  data of different  
296 compartments confirm the isotopic enrichment in benthic species compared to pelagic ones  
297 for an equivalent trophic position and also highlight an enrichment of the detritic pelagic  
298 chains compared to phytoplanktonic ones. Recycling processes of the detritic organic matter  
299 could explained these enrichments (Hughes et al., 2000; David et al., 2006).

300         Considering the pelagic chains, a prey may not have a clear detritic or phytoplanktonic  
301 origin, but rather have a mixed origin of both. For zooplanktonic species which feed on the  
302 two sources, an intermediate source has been attributed to them. It would be interesting to  
303 determine the relative importance of these sources for these species. Fatty acids would allow  
304 an identification of the different nutritive pools and produce a better estimate (David et al.,  
305 2006). However, the differentiation of the diverse pelagic sources described in the present  
306 work provides a realistic estimation of the fish trophic levels and of the importance of the  
307 phytoplanktonic source in the functioning of the system, which may be underestimated in  
308 previous studies.

309         During the warm period, the trophic organization of the Gironde appears to be based  
310 both on detritic and phytoplanktonic sources. Moreover, the pelagic organic matter sources  
311 seem to be more used than benthic ones. These observations on the functioning of the Gironde  
312 confirms the assumption put forward for this period of the year in a previous work using a  
313 mass-balanced trophic model (Lobry et al., 2008).

314

315 4.2. *Validity of the fish trophic levels obtained*

316

317 By describing the bottom of the food chains and their sources we were able to  
318 calculate the trophic levels of some main fish groups (species, genus or size class) in the  
319 Gironde estuary from  $\delta^{15}\text{N}$  values. The data from fish stomach content analyses obtained in  
320 the nitrogen isotopic integrative period, or part of this period, led to a discussion about the  
321 validity of results.

322 In this study, *L. ramada* presents the lowest trophic level. Its feeding ecology differs  
323 from that of other species as it consumes a high level of primary producers (micro-algae) and  
324 detritus. Its grazing behaviour in brackish water, as previously described in other estuarine  
325 and coastal systems (Laffaille et al., 2002; Almeida, 2003), is confirmed here by its diet  
326 composed principally of benthic diatoms such as *Coscinodiscus*, *Melosira*, *Raphoneis*,  
327 *Girosigma* or *Navicula* (Almeida et al., 1993; Laffaille et al., 2002; Almeida, 2003).

328 Among the fish studied, *Pomatoschistus* spp. and the two flatfish *S. solea* and *P. flesus*  
329 have intermediate trophic levels. The genus *Pomatoschistus* is described in this study as a  
330 small crustacean feeder, which is in agreement with a previous work on the Gironde  
331 (Pasquaud et al., 2004) or other estuaries (e.g. Hamerlynck and Cattrijsse, 1994; Salgado et  
332 al., 2004; Leitão et al., 2006). *P. flesus* feeds especially on primary consumers, including a  
333 very large quantity of molluscs (*C. edule*) which were not found in their stomach contents the  
334 previous year (Pasquaud et al., 2008). Several studies (e.g. Summers, 1980; Hampel et al.,  
335 2005) indicate that the diet of *P. flesus* is very flexible, depending on benthic prey availability  
336 in the environment. However, some authors emphasize this fish species' strong preference for  
337 molluscs (Jones, 1962; Vinagre et al., 2008). *S. solea* tends to eat carnivorous prey such as  
338 polychaetes, *Gammarus* spp. and some decapods, as previously described in estuaries  
339 (Vinagre et al., 2005; Pasquaud et al., 2008). The diet of the two flatfish species is

340 distinguished from that of *Pomatoschistus* by fish consumption and this may explain their  
341 higher trophic levels. The lower trophic level of *S. solea* compared to *P. flesus* may be due to  
342 the fact that vegetal debris is found in its stomach contents.

343 *A. anguilla*, *D. punctatus*, *D. labrax* and *A. regius* have the highest trophic levels.  
344 They could be considered as the top predators of this system because of their carnivorous  
345 feeding behaviour, feeding on a high diversity of crustaceans (amphipods, isopods, shrimps,  
346 mysids), and especially because of their piscivorous trend. The diets of these species show  
347 similarities with the results of the previous year in the Gironde estuary (Pasquaud et al., 2008)  
348 and with those observed in other estuarine systems (Costa, 1988; Cabral and Costa, 2001;  
349 Cabral and Ohmert, 2001). The quantitative differences could be explained by variations in  
350 prey availability in the environment.

351 This study also shows an increase in trophic levels in parallel with an increase in the  
352 size of individuals for the two species tested, *D. labrax* and *A. regius*. The same observation  
353 was made from the analysis of  $\delta^{15}\text{N}$  values (Pasquaud et al., 2008). This result suggests that a  
354 species can play different functions in the ecosystem and emphasizes the importance of  
355 considering size criteria in studies of trophic dynamics (Garrison and Link, 2000).

356 Thus the trophic levels of fish groups, calculated from  $\delta^{15}\text{N}$  values, are representative  
357 of their feeding ecology. This method allows us to correct the work of Pasquaud et al. (2008),  
358 and therefore redefine the trophic position of the benthic fish.

359

## 360 **5. Conclusions**

361

362 This study demonstrates the necessity of a good knowledge of estuarine trophic webs,  
363 i.e. qualitative and quantitative fish trophic relationships, benthic/pelagic food chains, sources  
364 of organic matter, in order to calculate fish trophic levels from  $\delta^{15}\text{N}$  values and validate them.

365           Due to the considerable variability of these systems, it is essential to use  
366 complementarily stable isotope and stomach contents analyses during the whole integration  
367 period in fish. With this method, the variability of the feeding ecology during the considered  
368 period is taken into account and a possible inter-annual variability in the feeding ecology of  
369 some species (e.g. *P. flesus*) is palliated.

370           This approach developed to identify fish trophic levels in estuaries appears particularly  
371 appropriate, giving more accurate results than those produced by the Ecopath model  
372 developed in the Gironde estuary (see Lobry et al., 2008). This type of data could help to  
373 validate trophic models (Dame and Christian, 2008).

374           For future research, it would be interesting to estimate more precisely the different  
375 matter fluxes at the food web base and to study in details the recycling processes in order to  
376 see the relative importance of the sources in the system and thus obtain a better understanding  
377 of the functioning of estuarine fish food webs.

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379

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577                   **Captions**  
578

579   Fig. 1. Map of the Gironde estuary showing the fish sampling stations (stars) for dietary and  
580   stable isotope analyses.

581  
582   Fig. 2. Synthesis of the Gironde estuary trophic web knowledge during the investigation  
583   period. Relationships between fish (simple line box) and their preys (dashed box) result from  
584   data of fish stomach content analyses (present study). The thicker the arrow, the greater the  
585   trophic link. Relationships between preys and sources of organic matter (double lines box)  
586   were established from bibliographic work. Excepted for *S. laticauda*, all these data come from  
587   the Gironde estuary. The different compartments were localised according to their trophic  
588   position, from TL results for fish, from feeding ecology information for prey. The mean  $\delta^{15}\text{N}$   
589   signature (‰) of each taxon, obtained in that study, is indicated in grey.

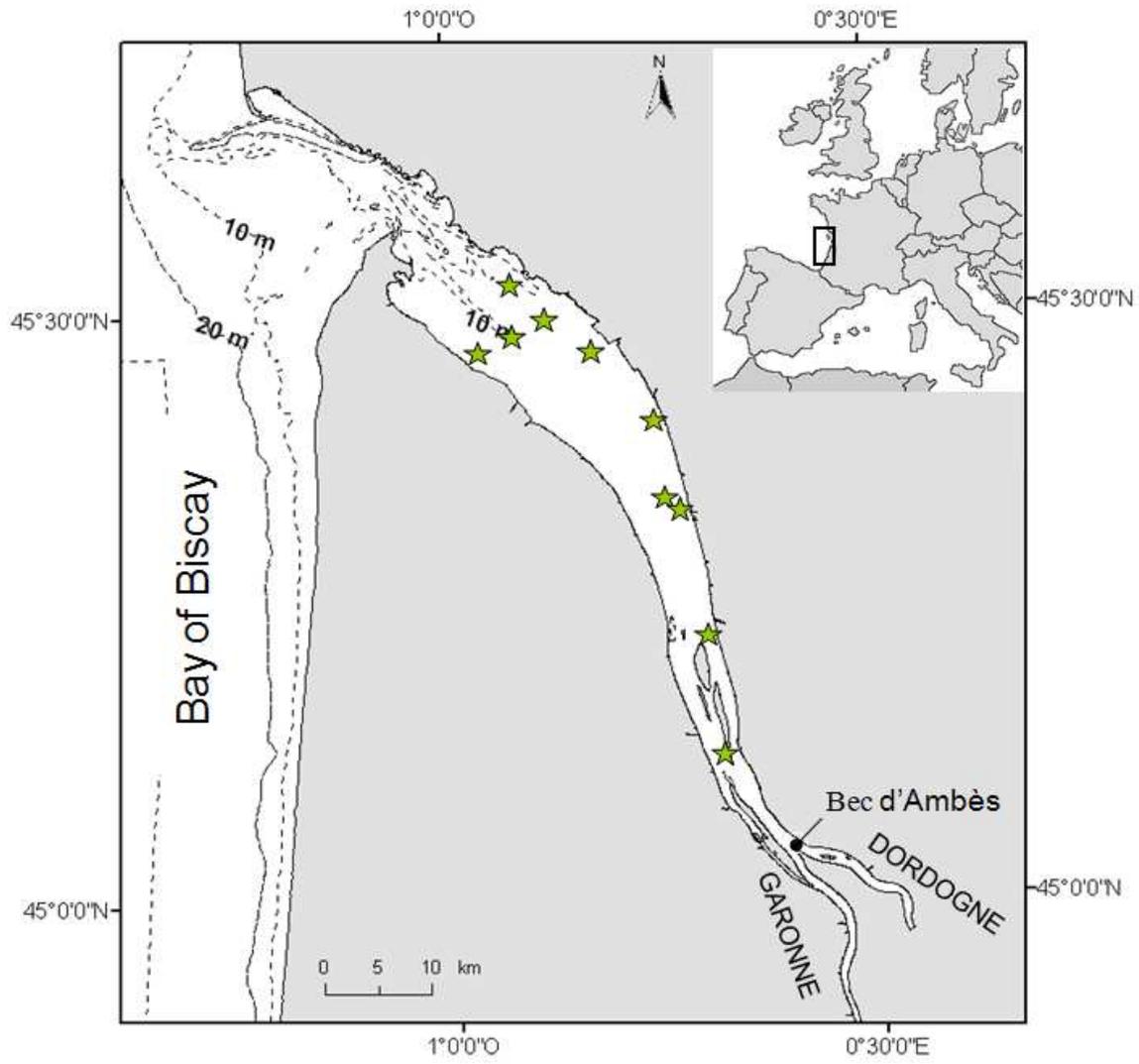
590  
591   Table 1. Size class and total number (n) of fish analysed for the Stable Isotope Analyses (SIA)  
592   and the Stomach Content Analyses (SCA).

593  
594   Table 2. Volumetric percentage diet composition of fish collected during their growth period  
595   in the Gironde estuary; n: total number of full stomachs; \*: small size class; \*\*: large size  
596   class and bold type denotes preferential preys (%V>10%).

597  
598   Table 3. Diet of the different preys examined in fish Stomach Content Analysis (SCA) and  
599   trophic chain to which preys belong.

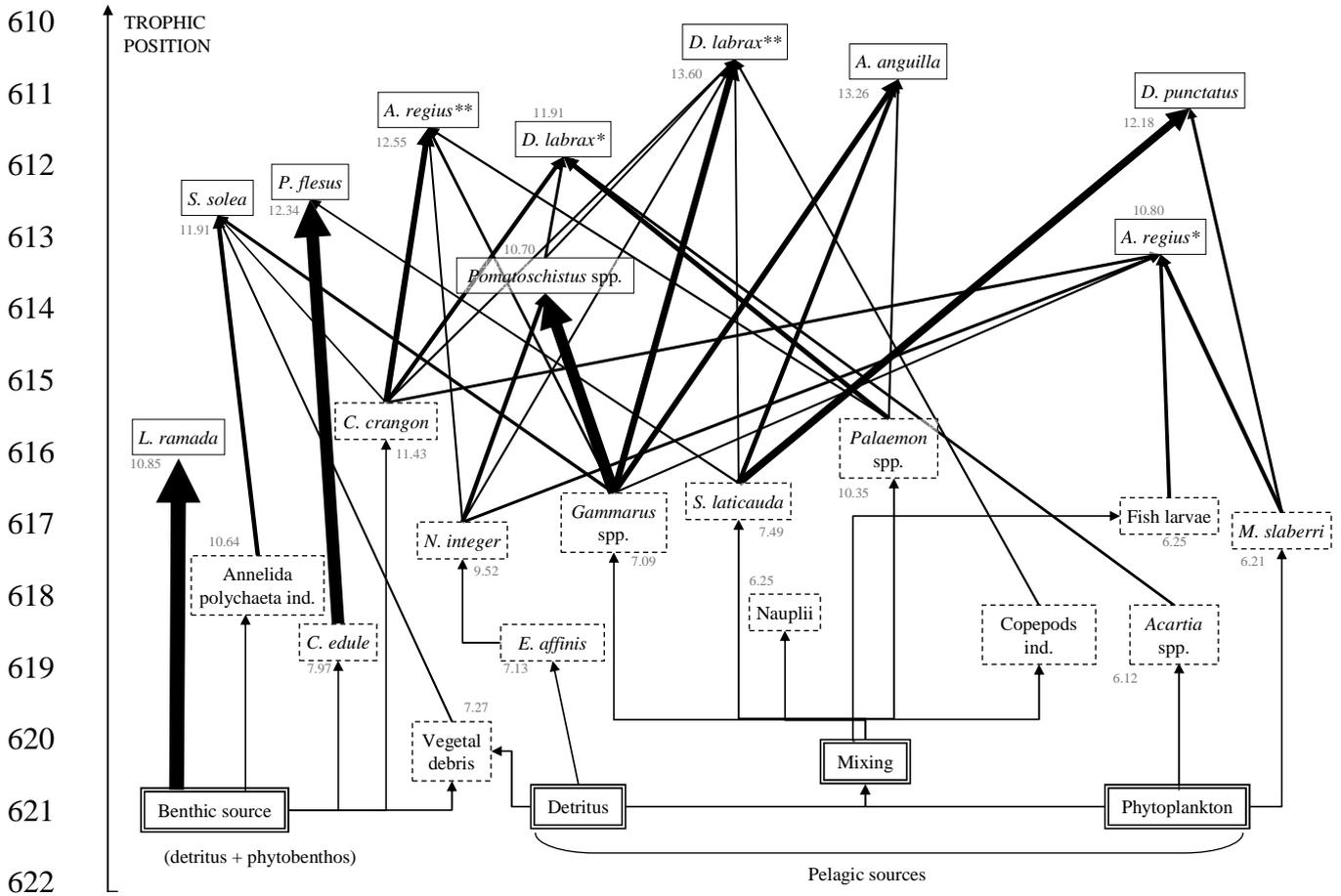
600  
601   Table 4. Volumetric percentage diet composition of fish species (or size class) on each trophic  
602   chain (%V = volumetric percentage of - B: preys dependent of the sediment as organic matter,

603 - P: preys dependent of the pelagic source, - H: preys dependent of the phytoplankton,- D:  
604 preys dependent of the detritus, - I: preys dependent of the intermediate pelagic source), mean  
605  $\delta^{15}\text{N}$  signatures (‰,  $\pm$  Standard Deviation) of organisms,  $\delta^{15}\text{N}_{\text{base}}$  estimation (‰,) of the  
606 trophic chain base to which fish species belong. TL: Trophic level of representative baseline  
607 organisms and each fish species or size class. \* = small size class and \*\* = large size class.



608

609 Fig. 1.



623 Fig. 2.

624 Table 1.

Species	n (SCA)	size class (mm) for SCA	n (SIA)	size class (mm) for SIA
<i>Anguilla anguilla</i>	36	267-643	21	267-760
<i>Dicentrarchus labrax</i> **	13	195-231	2	180-200
<i>Dicentrarchus labrax</i> *	6	79-109	4	70-100
<i>Dicentrarchus punctatus</i>	13	75-183	5	75-189
<i>Argyrosomus regius</i> **	82	237-309	9	220-300
<i>Argyrosomus regius</i> *	35	53-166	11	54-160
<i>Platichthys flesus</i>	22	147-333	11	121-333
<i>Solea solea</i>	52	117-197	12	90-198
<i>Pomatoschistus</i> spp.	35	57-65	7	29-65
<i>Liza ramada</i>	17	65-112	8	78-188

625

Species	<i>L. ramada</i>	<i>S. solea</i>	<i>P. flesus</i>	<i>Pomatoschistus</i> spp.	<i>D. labrax</i> *	<i>A. regius</i> *	<i>A. regius</i> **	<i>D. punctatus</i>	<i>A. anguilla</i>	<i>D. labrax</i> **
size (mm)	65-112	117-197	147-333	57-65	79-109	53-166	237-309	75-183	267-643	195-231
n	17	52	17	28	6	35	82	13	36	8
<b>Bivalves</b>										
<i>Cerastoderma edule</i>			<b>72.62</b>							
<i>Macoma balthica</i>		0.10	0.53							
<i>Littorina saxatilis</i>			0.01							
<b>Annelida polychaetes</b>										
<i>Nereis</i> spp.					3.30			7.57		
<i>Hediste diversicolor</i>		1.29								
<i>Neanthes succinea</i>		<b>13.69</b>					0.40			
<i>Polydora</i> spp.		4.83								
<i>Nephtys</i> spp.			0.31							
<i>Notomastus latericeus</i>			0.38							
<i>Heteromastus filiformis</i>			0.19							
<i>Pectinaria koreni</i>		<b>12.02</b>								
Polychaetes undetermined		6.09							0.27	
<b>Crabs</b>										
<i>Carcinus maenas</i>		0.32							2.08	
<i>Pachygrapsus marmoratus</i>									1.39	
<i>Liocarcinus</i> spp.		0.50								
<b>Amphipods</b>										
<i>Gammarus</i> spp.		<b>19.46</b>		<b>66.67</b>		<b>11.87</b>	<b>14.92</b>	8.24	<b>33.71</b>	<b>38.89</b>
<i>Bathyporeia</i> spp.		0.51		3.57			0.05	0.15		
<i>Corophium volutator</i>		3.61	5.88					1.87		
Amphipod undetermined				3.57						
<b>Isopods</b>										
<i>Synidotea laticauda</i>		4.83	<b>11.68</b>			2.37	8.13	<b>44.30</b>	<b>25.59</b>	<b>11.90</b>
<i>Sphaeroma serratum</i>					9.23	3.25				
<i>Cyathura carinata</i>		6.08	0.04							
<i>Idotea</i> spp.		1.48			0.18					
<b>Shrimps</b>										
<i>Crangon crangon</i>		<b>10.35</b>			<b>25.00</b>	<b>17.96</b>	<b>35.76</b>		7.40	<b>11.72</b>
<i>Palaemon</i> spp.		2.36			<b>28.30</b>	0.69	<b>13.02</b>	0.10	<b>12.75</b>	
<b>Mysids</b>										
<i>Mesopodopsis slabberi</i>					0.33	<b>23.52</b>	3.88	<b>18.37</b>	0.10	
<i>Neomysis integer</i>				<b>26.19</b>		<b>17.16</b>	<b>12.15</b>	3.31	9.10	<b>12.89</b>
<i>Shistomysis</i> spp.										
<i>Gastrosaccus</i> spp.							0.05			
Mysids undetermined							0.95		0.09	
<b>Copepods ind.</b>										
<i>Acartia</i> spp.	1.73				<b>16.67</b>	0.03				
<i>Eurytemora affinis</i>						0.63				
<i>Acanthocyclops trajani</i>								8.33		
<i>Euterpina acutifrons</i>	2.60									
Copepods undetermined	0.57									<b>12.50</b>

628 Table 2 (continued)

Species	<i>L. ramada</i>	<i>S. solea</i>	<i>P. flesus</i>	<i>Pomatoschistus</i> spp.	<i>D. labrax</i> *	<i>A. regius</i> *	<i>A. regius</i> **	<i>D. punctatus</i>	<i>A. anguilla</i>	<i>D. labrax</i> **
size (mm)	65-112	117-197	147-333	57-65	79-109	53-166	237-309	75-183	267-643	195-231
n	17	52	17	28	6	35	82	13	36	8
<b>Metazooplankton</b>										
Mysids						0.25	0.59			
Mollusc larvae										
Cypris larvae										
Nauplii	0.41									
Fish larvae						<b>20.78</b>				
<b>Fish</b>		0.87	6.08		<b>16.67</b>		1.52	7.58	2.01	<b>12.10</b>
<i>Pomatoschistus</i> spp.						1.08	7.04		2.20	
<i>Sprattus sprattus</i>							0.00		1.14	
<i>Solea solea</i>							0.15			
<i>Engraulis encrasicolus</i>							0.84			
<b>Divers</b>										
Eggs			0.07	0.16	0.33	0.41	0.06	0.01	0.00	
Vegetal debris		<b>11.59</b>	2.20	0.08			0.48	0.17	2.16	
Pollen										
Organic debris	<b>86.53</b>									
<b>Micro-algae</b>										
<i>Coscinodiscus</i> sp.	6.45									
<i>Navicula</i> sp.	0.23									
<i>Aulacoseira</i> sp.	0.11									
<i>Skeletonema</i> sp.	0.04									
<i>Plagiogramma</i> sp.	0.14									
<i>Gyrosigma</i> sp.	0.02									
<i>Thalassiosira</i> sp.	0.09									
<i>Thalasionema</i> sp.	0.01									
<i>Pediastrum</i> sp.	0.21									
<i>Diatoma</i> sp.	0.01									
Dinoflagellate cyst	0.37									
<i>Prorocentrum</i> sp.	0.01									
<i>Raphoneis</i> sp.	0.01									
<i>Nitzschia</i> sp.	0.15									
<i>Fragilaria</i> sp.	0.01									
<i>Cyclotella</i> sp.	0.30									

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Table 3

Species	Diet	References	Gironde data (*)	Source of organic matter
<b>Bivalves</b>				
<i>Cerastoderma edule</i>	active suspension feeder	Bachelet, 1981; Sauriau et al., 1989; Ysebaert & Herman, 2002	*	sediment
<i>Macoma balthica</i>	surface deposit and filter-feeder	Bachelet, 1981; Sauriau et al., 1989; Castel et al., 1994; Ysebaert & Herman, 2002	*	sediment
<i>Littorina saxatilis</i>	grazer	Laurand & Riera, 2006		sediment
<b>Annelid polychaetes</b>				
<i>Neanthes</i> spp.	carnivorous, omnivorous	Bachelet, 1981	*	sediment
<i>Hediste diversicolor</i>	carnivorous, omnivorous	Fauchald & Jumars, 1979; Cammen, 1980; Ysebaert & Herman, 2002		sediment
<i>Neanthes succinea</i>	carnivorous, omnivorous	Fauchald & Jumars, 1979; Ysebaert & Herman, 2002		sediment
<i>Polydora</i> spp.	surface deposit feeder	Fauchald & Jumars, 1979		sediment
<i>Nephtys</i> spp.	carnivorous, omnivorous	Fauchald & Jumars, 1979		sediment
<i>Notomastus latericeus</i>	subsurface deposit feeder	Fauchald & Jumars, 1979; Grall et al., 2006		sediment
<i>Heteromastus filiformis</i>	subsurface deposit feeder	Fauchald & Jumars, 1979; Ysebaert & Herman, 2002		sediment
<i>Pectinaria koreni</i>	subsurface deposit feeder	Fauchald & Jumars, 1979; Thiébaud et al., 1998		sediment
<b>Crabs</b>				
<i>Carcinus maenas</i>	carnivorous, omnivorous	Cohen et al., 1995; Grosholz & Ruiz, 1996		sediment
<i>Pachygrapsus marmoratus</i>	carnivorous, omnivorous	Cannicci et al., 2002		sediment
<i>Liocarcinus</i> spp.	carnivorous, omnivorous	Grall et al., 2006		sediment
<b>Amphipods</b>				
<i>Gammarus</i> spp.	carnivorous, omnivorous	Unpublished data	*	intermediate
<i>Bathyporeia</i> spp.	suspension and surface deposit feeder	Herman et al., 2000; Wieking & Kröncke, 2005		sediment
<i>Corophium volutator</i>	deposit and filter feeder	Castel et al., 1994; Gerdol & Hugues, 1994, Ysebaert & Herman, 2002	*	sediment
<b>Isopods</b>				
<i>Synidotea laticauda</i>	phytoplanktivorous, detritivorous			intermediate
<i>Sphaeroma serratum</i>	phytoplanktivorous, detritivorous			sediment
<i>Cyathura carinata</i>	carnivorous	Wägele et al., 1981; Olafsson & Persson, 1986		sediment
<i>Idotea</i> spp.	phytoplanktivorous, detritivorous			intermediate
<b>Shrimps</b>				
<i>Crangon crangon</i>	carnivorous, macro-benthivorous	Marchand, 1981		sediment
<i>Palaemon</i> spp.	carnivorous, mysid feeder	Marchand, 1981; Sorbe, 1983; Castel et al., 1994; Mouny et al., 1998	*	intermediate
<b>Mysids</b>				
<i>Mesopodopsis slabberi</i>	phytoplanktivorous	Unpublished data	*	phytoplankton
<i>Neomysis integer</i>	detritivorous	Castel et al., 1994; Focke & Mees, 1999	*	detritus
<i>Shistomysis</i> spp.	deposit and filter feeder	Unpublished data	*	phytoplankton
<i>Gastrosaccus</i> spp.	deposit and filter feeder	Unpublished data	*	phytoplankton

Table 3 (Continued)

Species	Diet	References	Gironde data (*)	Source of organic matter
<b>Copépods</b>				
<i>Acartia</i> spp.	phytoplanktivorous	Unpublished data	*	phytoplankton
<i>Eurytemora affinis</i>	detritivorous	Castel et al., 1994; Unpublished data	*	detritus
<i>Acanthocyclops trajani</i>	-	Unpublished data	*	sediment
<i>Euterpina acutifrons</i>	-	Unpublished data	*	intermediate
<b>Metazooplankton</b>				
Mysids	planktivorous	Castel et al., 1994	*	intermediate
Mollusc larvae	planktivorous	Castel et al., 1994	*	intermediate
Cypris larvae	planktivorous	Castel et al., 1994	*	intermediate
Nauplii	planktivorous	Castel et al., 1994	*	intermediate
Fish larvae	planktivorous	Castel et al., 1994	*	intermediate
<b>Fish</b>				
<i>Pomatoschistus</i> spp.	planktivorous	present work	*	intermediate
<i>Sprattus sprattus</i>	planktivorous	Pasquaud et al., 2008	*	intermediate
<i>Solea solea</i>	hyperbenthophagous, piscivorous	present work	*	sediment
<i>Engraulis encrasicolus</i>	planktivorous	Pasquaud et al., 2008	*	intermediate
<b>Divers</b>				
Eggs	-			detritus
Vegetal debris	-			detritus
Pollen	-			detritus

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Table 4.

Species	SCA				Trophic data		SIA		
	V%B	V%P			Trophic chain (source)	$\delta^{15}\text{N} \pm \text{SD}$	$\delta^{15}\text{N}_{\text{base}}$ estimation	TL <sub>base</sub> estimation	TL
		%V <sub>H</sub>	%V <sub>D</sub>	%V <sub>I</sub>					
<i>C. edule</i>					benthic	7.97 ± 0.39		2.00	
<i>Acartia</i> spp.					pelagic (phytoplanktonic)	6.12 ± 1.51		2.00	
<i>E. affinis</i>					pelagic (detritic)	7.13 ± 2.67		2.00	
Intermediate					pelagic	6.63 ± 0.72		2.00	
<i>D. labrax</i> **	11.72	0.00	12.89	75.39	mixed	13.60 ± 0.12	6.85		3.98
<i>A. anguilla</i>	11.14	0.10	11.27	77.50	mixed	13.26 ± 0.69	6.83		3.89
<i>D. punctatus</i>	17.92	18.37	3.48	60.22	mixed	12.18 ± 0.90	6.79		3.58
<i>A. regius</i> **	36.36	3.93	12.69	47.02	mixed	12.55 ± 0.68	7.16		3.58
<i>D. labrax</i> *	37.53	17.00	0.33	45.15	mixed	11.91 ± 0.81	7.05		3.43
<i>P. flesus</i>	79.96	0.00	2.27	17.77	benthic	12.34 ± 0.95	7.71		3.36
<i>S. solea</i>	59.41	0.00	11.59	29.00	benthic	11.91 ± 1.01	7.48		3.30
<i>A. regius</i> *	21.21	23.55	18.20	37.04	mixed	10.80 ± 1.07	6.88		3.15
<i>Pomatoschistus</i> spp.	3.57	0.00	26.44	70.24	mixed	10.70 ± 0.50	6.83		3.14
<i>L. ramado</i>	94.68	1.73	0.00	3.58	benthic	10.85 ± 1.43	7.89		2.87

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