Determination of fish trophic levels in an estuarine system

Stéphanie Pasquaud, Marion Pillet, V. David, B. Sautour, Pierre Elie

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

2

3 S. Pasquaud[a,*], M. Pillet[a], V. David[b], B. Sautour[c], P. Elie[a]

4 [a] Cemagref, groupement de Bordeaux, Estuarine Ecosystems and Diadromous Fish Research

5 Unit, 50 avenue de Verdun, 33612 Cestas Cedex, France

6 stephanie.pasquaud@cemagref.fr

7 pillet.marion@gmail.com

8 pierre.elie@cemagref.fr

9 [b] UMR LIENSs 6250 CNRS/Université de La Rochelle, Institut du Littoral et de

10 l'Environnement, 2 rue Olympes de Gouges, 17000 La Rochelle, France

11 valerie.david@univ-lr.fr

12 [c] UMR 5805 EPOC – OASU, Université de Bordeaux 1, 2 Rue du Professeur Jolyet, 33120

13 Arcachon, France

14 b.sautour@epoc.u-bordeaux1.fr

[*] Corresponding author. Tel. +33 5 57 89 09 80 – Fax. +33 5 57 89 08 01

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 δ^{15}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; δ^{15}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 $^{13}C/^{12}C$ 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 $^{15}N/^{14}N$ 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 $\delta^{13}C$ and $\delta^{15}N$ 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, $\delta^{15}N$ 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 $\delta^{15}N$ level than *Argyrosomus regius* which tends to be ichtyophagous. These

69   observations can be explained by an enrichment of the $\delta^{15}N$ 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 $\delta^{15}N$ 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 $\delta^{15}N$ value taking into account

79   the proportion of benthic/pelagic prey in their feeding and the $\delta^{15}N$ 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 km². 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^{-3} in May) and an important drought period in summer (about 250 m.s^{-3}

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^{-1} (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

6

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 δ^{15}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 δ^{15}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 δ^{15}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 δ^{15}N values to characterize suspended matter, i.e. pelagic source.

160   Consequently, averaged δ^{15}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 δ^{15}N

163   signature of a benthic primary consumer, *Cerastoderma edule*, was used to characterize the

164   benthic baseline.

165

8

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).

9

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 ichtyophagous. 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 carnivororous (*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

11

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 $\delta^{15}N$ values, we observed an enrichment of the $\delta^{15}N$ 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 $\delta^{15}N$ signatures

248   compared to the ones using a phytoplanktonic source.

249   To identify a $\delta^{15}N_{base}$ 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 $\delta^{15}N$ value of *C. edule*, used to characterize the benthic organic matter, is 7.97 $\pm$

257   0.39‰ and for *E. affinis* (pelagic/detritic food chain) and *Acartia* spp.

258   (pelagic/phytoplanktonic food chain) the values are 7.13 $\pm$ 2.67‰ and 6.12 $\pm$ 1.51 ‰

259   respectively. We assume that these three representative baseline organisms are primary

260   consumers and have a position of 2. Fish $\delta^{15}N$ signatures have higher values, varying between

261   10.70 $\pm$ 0.50‰ for *Pomatoschistus* spp. and 13.60 $\pm$ 0.12‰ for large *D. labrax*.

262   The $\delta^{15}N_{base}$ 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 $\delta^{15}N$ 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

13

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 δ^{15}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 $\delta^{15}N$ 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

15

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 δ^{15}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 δ^{15}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 δ^{15}N values and validate them.

16

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.

378   **Acknowledgements**

379

385 **References**

386

387 Akin, S., Winemiller, K.O., 2008. Body size and trophic position in a temperature estuarine

388 food web. Acta Oecologica-International Journal of Ecology 33, 144-153.

389 Almeida, P.R., 2003. Feeding ecology of *Liza ramada* (Risso, 1810) (Pisces, Mugilidae) in a

390 south-western estuary of Portugal. Estuarine, Coastal and Shelf Science 57, 313-323.

391 Almeida, P.R., Moreira, F., Costa, J.L., Assis, C.A., Costa, M.J., 1993. The feeding strategies

392 of *Liza ramada* (Risso, 1826) in fresh and brackish water in the River Tagus, Portugal.

393 Journal of Fish Biology 42, 95-107.

394 Bachelet, G., 1981. Données préliminaires sur l'organisation trophique d'un peuplement

395 benthique marin. Vie et Milieux 31, 205-213.

396 Cabral, H., Costa, M.J., 2001. Abundance, feeding ecology and growth of 0-group sea bass,

397 *Dicentrarchus labrax*, within the nursery areas of the Tagus estuary. Journal of the Marine

398 Biological Association of the United Kingdom 81, 679-682.

399 Cabral, H.N., Ohmert, B., 2001. Diet of juvenile meagre, *Argyrosomus regius*, within the

400 Tagus estuary. Cahiers de Biologie Marine 42, 289-293.

401 Cammen, L.M., 1980. A Method for Measuring Ingestion Rate Deposit Feeders and its Use

402 with the Polychaete *Nereis succinea*. Estuaries 3, 55-60.

403 Cannicci, S., Gomei, M., Boddi, B., Vannini, M., 2002. Feeding Habits And Natural Diet of

404 the Intertidal Crab Pachygrapsus marmoratus: Opportunistic Browser or Selective Feeder?

405 Estuarine, Coastal and Shelf Science 54, 983-1001.

406 Castel, J., Bachelet, G., Rochard, E., Elie, P., 1994. La biologie de l'estuaire, Estuaire de la

407 Gironde, livre blanc. Agence de l'Eau Adour Garonne, IFREMER, pp. 115.

408 Church, M.R., Ebersole, J.L., Rensmeyer, K.M., Couture, R.B., Barrows, F.T., Noakes,

409 D.L.G., 2009. Mucus: a new tissue fraction for rapid determination of fish diet switching

410 using stable isotope analysis. Canadian Journal of Fisheries and Aquatic Sciences 66, 1-5.

411 Cohen, A.N., Carlton, J.T., Fountain, M.C., 1995. Introduction, dispersal and potential

412 impacts of the green crab *Carcinus maenas* in San Francisco Bay, California. Marine Biology

413 122, 225-237.

414 Costa, M.J., 1988. Ecologie alimentaire des poissons de l'estuaire du Tage. Cybium 12, 301-

415 320.

416 Cupp, E.E., 1943. Marine plankton diatoms of the west coast of North America. University of

417 California press Berkeley and Los Angeles.

418 Dame, J.K., Christian, R.R., 2008. Evaluation of ecological network analysis: Validation of

419 output. Ecological Modelling 210, 327-338.

420 Davenport, S.R., Bax, N.J., 2002. A trophic study of a marine ecosystem off southeasten

421 Australia using stable isotopes of carbon and nitrogen. Canadian Journal of Fisheries and

422 Aquatic Sciences 59, 514-530.

423 David, V., Sautour, B., Chardy, P., 2007. Successful colonization of the calanoid copepod

424 *Acartia tonsa* in the oligo-mesohaline area of the Gironde estuary (SW France) - Natural or

425 anthropogenic forcing? Estuarine Coastal and Shelf Science 71, 429-442.

426 David, V., Sautour, B., Galois, R., Chardy, P., 2006. The paradox high zooplankton biomass-

427 low vegetal particulate organic matter in high turbidity zones: What way for energy transfer?

428 Journal of Experimental Marine Biology and Ecology 333, 202-218.

429 De Niro, M.J., Epstein, S., 1978. Influence of diet on the distribution of carbon isotopes in

430 animals. Geochimica et Cosmochimica Acta 42, 495-506.

431 De Niro, M.J., Epstein, S., 1981. Influence of the diet on the distribution of nitrogen isotopes

432 in animals. Geochimica et Cosmochimica Acta 45, 341-351.

433   Fauchald, K., Jumars, P.A., 1979. The diet of worms: a study of polychaete feeding guilds.

434   Oceanographic marine biology. Annual Review 17, 193-284.

435   Fockedey, N., Mees, J., 1999. Feeding of the hyperbenthic mysid *Neomysis integer* in the

436   maximum turbidity zone of the Elbe, Westerschelde and Gironde estuaries. Marine Systems

437   22, 207-228.

438   Fry, B., Sherr, E.B., 1984. δ^{13}C measurements as indicators of carbon flow in marine and

439   freshwater ecosystems. Contributions in Marine Science 27, 13-47.

440   Garrison, L.P., Link, J.S., 2000. Dietary guild structure of the fish community in the

441   Northeast United States continental shelf ecosystem. Marine Ecology Progress Series 202,

442   231-240.

443   Gerdol, V., Hughes, R.G., 1994. Feeding behaviour and diet of *Corophium volutator* in an

444   estuary in southeastern England. Marine Ecology Progress Series 114, 103-108.

445   Grall, J., Le Loc'h, F., Guyonnet, B., Riera, P., 2006. Community structure and food web

446   based on stable isotopes (δ^{15}N and δ^{13}C) analysis of a North Eastern Atlantic maerl bed.

447   Journal of Experimental Marine Biology and Ecology 338, 1-15.

448   Grosholz, E.D., Ruiz, G.M., 1996. Predicting the impact of introduced marine species:

449   Lessons from the multiple invasion of the European green crab *Carcinus maenas*. Biological

450   Conservation 78, 59-66.

451   Guelinckx, J., Maes, J., Van Den Driessche, P., Geysen, B., Dehairs, F., Ollevier, F., 2007.

452   Changes in delta C-13 and delta N-15 in different tissues of juvenile sand goby

453   *Pomatoschistus minutus*: a laboratory diet-switch experiment. Marine Ecology-Progress

454   Series 341, 205-215.

455   Hamerlynck, O., Cattrijsse, A., 1994. The food of *Pomatoschistus minutus* (Pisces, Gobiidae)

456   in Belgian coastal waters, and a comparison with the food of its potential competitor *P.*

457   *lozanoi*. Journal of Fish Biology 44, 753-771.

458   Hampel, H., Cattrijsse, A., Elliott, M., 2005. Feeding habits of young predatory fishes in

459   marsh creeks situated along the salinity gradient of the Schelde estuary, Belgium and The

460   Netherlands. Helgoland Marine Research 59, 151-162.

461   Herman, P.M.J., Middelburg, J.J., Widdows, J., Lucas, C.H., Heip, C.H.R., 2000. Stable

462   isotopes as trophic tracers: combining field sampling and manipulative labelling of food

463   resources for macrobenthos. Marine Ecology Progress Series 204, 79-92.

464   Hesslein, R.H., Capel, M.J., Fox, D.E., Hallard, K.A., 1991. Stable isotopes of sulfur, carbon,

465   and nitrogen as indicators of trophic level and fish migration in the lower Mackenzie river

466   basin, Canada. Canadian Journal of Fisheries and Aquatic Sciences 48, 2258-2265.

467   Hesslein, R.H., Hallard, K.A., Ramlal, P., 1993. Replacement of Sulfur, carbon and nitrogen

468   in tissue of Growing broad Whitefish (*Coregonus nasus*) in response to a change in diet

469   traced by delta 34S, delta 13C and delta 15N. Canadian Journal of Fisheries and Aquatic

470   Sciences 50, 2071-2076.

471   Hughes, J.E., Deegan, L.A., Peterson, B.J., Holmes, R.M., Fry, B., 2000. Nitrogen flow

472   through the food web in the oligohaline zone of a new England estuary. Ecology 81, 433-452.

473   Hyslop, E.J., 1980. Stomach contents analysis - a review of methods and their application.

474   Journal of Fish Biology 17, 411-429.

475   Jones, N.S., 1962. The bottom fauna and the food of flatfish of the Cumberland Coast. Journal

476   of Animal Ecology 21, 182-205.

477   Kwak, T., Zedler, J.B., 1997. Food web analysis of southern California coastal wetlands using

478   multiple stable isotopes. Oecologia 110, 262-277.

479   Laffaille, P., Feunteun, E., Lefebvre, C., Radureau, A., Sagan, G., Lefeuvre, J.-C., 2002. Can

480   Thin-lipped Mullet Directly Exploit the Primary and Detritic Production of European

481   Macrotidal Salt Marshes? Estuarine, Coastal and Shelf Science 54, 729-736.

482   Laurand, S., Riera, P., 2006. Trophic ecology of the supralittoral rocky shore (Roscoff,

483   France): A dual stable isotope (δ^{13}C, δ^{15}N) and experimental approach. Journal of Sea

484   Research 56, 27-36.

485   Leitão, R., Martinho, E., Neto, J.M., Cabral, H., Marques, J.C., Pardal, M.A., 2006. Feeding

486   ecology, population structure and distribution of *Pomatoschistus microps* (Kroyer, 1838) and

487   *Pomatoschistus minutus* (Pallas, 1770) in a temperate estuary, Portugal. Estuarine Coastal and

488   Shelf Science 66, 231-239.

489   Lobry, J., David, V., Pasquaud, S., Lepage, M., Sautour, B., Rochard, E., 2008. Diversity and

490   stability of an estuarine trophic network. Marine Ecology Progress Series 358, 13-25.

491   Marchand, J., 1981. Observations sur l'écologie de *Crangon crangon* (Linné) et *Palaemon*

492   *longirostris* H. Milne Edwards (Crustacea, Decapoda, Natantia) dans l'estuaire interne de la

493   Loire (France). Vie et Milieu 31, 83-92.

494   Mc Cuthan, J.H., Lewis, W.M., Kendall, C., Mc Grath, C.C., 2003. Variation in trophic shift

495   for stable isotope ratios of carbon, nitrogen, and sulfur. Oikos 102, 378-390.

496   Miller, T.W., 2006. Tissue-specific response of delta N-15 in adult Pacific herring (Clupea

497   pallasi) following an isotopic shift in diet. Environmental Biology of Fishes 76, 177-189.

498   Minagawa, M., Wada, E., 1984. Stepwise enrichment of ^{15}N along food chains: further

499   evidence and the relation between δ^{15}N and animal age. Geochimica et Cosmochimica Acta

500   48, 1135-1140.

501   Mouny, P., Dauvin, J.C., Bessineton, C., Elkaim, B., Simon, S., 1998. Biological components

502   from the Seine estuary: first results. Hydrobiologia 374, 333-347.

503   Ólafsson, E.B., Persson, L.-E., 1986. Distribution, life cycle and demography in a brackish

504   water population of the isopod *Cyathura carinata* (Kröyer) (Crustacea). Estuarine, Coastal

505   and Shelf Science 23, 673-687.

506   Padisak, J., Adrian, R., 1999. Biovolumen. In: Tümpling, W. and Friedrich, G. (Eds),

507   Biologische Gewässeruntersuchung. Fisher, G., pp. 334-368.

508   Pasquaud, S., Elie, P., Jeantet, C., Billy, I., Martinez, P., Girardin, M., 2008. A preliminary

509   investigation of the fish food web in the Gironde estuary, France, using dietary and stable

510   isotope analyses. Estuarine, Coastal and Shelf Science 78, 267-279.

511   Pasquaud, S., Girardin, M., Elie, P., 2004. Diet of gobies of the genus *Pomatoschistus* (*P.*

512   *microps* and *P. minutus*), in the Gironde estuary (France). Cybium 28, 99-106.

513   Perga, M.E., Gerdeaux, D., 2003. Using the $\delta^{13}C$ and $\delta^{15}N$ of whitefish scales for

514   retrospective ecological studies: Changes in isotope signatures during the restoration of Lake

515   Geneva, 1980-2001. Journal of Fish Biology 63, 1197-1207.

516   Perga, M.E., Gerdeaux, D., 2005. 'Are fish what they eat' all year round? Oecologia 144, 598-

517   606.

518   Persson, A., Hansoon, L.A., 1999. Diet shift in fish following competitive release. Canadian

519   Journal of Fisheries and Aquatic Sciences 56, 70-78.

520   Pillay, T.V.R., 1952. A critique of the methods of study of food of fishes. Journal of the

521   Zoological Society of India 4, 185-200.

522   Post, D.M., 2002. Using stable isotopes to estimate trophic position: Models, methods, and

523   assumptions. Ecology 83, 703-718.

524   Prygiel, J., Coste, M., 2000. Guide méthodologique pour la mise en œuvre de l'indice

525   biologique Diatomées. Agences de l'eau, MATE, Cemagref Bordeaux, 134 pp.

526   Salgado, J.P., Cabral, H.N., Costa, M.J., 2004. Feeding ecology of the gobies *Pomatoschistus*

527   *minutus* (Pallas, 1770) and *Pomatoschistus microps* (Kroyer, 1838) in the upper Tagus

528   estuary, Portugal. Scientia Marina 68, 425-434.

529   Sauriau, P.G., Mouret, V., Rince, J.P., 1989. Organisation trophique de la malacofaune

530   benthique non cultivée du bassin ostréicole de Marennes-Oléron. Oceanologica Acta 12, 193-

531   204.

532   Sherwood, G.D., Rose, G.A., 2005. Stable isotope analysis of some representative fish and

533   invertebrates of the Newfoundland and Labrador continental shelf food web. Estuarine,

534   Coastal and Shelf Science 63, 537-549.

535   Sorbe, J.-C., 1983. Les décapodes natantia de l'estuaire de la Gironde (France). Contribution à

536   l'étude morphologique et biologique de *Palaemon longirostris*. H. Milne Edwards, 1837.

537   Crustaceana 44, 251-270.

538   Sottolichio, A., Castaing, P., 1999. A synthesis on seasonal dynamics of highly-concentrated

539   structures in the Gironde estuary. Comptes rendus de l'Académie des Sciences Série II

540   Fascicule a- Sciences de la Terre et des Planètes 329, 795-800.

541   Summers, R.W., 1980. The diet and feeding behaviour of the flounder *Platichthys flesus* (L.)

542   in the Ythan estuary, Aberdeeshire, Scotland. Estuarine and Coastal Marine Science 11, 217-

543   232.

544   Suring, E., Wing, S.R., 2009. Isotopic turnover rate and fractionation in multiple tissues of red

545   rock lobster (*Jasus edwardsii*) and blue cod (*Parapercis colias*): Consequences for ecological

546   studies. Journal of Experimental Marine Biology and Ecology 370, 56-63.

547   Thiébaut, E., Lagadeuc, Y., Olivier, F., Dauvin, J.C., Retière, C., 1998. Do hydrodynamic

548   factors affect the recruitment of marine invertebrates in a macrotidal area? Hydrobiologia

549   375-376, 165-176.

550   Tieszen, L.L., Boutton, T.W., Tesdahl, K.G., 1983. Fractionation and turnover of stable

551   carbon isotopes in animal tissues: implication for $\delta^{13}C$ analysis of diet. Oecologia 57, 32-37.

552 Vander Zanden, M.J., Cabana, G., Rasmussen, J.B., 1997. Comparing trophic position of

553 freshwater fish calculated using stable nitrogen isotope ratios (δ^{15}N) and literature dietary

554 data. Canadian Journal of Fisheries and Aquatic Sciences 54, 1142-1158.

555 Vander Zanden, M.J., Rasmussen, J.B., 1999. Primary consumer δ^{13}C and δ^{15}N and the

556 trophic position of aquatic consumers. Ecology 80, 1395-1404.

557 Vinagre, C., Cabral, H., Costa, M.J., 2008. Prey selection by flounder, Platichthys flesus, in

558 the Douro estuary, Portugal. Journal of Applied Ichthyology 24, 238-243.

559 Vinagre, C., Franca, S., Costa, M.J., Cabral, H.N., 2005. Niche overlap between juvenile

560 flatfishes, *Platichthys flesus* and *Solea solea*, in a southern European estuary and adjacent

561 coastal waters. Journal of Applied Ichthyology 21, 114-120.

562 Wada, E., Mizutani, H., Minagawa, M., 1991. The use of stable isotopes for food web

563 analysis. Critical Reviews in Food Science and Nutrition 30, 361-371.

564 Wägele, J.W., Welsch, U., Müller, W., 1981. Fine structure and function of the digestive tract

565 of *Cyathura carinata* (Krøyer) (Crustacea, Isopoda). Zoomorphology 98, 69-88.

566 West, J.M., Williams, G.D., Madon, S.P., Zedler, J.B., 2003. Integrating spatial and temporal

567 variability into the analysis of fish food web linkages in Tijuana Estuary. Environmental

568 Biology of Fishes 67, 297-309.

569 Wieking, G., Kröncke, I., 2005. Is benthic trophic structure affected by food quality? The

570 Dogger Bank example. Marine Biology 146, 387-400.

571 Winemiller, K.O., Akin, S., Zeug, S.C., 2007. Production sources and food web structure of a

572 temperate tidal estuary: integration of dietary and stable isotope data. Marine Ecology-

573 Progress Series 343, 63-76.

574 Ysebaert, T., Herman, P.M.J., 2002. Spatial and temporal variation in benthic macrofauna and

575 relationships with environmental variables in an estuarine, intertidal soft-sediment

576 environment. Marine Ecology Progress Series 244, 105-124.
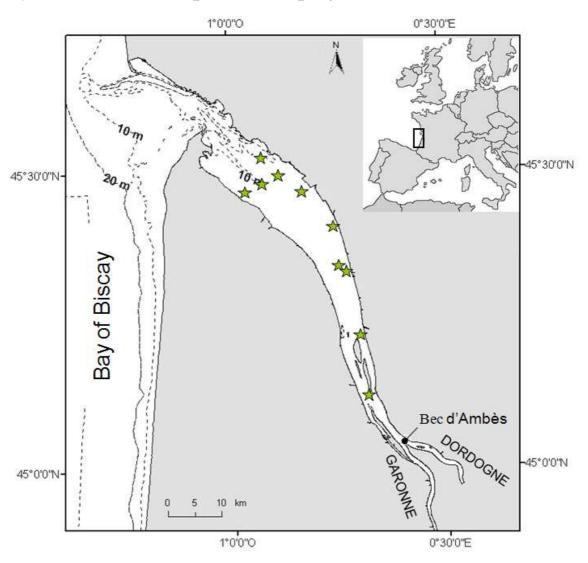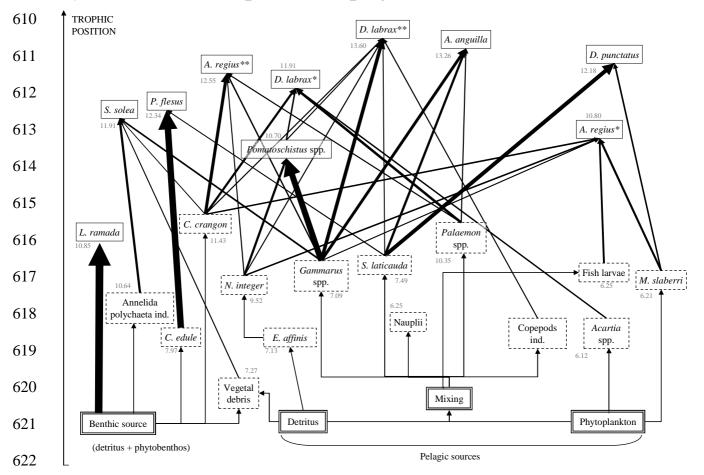
577   **Captions**

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 δ^{15}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    $\delta^{15}N$ signatures (‰, ± Standard Deviation) of organisms, $\delta^{15}N_{base}$ 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.

Fig. 2.

624   Table 1.

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

625

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

626
627 Table 2

628   Table 2 (continued)

Species	*L. ramada*	*S. solea*	*P. flesus*	*Pomatoschistus* spp.	*D. labrax* *	*A. regius* *	*A. regius* **	*D. punctatus*	*A. anguilla*	*D. labrax* **
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
Metazooplankton										
Mysids						0.25	0.59			
Mollusc larvae										
Cypris larvae										
Nauplii	0.41									
Fish larvae						**20.78**				
Fish		0.87	6.08		**16.67**		1.52	7.58	2.01	**12.10**
Pomatoschistus spp.						1.08	7.04		2.20	
Sprattus sprattus							0.00		1.14	
Solea solea							0.15			
Engraulis encrasicolus							0.84			
Divers										
Eggs			0.07	0.16	0.33	0.41	0.06	0.01	0.00	
Vegetal debris		**11.59**	2.20	0.08			0.48	0.17	2.16	
Pollen										
Organic debris	**86.53**									
Micro-algae										
Coscinodiscus sp.	6.45									
Navicula sp.	0.23									
Aulacoseira sp.	0.11									
Skeletonema sp.	0.04									
Plagiogramma sp.	0.14									
Gyrosigma sp.	0.02									
Thalassiosira sp.	0.09									
Thalasionema sp.	0.01									
Pediastrum sp.	0.21									
Diatoma sp.	0.01									
Dinoflagellate cyst	0.37									
Prorocentrum sp.	0.01									
Raphoneis sp.	0.01									
Nitzschia sp.	0.15									
Fragilaria sp.	0.01									
Cyclotella sp.	0.30									

629
630

33

Table 3

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

630

Table 3 (Continued)

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

631
632

35

Table 4.

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

632
633

36