Vegetated habitats trophically support early development stages of a marine migrant fish in a coastal lagoon

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Abstract

Understanding the trophic ecology of early developmental stages of commercial fish species migrating between sea and coastal lagoons is crucial to effective management of nursery habitats and resulting resources. While most information on diet shift of the gilthead seabream *Sparus aurata* comes from studies in experimental conditions, here we investigated the trophic niche dynamics of post-larvae, juveniles and sub-adults in natural environment, hypothesizing that habitat and resources use of marine and lagoonal sites will exhibit strong relationship with ontogeny of the species. Carbon and nitrogen stable isotopes showed evidence of a clear shift in the trophic niche features, trophic position and trophic pathways supporting *S. aurata* during growth. Main differences occurred between post-larvae and juveniles/sub-adults, perfectly matching their diet shift from zooplanktivorous to zoobenthivorous habits. The wider trophic niche of juveniles exploiting the variety of resources within the lagoon, compared to the narrower niche of marine post-larvae demonstrates the importance of the trophic role of the

27 lagoon as nursey ground, where aquatic macrophytes (seagrasses and macroalgae) provide the

28 main trophic support as sources of organic matter.

29

30 **Key words**: trophic niche, ontogenetic development, gilthead seabream, lagoon nursery areas,

31 stable isotopes

32

Introduction

34 Coastal lagoons are important nursery areas for many aquatic species, either vertebrates or

35 invertebrates, to which they provide suitable conditions for safe development and rapid growth

36 (Beck et al. 2001; Sheaves et al. 2015). A variety of lagoonal habitats, such as salt marshes,

37 intertidal creeks or seagrass meadows, constitute highly productive systems where plentiful

38 availability of food sources grants optimal foraging, as well as shallow waters assure ideal

39 shelter from predators (Vasconcelos et al. 2010; Tournois et al. 2017). As such, they play a

40 central role in maintaining high densities of juveniles, contributing to the biodiversity of

41 adjacent environments, either marine or freshwater (Tagliapietra et al. 2014), as well as the

42 connectivity among these systems (Able 2005; Sheaves et al. 2015). These aspects contribute

43 to delineate the ecosystem services of a lagoon and broadly speaking, of whole estuarine and

44 coastal ecosystems (Beck et al. 2001; Barbier et al. 2011), through indirect support to human

45 activities such as artisanal and industrial fishery.

46 Fish species using lagoonal habitats as nursery grounds are usually included in the ecological

47 group of marine migrants (Franco et al. 2008), which spend the early stages of life cycle within

48 such transitional waters, entering as post-larvae and leaving once they reach the sub-adult phase

49 that enables them to survive in the open sea. The gilthead seabream *Sparus aurata* Linnaeus,

50 1758 represents one of the marine migrants widely distributed in the Mediterranean Sea, in the

51 eastern coasts of the Atlantic Ocean and rarely also in the Black Sea (Bauchot and Hureau

52   1986). *S. aurata* is a euryhaline and eurythermal species whose juvenile stage development is

53   characterized by stepwise changes in anatomy, physiology, and behaviour, that is strictly linked

54   to ontogenetic changes in habitat and resource use (Cataldi et al. 1987; Tancioni et al. 2003;

55   Isnard et al. 2015). Research interest on this species is due not only to its high ecological value,

56   but also to its considerable commercial importance in fishery and aquaculture (FAO 2016).

57   Many studies, in the past decades, were conducted prevalently in controlled experimental

58   conditions, focussing on the morphological changes occurring during ontogeny of *S. aurata*.

59   These provided detailed knowledge about the development of body structures such as the

60   digestive tract (Elbal et al. 2004), the oral cavity and the way teeth differ with age (Cataldi et

61   al. 1987; Elgendy et al. 2016), the gonadal cycle (Zohar et al. 1978), osmoregulation (Bodinier

62   et al. 2010) or, more generally, the progressive changes of the body shape (Russo et al. 2007).

63   In these studies, authors agree that slight morphological variations during ontogeny promote

64   progressive capability to perform new behaviours related, above all, to feeding habits and

65   swimming ability (Russo et al. 2007; Elgendy et al. 2016). This was observed also for other

66   species in different aquatic systems. For instance, the June sucker *Chasmistes liorus*

67   (Cypriniformes) has been found to shift rapidly to double size preys during the ontogenetic

68   shift from larval to juvenile stage in the Utah Lake (Kreitzer et al. 2010), while the

69   Syngnathidae *Syngnathus typhle* shows a strong correlation between prey size ingested and

70   both total and snout length in Ria Formosa Lagoon (Oliveira et al. 2007). The diet of *S. aurata*

71   has been demonstrated to shift progressively from zooplankton during larval stage, to micro-,

72   meio- and macrozoobenthos once reached the juvenile and adult stages (Ferrari and Chieregato

73   1981; Cataldi et al. 1987; Russo et al. 2007; Elgendy et al. 2016).

74   Deepening the knowledge about the trophic ecology of the very first developmental stages of

75   fish in natural environments is important to encourage correct management of their essential

76   habitats and promote the most favourable conditions to the growth of adult populations. Few

77   studies, however, have deepened the relationship between ontogeny and resource use in natural

78   sites (see for example Escalas et al. 2015). More importantly, few studies have been conducted

79   in those habitats (both near-shore and transitional ones) where fish settle for a while during

80   their migration from the sea to coastal lagoons and *vice versa*, hence considering the

81   importance of spatial change.

82   As a consequence, there is a lack of quantitative information on the trophic niche dynamics of

83   *S. aurata*, either in terms of width and other related quantitative metrics, or in terms of trophic

84   pathways leading to the species during early development stages. New insights into trophic

85   niche dynamics can be provided by the use of carbon and nitrogen stable isotope analysis,

86   which has been extensively applied to trace the pathways of organic matter in a wide variety

87   of systems, and allows quantitative description of the trophic niche of species across time and

88   space (Layman et al. 2007; Jackson et al. 2011).

89   We investigated the trophic niche dynamics of *S. aurata* hypothesizing that habitat and

90   resources use of marine and lagoonal sites will exhibit strong relationship with ontogeny of the

91   species. Particularly, the aim of this study was to determine: (1) isotopic shift of *S. aurata*

92   muscle tissue occurring during growth; (2) ontogenetic variations of the isotopic niche features

93   and species' trophic position across nursery sites placed along the migration route from the sea

94   to a coastal lagoon; (3) trophic pathways supporting the different early development stages of

95   *S. aurata* within a coastal lagoon.

96

97   **Material and methods**

98   *Study area and sampling*

99   The study was carried out in the Lagoon of Venice as it represents an important nursery area

100 of *S. aurata* (Franzoi et al. 2002; Provincia di Venezia 2009). The Lagoon of Venice is located

101 in the Northern Adriatic Sea (Mediterranean Sea) and extends over 550 km^2; it is connected to

102 the sea by three wide mouths and can be hydrologically subdivided in three main sub-basins

103 (northern, central and southern, Solidoro et al. 2004). The water body has a mean depth of

104 about 1 ± 0.3 m (Bonfà et al. 2004), but it is crossed by deeper shipping channels that extend

105 towards the three openings and allow water exchange, while the tidal range is about ± 0.7 m

106 (Solidoro et al. 2010). The high variability of environmental conditions favours a high habitat

107 heterogeneity that characterizes the whole lagoon (Solidoro et al. 2010), that is designated as a

108 Special Protection Area (SPA, IT3250046).

109 To collect *S. aurata* at different life stages during their migration route between the lagoon and

110 the adjacent sea, sampling trips were conducted in spring 2014 and summer 2015 in lagoonal

111 and marine sites (Fig. 1). Two salt marsh sites were located within the northern sub-basin of

112 the lagoon, at different distances from the nearby mouth, named SM1 and SM2 (distance to

113 mouth respectively 2.2 and 5 km). In particular, fish were collected both at the outer edge and

114 in the intertidal creek of SM1 (named respectively SM1e and SM1c) and also in the intertidal

115 creek of SM2 (named SM2c). One marine/nearshore site adjacent to the lagoon (named MA)

116 was located in proximity of the northern mouth (distance to mouth 1.8 km); two sites were

117 located in the deep channels of the northern and central mouths of the lagoon (respectively

118 named MO1 and MO2) where larger individuals were expected to be collected during their

119 passage from or to open sea. Post-larvae and juveniles were collected through a small beach

120 seine net (8-10 m long, 2 mm mesh size), sub-adults were collected by angling, using a fishing

121 hook and line.

122   Fig. 1

123

124 Sources of organic matter were collected in triplicate in spring 2014 and 2015 at the outer edge

125 of the two salt marsh sites, SM1e and SM2e. Seagrasses, macroalgae and halophytes were

126 collected randomly by hand in triplicate. Only the most abundant halophytes species were

127　collected and considered for trophic assessments. Sedimentary organic matter (SOM) was

128　obtained by sampling three replicates of superficial sediment cores (3 cm Ø). In the same sites,

129　2 L of superficial water were collected for the analysis of particulate organic matter (POM).

130　Water was sampled in spring as proxy of lagoonal POM (l-POM), since it provides isotopic

131　signal of autochthonous inputs of organic matter, while additional water samples were collected

132　in autumn (rainy season) of both years (2014 and 2015) as proxy of terrigenous POM (t-POM),

133　since it is influenced by the increase in freshwater inputs and land runoff (Berto et al. 2013).

134　Zooplankton was also sampled to estimate the trophic position of *S. aurata* specimens (see

135　details later in the text) and was gathered by horizontal haul with a small plankton net (mesh

136　size 160 μm, mouth diameter 30 cm and length 90 cm). All the samples collected were

137　maintained refrigerated in thermic boxes until arrival at the laboratory and stored at -20°C prior

138　to the processing.

139

140　*Sample processing and laboratory analysis*

141　Fish were identified at species level and measured for standard length (SL) and total weight

142　(TW). According to the trophic ontogenetic shift previously observed (e.g. Cataldi et al. 1987;

143　Elbal et al. 2004), the size at first maturation (330-400 mm for females and 200-300 mm for

144　males according to Bauchot and Hureau 1986) and the size range of all specimens collected,

145　three size classes were defined and assigned to the following life stages: post-larvae, 14<SL<20

146　mm; juveniles, 20<SL<40 mm; sub-adults, 160< SL<210 mm.

147　Whenever available, at least 15 individuals for each life stage were dissected using scalpel and

148　tweezers to extract the dorsal muscle. In addition, in sub-adult individuals collected in 2015,

149　dorsal scales were removed and interpreted under a stereomicroscope for determination of the

150　age class, in order to check their assignment to the same cohort (1+) of the population sampled

151　in 2014. Primary producers (seagrasses, macroalgae and halophytes), water samples and

152   sediment cores were processed following Vizzini et al. (2016, 2017). Zooplankton samples

153   were concentrated in microcentrifuge tubes after removal of extraneous material under

154   binocular microscopy. After processing, all samples were oven dried, prepared for stable

155   isotope analysis (see Vizzini et al. 2016, 2017 for details) and analysed for $\delta^{13}C$ and $\delta^{15}N$ using

156   an isotope ratio mass spectrometer (Thermo-Electron Delta Plus XP) coupled to an elemental

157   analyser (Thermo-Electron Flash EA1112). Carbon and nitrogen stable isotope ratios were

158   reported based on the following equation: $\delta X = [(R_{sample}/R_{standard})-1] \times 10^3$, where the δ notation

159   expresses the parts per mil deviation from the reference international standards (Pee Dee

160   Belemnite and atmospheric N_2, respectively for carbon and nitrogen), X is ^{13}C or ^{15}N and R is

161   the relative $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratio. The analytical precision of the measurement based on the

162   standard deviation of replicates of internal standards (International Atomic Energy Agency

163   IAEA-CH-6 for $\delta^{13}C$ and IAEA-NO-3 for $\delta^{15}N$) was 0.1 and 0.2‰ for $\delta^{13}C$ and $\delta^{15}N$

164   respectively. Analysis was conducted for each specimen, except for small post-larvae collected

165   in MA that were pooled by five individuals to reach the minimum analytical weight.

166

167   *Data analysis*

168   Carbon and nitrogen isotopic data of *S. aurata* were analysed separately. Normality and

169   homogeneity of variances were tested using respectively Shapiro-Wilk and Bartlett tests. Since

170   none of the data showed suitable conditions for using parametric tests, non-parametric Kruskal-

171   Wallis test and relative post-hoc tests were used to assess differences in multiple comparisons

172   among fish life stages or sites.

173   Trophic positions (TP) were estimated using the following equation according to Post (2002):

174    $TP_f = [(\delta^{15}N_f - \delta^{15}N_b)/\Delta_n] + \lambda$. $\delta^{15}N_f$ and $\delta^{15}N_b$ are respectively the nitrogen isotopic signature

175   of the fish species of interest and that of a baseline, for which we used the mean value of

176   zooplankton samples (8.6‰). Δ_n is the trophic enrichment expected for each trophic level

177   (2.26‰, as specifically calculated by Isnard et al. (2015) for *S. aurata* juveniles) and λ is the

178   trophic position of the baseline. According to the ontogenetic shift of the species, *S. aurata* is

179   a direct consumer of zooplankton until approximately the size of 20-30 mm in SL (Ferrari and

180   Chieregato 1981), therefore we used $\lambda=1$ for individuals of the first size class and $\lambda=2$ for

181   bigger individuals. Comparisons of isotopic niche position within the isotopic space among life

182   stages or sites were based on non-parametric permutational MANOVA (PERMANOVA,

183   Anderson et al. 2008) test of the bivariate $\delta^{13}C$-$\delta^{15}N$ centroids, which represent the average

184   niche position (Muller and Strydom 2017). PERMANOVA was performed on Euclidean

185   distance matrix of normalised bivariate isotope data.

186   To assess trophic niche variability across life stages and sites, carbon and nitrogen stable

187   isotope data of each group (life stages or site) were used to estimate the standard ellipse area

188   corrected for small sample size (SEAc) and the Bayesian standard ellipse area (SEAb). The

189   SEAc was set to contain 60% of the data and provided a unique value, the SEAb output derived

190   from 100000 posterior iterations and was reported as mode along with 95% credible interval

191   (Jackson et al. 2011). Layman isotopic metrics (Layman et al. 2007) were estimated to quantify

192   the following descriptors of the isotopic niche: $\delta^{15}N$ Range (NR), which is the difference

193   between the most enriched and most depleted $\delta^{15}N$ values and estimates the trophic length of

194   the population; $\delta^{13}C$ Range (CR), which is the difference between the most enriched and the

195   most depleted $\delta^{13}C$ values and provides a measure of the diversity of basal resources used;

196   mean Distance to Centroid (CD), which is the average Euclidean distance of each species to

197   the centroid $\delta^{13}C$-$\delta^{15}N$ and describes the trophic diversity and species spacing within the

198   isotopic space; mean Nearest Neighbour Distance (NND), and its Standard Deviation

199   (SDNND), which is the Euclidean distance of each individual to the nearest neighbour, and

200   describes trophic redundancy, providing information on individuals density and packing within

201   the population. Additionally, in order to perform comparisons among life stages and sites, all

202 Layman metrics were bootstrapped (n = 10000) and then presented as mode along with 95%

203 credible interval (Jackson et al. 2011; Jackson et al. 2012). Bootstrapped metrics are indicated

204 with a subscript 'b'. Differences in SEAb and the bootstrapped metrics among life stages and

205 sites were tested through pair-wise comparisons, by calculating the probability that the metric

206 of one group is larger than that of the other, hence a significant difference was regarded as a

207 probability of at least 95% (Jackson et al. 2011).

208 Lastly, Bayesian mixing models were used to estimate the trophic pathways leading to *S.*

209 *aurata* during the different stages of development within the Lagoon of Venice. We ran four

210 separate models, combining the same consumers' dataset (*S. aurata* grouped by life stages)

211 with the organic matter sources collected in each lagoonal site (SM1e and SM2e) in the

212 corresponding year. The *a priori* assumption was that, during its residency within the lagoon,

213 the species could potentially populate both the salt marshes sampled. In each model we

214 included all the sources of organic matter: l-POM, t-POM, SOM, as well as all primary

215 producers' species (algae, seagrasses and halophytes), after testing for their significant

216 difference through PERMANOVA analysis, using a three factor design (factors Year, Site and

217 Source). PERMANOVA was run on Euclidean distance matrix of bivariate isotope data δ^{13}C-

218 δ^{15}N of all sources. When sources did not show significant differences they were grouped

219 before running the mixing model. Then, the contribution of different species of primary

220 producers belonging to the same category (algae, seagrasses and halophytes) where summed *a*

221 *posteriori* whenever necessary to simplify the graphical output. Results obtained for the four

222 models were then summarized and presented by life stage. Trophic enrichment factors (TEFs)

223 used in the model were respectively 1.26‰±0.67 for δ^{13}C and 2.26‰±0.75 for δ^{15}N (specific

224 for *S. aurata*, Isnard et al. 2015), that were multiplied by two to consider fish as second level

225 consumer.

226   All statistical tests and models but PERMANOVA, were performed in R (R Core Team, 2015).

227   SEAc, SEAb, and the overlap between ellipses were elaborated with SIBER package v2.0.2

228   (Stable Isotope Bayesian Ellipses in R) (Jackson et al. 2011). Layman isotopic metrics (Layman

229   et al. 2007) and mixing models were run using SIAR 4.2.2 (Parnell et al. 2010). PERMANOVA

230   was run in PRIMER 6 & PERMANOVA+ (PRIMER-E Ltd, Plymouth, UK).

231

232   **Results**

233   *Demographic characteristics*

234   Throughout all the sampling sites, 158 individuals of *Sparus aurata* were collected (Table 1).

235   Their distribution within the study area showed a clear pattern in accordance with

236   developmental stage. Post-larvae were found in spring 2014 both in the marine and the lagoon

237   sites (respectively MA and SM1e). Later in the same season, juveniles were collected mainly

238   within the intertidal creeks of the salt marshes (SM1c and SM2c). Lastly, sub-adults were

239   caught at both mouths (MO1 and MO2) of the lagoon in summer 2015. Age determined through

240   dorsal scales extracted from sub-adults confirmed that individuals sampled in 2015 were 1+

241   old, therefore belonged to the same cohort as juvenile population sampled in 2014.

242   Table 1

243

244   *Ontogenetic shift of isotopic signatures, isotopic niche and trophic position*

245   Isotopic signatures of all the individuals of *Sparus aurata* ranged widely between -21.3‰ and

246   -12.8‰ for $\delta^{13}C$ and between 8.1‰ and 14.5‰ for $\delta^{15}N$ (Fig. 2). The relationship between

247    $\delta^{13}C$ and size (SL) for the early stage of development (from post-larvae to juveniles) was

248   described by a logarithmic curve, which explained 74% of the relationship (Fig. 2a): post-

249   larvae showed a steep enrichment as they grew up to approximately 20 mm (SL) and then

250   stabilize around values within the range between -15.9‰ and -12.8‰. Regarding the $\delta^{15}N$, the

251 same trend as the δ^{13}C characterized the post larvae and juveniles up to 31 mm and was

252 described by a logarithmic curve, which explained 58% of the relationship (Fig. 2c). Lastly,

253 sub-adults showed quite stable δ^{13}C, but large variability for δ^{15}N without any remarkable trend

254 (Fig 2b and d).

255 Fig. 2

256 Distribution of standard ellipse areas (SEAcs) of the different *S. aurata* life stages within the

257 isotopic space (δ^{13}C-δ^{15}N biplot) showed a clear ontogenetic shift of the corresponding isotopic

258 niches (Fig. 3), which varied in position, shape and size. Comparisons of niche positions based

259 on δ^{13}C-δ^{15}N centroids confirmed that post-larvae were separated from both juveniles and sub-

260 adults (PERMANOVA p-value = 0.001, Table 1S), which instead shared the centroid location

261 (p-value = 0.222). Indeed, overlap between juveniles and sub-adults accounted for 39% and

262 50% of their respective SEAc.

263 As the fish size increased, isotopic niche moved along both axes, with a significant shift from

264 post-larvae to juveniles, towards more enriched isotopic signatures of both δ^{13}C and δ^{15}N

265 (*Kruskal-Wallis post-hoc test*, p-value<0.05). Then, sub-adults stabilized around similar δ^{15}N

266 range of the juveniles, but displayed a slight depletion in δ^{13}C.

267 Fig. 3

268 Niche width reported as SEAb for post-larvae (mode 4.0‰^{2}) was significantly larger than that

269 of juveniles (2.0‰^{2}, probability of difference > 95%) but not of sub-adults (2.5‰^{2}, probability

270 of difference < 95%) (Table 2). Post-larvae showed the widest ranges of both nitrogen range

271 and trophic diversity (respectively NR_b and CD_b) compared to both juveniles and sub-adults.

272 Conversely, carbon range (CR_b) of post-larvae, which accounts for the diversity of basal

273 sources used, was comparable to that of juveniles but significantly higher than sub-adults

274 (Table 2). No significant differences occurred for trophic redundancy (NND_b) and evenness of

275 the individuals spacing ($SDNND_b$) among life stages (Table 2).

276   Table 2

277

278   *Inter-site variability of the isotopic niche*

279   Position, shape and size of *Sparus aurata* isotopic niches showed a remarkable variability also

280   across the sites sampled (Fig. 3). Following the migration route of the species, a significant

281   shift of the isotopic niche centroid occurred from the marine site (MA) to both salt marsh sites

282   (SM1e and SM2c), and within the same salt marsh, from the edge (SM1e) to the intertidal creek

283   (SM1c). Such shift occurred towards a more carbon and nitrogen-enriched area of the isotopic

284   space (PERMANOVA p-values<0.05 in all cases mentioned, see Table 2S), where layed also

285   the niches of *S. aurata* captured at the lagoon mouths (MO1 and MO2).

286   Post-larvae caught in the marine site MA, just before entering the lagoon, showed the smallest

287   isotopic niche width (SEAb $0.11‰^2$). Accordingly, their niche extended over the narrowest

288   ranges of carbon and nitrogen (respectively CR_b and NR_b), displayed the lowest trophic

289   diversity (CD_b) and the highest trophic redundancy (lowest NND_b) compared to all the other

290   sites (Table 3). Conversely, post-larvae found at the intermediate salt marsh SM1e showed a

291   significantly wider isotopic niche (SEAb $5.0‰^2$, associated probability >95%, Table 3),

292   spanning over the most extended ranges of both carbon and nitrogen (respectively 5.5‰ for

293   CR_b and 4.3‰ for NR_b) and showing the highest level of trophic diversity (CD_b 1.4‰).

294   Juveniles caught at both the intertidal creeks sampled, SM1c and SM2c, shared a similar

295   position of the trophic niche, but niche in SM1c showed a significantly higher width (SEAb)

296   and trophic diversity (CD_b) than in SM2c. Lastly, in the two sites located at the deep channels

297   of the lagoon mouths (MO1 and MO2), where only sub-adults were caught, *S. aurata* had

298   overlapped isotopic niches but significantly different widths. In fact, sub-adults at MO1

299   showed significantly smaller niches than sub-adults of MO2 and clustered on a more restricted

300   carbon range (CR, although not significant). However, sub-adults of both mouth sites showed

301 comparable values of the other metrics, indicating similar trophic structure in terms of

302 diversity, redundancy and evenness of species spacing. Overall, the extent of SEAc overlapped

303 among the juveniles of SM1c-SM2c and the sub-adults of the MO1-2 ranged between 21 and

304 81%.

305   Table 3

306 Trophic positions (TP) calculated at the individual level exhibited an increasing trend,

307 following both the migration and the growth of the species (Fig. 4). Overall, *S. aurata* post-

308 larvae had the lowest TP in MA (mean 0.9±0.1). Then it progressively raised during

309 development to bigger post-larvae, juveniles and the parallel shift into the inner sites of the

310 lagoon (mean values 1.8±0.7 in SM1e, 3.2± 0.5 in SM1c and 3.7±0.3 in SM2c). Lastly, TP

311 reached the highest values for sub-adults (MO1 and MO2, mean respectively 3.9±0.6 and

312 3.7±0.5).

313   Fig. 4

314

315 *Sources of organic matter and mixing models results*

316 Sites sampled for organic matter sources (SM1e and SM2e) showed different macrophytes

317 composition (see Supplementary material, Tab. 3S for complete species list). In terms of

318 species recorded, the most confined site (SM1e) showed a higher diversity of both seagrasses

319 and macroalgae compared to the less confined salt marsh (SMe2). The only species found in

320 common to both sites were the seagrass *Zostera noltei* Hornemann 1832 in 2014, the red

321 macroalga *Agardhiella subulata* Kraft & Wynne 1979 in 2015, and the green alga *Ulva rigida*

322 Agardh 1823 in both years. Regarding halophytes, three species were the most abundant in the

323 sites investigated, with *Sarcocornia fruticosa* (L.) A. J. Scott present in all of them.

324 Overall, all organic matter sources collected showed similar isotopic structure among sites and

325 years, with both carbon and nitrogen isotopic ratios falling within similar ranges (Fig. 5). In

326   particular, overall $\delta^{13}C$ ranges in 2014 were between -27.7 and -10.27‰ in SM1e and between

327   -27.8 and -11.4‰ in SM2e, while in 2015 $\delta^{13}C$ ranged between -28.4 and -8.8‰ in SM1e and

328   between -27.2 and -10.7‰ in SM2e. As regards $\delta^{15}N$, overall range in 2014 spanned between

329   2.0 and 12.7‰ in SM1 and between 2.5 and 10.6‰ in SM2e, whereas overall $\delta^{15}N$ ranges in

330   2015 were between 3.9 and 10.3‰ in SM1e and between 3.3 and 9.6‰ in SM2e. However,

331   results of PERMANOVA highlighted significant differences among bivariate isotope signature

332   of the sources for the interaction of all the factors Year, Site and Source (Table 4Sa).

333   Accordingly, pairwise tests highlighted significant differences between years for most of the

334   sources within each site (Table 4Sb), and also in the majority of the cases between sites within

335   each year and most of sources in common (Table 4Sc). Lastly, significant differences emerged

336   also between most of sources within year and sites (Table 4Sd), with the only exception of two

337   macroalgae sources at site SM1e in 2014, which were grouped before running the mixing

338   model.

339   Fig. 5

340   Results of mixing models provided evidence that the contribution of the potential sources of

341   organic matter to the trophic pathway of *Sparus aurata* clearly varied across life stages,

342   particularly between post-larvae and other classes, with slight differences across sites (see

343   Supplementary material, Fig. 1S). Overall results summarized by life stage (Fig. 6) showed

344   that a mixture of l- and t-POM, SOM seagrasses, macroalgae, and also halophytes, accounting

345   for about 17% each, drove the trophic pathway of post-larvae within the lagoon. Conversely,

346   the main sources of organic matter supporting juveniles and sub-adults were mainly

347   macroalgae followed by seagrasses, accounting respectively for about 69% and 20% for

348   juveniles and 37% for both sources for sub-adults.

349   Fig. 6

350

Discussion

Trophic niche variability across life stage

Isotopic signatures, especially $\delta^{15}N$, are commonly recognized to increase with body size in fishes due to multiple reasons: changes in preys' size (Fry et al. 1999; Vander Zanden et al. 2000; Badalamenti et al. 2002), alteration of catabolism due to starvation (Sweeting et al. 2007; Varela et al. 2015), growth rate (Trueman et al. 2005 among others). The shift towards more enriched values of $\delta^{15}N$ and $\delta^{13}C$ observed during the earliest stages of growth and development of *Sparus aurata* (post-larvae), subtends a shift in resource use that is strictly linked to the trophodynamic of the gilthead seabream. In fact, such development stage realistically corresponds to the transition phase from a pelagic, planktivorous feeding habit, typical of the post-larvae living in (or entering from) the open sea, to a benthic diet, typical of when they enter into the shallow environments of the lagoon (Ferrari and Chieregato 1981; Russo et al. 2007). The shift in resource use has been found strictly linked also to the development of oral cavity and teeth during growth. As reported by Cataldi et al. (1987) and Elbal et al. (2004), post-larvae up to 20-25 mm in standard length show canine-like teeth that allow ingestion of only small planktonic preys, while the gastrointestinal tract develops in its definitive morphology. In juveniles around 35 mm standard length, three concentric rows of canine teeth and the presence of "transitional teeth" that will develop in molars (Cataldi et al. 1987), promote a benthivorous diet (Russo et al. 2007). In adults, strong molars in addition to canines allow predation on hard preys such as decapods, gastropods and bivalves (Francescon et al. 1987; Elgendy et al. 2016). Indeed, the evident isotopic shift reported in this study for the first size class can also be addressed to the higher turnover rate occurring in the muscles of younger individuals (Bosley et al. 2002; Heady and Moore 2013). Hence, diet shifts in early juveniles are generally more visible than in older juveniles, whose turnover rates decrease with

age and longer time is needed to integrate the information into the muscle tissues (Martínez Del Rio et al. 2009; Layman et al. 2012).

Ontogenetic changes reflected on trophic niche as described by the estimates of the isotopic niche: during different development stages *S. aurata* occupied different isotopic niches within the bi-plot δ^{13}C-δ^{15}N. The main difference emerged between post-larvae and the older individuals, both in terms of occupied space and width, highlighting two completely different feeding habits. Furthermore, the absence of overlap between these two main groups suggests that they evidently rely on different pathways and resources. The threshold of change was well defined around 20 mm (standard length) and was similar to what found from Ferrari & Chieregato (1981) for populations of the Po River Delta in the Adriatic Sea.

The width of the isotopic niche, higher for post-larvae and narrower for older size classes, needs to be described together with its metrics (Layman et al. 2007). According to previous studies on trophic niche, generalist species show a wider niche width in comparison to specialist species, as the first ones rely on a wider variety of sources, whilst the second ones feed on restricted types of preys (Bearhop et al. 2004; Layman et al. 2007). Thus, this reasoning would erroneously lead us to conclude that post-larvae just settled within the lagoon (SM1e) are somewhat more generalist than juveniles and sub-adults (SM1c-2c-MO1) given the differences found in the niche widths. However, in our study, post-larvae showed a high individual specialization (Post 2003; Matthews and Mazumder 2004) that explains a higher trophic diversity to which is attributable the significant increase in isotopic niche width found. In fact, an "intra-class" shift is clearly visible within post-larval stage, that not only is due to the ability of slightly bigger individuals to feed on slightly bigger preys (Russo et al. 2007; Cummings et al. 2012), but more importantly is related to the migration of the species within the lagoon. This change of habitat, in turn, induces a transition from a planktonic diet to a benthic diet. In this sense, there is an high habitat effect in determining the features of the

400   trophic niche of the post-larvae, that once in the lagoon, colonize shallow water areas assuming

401   progressively a benthic habitus. This results in a notable difference in trophic diversity of the

402   different life stages. In fact, the isotopic distance of individuals from the centroid of the isotopic

403   niche (CD, representing the trophic diversity), was found almost double for post-larvae than

404   for the older life stages. On the other hand, the distribution of juveniles and sub-adults within

405   their isotopic niches followed a progressive lower trophic redundancy and increasing spacing.

406   This is in agreement with the shift towards a more diversified feeding behaviour of the older

407   individuals of the species (Francescon et al. 1987; Hadj Taieb et al. 2013) and also explains the

408   variability of δ^{15}N observed in sub-adults. Consequently, caution must be taken when analysing

409   isotopic niches just based on the width, especially when dealing with species undergoing

410   ontogeny (Hammerschlag-Peyer et al. 2011).

411

412   *Trophic niche variability across sites*

413   Due to the migratory movements of *Sparus aurata* between the open-sea and costal lagoons

414   and its commercial value, it is important to undestand how lagoon nursery sites support

415   trophically the different stages of the species development (Isnard et al. 2015; Tournois et al.

416   2017). Stable isotope ratios, especially δ^{13}C, have been widely used to follow organism

417   movements between different feeding sites (Rubenstein and Hobson 2004; Hobson and

418   Wassenaar 2008; Graham et al. 2010; Carlisle et al. 2012), as different basal sources vary

419   greatly in carbon isotopic signature and the trophic fractionation between trophic levels is small

420   (Post 2002; Caut et al. 2009; Isnard et al. 2015). In addition, quantitative isotopic niche and

421   relative metrics allowed a good description of the trophic niche ontogeny during migratory

422   pattern (Muller and Strydom 2017). *S. aurata* followed a progressive shift of the trophic niche

423   towards more δ^{13}C-enriched areas of the isotopic space, as the species moved from the sea

424   towards inner sites of the Venice Lagoon, and then a return to δ^{13}C-depleted but more δ^{15}N-

425 enriched area, once *S. aurata* reached the sub-adult stage. This pattern indicated a clear

426 correspondence between the migration movements and the diet shift during ontogeny,

427 confirming the progressive shift from pelagic to benthic resources use (Ferrari and Chieregato

428 1981; Russo et al. 2007). Likewise, the width of the isotopic niche increased and trophic

429 positions raised progressively following the same direction.

430 Overall, we found minor differences in trophic niche features between sub-adults coming from

431 different deep channel sites (the two mouths MO1 and MO2) compared to the considerable

432 differences between juveniles, even on a very small spatial scale, coming from lagoonal

433 shallow water sites (e.g. different portions, edge and intertidal creeks, of the same salt marsh).

434 Particularly, post-larvae collected in spring before entering the lagoon, hence with a diet based

435 mainly on marine zooplankton (Ferrari and Chieregato 1981; Cataldi et al. 1987; Elbal et al.

436 2004), showed the narrowest isotopic niche width typical of specialist feeders. Then, once

437 settled in the shallow waters of the lagoon, where habitats are characterized by high

438 productivity and a wider availability of resources, the species underwent rapid growth. As a

439 consequence, the enhanced feeding ability led to significant changes of the trophic niche that

440 became wider, raised its trophic position and increased its trophic diversity.

441 Interestingly, post-larvae and juveniles collected from different parts of the same salt marsh,

442 respectively from the outer edge and the inner creek, displayed similar isotopic niches in terms

443 of width (SEAb), but a different position in the $\delta^{13}C$ vs. $\delta^{15}N$ bi-plot. The shift of juveniles

444 towards more ^{13}C-enriched values indicates a diet shift from zooplankton to zoobenthos

445 (Ferrari and Chieregato 1981; Salvatrice Vizzini et al. 2005), and niche features higher

446 specialisation, reduced trophic diversity, increased redundancy and reduced evenness. Lastly,

447 the trophic varability of sub-adults catched at the two mouths of the lagoon (central and

448 northern) can be related to the high trophic plasticity and increased mobility of the species, that

449 during its permanence inside and outside the lagoon may have taken advantage of different

450　feeding opportunities, as typical for generalist and opportunistic species (Elliott et al. 2007;

451　Franco et al. 2008). Indeed, although the isotopic niche of *S. aurata* catched in proximity of

452　the central mouth was wider than that of the individuals catched in the northern mouth, the

453　metrics confirmed a similar trophic organization of individuals, with similar levels of trophic

454　diversity, redundancy and evenness of the species within the isotopic space.

455

456　*Trophic role of the lagoon as a nursery area for* Sparus aurata

457　Mixing model estimates provided a quantitative description of the shift in basal sources

458　supporting the early development stages of *Sparus aurata*. Despite slight differences due to the

459　variability of the basal sources occurring in the sites taken into account (SM1e and SM2e), the

460　overall picture of the results highlights a difference between the trophic pathways leading to

461　the post larval stage and the ones leading to juveniles and sub-adults.

462　The mixture of particulate organic matter of lagoonal and terrestrial origin plays a major role

463　exclusively for post-larvae independently from the lagoon site considered (Escalas et al. 2015)

464　and reflects their ability to exploit zooplankton (Russo et al. 2007), of which POM is partially

465　composed (micro-zooplankton). The non-negligible contribution of sedimentary organic

466　matter to post-larvae, instead, could be attributable to the frequent resuspension events that

467　usually characterize such shallow environments (Vizzini et al. 2005; Vizzini and Mazzola

468　2008). In accordance with different studies investigating trophic dynamics in coastal lagoons

469　(*e.g.* Vaslet et al. 2011; Vizzini 2009), our results highlighted the importance of macrophytes

470　(both macroalgae and seagrasses) as predominant contributor to the organic matter pathways

471　supporting the target species, especially for juveniles and sub-adults.

472　Conversely, halophytes structuring the salt marsh edges did not contribute to the trophic

473　pathway of the species, meaning that their role is mainly structural as habitat former rather than

474　trophic. Overall, such crucial difference emerged among the two trophic pathways supporting

475   the post-larvae from the following life stages is in accordance to their diet shifting from

476   zooplanktivorous to zoobenthivorous habit (Ferrari and Chieregato 1981; Cataldi et al. 1987;

477   Russo et al. 2007).

478   Overall, the most evident and significant differences among the development stages of *Sparus*

479   *aurata* occur between post-larvae and the older stages. Given the same size, morphology and

480   development stage of *S. aurata*, it is the transition from the water column to the shallow water

481   habitat itself that seems to determine a clear change in the trophic niche. Whereas trophic

482   differences found between individuals caught within lagoon habitats could be driven by a

483   different time of arrival in those habitats, but also by a wider diversity of the resources used,

484   which in turn is given both by the diversity of the preys available and by the spatial

485   heterogeneity of their distribution. Indeed, lagoon shallow water habitats are characterized by

486   much more varied and heterogeneous trophic complexity than the pelagic marine environment

487   (where post-larvae live until the arrival to more near-shore sites).

488

489   **Conclusion**

490   Overall, this study emphasises the importance of lagoon shallow water habitats as nursery areas

491   for the early stages of the gilthead seabream *S. aurata* life cycle, whose role should be taken

492   into account when discussing the implications that may result in terms of coastal management.

493   Special attention should be paid to the conservation strategies of those lagoonal habitats, such

494   as the salt marshes, that are increasingly threatened by the effects of anthropic pressures and

495   climate change (Bellafiore et al. 2014), and whose irreversible regression would result in loss

496   of crucial ecological function of lagoon systems. Despite their negligible trophic contribution,

497   in fact, salt marshes play a key role in increasing the habitat heterogeneity of the system,

498   structuring ideal nursery grounds for juveniles of several migrant species. The main trophic

499   role, instead, is played by the aquatic macrophytic component, as macroalgae and seagrasses

500 enter the trophic pathways providing more than the 60% of trophic support as sources of

501 organic matter for juveniles and sub-adults. Therefore, high structural complexity (high habitat

502 heterogeneity even at small spatial scale), together with a high trophic complexity (many

503 trophic contributors) lay the foundations for the coastal lagoon system to support many among

504 the most productive ecosystem services, such as fishery.

505

506 **Acknowledgments**

512

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514 **References**

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731 **Tables**

732 **Table 2** SEAc (Standard Ellipse Area corrected, ‰^{2}), mode of the SEAb (Bayesian Standard Ellipse
733 Area , ‰^{2}) and bootstrapped ($_b$) isotopic metrics: NR, Nitrogen Range, CR, Carbon Range, CD,
734 Distance to Centroid, NND, Nearest Neighbour Distance, SDNND, Standard deviation of the Nearest
735 Neighbour Distance (‰). Relative 95% credible intervals calculated for each life stage of *S. aurata*
736 are in brackets. Same letters in apex indicate not significant differences (p > 0.05) based on the
737 probability associated with paired comparisons

	post-larvae	juveniles	sub-adults
SEAc	3.1	2.2	2.9
SEAb	4.0^a (2.3 - 7.5)	2.0^b (1.1 - 3.5)	2.5^{ab} (1.2 - 4.8)
NR$_b$	4.9^a (2.8 - 4.9)	3.0^b (1.2 - 3.0)	3.6^b (1.8 - 3.6)
CR$_b$	5.4^a (2.3 - 5.4)	3.0^{ab} (1.1 - 3.0)	2.3^b (0.7 - 2.3)
CD$_b$	1.9^a (1.1 - 2.6)	0.9^b (0.5 - 1.3)	1.2^b (0.7 - 1.5)
NND$_b$	0.3^a (0.1 - 0.7)	0.3^a (0.1 - 0.6)	0.5^a (0.1 - 0.8)
SDNND$_b$	0.5^a (0.1 - 1.1)	0.5^a (0.1 - 0.7)	0.6^a (0.2 - 0.8)

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740 **Table 3** SEAc (Standard Ellipse Area corrected, ‰^{2}), mode of the SEAb (Bayesian Standard Ellipse

741 Area, ‰^{2}) and bootstrapped ($_b$) isotopic metrics: NR, Nitrogen Range, CR, Carbon Range, CD, Distance

742 to Centroid, NND, Nearest Neighbour Distance, SDNND, Standard deviation of the Nearest Neighbour

743 Distance (‰) with relative 95% credible intervals calculated for *S. aurata* in each site sampled. Same

744 letters in apex indicate not significant differences (p > 0.05) based on the probability associated with

745 paired comparisons

	MA	SM1e	SM1c	SM2c	MO1	MO2
SEAc	0.1	3.4	3.7	1.6	1.5	4.3
SEAb	0.1^a	5.0^b	3.2^b	1.1^c	1.0^c	2.9^{bc}
	(0.1 - 0.4)	(3.0 - 17.3)	(2.0 - 7.5)	(0.7 - 3.4)	(0.6 - 3.3)	(1.7 - 10.4)
NRb	0.7^a	4.2^{ab}	2.4^b	2.3^{ab}	3.3^{ab}	3.2^{ab}
	(0 - 0.7)	(0 - 4.3)	(0.8 - 2.4)	(0.1 - 2.3)	(0 - 3.3)	(0 - 3.2)
CRb	0.2^a	5.5^{abc}	5.4^b	1.6^c	0.7^{ac}	2.2^{ac}
	(0 - 0.5)	(0 - 5.3)	(1.7 - 5.4)	(0.3 - 1.6)	(0 - 0.7)	(0 - 2.3)
CDb	0.3^a	1.6^{abc}	1.4^b	0.7^c	0.7^{abc}	1.2^{bc}
	(0 - 0.4)	(0 - 3.3)	(0.7 - 2.1)	(0.2 - 1.)	(0 - 1.5)	(0 - 1.6)
NNDb	0.2^a	1.2^a	0.5^a	0.5^a	0.9^a	1.5^a
	(0 - 0.5)	(0 - 3.531)	(0.1 - 1.1)	(0 - 1.0)	(0 - 1.6)	(0 - 2.3)
SDNNDb	0.1^a	2.1^{ab}	0.9^b	0.5^{ab}	0.2^{ab}	0.6^{ab}
	(0 - 0.5)	(0 - 4.0)	(0.2 - 1.2)	(0 - 1.0)	(0 - 1.9)	(0 - 2.0)

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750   **Fig. 1** Study area and sampling sites. Lagoon sites SM1-2 are salt marshes located at increasing distance

751   from the Northern mouth. MA is the marine site adjacent to the lagoon. MO1 and MO2 are the deep

752   channels sites corresponding respectively to the Northern and Central mouths

753   **Fig. 2** Relationship between standard length (SL) and isotopic signatures (δ^{13}C and δ^{15}N) of *S. aurata*

754   for post-larvae and juveniles (a, c) and sub-adults (b, d). Trend curves are showed, and relative equation

755   and R^2 values reported in each panel

756   **Fig. 3** δ^{13}C (‰) *vs.* δ^{15}N (‰) of *Sparus aurata* grouped by size class and site. Colours remark size

757   classes: post-larvae (light grey), juveniles (intermediate grey) and sub-adults (dark grey). Symbols

758   remark sites: MA, marine (inverted triangle); SM1e, edge of the intermediate salt marsh (circle); SM1c,

759   intertidal creek of the intermediate salt marsh (diamond); SM2c, intertidal creek of the salt marsh

760   nearest to the mouth (triangle); MO1, northern mouth (plus) and MO2, central mouth of the Venice

761   Lagoon (square). Ellipses represent isotopic niches: full ellipses enclose the corrected Standard Ellipse

762   Areas (SEAc) by size class, while solid lines enclose the SEAcs by site

763   **Fig. 4** Boxplots showing trophic positions (TP) of *S. aurata* grouped by life stage and site. In each box,

764   horizontal line is the median, lower and upper sides of the box are 25th and 75th percentiles, lower and

765   upper whiskers are min and max values, diamond is the mean, circles are outliers

766   **Fig. 5** Mean ($\pm$ s.d.) δ^{13}C (‰) *vs.* δ^{15}N (‰) of *S. aurata* by life stage and sources of organic matter

767   sampled each year (2014 and 2015) in each site located at different distance from the mouth: SM1e,

768   farther; SM2e, closer

769   **Fig. 6** Summarized output of mixing models, with each box containing the percentage contribution of

770   a source obtained from the two models run for each salt marsh site (SM1e-2e) for the same life stage

771   class of *S. aurata*. Boxes present the relative credible intervals of 95% (light grey), 75% (intermediate

772   grey) and 50% (dark grey) and mode (black circle). Among sources, l-POM is lagoonal-particulate

773   organic matter, t-POM is terrigenous-particulate organic matter, SOM is sedimentary organic matter,

774   Algae is macroalgae, Seag is seagrass and Halo is halophytes. See Figure 1S for outputs of the separate

775   models