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1 **Diet and trophic interactions of Mediterranean planktivorous fishes**

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21 **Highlights**

- 22 • Diets of seven Mediterranean planktivorous fishes: *Engraulis encrasicolus*,
23 *Sardina pilchardus*, *Sardinella aurita*, *Sprattus sprattus*, *Cepola macrophthalma*,
24 *Chromis chromis* and *Boops boops*, are detailed.
- 25 • Copepods, decapod larvae and fish eggs were their main prey.
- 26 • Prey groups, size distribution and average size differed among fish species.
- 27 • Stomach content and stable isotope compositions showed contrasted results in
28 terms of dietary niches, overlap and prey diversity among fishes.
- 29 • Stable isotope compositions displayed more similarities in diet between studied
30 fish species than stomach content analysis, suggesting potential trophic
31 competition, particularly among Engraulidae and Clupeidae.
- 32 • Even in coastal zones, small pelagic fish did not seem to compete much with the
33 main pelago-demersal planktivorous fish species for their food resources.

34

35 **Keywords**

36 Small pelagic fish; diet composition; prey-predator size-ratio; trophic niche overlap;
37 food competition

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41 **Abstract**

42 Diet and trophic interactions of seven species of planktivorous fishes: European
43 anchovy *Engraulis encrasicolus*, European sardine *Sardina pilchardus*, round
44 sardinella *Sardinella aurita*, European sprat *Sprattus sprattus*, red bandfish *Cepola*
45 *macrophthalma*, damselfish *Chromis chromis* and bogue *Boops boops*, were studied in
46 the Bay of Marseille (North-Western Mediterranean Sea) from March to September
47 2017. Taxonomic composition and size distribution of prey were studied using
48 stomach content analysis, and compared to prey availability determined by continuous
49 zooplankton sampling at a fixed point. Frequently consumed items included copepods,
50 decapod larvae and fish eggs. Comparatively, *E. encrasicolus* consumed more
51 calanoid copepods (i.e. *Centropages* spp.), *S. pilchardus*, *S. aurita* and *S. sprattus*
52 consumed more harpacticoid copepods (i.e. *Microsetella/Macrosetella* spp.), *C.*
53 *macrophthalma* consumed more decapod larvae, *C. chromis* consumed more
54 pteropods and *B. boops* consumed more benthic polychaetes. *Sardina pilchardus*
55 consumed the widest diversity of prey. Prey size distribution and average prey size
56 significantly differed among species. The prey-predator size ratio (PPSR) was highest
57 for *B. boops* and lowest for *S. aurita*. Carbon and nitrogen stable isotope composition
58 of species overlapped, probably due to the ingestion of particulate organic matter
59 from the same sources at the base of the planktonic food web in the Bay of Marseille.
60 Furthermore, trophic niche overlap supported the hypothesis of potential trophic
61 competition between Engraulidae and Clupeidae.

62 1. Introduction

63 Small planktivorous fish represent a fundamental trophic group in many ecosystems
64 (Peck et al. 2020) as they feed on diverse plankton groups and are forage species for
65 many predators including mackerel, hake and tuna (Palomera et al. 2007; Bănaru et al.
66 2013, 2019). Small planktivorous fishes also have a high commercial value as they
67 represent more than 50% of the annual landings in the Gulf of Lion and more widely
68 in the Mediterranean Sea (Bănaru et al. 2013; FAO 2020).

69 Bottom-up changes in the pelagic food web induced by climate change via
70 hydrological processes are suspected to be one of the major drivers of planktivorous
71 fish populations, including in the North-Western Mediterranean Sea (NWMS)
72 (Moullec et al. 2016 and references therein; Bănaru et al. 2019; Feuilloley et al.
73 2020). Moreover, the NWMS has also been impacted by changes in the management
74 of sewage water, leading to a reduction of nutrient inputs (Raimbault et al. 2021) that
75 may potentially impact plankton communities, as demonstrated in other
76 Mediterranean areas (Pagès et al. 2020). Zooplankton play a pivotal role in marine
77 ecosystems, as they support fisheries production and mediate fluxes of nutrients and
78 chemical elements (Banse 1995). Mesozooplankton of 200 μm to 1000 μm in length,
79 mainly consisting of copepods, constitute the main prey of adult planktivorous fish,
80 and the bottom-up control hypothesis involves changes in the quantity or quality of
81 these prey (Le Bourg et al. 2015). In addition, recent studies have demonstrated that
82 the quality of plankton depends on both taxonomic group and size class
83 (Harmelin-Vivien et al. 2019; Chen et al. 2019a).

84 In coastal areas, the changes described above may have contributed to the reduction of
85 planktivorous fish biomass and their relative proportions in fish communities
86 (Ourgaud et al. 2015; Cresson et al. 2019). In the NWMS, it has been hypothesized

87 that changes in both taxonomic composition and individual size of zooplankton may
88 underlie recently observed changes in the diet of some planktivorous fish species such
89 as the European pilchard and the European anchovy (Le Bourg et al. 2015; Chen et al.
90 2019a). A recent decline in planktivorous fish biomass, abundance, body size and
91 relative body condition has been observed and may have led to changes in fish diet
92 (STECF 2015; Ourgaud et al. 2015; GFCM 2019; Saraux et al. 2019; Cresson et al.
93 2019; Chen et al. 2021). In the changing environmental context of the Mediterranean
94 Sea, this bottom-up control hypothesis highlights the need to study the composition of
95 zooplanktivorous fish diet and trophic interactions, and to consider zooplankton prey
96 groups and size in order to better estimate their role in the pelagic food webs.
97 Knowledge of dietary specialization, including potential sharing of food resources
98 among co-occurring members of the fish community, would help determine functional
99 roles and to identify species for which potential competitive interactions during
100 periods of prey scarcity may occur (Burbank et al. 2019). This is essential for
101 ecosystem management and conservation (Bachiller et al. 2021).

102 In the NWMS, previous studies on planktivorous fish diet have focused on the
103 taxonomic composition of stomach contents (Stergiou and Karpouzi 2002; Costalago
104 and Palomera 2014). Some of these studies were based on the isotopic composition of
105 muscle tissue and of the prey of planktivorous fish (Cresson et al. 2014; Costalago et
106 al. 2014; Brosset et al. 2016), and some of them also provided information on prey
107 size structure (Le Bourg et al. 2015; Albo-Puigserver et al. 2019; Chen et al. 2021).
108 Combining stable isotope analyses (SIA) and stomach content analyses (SCA) offers
109 different and complementary insights into trophic relationships. While SCA provide
110 information on recently consumed prey (hours to one day), SIA (measurement of $\delta^{13}\text{C}$
111 and $\delta^{15}\text{N}$ values) of muscle tissues [provides](#) information on food sources integrated
112 over time (weeks to months) (Vander Zanden et al. 2015). An underlying assumption

113 is that isotopic compositions accurately reflect species dietary breadths over a period
114 of time defined by tissue-specific turnover rates.

115 The present study focused on the diet and trophic interactions of seven planktivorous
116 fish species: European anchovy *Engraulis encrasicolus* (Linnaeus, 1758)
117 (Engraulidae), European sardine *Sardina pilchardus* (Walbaum, 1792) (Clupeidae),
118 round sardinella *Sardinella aurita* (Valenciennes, 1847) (Clupeidae), European sprat
119 *Sprattus sprattus* (Linnaeus, 1758) (Clupeidae), red bandfish *Cepola macrophthalma*
120 (Linnaeus, 1758) (Cepolidae), damselfish *Chromis chromis* (Linnaeus, 1758)
121 (Pomacentridae) and bogue *Boops boops* (Linnaeus, 1758) (Sparidae) in the Bay of
122 Marseille, North-Western Mediterranean Sea. The study was performed from March
123 to September 2017. These species represent a range in body sizes, shapes, and habitat
124 occupations (entire water column in the case of pelagic Engraulidae and Clupeidae
125 species, benthic and pelagic environments for the demersal *B. boops* and *C. chromis*,
126 and burrows in soft-bottoms for *C. macrophthalma* (Stergiou and Karpouzi 2002)).
127 Several previous studies have focused on diet, notably in commercially important *E.*
128 *encrasicolus* and *S. pilchardus*, which demonstrated diet changes and reduced prey
129 diversity (Le Bourg et al. 2015; Brosset et al. 2016). Using a multi-proxy approach,
130 Bachiller et al. (2020, 2021) highlighted a trophic latitudinal gradient in *E.*
131 *encrasicolus* and *S. pilchardus* in the Western Mediterranean Sea and a trophic niche
132 overlap between *S. pilchardus* and *S. aurita*. Information on other pelago-demersal
133 planktivorous species from the Gulf of Lion is lacking.

134 Our aim here was to determine the food resources consumed by planktivorous fish.
135 Our hypothesis is that competition for food resources may occur between the NWMS
136 planktivorous species, leading to the observed changes in small pelagic fish
137 populations. The information provided by our study will [improve food web modeling](#),
138 which is increasingly applied to predict fish population and ecosystem dynamics. We

139 investigated the diet of pelago-demersal planktivorous fish by combining SCA and
140 SIA. Specifically, we aimed: (1) to analyze fish diet in terms of prey taxonomic
141 groups and prey sizes over the entire sampling period, (2) to estimate the trophic
142 overlap among these species using SCA and SIA, and (3) to compare the dietary
143 diversity and overlap of trophic niches using both methods, to test the hypothesis of
144 potential trophic competition.

145

146 **2. Material and methods**

147 *2.1. Study site*

148 The Bay of Marseille (South of France) is located in the eastern part of the Gulf of
149 Lion in the NWMS. In this bay, phyto- and zooplankton communities benefit from
150 diverse marine and terrestrial nutrient input (Bănaru et al. 2014; Chen et al. 2019a).
151 The Bay of Marseille is influenced from the south by the intrusions of the offshore
152 Northern Current (Petrenko et al. 2005), and occasionally from the west by the
153 intrusions of the Rhone River plume, and by the coastal Huveaune River, depending on
154 wind and rain events (Cresson et al. 2012; Frayssé et al. 2014). The bay is also subject
155 to anthropogenic and terrestrial inputs from the Marseille sewage treatment plant at
156 Cortiou (Millet et al. 2018) (Fig. 1).

157 *2.2. Sampling and sample processing*

158 Fish were sampled through a partnership with local fishermen working with lampara
159 net, gillnets and trawling (Fig. 1). Where possible, 30 individuals of each species were
160 sampled in the early morning, each month from March 2017 to September 2017.
161 Alongside this monthly sampling, data were also analyzed collectively - and not
162 seasonally - because the number of individuals sampled each month widely differed
163 between species. The potential impact of seasonality on the results is considered
164 separately (see section 4.4.). The number of samples for body length measurements,

165 SCA and SIA are listed in Table 1.

166 Once caught, fish were immediately put on ice and kept at -20°C upon arrival at the
167 laboratory to inhibit digestion of the stomach contents.

168 Zooplankton was collected monthly at the Solemio station (43.24°N; 5.29°E)
169 simultaneously with the SOMLIT (Service d'Observation en Milieu Littoral)
170 long-term national program for littoral observation (Fig. 1). Zooplankton was sampled
171 vertically from 50 m depth (close to the bottom) to the surface with an 80-µm mesh
172 size net (50 cm opening diameter). Zooplankton samples were preserved in a buffered
173 4% formaldehyde - seawater solution.

174 In the laboratory, total body length (TL) of each fish was measured to the nearest
175 millimeter. Each individual was sexed by examination of the gonads. Digestive tracts
176 were collected and preserved individually in a buffered 4% formaldehyde - seawater
177 solution for later SCA. Dorsal white muscle was sampled from each fish and frozen at
178 -20°C for later SIA.

179 2.3. *Analysis of fish diet and plankton composition*

180 The number of analyzed individuals for total body length measurements, the
181 minimum, maximum and mean total body length (cm), the proportion of empty
182 stomachs and the degree of repletion per species are displayed in Table 1. The mean
183 degree of stomach repletion (SRD) was estimated visually using a semi-quantitative
184 scale of prey volume in stomach: 0 = empty, 1 = > 0 – 25 % full, 2 = 25 - 50% full, 3
185 = 50 - 75% full, 4 = 75 - 100% full. Empty stomachs were not considered in diet
186 index calculations.

187 Zooplankton prey were identified and sorted for further taxonomic and size analyses.

188 The taxonomic groups of zooplankton found in both fish stomachs and plankton net
189 samples were identified and the number of individuals in each group were counted
190 using a stereomicroscope (Leica M205C) as described by Chen et al. (2021). The

191 main copepod groups were identified using the website 'Marine Planktonic Copepods'
192 (<https://copepodes.obs-banyuls.fr/en/loc.php?loc=14>) (Razouls et al. 2005).

193 The maximum length (sum of prosome and urosome lengths for copepods) was
194 measured on the first 30 individuals of representative prey groups found in each
195 stomach and zooplankton net sample. Size measurement allowed the calculation of a
196 prey-predator size ratio (PPSR) between each fish and its prey, achieved by
197 determination of the most consumed prey size range by species and comparison with
198 the size of the available zooplankton prey in the 80- μm mesh size net. For graphic
199 representation, PPSR values were multiplied by 1000. Dry weight (DW) of each
200 organism was estimated from length-weight relations gathered from the literature
201 (Table S1). The degree of stomach fullness (SFD) was used to assess feeding intensity
202 (as described by Bachiller et al. (2016)). *The SFD, expressed in $\mu\text{g}\cdot\text{cm}^{-1}$, is defined as*
203 *the sum of prey dry weight in the stomach divided by the total length of the fish.*

204 2.3.1. Trophic indices

205 Diet composition based on stomach content analyses (SCA) was analyzed using several
206 complementary indices: frequency of occurrence (O%: percentage of non-empty
207 stomachs containing a given prey item), numerical percentage (N%: mean percentage
208 per stomach of the number of a given prey item related to the total number of all prey in
209 each stomach), dry weight percentage (W%: mean percentage per stomach of the
210 weight of a given prey item related to the total weight of all prey in each stomach).
211 These indices were used to calculate the index of relative importance (IRI) following
212 the formula (Pinkas et al. 1971):

$$\text{IRI} = \text{O}\% \times (\text{N}\% + \text{W}\%)$$

213 and then expressed as a percentage (IRI%) (Cortés 1997).

214 For consistency, IRI was also used to compute trophic niche width and dietary overlap.

215 To compare the community composition of zooplankton sampled with nets and the

216 zooplanktonic prey in fish diet, IRI% was also calculated by month for zooplankton
217 sampled with the 80- μ m mesh size plankton net. Here, IRI% integrates information on
218 the abundance, occurrence and biomass of zooplankton groups sampled with nets.

219 2.3.2. *Diversity indices*

220 The Shannon diversity index was determined to quantify the diversity of prey in fish
221 stomachs and then for zooplankton net samples, using the formula:

$$H' = - \sum_{i=1}^s p_i \ln p_i$$

222 where p_i is the mean proportion (IRI%) of the prey group i , and S is the total number
223 of prey groups.

224 The Pielou evenness index was computed to determine the evenness of prey group
225 distribution in the diets of the studied species, using the formula: $J = H' \div \ln(S)$

226 where H' is the Shannon diversity index and S is the total number of prey groups
227 found in stomach contents per species. This index was also estimated for the 80- μ m
228 mesh size plankton net.

229 Trophic niche breadth and prey diversity in relation to increasing fish size was
230 analyzed following the method proposed by Bachiller and Irigoien (2015).

231 2.4. *Stable isotope analysis (SIA)*

232 For the SIA, where possible, at least three fish were analyzed per month and per
233 species among those previously analyzed for SCA (Table 1). Fish muscle tissues were
234 freeze-dried, ground into a fine powder and a subsample was packed into a 5 \times 8 mm tin
235 cup. Carbon and nitrogen stable isotope analyses were performed using a
236 continuous-flow isotope-ratio mass spectrometer (Delta V Plus, Thermo Scientific)
237 with a Conflo IV interface coupled to an elemental analyzer (Flash EA 1112, Thermo
238 Scientific) equipped with the Smart EA option. Analyses were conducted at the Littoral,
239 Environment and Societies Joint Research Unit stable isotope facility (CNRS -

240 University of La Rochelle, France). The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values are expressed in δ
241 notation as deviations from standards (Vienna Pee Dee Belemnite for $\delta^{13}\text{C}$ and N_2 in air
242 for $\delta^{15}\text{N}$), in ‰, according to the formula:

$$243 \quad \delta X = \left[\left(\frac{R_{\text{sample}}}{R_{\text{standard}}} \right) - 1 \right] \times 1000$$

244 where X is ^{13}C or ^{15}N , R_{sample} is the isotopic ratio of the sample and R_{standard} is the
245 isotopic ratio of the standard. Calibration was carried out using reference materials
246 (USGS-24, -61, -62, IAEA-CH6, -600 for carbon; USGS-61, -62, IAEA-N2, -NO-3,
247 -600 for nitrogen). The analytical precision of the measurements was <0.15‰ for
248 carbon and nitrogen based on analyses of USGS-61 and USGS-62 used as laboratory
249 internal standards.

250 The $\delta^{13}\text{C}$ values were corrected for the effect of lipids when the C/N ratio was higher
251 than 3.5 according to the formula developed by Post et al. (2007):

$$\delta^{13}\text{C}_{\text{normalised}} = \delta^{13}\text{C}_{\text{sample}} - 3.32 + 0.99 \times \text{C/N}$$

252 Individual and mean trophic level (TL) of fish species (TL_{fish}) were calculated using
253 $\delta^{15}\text{N}$ values according to the following formula (Vander Zanden and Rasmussen
254 2001):

$$TL_{\text{teleost}} = \lambda + (\delta^{15}\text{N}_{\text{teleost}} - \delta^{15}\text{N}_{\text{baseline}}) \div 3.4$$

255 where $\delta^{15}\text{N}_{\text{fish}}$ is the $\delta^{15}\text{N}$ value of the consumer, λ the trophic level of the baseline
256 and $\delta^{15}\text{N}_{\text{baseline}}$ the $\delta^{15}\text{N}$ value of the baseline. The mean annual $\delta^{15}\text{N}$ value of the
257 200-300 μm size class of zooplankton sampled in the framework of SOMLIT (see
258 section 2.2.) (Chen et al. 2019a) was considered as the baseline for primary
259 consumers (i.e. trophic position $\lambda = 2$) and a trophic fractionation factor of 3.4 was
260 used for the computations (Post 2002).

261 2.5. Resource partitioning

262 The total dietary trophic niche width (DNW) based on stomach content analysis was

263 estimated to compare fish species trophic diversity (Schoener 1970): $DNW = e^{H'}$

264 with $H' = -\sum(IRI_i \times \log_2 IRI_i)$.

265 Overlap in species diet (T%; Schoener 1970) was calculated according to the formula:

$$T\% = (1 - 0.5\sum|IRI_{xi}\% - IRI_{yi}\%|) \times 100$$

266 where $IRI_{xi}\%$ and $IRI_{yi}\%$ are IRI% for the prey item i in the fish species x and y .

267 In theory, T% varies from zero (when the two groups of fish feed on totally different
268 prey items) to one (when they consume the same prey items in the same proportions).

269 Index values higher than or equal to 0.6 are considered significantly elevated (Keast
270 1978).

271 2.6. Statistical analyses

272 Data normality and homogeneity were tested using Shapiro-Wilk and Levene tests.

273 Non-parametric analysis of variance Kruskal–Wallis tests were used to examine
274 differences in total body length, average prey size, PPSR, and isotopic compositions
275 ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) among species. Dunn's multiple non-parametric post-hoc tests were
276 used to pinpoint which specific means significantly differed from the others.

277 Spearman's correlation was used to test the relationship between the total body length
278 and stable isotopic compositions ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of fish species. Differences in diet

279 composition between species were tested using one-way analysis of similarity
280 (ANOSIM). SIMPER analysis provided the average dissimilarity of their diets. The

281 prey groupings were based on Bray-Curtis mean dissimilarities. Differences of prey
282 size distribution among species were tested with the Chi-square test. The Bray-Curtis

283 dissimilarity was also calculated for interspecific pairwise comparisons of prey size
284 distribution. Changes in the range of the Shannon-Wiener diversity index (H') and

285 **PPSR** of each individual predator with increasing size were analyzed according to the

286 methods described in Scharf et al. (2000) and Bachiller and Irigoien (2013, 2015). To

287 represent interspecific differences in dietary and isotopic compositions, non-metric

288 multi-dimensional scaling (nMDS) was carried out on the similarity matrix of the diet
289 (IRI%) and the stable isotopic compositions ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of fishes, respectively.
290 The differences between the isotopic niche and the dietary niche overlaps were tested
291 with a χ^2 test and Spearman's rank order correlation. Data processing was carried out
292 using the software R (R Core Team 2021).

293

294 **3. Results**

295 *3.1. Prey availability*

296 Available prey were often smaller than 1.5 mm with the most abundant prey ranging
297 in size from 0.5 to 0.8 mm (36.4%) (Fig. 2). Zooplankton was dominated by copepods
298 (IRI% = 84.8%), especially calanoid copepods (IRI% = 72.3%) (Fig. 3). Other
299 zooplankton groups such as Monstrilloid and Sapphirinid copepods, fish larvae,
300 tintinnids, echinoderm and cephalopod larvae, and other microzooplankton groups
301 were also observed in net samples and contributed to overall diversity (Shannon
302 diversity index = 1.00 ± 0.25 (mean \pm standard deviation)).

303 *3.2. Biometry and stomach fullness of caught fish*

304 Body size was measured from a total of 715 fish, SCA was measured from 446
305 individuals and SIA from 173 individuals (Table 1). Total body length (Table 2)
306 differed significantly among species ($H = 129.4$, $P < 0.001$). *Cepola macrophthalma*
307 was the longest (29.3 ± 6.8 cm), followed by *S. aurita*, *B. boops*, *S. pilchardus*, *C.*
308 *chromis* and *E. encrasicolus*, the lowest mean total length being recorded in *S.*
309 *sprattus* (11.2 ± 1.0 cm). The proportion of empty stomachs was lowest for *S.*
310 *pilchardus* (2.1%) and highest for *C. chromis* (20.6%), and stomach content was
311 about half full for most species (Table 2). The SFDs varied significantly between
312 species ($H = 36.64$, $P < 0.001$), with highest values for *C. macrophthalma* and *C.*
313 *chromis* and lowest values for *S. pilchardus* (Table 2).

314 3.3. Prey group composition

315 For each species, trophic niche breadth based on prey diversity (i.e. H') did not vary
316 with fish size (Fig. S1) or between sexes (not displayed, $P > 0.05$ for all species).

317 The average diet composition differed significantly among species (ANOSIM $R =$
318 0.15 , $P < 0.001$) (Table 3, Fig. 3).

319 *Engraulidae* and *Clupeidae* species mainly preyed on copepods ($IRI\% > 86$).

320 Although small harpacticoid (*Microsetella/Macrosetella* spp. and *Euterpinidae*) and
321 cyclopoid (*Oncaeidae* and *Corycaeidae*) copepods were not the most abundant in the
322 environment, they were present in high proportions as prey (Fig. 3, Table S2).

323 *Engraulis encrasicolus* and *S. aurita* consumed higher proportions of calanoid
324 copepods than *S. pilchardus* and *S. sprattus*. *Cepola macrophthalma* consumed a high
325 proportion of decapod larvae in addition to calanoid copepods (*Acartiidae* and
326 *Calanidae*). *Chromis chromis* and *B. boops* both consumed other prey that were not
327 abundant in the environment during the same sampling periods. *Chromis chromis*
328 consumed a high proportion of gastropods (pteropods), cypris larvae and ostracods,
329 while *B. boops* consumed benthic polychaetes and cypris larvae. Both species
330 ingested high proportions of amphipods and decapod larvae.

331 The SIMPER analysis (Table 3) showed that the $IRI\%$ of copepods contributed the
332 most ($> 60\%$) to the dissimilarities of diet between the studied species, but the $IRI\%$
333 of other prey such as gastropod and decapod larvae was also high ($> 5\%$).

334 Bray-Curtis dissimilarity indices on prey composition were lowest between *S.*
335 *pilchardus* and *S. aurita* (Table 4), while the highest values were found between *S.*
336 *sprattus* and both *C. macrophthalma* and *C. chromis*.

337 Prey diversity ($\chi^2 = 26$) and evenness ($\chi^2 = 24$) indices differed significantly among
338 fish species ($P < 0.001$ for both). *Sprattus sprattus* exhibited the highest prey diversity
339 ($H' = 0.95 \pm 0.08$) and *C. chromis* presented the highest prey evenness ($J = 0.76 \pm$

340 0.11). *Cepola macrophthalma* showed the lowest values for both indexes ($H' = 0.29 \pm$
341 0.11 , $J = 0.32 \pm 0.14$) (Table 5). The zooplankton composition of the 80- μm mesh size
342 net generally showed a higher group diversity but had about the same evenness index
343 as fish stomach content (Table 5).

344 3.4. Prey size structure

345 Prey size distribution differed significantly among species ($\chi^2 = 405.23$, $P < 0.001$),
346 with one or two modes of prey size between 0.1 and 2 mm (Fig. 4). *Sardina*
347 *pilchardus* and *S. sprattus* both presented a high consumption of prey ranging
348 between 0.5 and 1.0 mm in size, while *S. aurita*, *C. macrophthalma*, *C. chromis* and *B.*
349 *boops* also consumed a high proportion of prey > 2 mm (up to 40% for *B. boops*).

350 Average prey size and PPSR differed significantly among species ($H = 54$ and $H = 61$,
351 respectively, with $P < 0.001$ for both). *Boops boops* presented the highest values for
352 average prey size (8.54 ± 6.31 mm), and *S. pilchardus* the lowest (0.99 ± 0.21 mm)
353 (Fig. 5A). *Boops boops* also had the highest average value for PPSR, and *C.*
354 *macrophthalma* the lowest (Fig. 5B). A significant increase of trophic niche breadth
355 based on PPSR with increasing fish size was observed in *B. boops* (Fig. S2). For other
356 species, no change in mean prey size or of trophic niche breadth based on PPSR and
357 prey diversity was observed with increasing body size.

358 Prey size distribution was significantly different between *B. boops*, *S. pilchardus* and
359 *S. sprattus* (Table 6) and the most similar between the Clupeidae (< 0.30).

360 3.5. Stable isotope composition

361 Mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values and the estimated trophic level differed significantly
362 among species ($\delta^{13}\text{C}$: $H = 35.67$, $P < 0.001$; $\delta^{15}\text{N}$: $H = 61.52$, $P < 0.001$, TL: $H =$
363 61.50 , $P < 0.001$). *Sprattus sprattus* had the highest $\delta^{13}\text{C}$ values and *B. boops* and *S.*
364 *pilchardus* had the lowest (Table 7). *Boops boops* and *C. macrophthalma* had the
365 highest $\delta^{15}\text{N}$ values and estimated trophic levels, while *S. sprattus* and *E. encrasicolus*

366 presented the lowest. Spearman's correlation tests highlighted significant negative
367 correlations between $\delta^{13}\text{C}$ values and total body length for *S. pilchardus* (Table 7).
368 Significant positive correlations were found between $\delta^{15}\text{N}$ values and both estimated
369 trophic levels and fish size for *C. chromis* and *B. boops*, while significant negative
370 correlations were found for *E. encrasicolus*.

371 3.6. Dietary niche widths and diet overlaps

372 *Chromis chromis* had the highest DNW and *C. macrophthalma* the lowest (Table 8).
373 Higher and significant dietary niche overlaps were found between *S. pilchardus*, *S.*
374 *aurita* and *E. encrasicolus*, while overlap was also observed between *E. encrasicolus*
375 and *C. macrophthalma*.

376 The nMDS analysis based on SCA showed no ellipse overlaps between *S. sprattus*
377 and the other species except for *S. pilchardus*. *Sprattus sprattus* differed the most
378 from *E. encrasicolus*, *S. aurita* and *C. macrophthalma* (Fig. 6A). *Sardina pilchardus*,
379 *C. chromis* and *B. boops* had an intermediate position between the two groups. The
380 nMDS analysis based on isotopic compositions displayed more extensive ellipse
381 overlaps among all fish species, with *S. sprattus* on one side, close to *E. encrasicolus*
382 and *S. aurita*, while the other species appeared on the other side of the biplot (Fig. 6B).
383 With both SCA and SIA, *S. sprattus* and *C. macrophthalma* were the most distant
384 species.

385

386 4. Discussion

387 This study characterized the diet of seven Bay of Marseille fish species with the
388 complementary use of stomach content (SCA) and stable isotope (SIA) analyses, and
389 also considered the size of prey consumed. Diet studies generally focus on
390 composition of prey species, data on prey size tending to be rare, despite prey size being
391 a defining factor in pelagic fish growth (Thoral et al. 2021). For planktivorous species,

392 inter-specific differences in diet and prey size depend on the gill raker density as well as
393 the number of gill arches, whereas gape height determines the maximum size of prey
394 ingested (Velip and Rivonker 2018). Moreover, prey energy content is generally
395 closely linked to both species/groups composition and size (Harmelin-Vivien et al.
396 2019; Chen et al. 2019a). We adopted this approach in the context of recently
397 observed changes to small pelagic fish populations in the NWMS, assumed to be
398 related to changes in the composition of zooplankton, fish diet and trophic
399 interactions (Brosset et al. 2016; Saraux et al. 2019).

400 *4.1. Prey group and prey size composition*

401 Our study shows that diets differed from ambient prey availability, suggesting
402 differential prey selection (Karachle and Stergiou 2013; Costalago and Palomera 2014;
403 Chen et al. 2021). Dietary differences may be related to body shape, size, jaw
404 morphology and habitat (Karachle and Stergiou 2013; Frédérick et al. 2014). In our
405 study, differences in prey composition and diversity, prey size structure and dietary
406 overlaps allowed the differentiation of two main groups, which also differed in mean
407 size, morphology and foraging habitats. The first group included the strictly pelagic
408 Clupeidae (*S. pilchardus*, *S. aurita* and *S. sprattus*), which preyed mostly on small
409 sized copepods. The second group contained the benthic-demersal Cephalopoda (*C.*
410 *macrophthalma*), Pomacentridae (*C. chromis*) and Sparidae (*B. boops*), which
411 consumed larger copepods, other zooplankton groups and varying proportions of
412 benthic prey. The Engraulid *E. encrasicolus* was intermediate due to the wide
413 spectrum of planktonic prey consumed.

414 *Engraulis encrasicolus*, *S. pilchardus* and *S. sprattus* consumed mainly small and
415 medium size copepods, such as harpacticoid (*Microsetella/Macrosetella* spp.,
416 *Euterpina acutifrons*) and cyclopoid (Oncaeiidae, Corycaeiidae) copepods, in
417 accordance with previous observations from the literature (Costalago et al. 2014; Le

418 Bourg et al. 2015; Albo-Puigserver et al. 2019; Bachiller et al. 2020, 2021). *Sprattus*
419 *sprattus*, which had the highest relative proportion of copepods in its stomach contents,
420 fed on a higher proportion of cyclopoid copepods than the other Clupeidae species, as
421 reported by Le Bourg et al. (2015). Bachiller et al. (2020) also observed a latitudinal
422 gradient of diet variation in *E. encrasicolus* and *S. pilchardus* along the NWMS coast.
423 Our results seem to confirm poorer feeding conditions previously described for
424 northern Mediterranean populations, with smaller prey consumed compared to the
425 southern populations which consumed high proportions of decapods, malacostracans,
426 and euphausiids (Bachiller et al. 2020). In terms of prey size, the preferred prey size
427 range of *E. encrasicolus*, *S. pilchardus* and *S. sprattus* was wider in the Bay of
428 Marseille (0.4 – 0.9 mm) than in the Gulf of Lion (0.2 – 0.5 mm) (Le Bourg et al. 2015),
429 with larger prey (1.1 – 1.5 mm) consumed in higher proportions. The larger plankton
430 prey size in the Bay of Marseille was probably linked to the high production induced by
431 terrestrial inputs (Frayse et al. 2014). Mean PPSR values of these three species were
432 higher in the Bay of Marseille (11.50 ± 1.94 , 44.83 ± 15.76 , and 11.09 ± 3.68 ,
433 respectively) than in the Gulf of Lion (7.32 ± 6.57 , 5.20 ± 1.86 , and 6.21 ± 1.61 ,
434 respectively) during the summer months, possibly due to temporal variation in prey size
435 (Chen et al. 2021). It seems important therefore that PPSR comparisons should be
436 undertaken contemporaneously.

437 The diet of *S. aurita* was similar to that of *S. pilchardus* in the Bay of Marseille, but
438 presented higher prey diversity and evenness. Bachiller et al. (2021) also observed
439 more diet similarities between *S. aurita* and *S. pilchardus* than with *E. encrasicolus*.
440 This was likely due to their similar filtering apparatus structure, with smaller gill raker
441 spaces than in *E. encrasicolus* (Costalago and Palomera 2014). Bachiller et al. (2021)
442 found a high proportion of salps, appendicularians, chaetognaths and polychaetes in *S.*
443 *aurita*, but not in *S. pilchardus*. These dietary differences may suggest more

444 opportunistic feeding in *S. aurita*, as observed in the Aegean Sea (Lomiri et al. 2008).
445 The prey size distribution of *S. aurita* observed in the Bay of Marseille was similar to
446 that observed in the Ebro River Delta by Albo-Puigserver et al. (2019). Although *S.*
447 *aurita* had a higher average prey size than the other Clupeidae, it presented the lowest
448 PPSR due to its larger body size. The high consumption of *Microsetella/Macrosetella*
449 spp. copepods by the four small sized pelagic fish (Engraulidae and Clupeidae) may be
450 related to the high input of aggregates in the Bay of Marseille with which these
451 copepods were associated (Chen et al. 2021 and references therein).

452 Among benthic-demersal species, *C. macrophthalma* mainly preyed on decapod larvae,
453 calanoid (Acartiidae and Calanidae) copepods, and small proportions of gelatinous
454 zooplankton with their associated *Phronima* spp. amphipods. *Cepola macrophthalma* is
455 considered a benthic opportunist preying mainly on diverse zooplankton groups, but
456 also ingesting some benthic organisms (Stergiou 1993). Similarly, *C. chromis*
457 consumed both planktonic and benthic organisms (Mapstone and Wood 1975; Bell and
458 Harmelin-Vivien 1983; Dulčić 2007; Pinnegar et al. 2007; Pinnegar 2018). We
459 observed that *C. chromis* consumed high proportions of pteropods, copepods, decapod
460 larvae, and amphipods. Pteropods are prey that contain high protein and lipid contents
461 (Harmelin-Vivien et al. 2019), but were rare in the diet of other species in our study.
462 The high proportion of pteropods in *C. chromis* diet may be explained by powerful jaws
463 specialized in ram suction feeding like other phylogenetically close pomacentrid
464 species (Frédérich et al. 2013, 2014; Chen et al. 2019b). *Boops boops* consumed a
465 wide diversity of benthic prey in addition to zooplanktonic crustaceans, as previously
466 observed in the Bay of Marseille (Bell and Harmelin-Vivien 1983; Cresson et al. 2014).
467 This demersal species can be considered an opportunistic omnivore (Stergiou and
468 Karpouzi 2002; El-Hawet et al. 2005). In the present study *B. boops* consumed mainly
469 annelids, gelatinous zooplankton and pteropods. Despite the generally low energy

470 content of small gelatinous zooplankton (Harmelin-Vivien et al. 2019; Chen et al.
471 2019a), this diet is reflective of its opportunistic predation behavior. *Cepola*
472 *macrophthalma*, together with *C. chromis* and *B. boops*, but also *S. aurita*, consumed a
473 high proportion of prey > 2 mm in size, related to both larger body, and thus a larger
474 mouth size for *C. macrophthalma* and *S. aurita*, and to the powerful jaws for *C. chromis*
475 (Frédérich et al. 2013, 2014; Chen et al. 2019b).

476 While Bachiller and Irigoien (2013) observed an increase in trophic niche breadth
477 based on PPSR in *E. encrasicolus* and *S. pilchardus*, and a decrease with growth in *S.*
478 *sprattus*, a corresponding increase with increasing fish size was observed only for *B.*
479 *boops* in the present study, possibly reflecting the poorer feeding conditions in the Bay
480 of Marseille compared to those of the Atlantic Sea.

481 4.2. Stable isotope composition of planktivorous fish species

482 Stable isotope analysis revealed that strictly pelagic planktivorous fish (i.e. *E.*
483 *encrasicolus*, *S. pilchardus*, *S. aurita*, *S. sprattus*) demonstrated generally higher
484 mean $\delta^{13}\text{C}$ and lower mean $\delta^{15}\text{N}$ values than benthic-demersal species (*C.*
485 *macrophthalma*, *B. boops*), excluding *C. chromis*. This is consistent with previous
486 observations in the Gulf of Lion (Le Bourg et al. 2015) and the Ebro River Delta
487 (Albo-Puigserver et al. 2019). Similar mean $\delta^{15}\text{N}$ values between *E. encrasicolus* and
488 *S. sprattus*, and between *S. pilchardus* and *S. aurita* respectively, indicate close trophic
489 level consistency between these species (Hussey et al. 2014). However, $\delta^{13}\text{C}$ values
490 differed significantly between species pairs, with *S. sprattus* presenting higher $\delta^{13}\text{C}$
491 values than *E. encrasicolus*, and *S. aurita* presenting higher values than *S. pilchardus*,
492 allowing some isotopic segregation of these four species in spite of high isotopic
493 overlaps. The values reported were similar to those observed for these species in the
494 Gulf of Lion in 2010-2014, but differed from those recorded in 2004-2005 (Brosset et
495 al. 2016). This may be linked to changes in zooplankton community structure and/or in

496 the organic matter composition at the base of the pelagic food webs. *Sardina pilchardus*
497 also presented higher relative $\delta^{15}\text{N}$ values than *E. encrasicolus* and *S. aurita* in our
498 studies, corresponding to the observations in the nearby Western Mediterranean Sea. In
499 our study, this observation can be related to the higher proportion of eggs in the diet of
500 *S. pilchardus*, as isotopic compositions of fish eggs are generally similar to those of
501 adult fish (Chen 2019). The higher $\delta^{15}\text{N}$ values and trophic levels of *B. boops* and *C.*
502 *macrophthalmus* may be related to their consumption of crustacean larvae, chaetognaths
503 and benthic organisms. The higher dispersion of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values observed in these
504 two benthic-demersal species may result from a higher diversity of sources of organic
505 matter at the base of the pelagic and the benthic food webs in the Bay of Marseille
506 (Cresson et al. 2012).

507 While no relationship was detected between fish size and diet composition, or between
508 fish size and trophic niche breadth, changes in fish isotopic compositions occurred with
509 size for some species. We observed a decrease of $\delta^{13}\text{C}$ values with growth in *S.*
510 *pilchardus* and of $\delta^{15}\text{N}$ values in *E. encrasicolus*, while $\delta^{15}\text{N}$ values of *C. chromis* and
511 *B. boops* increased. These alterations may be linked to changes in diet or foraging
512 zones, or to zooplankton community changes related to changes of primary producers,
513 and thus of carbon food sources, as observed in the Gulf of Lion (Costalago et al.
514 2012). The increase of $\delta^{15}\text{N}$ values and of trophic niche breadth (i.e. PPSR) with growth
515 of *B. boops* may be related to the higher ability of this species to capture larger prey
516 when growing. In contrast to our observations, Bachiller et al. (2021) showed
517 increasing $\delta^{13}\text{C}$ values with size for *E. encrasicolus* and *S. aurita* and decreasing $\delta^{15}\text{N}$
518 values with size for *S. pilchardus*. This may be related to differences in foraging
519 patterns with growth, as larger prey and better body condition were observed in the fish
520 populations from the southern areas of the Mediterranean Sea (Bachiller et al. 2020).

521 4.3. Differences in trophic interactions revealed by SCA and SIA

522 This study highlighted differences and complementarity between SCA and SIA, as the
523 two methods demonstrated contrasting results. We found a higher degree of dietary
524 overlap with the SIA than with the SCA between *S. pilchardus*, *S. aurita* and *S. sprattus*.
525 However, even if the different species ingested different zooplankton groups, these
526 prey may have similar isotopic composition due to similar POM sources at the base of
527 the food web and similar trophic levels (Bănaru et al. 2014). Le Bourg et al. (2015)
528 demonstrated that in the last decade *S. pilchardus* and *E. encrasicolus* had similar diets
529 to *S. sprattus* in the Gulf of Lion. However, *E. encrasicolus* appeared to be less in
530 competition with the Clupeidae species in the Bay of Marseille. The results of SCA also
531 suggested less competition among benthic-demersal fish, indicating specialization on
532 different prey groups and sizes, despite the high similarities of their isotopic
533 compositions. In addition, we found some species (i.e. *S. aurita*, *C. macrophthalma*, *C.*
534 *chromis* and *B. boops*) to be highly opportunist, in agreement with the observations of
535 other authors (El-Maremie and El-Mor 2015; Pinnegar 2018; Albo-Puigserver et al.
536 2019; Bachiller et al. 2020, 2021). By contrast, *E. encrasicolus*, *S. pilchardus* and *S.*
537 *sprattus* fed mainly on copepods and seemed to be less opportunist feeders. However,
538 with sufficient food availability, dietary overlap does not necessarily imply
539 competition for food resources (Coe et al. 2001). Our data thus tend to show a higher
540 potential for competition between the Engraulidae and Clupeidae species than
541 between the other species studied. Data on the quality and quantity of zooplankton
542 available as prey remain essential to confirm or disprove the hypothesis on the
543 limitation of food resources.

544 4.4. Methodological issues and improvements

545 [The main difficulty for multi-species fish diet studies](#) is in simultaneously obtaining
546 both plankton prey and fish samples. Here, monthly fish and zooplankton sampling

547 was carried out several days and a few kilometers apart. However, as some of our
548 target species consumed high proportions of large prey (> 2 mm), certain prey
549 categories that the fish were feeding on locally may have been missed. Combining
550 multiple nets to capture a wider range of prey size and categories would allow a more
551 precise comparison of diet with available zooplankton (Costalago and Palomera 2014;
552 Albo-Puigserver et al. 2019; Bachiller et al. 2020).

553 It was not possible to collect monthly data for all our study species, as not all belong
554 to targeted fisheries, necessitating time-aggregation of data. For example, a large
555 sample of *S. pilchardus* was compared to small samples (< 30) of *S. sprattus*, *C.*
556 *macrophthalmus*, *C. chromis*, and *B. boops*. Planktivorous species diets may also vary
557 monthly/seasonally (Chen et al. 2021) along with the isotopic compositions of the
558 primary producers (i.e. baseline), as previously shown in the Bay of Marseille by
559 Bănaru et al. (2014). For improved fisheries management, better temporal sampling
560 would better determine whether the observed differences of fish isotopic compositions
561 are truly related to dietary differences or to changes in the isotopic compositions of
562 the primary food sources. Furthermore, global climate change, and associated changes
563 in water convection, and urban and river inputs, could drastically alter the nutrient
564 inputs at the base of the food web, thereby altering the availability of different
565 plankton prey groups.

566 Nevertheless, our extensive survey provided the best possible results. Most previous
567 studies in the Mediterranean Sea have focused on the feeding of *E. encrasicolus*, *S.*
568 *pilchardus* and *S. sprattus* with samples obtained from oceanographic surveys in
569 targeted areas and periods, and have faced similar difficulties in relating plankton
570 availability to fish feeding (Costalago et al. 2012, 2014; Le Bourg et al. 2015;
571 Bachiller et al. 2020). In addition, as the commercial value of species tends to define
572 research priorities, more scientific knowledge is available for *E. encrasicolus* and *S.*

573 *pilchardus*, but remains more limited for *S. aurita*, *S. sprattus* (Palomera et al. 2007)
574 and the other species in this study.

575 Finally, better harmonization between fish and zooplankton sampling might include
576 the simultaneous use of acoustic systems to detect fish species at given feeding depths
577 (prior to capture) and of multiple net stratified-horizontal tows (such as the multiple
578 plankton sampler Hydro-Bios) to target zooplankton at the same depths. In addition,
579 as different prey types have different digestion rates, the rapid conservation of fish
580 samples immediately following capture could also ensure better preservation of
581 stomach contents. The use of DNA barcoding can also improve the prey
582 identification, especially gelatinous prey (Lamb et al. 2019; Bachiller et al. 2021).

583

584 **5. Conclusion**

585 Similarities in the dietary and isotopic composition revealed by this study suggest
586 higher potential trophic competition among strictly pelagic species under conditions
587 of scarce food resources, while little competition was evident among benthic-demersal
588 species.

589 Our results highlight that inter-specific similarities in isotopic composition does not
590 necessarily imply similar diets. While SCA differed significantly between species,
591 similar inter-specific isotopic compositions may result from similar POM sources at the
592 base of the food web. Long-term monitoring of zooplankton communities and
593 planktivorous fish diets is necessary to improve our understanding of the variability of
594 species interactions in the context of climate change and to underpin ecosystem-based
595 studies for fisheries management in the NWMS.

596

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619

620 **Declarations**

621 **Conflict of interest**

622 The authors declare that they have no conflicts of interest.

623 **Ethical approval**

624 All applicable national and/or institutional guidelines for sampling, care, and

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626

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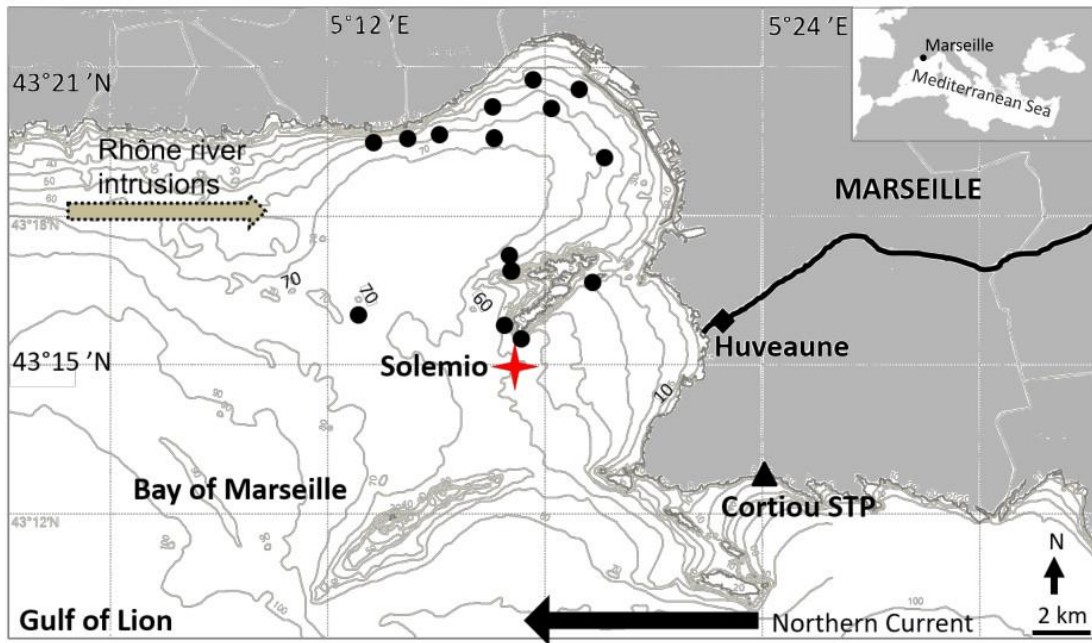
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847

848 Fig. 1. Planktivorous fish (black dots) and zooplankton sampling sites (Solemio, red star) in the Bay of
849 Marseille, NW Mediterranean Sea. Huveaune river (bold line), Cortiou sewage treatment plant (black
850 triangle).

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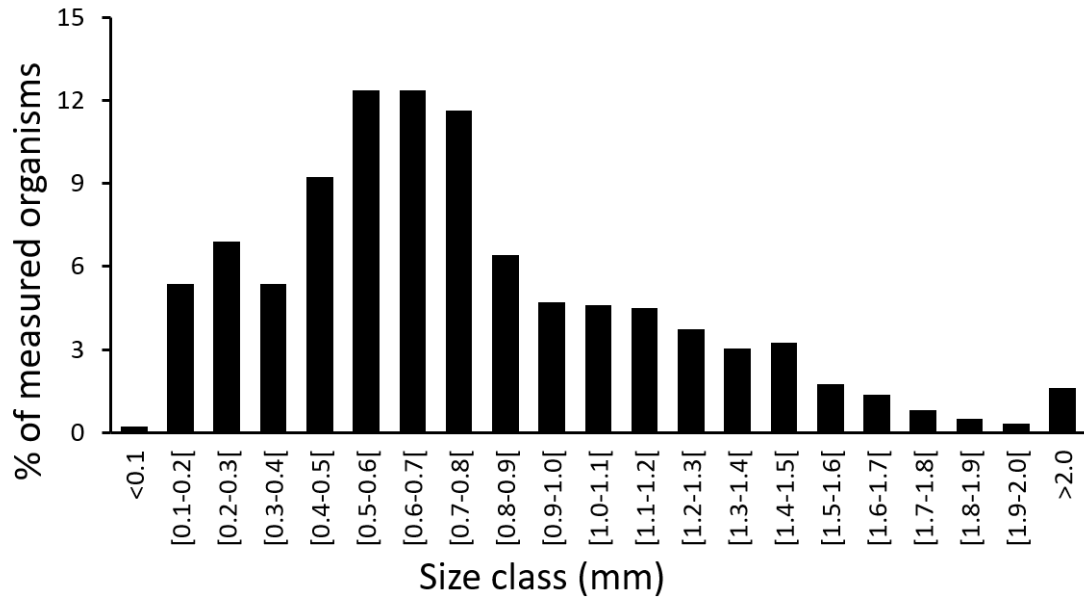


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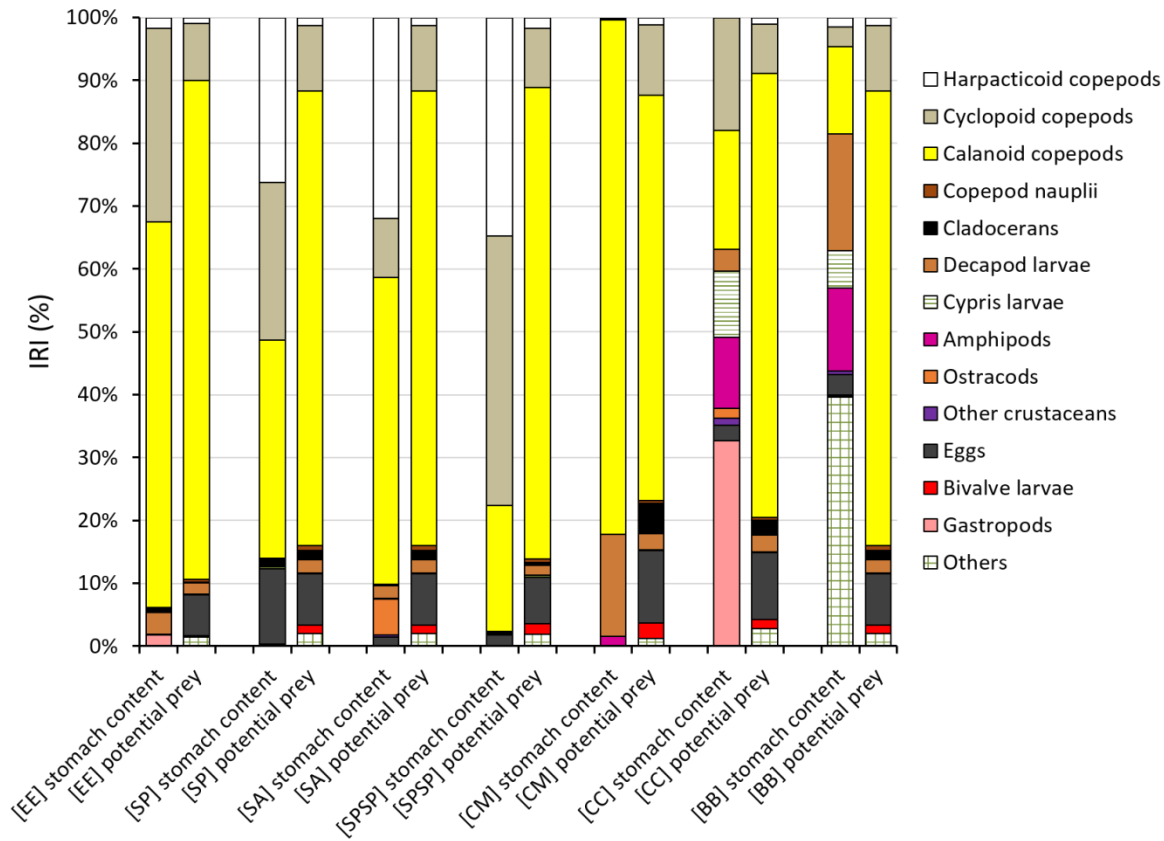
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855 Fig. 2. Size spectrum (0.1 mm size classes) of zooplankton sampled in the Bay of Marseille between
856 March and September 2017 with an 80- μ m mesh size net. Zooplankton (N = 2394) from 14 different
857 samples were measured.



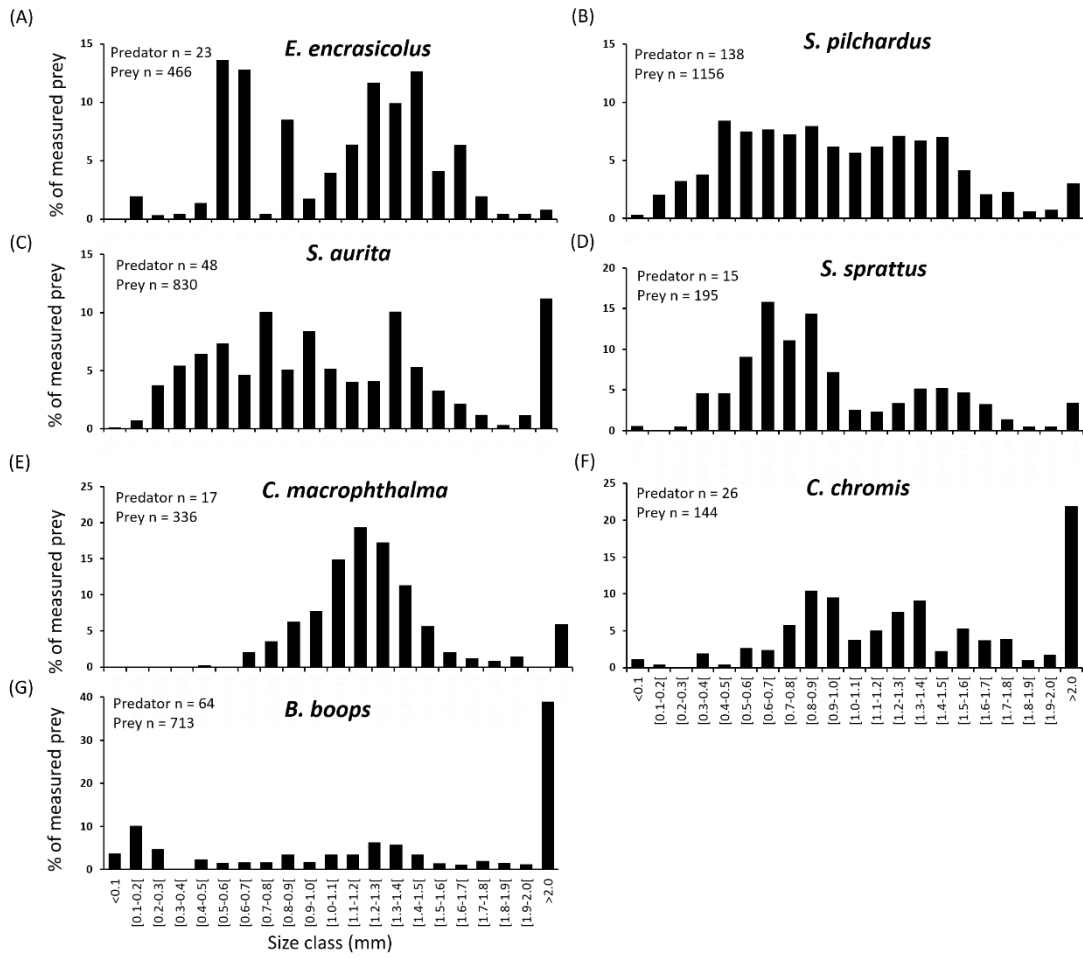
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Fig. 3. Average index of relative importance (IRI%) of zooplankton in the stomach contents of each fish species compared to the composition of zooplankton sampled with the 80- μ m mesh size net (potential prey) from March to September 2017 in the Bay of Marseille. For abbreviations of species: [EE] = *E. encrasicolus*, [SP] = *S. pilchardus*, [SA] = *S. aurita*, [SPSP] = *S. sprattus*, [CM] = *C. macrophthalma*, [CC] = *C. chromis*, [BB] = *B. boops*.



1 Fig. 4. Proportion of consumed prey by 0.1 mm size classes for the studied species (A – G) in the Bay
 2 of Marseille (March to September 2017). “Predator n” is the number of stomachs of fish analyzed.
 3 “Prey n” is the number of measured prey items in their stomach content.

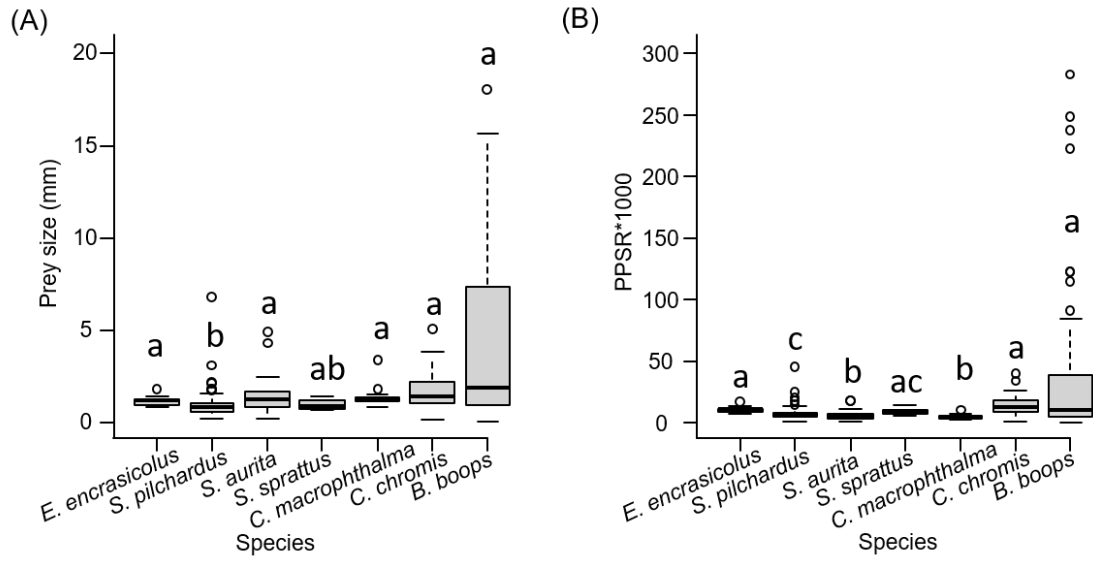
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6 Fig. 5. (A) Average prey size (\pm standard deviation) and (B) average predator-prey size
 7 ratio (PPSR, values multiplied by 1000) by species in the Bay of Marseille (March to
 8 September 2017). Superscript letters represent post-hoc groups and species with
 9 same letters are not significantly different ($P > 0.05$).

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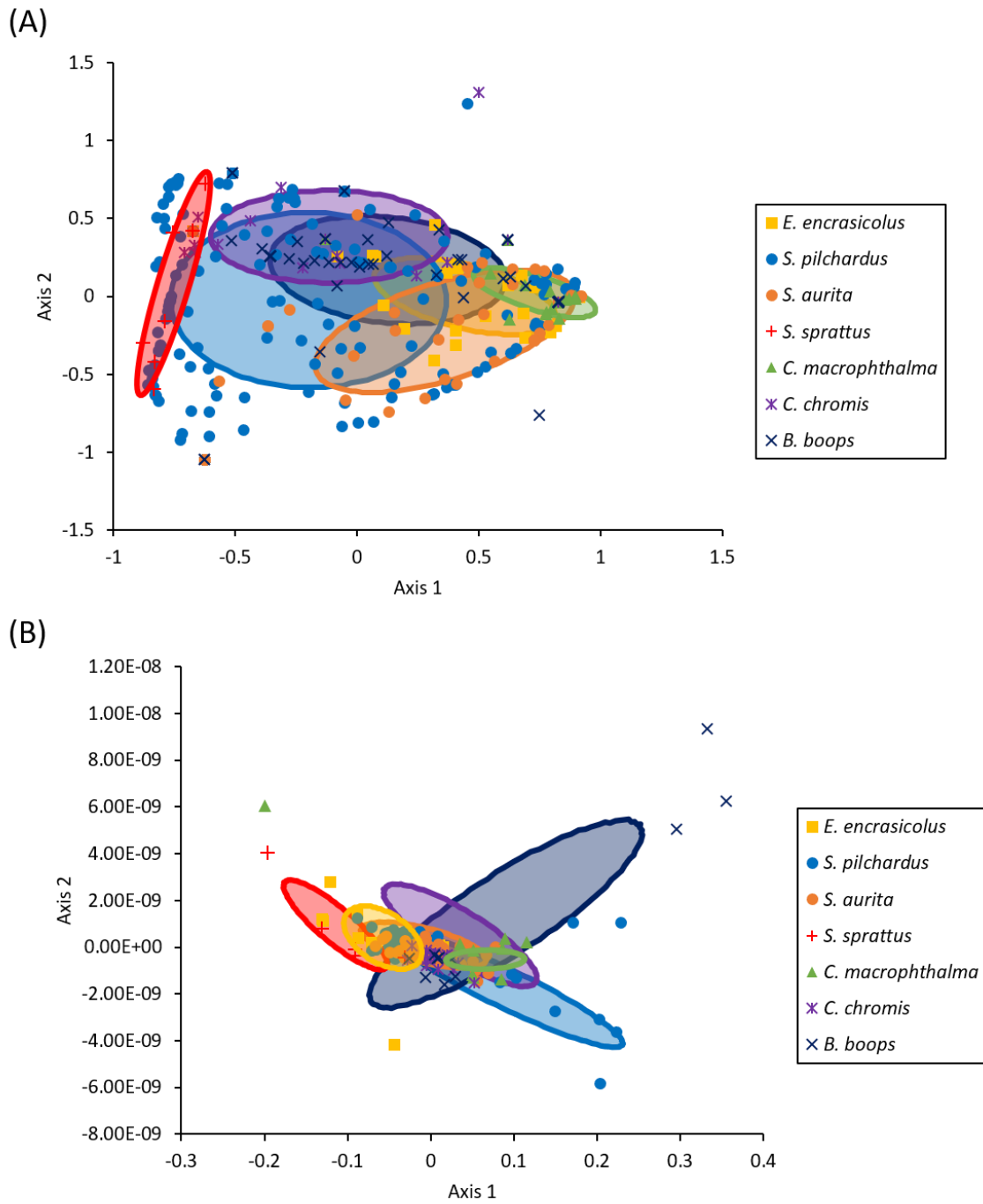


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14 Fig. 6. Non-metric multi-dimensional scaling (nMDS) plot of the diet composition similarities (A)
15 based on stomach content analysis (IRI%, Bray-Curtis) and (B) based on isotope compositions ($\delta^{13}\text{C}$
16 and $\delta^{15}\text{N}$ values, in ‰, Euclidean) of the studied planktivorous teleost species in the Bay of Marseille
17 (March to September 2017).



20 **Table 1.** Number of samples of planktivorous fish for total length measurements (TL), stomach content
 21 analyses (SCA) and stable isotope analyses (SIA) by month.

Month	<i>E. encrasicolus</i>			<i>S. pilchardus</i>			<i>S. aurita</i>			<i>S. sprattus</i>		
	TL	SCA	SIA	TL	SCA	SIA	TL	SCA	SIA	TL	SCA	SIA
Mar.	4	4	4	97	44	11	7	10	5	0	0	0
Apr.	0	0	0	64	44	8	6	10	4	0	0	0
May	4	4	4	45	44	6	6	7	5	7	7	4
Jun.	0	0	0	28	28	8	26	8	9	0	0	0
Jul.	31	15	9	21	21	7	36	12	10	0	0	0
Aug.	0	0	0	35	20	8	30	17	8	0	0	0
Sep.	0	0	4	44	23	9	30	13	9	8	8	4
Total	39	23	21	334	224	57	141	77	50	15	15	8

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23

24 **Table 1 (continued).** Number of samples of planktivorous fish for total length measurements (TL),
 25 stomach content analyses (SCA) and stable isotope analyses (SIA) by month.

Month	<i>C. macrophthalma</i>			<i>C. chromis</i>			<i>B. boops</i>		
	TL	SCA	SIA	TL	SCA	SIA	TL	SCA	SIA
Mar.	0	0	0	0	0	0	33	11	11
Apr.	0	0	0	0	0	0	6	2	0
May	0	0	0	3	3	3	23	9	0
Jun.	17	17	9	18	18	9	16	6	0
Jul.	0	0	0	4	4	4	13	8	0
Aug.	0	0	0	1	1	1	27	22	0
Sep.	0	0	0	0	0	0	25	6	0
Total	17	17	9	26	26	17	143	64	11

26

27

28 **Table 2.** Details of biometric measurements (n), minimum (min), maximum (max) and mean (\pm SD, standard deviation) of total length (cm), proportion of empty stomachs,
 29 mean degree of stomach repletion (SRD) (0 = empty, 5 = full) (\pm SD) and stomach filling degree (SFD) (\pm SD) of the studied planktivorous fish species. Superscript letters
 30 represent post-hoc groups after Kruskal-Wallis tests comparing the total body length ($\chi^2 = 1059.90$, $P = 0.002$). Species with the same letters have similar sizes ($P > 0.05$).

31

Species	Family	n	Total length (cm)				Empty stomachs (%)	SRD	SFD
			min	max	mean	SD			
<i>E. encrasicolus</i>	Engraulidae	39	10.5	12.7	11.6 ^b	0.6	6.3	2.1 \pm 1.2	21.1 \pm 10.0 ^{bc}
<i>S. pilchardus</i>	Clupeidae	334	10.0	18.1	13.0 ^d	0.6	2.1	2.3 \pm 1.3	13.0 \pm 0.8 ^c
<i>S. aurita</i>	Clupeidae	141	15.5	27.5	23.4 ^c	1.3	19.7	2.4 \pm 1.3	30.2 \pm 26.4 ^{ab}
<i>S. sprattus</i>	Clupeidae	15	10.1	12.5	11.2 ^b	1.0	15.1	1.8 \pm 1.1	50.4 \pm 3.9 ^a
<i>C. macrophthalma</i>	Cepolidae	17	21.2	43.9	29.3 ^c	6.8	18.6	3.0 \pm 1.3	68.9 \pm 17.5 ^a
<i>C. chromis</i>	Pomacentridae	26	9.3	13.8	11.7 ^b	0.6	20.6	2.1 \pm 1.1	68.6 \pm 38.0 ^a
<i>B. boops</i>	Sparidae	143	15.1	35.0	19.9 ^a	1.8	13.6	2.2 \pm 0.9	22.6 \pm 6.9 ^{bc}

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34

35 **Table 3.** Results of SIMPER analysis of prey groups that contributed to the dissimilarities (based on Bray-Curtis distances) of the diet of the studied planktivorous teleost
 36 species in the Bay of Marseille. Contrib. = Contribution, Cumulative % = cumulative contribution.

37

Prey groups	Average dissimilarity	Contrib. %	Cumulative %	<i>E.</i> <i>encrasicolus</i>	<i>S.</i> <i>pilchardus</i>	<i>S.</i> <i>aurita</i>	<i>S.</i> <i>sprattus</i>	<i>C.</i> <i>macrophthalmalma</i>	<i>C.</i> <i>chromis</i>	<i>B.</i> <i>boops</i>
Calanoid copepods	17.64	28.25	28.25	61.40	34.70	48.80	0.00	81.80	18.90	13.90
Cyclopoid copepods	12.05	19.30	47.55	30.80	25.10	9.36	60.70	0.10	17.80	3.09
Harpacticoid copepods	8.67	13.89	61.44	1.74	26.20	31.90	31.20	0.24	0.10	1.54
Others	5.69	9.12	70.56	0.00	0.33	0.00	0.00	0.00	0.00	39.60
Gastropods	4.84	7.75	78.31	1.74	0.00	0.01	0.00	0.00	32.70	0.19
Decapod larvae	4.27	6.83	85.14	3.44	0.08	2.01	0.00	16.20	3.49	18.60
Amphipods	3.03	4.86	90.00	0.00	0.02	0.09	0.00	1.60	11.30	13.10
Cypris larvae	2.08	3.34	93.33	0.01	0.33	0.00	0.00	0.00	10.50	5.97
Teleost eggs	2.08	3.32	96.66	0.03	12.00	1.46	2.66	0.00	2.45	3.24
Ostracods	0.97	1.55	98.21	0.00	0.00	5.71	0.00	0.00	1.60	0.03
Other crustaceans	0.65	1.04	99.25	0.00	0.01	0.30	3.59	0.00	1.16	0.55
Cladocerans	0.41	0.66	99.90	0.78	1.12	0.23	1.85	0.00	0.00	0.00
Bivalve larvae	0.03	0.05	99.96	0.10	0.00	0.01	0.00	0.00	0.00	0.17
Copepod nauplii	0.03	0.04	100.00	0.00	0.14	0.06	0.00	0.00	0.00	0.00

38

39

40 **Table 4.** Bray-Curtis' dissimilarity indices for the pairwise comparisons of the composition of stomach
 41 content between the studied planktivorous fish species. The most significant (> 0.6) values of the index
 42 are in bold.

43

Species	<i>E.</i> <i>encrasicolus</i>	<i>S.</i> <i>pilchardus</i>	<i>S.</i> <i>aurita</i>	<i>S.</i> <i>sprattus</i>	<i>C.</i> <i>macrophthalma</i>	<i>C.</i> <i>chromis</i>
<i>S. pilchardus</i>	0.61					
<i>S. aurita</i>	0.59	0.28				
<i>S. sprattus</i>	0.50	0.42	0.37			
<i>C. macrophthalma</i>	0.76	0.78	0.77	0.83		
<i>C. chromis</i>	0.68	0.79	0.76	0.83	0.54	
<i>B. boops</i>	0.72	0.67	0.62	0.74	0.63	0.61

44

45 **Table 5.** Shannon's diversity (H') and Pielou's evenness index (J) calculated according to %IRI of the
 46 diet of the studied planktivorous fish species and for the zooplankton sampled with the 80 µm mesh
 47 size net in the Bay of Marseille (March to September 2017). Superscript letters represent post-hoc
 48 groups after Kruskal-Wallis tests. Values with the same post-hoc letters are not significantly different
 49 (P > 0.05).

Species	H'	H'	J	J
	Stomach content	Zooplankton	Stomach content	Zooplankton
<i>E. encrasicolus</i>	0.69 ± 0.05 ^{bc}	0.85 ± 0.32	0.61 ± 0.15 ^{ab}	0.35 ± 0.12
<i>S. pilchardus</i>	0.61 ± 0.08 ^{bc}	1.00 ± 0.25	0.63 ± 0.11 ^{bc}	0.41 ± 0.10
<i>S. aurita</i>	0.69 ± 0.32 ^{bc}	1.00 ± 0.27	0.61 ± 0.22 ^{ab}	0.41 ± 0.11
<i>S. sprattus</i>	0.95 ± 0.08^c	1.83 ± 0.24	0.67 ± 0.07 ^{abc}	0.60 ± 0.07
<i>C. macrophthalma</i>	0.29 ± 0.11^a	1.25 ± 0.08	0.32 ± 0.14^a	0.49 ± 0.05
<i>C. chromis</i>	0.71 ± 0.33 ^c	1.08 ± 0.26	0.76 ± 0.11^c	0.44 ± 0.10
<i>B. boops</i>	0.47 ± 0.29 ^{ab}	1.00 ± 0.25	0.48 ± 0.21 ^{ab}	0.41 ± 0.10

50

51 **Table 6.** Bray-Curtis' dissimilarity indices for the pairwise comparisons of the prey size distribution
 52 between the studied planktivorous fish species. The highest significant (> 0.6) values of the index are in
 53 bold.

54

Species	<i>E.</i> <i>encrasicolus</i>	<i>S.</i> <i>pilchardus</i>	<i>S.</i> <i>aurita</i>	<i>S.</i> <i>sprattus</i>	<i>C.</i> <i>macrophthalma</i>	<i>C.</i> <i>chromis</i>
<i>S. pilchardus</i>	0.49					
<i>S. aurita</i>	0.34	0.25				
<i>S. sprattus</i>	0.44	0.20	0.26			
<i>C. macrophthalma</i>	0.44	0.53	0.35	0.53		
<i>C. chromis</i>	0.48	0.39	0.27	0.37	0.31	
<i>B. boops</i>	0.63	0.61	0.48	0.62	0.54	0.39

55

56 **Table 7.** Mean (\pm standard deviation) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values and trophic levels (TL) of the planktivorous fish species
 57 in the Bay of Marseille. Spearman's rho and associated P-value testing the rank correlation between the total body
 58 length and $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and TL are indicated. n = sample size. Superscript letters represent post-hoc groups after
 59 Kruskal-Wallis test comparing the mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values and the TLs. Values with the same post-hoc letters are
 60 not significantly different ($P > 0.05$). Significant rho correlation values are in bold.

61

Species	n	$\delta^{13}\text{C}$ (‰)	rho	P	$\delta^{15}\text{N}$ (‰)	rho	P	TL	rho	P
<i>E. encrasicolus</i>	17	$-19.8 \pm 0.1^{\text{ab}}$	-0.12	0.639	$7.2 \pm 0.6^{\text{c}}$	-0.66	0.004	$2.9 \pm 0.2^{\text{c}}$	-0.66	0.004
<i>S. pilchardus</i>	60	$-20.0 \pm 0.3^{\text{a}}$	-0.45	0.001	$8.3 \pm 0.4^{\text{bd}}$	0.04	0.755	$3.2 \pm 0.1^{\text{bd}}$	0.04	0.755
<i>S. aurita</i>	50	$-19.6 \pm 0.2^{\text{bc}}$	-0.08	0.595	$8.0 \pm 0.3^{\text{d}}$	0.09	0.521	$3.1 \pm 0.1^{\text{d}}$	0.09	0.521
<i>S. sprattus</i>	5	$-19.5 \pm 0.6^{\text{c}}$	0.00	1.000	$6.9 \pm 1.2^{\text{cd}}$	0.00	1.000	$2.8 \pm 0.3^{\text{cd}}$	0.00	1.000
<i>C. macrophthalma</i>	9	$-21.1 \pm 2.5^{\text{bc}}$	0.37	0.169	$9.3 \pm 0.4^{\text{a}}$	0.33	0.211	$3.5 \pm 0.1^{\text{a}}$	0.33	0.211
<i>C. chromis</i>	17	$-19.7 \pm 0.1^{\text{bc}}$	0.06	0.815	$8.3 \pm 0.2^{\text{ab}}$	0.49	0.044	$3.2 \pm 0.1^{\text{ab}}$	0.49	0.044
<i>B. boops</i>	11	$-20.2 \pm 0.4^{\text{a}}$	-0.30	0.370	$10.6 \pm 3.8^{\text{a}}$	0.85	0.001	$3.9 \pm 1.1^{\text{a}}$	0.85	0.001

62

63 **Table 8.** Dietary trophic niche width (DNW) based on stomach content analysis of the studied planktivorous fish
 64 species and pairwise comparisons of dietary niche overlap on the upper diagonal. The highest dietary niche width
 65 values and the high niche overlaps (> 0.60) are in bold.

66

Species	DNW	<i>S. pilchardus</i>	<i>S. aurita</i>	<i>S. sprattus</i>	<i>C. macrophthalma</i>	<i>C. chromis</i>	<i>B. boops</i>
<i>E. encrasicolus</i>	2.3	0.62	0.62	0.33	0.65	0.42	0.22
<i>S. pilchardus</i>	3.6		0.72	0.55	0.35	0.40	0.23
<i>S. aurita</i>	3.2			0.43	0.51	0.34	0.22
<i>S. sprattus</i>	2.6				0.00	0.22	0.08
<i>C. macrophthalma</i>	2.2					0.24	0.32
<i>C. chromis</i>	5.4						0.41
<i>B. boops</i>	4.1						

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68

69 **Table S1.** Regression equations of dry weight (DW) against total length (TL) used for zooplankters found in the
 70 field and/or in *S. pilchardus* stomach content during this study.

71

Zooplankton group		DW (μg) -TL (μm)	Reference
Crustaceans			
Copepods			
Calanoids			
	<i>Pleuromamma</i>	Log DW = 2.285 log TL - 5.965	Mauchline, 1998
	<i>Calanus</i>	Log DW = 2.790 log TL - 7.370	Mauchline, 1998
	Centropagidae	Log DW = 2.243 log TL - 5.568	Mauchline, 1998
	Candaciidae	DW = 106.2	Pitois and Fox, 2006
	Temoridae	Log DW = 2.179 log TL - 5.567	Mauchline, 1998
	Acartidae	Log DW = 3.208 log TL - 7.644	Mauchline, 1998
	Lucicutidae	DW = 16.9	Delpy, 2013
	<i>Isias</i>	Log DW = 2.33 log TL - 3.034	Dumont et al., 1975
	Clauso/Paracalanidae	Log DW = 2.738 log TL - 6.934	Mauchline, 1998
Cyclopoids			
	Oncaeidae	DW = 1.34	Borme et al. 2009
	Corycaeidae	DW = 62	Pitois and Fox, 2006
	Oithonidae	DW = 2.2	Pitois and Fox, 2006
Harpacticoids			
	<i>Microsetella/Macrosetella</i>	Log DW = 2.648 Log TL - 17.937	Satapoomin, 1999
	Euterpinidae	Log DW = 2.857TL - 7.857	Ara, 2001
	<i>Distiocus</i>	Log DW = 4.400TL + 0.176	Dumont et al., 1975
	<i>Clytemnestra</i>	DW = 0.3	Delpy, 2013
Cladocerans	<i>Evadne</i>	Log DW = 0.033TL - 7.785	van der Lingen 2002
	<i>Podon</i>	DW = 1.6	Fonda Umani et al. 1979
Ostracods	Ostracods	DW = 6.036	Borme et al. 2009
Amphipods	<i>Phronima</i>	DW = 40; DW = 770	Le Bourg et al. 2015
	Other amphipods	DW = 40	Le Bourg et al. 2015
Larvae			
Crustaceans	Copepod nauplii	Log DW = 2.848 log TL - 7.265	Borme et al. 2009
	Cirriped nauplii	DW = 1.1	Borme et al. 2009
	Cirriped cypris	DW = 5.4	Hopkins, 1966
	Decapods Zoea	DW = 27.798	Borme et al. 2009
	Euphausiids	Log DW = 2.8 log TL + 0.456	Lindley et al., 1999
	Isopods	Log DW = 4.51 log TL - 11.64	Hopkins 1966
	Mollusks	Bivalve	DW = 3.758
	Pteropods	DW = 0.6	Borme et al. 2009
Siphonophores			
		DW = 5.0	Hopkins 1966
Ophiuroids			
		Log DW = 2.22 log TL - 2.24	Robinson et al. 2010
Polychaetes			
		Log DW = 1.92 log TL + 0.17	Galasso et al. 2018
Chaetognaths	<i>Sagitta</i> spp.	Log DW = 1.875- 1.442 log TL	Vergara-Soto et al. 2010
Teleost eggs	Anchovy eggs	DW = 30.1	Borme et al. 2009

73

74 **Table S2-1.** Mean stomach content composition of the studied planktivorous fish species in the Bay of Marseille from March to September 2017. O% =
 75 percentage of prey occurrence, N% = percentage of prey numerical abundance, W% = percentage of prey weight. IRI% = percentage of relative importance
 76 index. The highest percentages by species are in bold.

Prey category	<i>E. encrasicolus</i>				<i>S. pilchardus</i>				<i>S. aurita</i>				<i>S. sprattus</i>			
	O%	N%	W%	IRI%	O%	N%	W%	IRI%	O%	N%	W%	IRI%	O%	N%	W%	IRI%
Harpacticoid copepods	0.3	3.0	3.0	1.7	0.6	26.2	23.4	26.2	0.7	32.3	31.2	31.9	0.5	30.3	26.4	34.7
Cyclopoid copepods	0.8	28.2	29.4	30.8	0.6	22.8	24.1	25.1	0.3	10.4	10.5	9.4	1.0	15.8	19.1	42.9
Calanoid copepods	0.9	57.7	56.5	61.4	0.6	32.5	33.4	34.7	0.7	42.7	43.6	48.8	0.2	49.1	49.3	20.1
Copepod nauplii	0.0	0.0	0.0	0.0	0.0	0.9	1.0	0.1	0.0	0.3	0.3	0.1	0.0	0.0	0.0	0.0
Cladocerans	0.2	2.1	2.1	0.8	0.1	2.6	2.6	1.1	0.1	0.6	0.6	0.2	0.1	1.0	1.1	0.3
Decapod larvae	0.4	4.6	4.6	3.4	0.0	0.5	0.5	0.1	0.3	3.2	3.3	2.0	0.0	0.0	0.0	0.0
Cypris larvae	0.0	0.1	0.1	0.0	0.0	1.4	1.4	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Amphipods	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.5	0.6	0.1	0.0	0.0	0.0	0.0
Ostracods	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	5.7	5.7	5.7	0.0	0.0	0.0	0.0
Other crustaceans	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.1	0.5	0.5	0.3	0.1	1.0	1.1	0.3
Eggs	0.0	0.4	0.4	0.0	0.3	11.7	12.1	12.0	0.1	3.6	3.6	1.5	0.4	1.5	1.7	1.8
Bivalve larvae	0.1	0.4	0.4	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Gastropods	0.3	3.4	3.4	1.7	0.0	0.1	0.1	0.0	0.0	0.1	0.1	0.0	0.0	1.2	1.2	0.0
Others	0.0	0.0	0.0	0.0	0.1	1.0	1.1	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

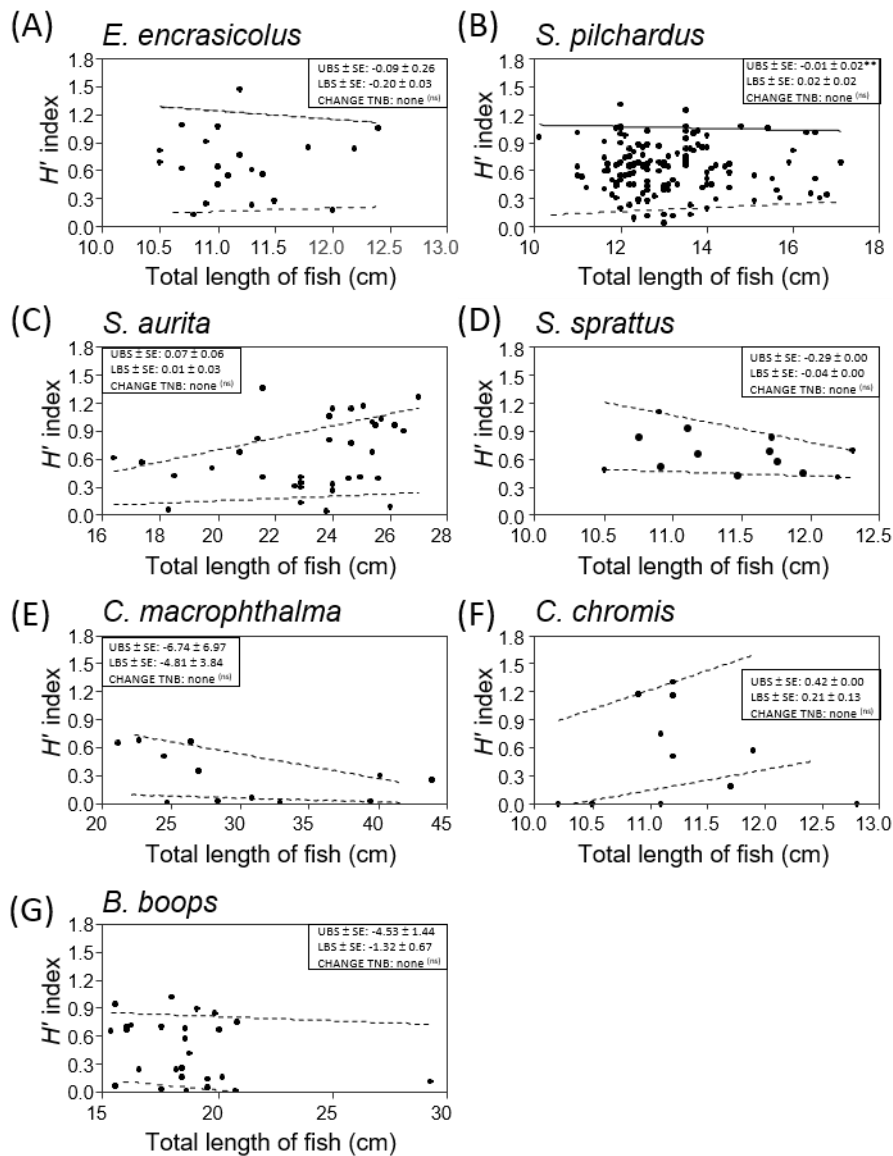
77 **Table S2-2.** Mean stomach content composition of the studied planktivorous fish species in the Bay of Marseille
 78 from March to September 2017. O% = percentage of prey occurrence, N% = percentage of prey numerical
 79 abundance, W% = percentage of prey weight. IRI% = percentage of relative importance index. The highest
 80 percentages by species are in bold.

Prey category	<i>C. macrophthalma</i>				<i>C. chromis</i>				<i>B. boops</i>			
	O%	N%	W%	IRI%	O%	N%	W%	IRI%	O%	N%	W%	IRI%
Harpacticoid copepods	0.3	0.6	0.6	0.2	0.1	0.4	0.2	0.1	0.2	4.1	1.5	1.5
Cyclopoid copepods	0.1	0.6	0.6	0.1	0.7	19.0	19.2	17.8	0.1	5.5	2.1	3.1
Calanoid copepods	0.9	72.5	74.7	81.8	0.6	20.3	20.3	18.9	0.4	13.6	4.7	13.9
Copepod nauplii	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Cladocerans	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Decapod larvae	0.8	19.5	17.3	16.2	0.3	4.9	4.9	3.5	0.3	22.5	8.6	18.6
Cypris larvae	0.0	0.0	0.0	0.0	0.3	10.7	10.7	10.5	0.1	6.3	1.2	6.0
Amphipods	0.2	6.8	6.8	1.6	0.3	6.9	7.0	11.3	0.2	14.5	8.7	13.1
Ostracods	0.0	0.0	0.0	0.0	0.3	1.8	1.8	1.6	0.0	0.3	0.2	0.0
Other crustaceans	0.0	0.0	0.0	0.0	0.1	1.8	1.8	1.2	0.1	0.7	5.5	0.5
Eggs	0.0	0.0	0.0	0.0	0.1	4.6	4.6	2.4	0.1	5.4	2.0	3.2
Bivalve larvae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.8	0.5	0.2
Gastropods	0.0	0.0	0.0	0.0	0.7	29.5	29.5	32.7	0.1	0.6	0.4	0.2
Others	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	28.8	64.6	39.6

81

82 **Fig. S1.** Scatter plots illustrating Shannon-Wiener diversity index (H') of prey as functions of predator
 83 total body length (cm) for the studied species in the Bay of Marseille from March to September 2017.
 84 Each point represents the prey diversity in the stomach content of a single fish. Dotted quantile
 85 regression lines represent changes in the range of diversity as trophic niche breadth (TNB) with
 86 increasing predator size. Upper- (UBS) and lower-bound slopes (LBS) are quantile regressions
 87 estimating 90th (upper) and 10th (lower) quantiles of diversity vs. predator length comparisons (**
 88 0.0001, ** P < 0.001, * P < 0.01, ' P < 0.1, none = not significant). SE is the standard error of each
 89 quantile regression. Results from quantile regressions of diversity vs. predator length comparisons are
 90 also presented. CHANGE TNB represents decreases or increases in trophic niche breadth based on
 91 statistically significant differences between UBS and LBS (P > 0.05 and thus no change in TNB for
 92 all).

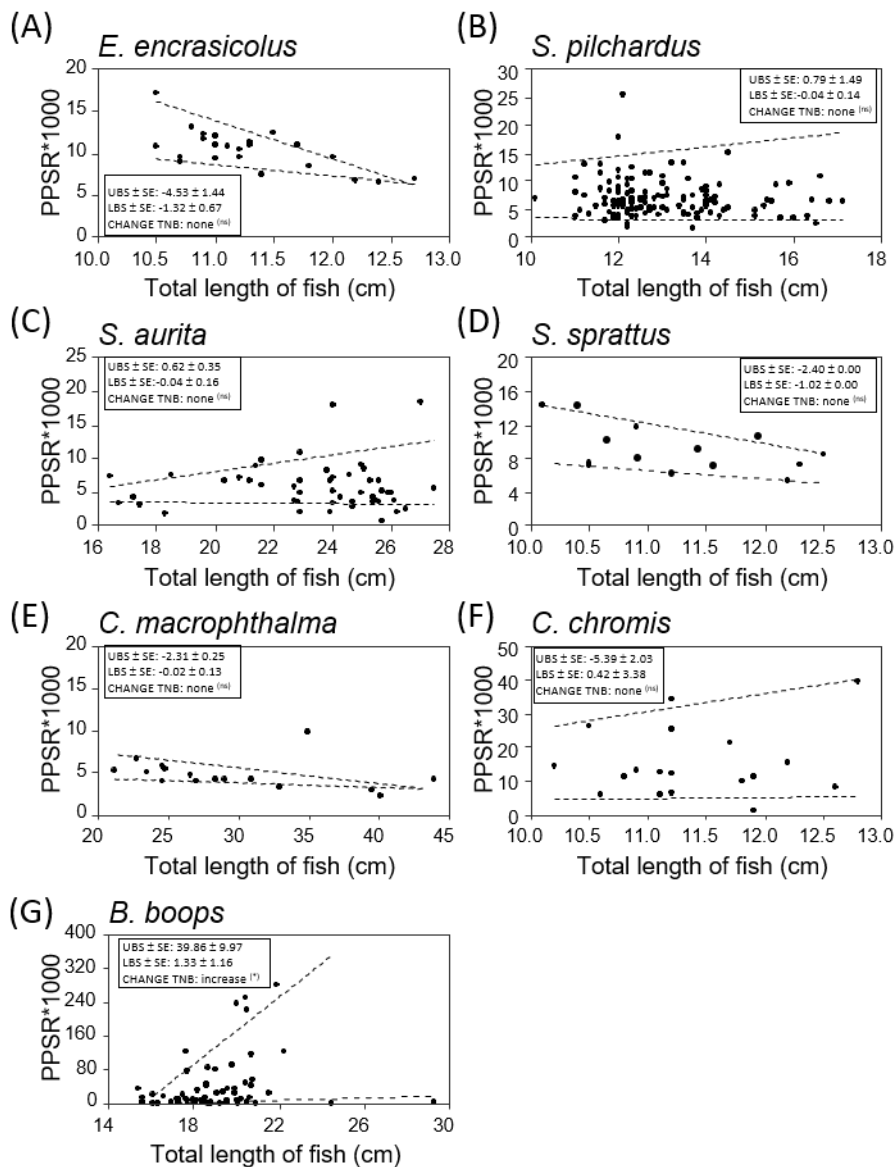
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96 **Fig. S2.** Scatter plots illustrating prey-predator size ratios (PPSR)*1000 as functions of predator total
 97 body length (cm) of the studied species in the Bay of Marseille from March to September 2017. Each
 98 point represents the PPSR*1000 in the stomach content of a single fish. Solid quantile regression lines
 99 represent significant changes in the range of the PPSR*1000 as trophic niche breadth (TNB) with
 100 increasing predator size while dotted quantile regression lines represent non-significant changes.
 101 Upper- (UBS) and lower-bound slopes (LBS) are quantile regressions estimating 90th (upper) and 10th
 102 (lower) quantiles of PPSR*1000 vs. predator length comparisons (*** P < 0.0001, ** P < 0.001, * P <
 103 0.01, ' P < 0.1, none = not significant). SE is the standard error of each quantile regression. Results
 104 from quantile regressions of PPSR*1000 vs. predator length comparisons are also presented. CHANGE
 105 TNB represents decreases or increases in trophic niche breadth based on statistically significant
 106 differences between UBS and LBS (P > 0.05). There was no change in TNB for all species, except for
 107 *B. boops* (P = 0.004).

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