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2 **New insight into the trophic position and ecological role of the European hake (*Merluccius*
3 *merluccius* L., 1758) in Central and Southern Tyrrhenian Sea (Central Mediterranean Sea).**

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5 **Feeding habits of *Merluccius merluccius*.**

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27 **ABSTRACT**

28 This paper aims to investigate the ecological role of *Merluccius merluccius*, Linnaeus, 1758, in the
29 southern and central Tyrrhenian Sea (GSA 10, Resolution GFCM/33/2009/2 General Fisheries
30 Commission for the Mediterranean), analysing ontogenetic diet shift, geographical variations on
31 prey composition and feeding habits. A sample of 734 hake specimens between 6 cm and 73 cm of
32 total length (TL) were collected in 2018. To value the ontogenetic shifts in prey composition, five
33 size-classes were created from the sample and for each class were calculated quantitative feeding
34 indices. The cluster and MDS analysis, based on the % IRI, resulted in three trophic groups of hake
35 size classes. The most abundant preys for small hake (size class I) were the Euphausiids,
36 *Stylocheiron longicorne* and Mysidacea, while for hake with size over 10.5 cm of TL were
37 crustaceans and fish. *Engraulis encrasicolus* was the most abundant prey fish for hake, followed by
38 *Boops boops* and Myctophids. The mesopelagic fauna had a relevant role in the European hake diet
39 in the southern zone. The high presence of Euphausiids, Mysids, Myctophidae and Sternopychidae
40 in the gut content of juvenile hakes (6-23 cm) showed the importance of organic matter and energy
41 flowed from the mesopelagic environment to the epipelagic. Important is also the presence of
42 decapod crustaceans in hake with size over 36 cm TL considering that our study area includes an
43 important Gulf for the fishing of decapod crustacea.

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45 **KEYWORDS:**

46 *Merluccius merluccius*, feeding habits, central Mediterranean, ontogenetic diet shift, energy flow.

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53 RESEARCH HIGHLIGHTS:

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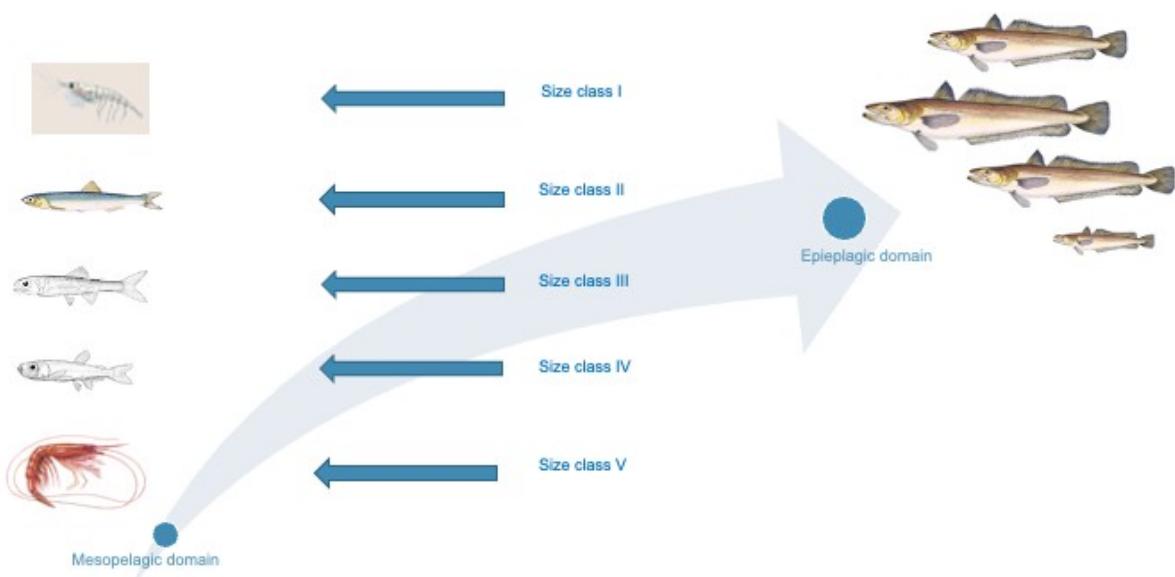
- 55 • *Merluccius merluccius* occupies a generalist niche with active predation and mixed diet
56 showing ontogenetic changing in prey composition. European hake diet shifted from
57 Euphausiids and Mysids for the smaller hakes (<14 cm TL) to fishes for the larger hakes.
58 Before the transition to the complete ichthyophagous phase, hake showed more generalized
59 feeding habits where mesopelagic and nektonic fish dominated the diet, while cephalopod
60 shad a lower incidence.

61

- 62 • The feeding habits of European hake in the Sicilian coast of the Tyrrhenian Sea is a clear
63 example of inverse energy flow between mesopelagic and epipelagic biocenosis. The high
64 occurrence of Euphausiids, Myctophids and Sternopychids found in gut contents of juvenile
65 hake showed the importance of energy and organic matter from deeper strata for the growth
66 of young hakes.

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68 GRAPHICAL ABSTRACT:



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70 **1. Introduction**

71 The Mediterranean is among the most exploited ecosystem of the world, with a very high anthropic
72 impact due to fisheries activity. To improve the multispecies approach of ecosystem-based fishery
73 management and the knowledge on relationships inside the marine trophic chain, it is essential to
74 broaden the knowledge on fish feeding ecology (Möllmann et al., 2014; Pikitch et al., 2004;
75 Stagioni et al., 2011; Zhou et al., 2010). The trophic connections and the ecological dynamics of the
76 target species in the aquatic environments are clarified by the careful study and description of their
77 diet and feeding habits (Angelini et al., 2016; Chipps & Garvey, 2006; Punt et al., 2016; Riccioni et
78 al., 2018). They should be increased to prevent a fishing-induced decline of marine food web
79 trophic levels (Shackell et al., 2010) and to set conscious exploitation across the trophic levels (S.
80 Garcia et al., 2014; S. M. Garcia et al., 2014). The prey-predator relationships, and how these
81 change in time, are the basis of multispecies population dynamics and complex ecosystems models
82 (Carrozzi et al., 2019). The apical predators play a crucial role in the ecosystem, and the research on
83 trophic relationships inside different habitats are focused on them (Leroux & Loreau, 2015), also for
84 the decreasing of piscivorous predators abundance in the Mediterranean due to the heavy
85 exploitation (Francesco Colloca et al., 2013) with consequences for the food chain not fully
86 understood (Lotze et al., 2011).

87 European hake (*Merluccius merluccius* Linnaeus, 1758) is an essential predator of the deeper
88 shelf–upper slope Mediterranean communities; the depth range inhabited by these is wide (20-1000
89 m) throughout the Mediterranean and the north-eastern Atlantic region (Carpentieri et al., 2005;
90 Fischer, 1987) and it is considered a nektobenthic species. Usually, it is possible to evidence
91 bathymetric segregation based on size, particularly the largest individuals live in water deeper than
92 200 m, while juveniles inhabit mostly the coastal shelf (Meiners, 2007). The European hake is an
93 opportunistic predator since its feeding habits change showing geographical differences in prey
94 richness and availability; this allows it to change the composition in species of its diet (Carrozzi et

95 al., 2019; Joan E. Cartes et al., 2009; Costas Papaconstantinou & Stergiou, 1995; Velasco & Olaso,
96 1998).

97 The trawl gears discard may change the diet of opportunistic predators due to the organisms killed
98 or injured by this fishing activity, which can become their food (Kaiser et al., 1994). The wide
99 variety of preys based on fish, crustaceans and cephalopods, show an opportunistic feeding strategy
100 (Anna Bozzano et al., 2005). Maybe, this high variety of feeding habits is due to its broad
101 bathymetric distribution (Carpentieri et al., 2005; J. E. Cartes et al., 2004; Joan E. Cartes et al.,
102 2009). As found in most marine species, hake experiences change in diet due to its ontogenetic
103 development; planktonic species, like euphausiids and mysids, are preyed by the juvenile
104 individuals (total length >11 cm), that feed totally on these, while the small fishes, cephalopods and
105 the nektobenthic decapods are preyed by the smaller adults' individuals (total length from 12 to 16
106 cm). The larger pelagic and nektobenthic fishes are the typical preys of larger hake specimens (up
107 to 20 cm). Furthermore, juvenile individuals perform nictemeral vertical migrations by moving
108 upward and downward through the water column (Orsi Relini et al., 1997).

109 Although many authors deal with this topic in all the Mediterranean, there are two studies in the
110 Central and Southern Tyrrhenian (Modica et al., 2015; Sinopoli et al., 2012). The purpose of our
111 paper is to investigate the diet, the feeding habits and the trophic ecology of hake in the Central and
112 Southern Tyrrhenian Sea, comparing our data to those in the bibliography. The choice to study this
113 area is due to the attendance of gulfs with high anthropogenic pressure, heavily exploited by a large
114 trawling fleet and one fishery exclusion zone which has been subject to a trawling ban since 1990
115 (Pipitone et al., 2000; Rinelli et al., 2004).

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117 **2. Materials and methods**

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119 *2.1. Study area*

120 The investigated area extends in southern and central Tyrrhenian Sea (GSA10) (Figure 1), from the
121 mouth of the Garigliano river, on the border with Lazio, to Capo S. Vito, the westernmost part of
122 norther Sicily, covering a 20225 km² area, with a bathymetric depth between 10 and 800m. The area
123 has a coastal extension of about 1129 km, which involves 5 regions: Lazio, Basilicata, Campania,
124 Calabria and Sicily. The choice to study this area is due to the attendance of gulfs heavily exploited
125 by a large trawling fleet and one fishery exclusion zone which has been subject to a trawling ban
126 since 1990 (Pipitone et al., 2000; Rinelli et al., 2004), with a south region that is typically
127 oligotrophic (POVERO et al., 1990).

128 Furthermore, the coasts bathed by the Tyrrhenian sea are among the most polluted of Mediterranean
129 area, like the eastern area of the Campania region ("Land of Fire") and many others from north to
130 south of the basin (Tamburrino et al., 2019; Triassi et al., 2019; Vichi et al., 2019), where decades
131 of waste dumping practices, associated with the widespread use of pesticides and fertilizers, the
132 incineration and the massive release of environmental contaminants, hurt the whole environment,
133 including the marine coastal ecosystem (Ariano et al., 2019; Esposito et al., 2018; Legambiente,
134 2003, 2007, 2018; Piazese et al., 2019; Rodhouse et al., 2015; Senior & Mazza, 2004).

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136 2.2. Stomach data

137 A sample of 734 European hakes, *Merluccius merluccius*, individuals ranging from 6 and 73 cm
138 total length (TL) was collected in GSA10 by the MEDITS 2018 bottom-trawl survey and by
139 commercial landings of the fishing fleets CAMPBIOL 2018 survey. Specimens collected were
140 frozen on board to prevent digestion of the stomach contents and taken to the laboratory, where
141 each individual was measured (total length, LT cm), weighed (total mass, M g) and sexed with the
142 relative maturity.

143 Stomach repletion status was detected using a five stages macroscopic scale (1 empty; 2 full < 50%;
144 3 full > 50%; 4 bursting; 5 everted, stomach inside the mouth). The stomachs were preserved in

145 ethanol 70% + glycerine 5%). For each prey (species or major Taxa) the digestion degree was taken
146 (1 = undamaged; 2 = almost digested; 3 = highly digested).

147 Each prey item was identified to the lowest taxonomic level possible, counted and weighed.

148 Prey items with a state of digestion advanced were grouped into undetermined fish, crustaceans and
149 cephalopods. To estimate the change in feeding during growth we calculated the vacuity index (VC)
150 obtained by $VC = (Ne/N) \times 100$, where Ne is the number of empty stomachs and N is the number of
151 total stomachs; in the VC we didn't take into account the everted stomachs. To evaluate the
152 contribution of each prey items were calculated: the percentage of abundance composition (%N),
153 the percentage of biomass composition (%W) and the frequency of occurrence (%F) (Hyslop,
154 1980). These indices are necessaries to calculate the index of Relative Importance,
155 $IRI = \%F \cdot (\%N + \%P)$ (Cortés, 1997) expressed as $\%IRI = \textcolor{red}{\dot{I}} \times 100$. To assess variation in diet-
156 related to growth, the samples were divided into eight-length classes included in three trophic
157 groups (Table 4).

158 To classify and ordinate of hake size classes were used both hierarchical cluster analysis and non-
159 metrical multidimensional scaling (NMDS) respectively. The percentage contribution of IRI was
160 chosen as a descriptor of diet similarity (K Robert Clarke & Warwick, 1994). The similarity
161 between biological samples was assessed by the similarity of Bray-Curtis, widely used in
162 multivariate analysis (K R Clarke & Gorley, 2006). The similarity percentage (SIMPER) was used
163 to obtain information about the importance of each prey species to the similarity in diet composition
164 among trophic clusters (CLARKE, 1993). The cluster analysis, nMDS and SIMPER tests were
165 performed using the PRIMER v6 (K Robert Clarke & Warwick, 1994).

166 FIGURE 1

167

168 3. Results

169 A sample of 734 hakes was collected during 2018 and 274 stomachs were analysed divided into five
170 size classes (Table 1). The total number of preys found was 120 belonging to 28 taxa.

171 TABLE 1

172 Analysing the vacuity index (VC) it is possible to notice an increase of this value from size class I
173 to II, this trend is due to decreasing feeding intensity when the species changed typology of prey
174 (Table 2).

175 The overall analysis of the diet, based on the %IRI, shown that fishes were the preferential prey (%
176 IRI = 95.29; %F = 80.5), followed by Mysids (%IRI = 2.57) and Euphausiids (% IRI = 1.25).

177 TABLE 2

178 TABLE 3

179 Table 3 shows the overall list of Taxa found in the stomachs of the hake specimens analysed in the
180 study area.

181 The Euphausiids *Stylocheiron longicorne*, G.O. Sars, 1883, and Mysidacea were the most important
182 prey for small hake (size class I < 10 cm TL) while crustaceans and fish are the preferred prey for
183 hake with size > 10.5 cm of TL. *Engraulis encrasicolus* (Linnaeus, 1758) was the most important
184 prey fish for hake, followed by *Boops boops* (Linnaeus, 1758) and Mictophid (Table 3).

185 TABLE 4

186 FIGURE 2

187 Cluster and MDS analysis based on the % IRI showed three trophic groups of hake size classes
188 (Figure 2 and Table 4). The first group (Group A) is formed by hake species below 14 cm TL (size
189 classes I and II); in this group, the unidentified Osteichthyes were the species that contributing most
190 to the within-group similarities (71.69%) followed by Mysids (28.31%). The high percentage of
191 Osteichthyes is due to the dominance of pieces of muscle or vertebrae with a high grade of
192 digestion found in the stomachs.

193 The second trophic group (Group B) was composed by size class III (14.5-17.5 cm), while the third
194 group (Group C) consisted by size classes over 18 cm TL (size classes IV-VIII) with a high
195 between group average similarity (73%) due to the dominance of unidentified Osteichthyes,
196 followed by *E. encrasicolus* (7.74%) (Table 3, Table 4).

197 The decapod crustacean *Solenocera membranacea* (Risso, 1816) was found as prey for hake with
198 size between 18 and 21 cm TL, *Processa acutirostris* (Nouvel & Holthuis, 1957) in hake with size
199 between 28.5 and 36 cm TL, while the other decapod crustaceans (*Plesionika martia*, A. Milne-
200 Edwards 1883, *Aristaeomorpha foliacea*, Risso 1827, *Pasiphaea sivado*, Risso 1816,
201 *Pseudosquillopsis cerisii*, Roux 1828, and *Aegaeon cataphractus*, Olivi 1792) were found only in
202 the VIII size class of the C group (Table 4). As regard of Cephalopods, we can see that unidentified
203 cephalopod paralarvae were present in the size class I of the A group, while *Rondeletiola minor*
204 (Naef, 1912) was found a spray of hake with size comprised between 21.5-24.5 cm of TL. About
205 the Osteichthyes the results show that mesopelagic species (*Lampanyctus crocodilus*, Risso 1810,
206 *Ceratoscopelus maderensis*, Lowe 1839, *Maurolicus muelleri*, Gmelin, 1789) was found in the hake
207 with size classes 11-14 cm TL in the trophic group A, while demersal and pelagic species as *Cepola*
208 *macrophthalma* (Linnaeus, 1758), *Conger conger* (Linnaeus, 1758), *B. boops*, *Capros aper*
209 (Linnaeus, 1758), *E. encrasiculus*, *Trachurus trachurus* (Linnaeus, 1758), *Microramphus*
210 *scolopax* (Linnaeus, 1758) were found principally in the hake with size classes V, VI and VII
211 (Figure 2, Table 4) of the C trophic group.

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215 **4. Discussion**

216 This study provides, for the first time, an analysis of hake diet and feeding habits in the entire area
217 of central and southern Tyrrhenian sea. Our findings are to be added to those of Modica et al., 2015,
218 and Sinopoli et al., 2012, who has studied the *M. merluccius* diet in the southern part of Tyrrhenian
219 (Calabria and Sicily coast). Modica et al., 2015, focused their attention on Juvenile hake and their
220 predation on Myctophidae and Sternopychidae in a study area that extended from Cape Suvero
221 (western Calabria) to Cape Saint Vito (northern Sicily). While Sinopoli et al., 2012, assessed the
222 effect of trawling on *M. merluccius* diet and trophodynamic, comparing stomach contents and stable

223 isotopes in three northern Sicily gulfs. According to Sinopoli et al., 2012 and other authors from
224 different Mediterranean areas (Anna Bozzano et al., 2005; Carpentieri et al., 2005; J. E. Cartes et
225 al., 2004; Joan E. Cartes et al., 2009), our data confirmed that *M. merluccius* is an active predator
226 with a mixed diet based on demersal-pelagic and benthic preys. It feeds on motile preys
227 (crustaceans or fishes), with a diet which shows an ontogenetic change with a consequential
228 differentiation on trophic level (Fanelli et al., 2018; Sinopoli et al., 2012).

229 Hake diet shifted from Euphausiids (*Meganyctiphanes norvegica*, M. Sars, 1857, *S. longicorne*) and
230 Mysids for the smaller hakes (<14 cm TL) to fishes for the larger hakes. Before the transition to the
231 complete ichthyophagous phase, hake showed more generalized feeding habits where mesopelagic
232 (*C. maderensis*, *M. muelleri*, *L. crocodilus*) and nektonic fish (*E. encrasiculus*, *B. boops*, *C. conger*,
233 ecc.) dominated the diet, while cephalopod shad a lower incidence. The genetic needs and/or the
234 differences in spatial distribution seem to be correlated with the size-related dissimilarities in diet
235 composition (Flamigni, 1983; Jukic & Arneri, 1984; Velasco & Olaso, 1998).

236 As highlighted by Modica et al., 2015, the mesopelagic fauna has a relevant role in the diet of *M.*
237 *merluccius* in the Tyrrhenian sea, especially in the southern zone. This is due to the geomorphologic
238 features of the area, characterized by abrupt shelf-slope breaks and steps slopes, such as Balearic
239 island and north side of Mallorca (Carpentieri et al., 2005; Joan E. Cartes et al., 2009; Modica et al.,
240 2015). In this kind of habitat, the abundance of mesopelagic preys in predator guts like hakes is
241 most enhanced than in areas with a wide continental shelf, like Central Tyrrhenian and Gulf of
242 Lyon (A. Bozzano et al., 1997; Ferraton et al., 2007). The geomorphologic features of Southern
243 Tyrrhenian, together with the water mass circulation of the area and upwelling (Gasparini et al.,
244 2005; Marullo et al., 2011; Sparnocchia et al., 1999), promote and increase the vertical migration of
245 mesopelagic fishes toward shallow waters, enhancing the contact between this community with the
246 demersal predator, like *M. merluccius*. Moreover, the shelf breaks are the hot spots for the nurseries
247 formation in all the Mediterranean. The high density of juveniles of different species including hake
248 is sustained by these habitats, thanks to the aggregation of zooplankton and micronekton enhanced

249 by the increase of primary production and convergence processes (J. E. Cartes, Hidalgo, et al.,
250 2009; F. Colloca et al., 2004; Fiorentino et al., 2003; Garofalo et al., 2011). The presence of *C.*
251 *maderensis*, together with Euphasiids and Mysids, in the gut content of MEDITS juvenile hake (6-
252 23 cm) showed the importance of organic matter and energy flowed from the mesopelagic
253 environment to the epipelagic. This energy conveyance by daily migration of zooplankton and
254 mesopelagic fishes (*C. maderensis*, *Diaphus sp.*, *L. crocodilus*, *M. muelleri*) was essential for
255 juvenile hakes growing. These preyed the mesopelagic species (most of them belong to “Deep
256 Scattering Layer”) during their nictimeral migration to superficial and shallower water. This was a
257 clear example of inverse energy flow from deeper to epipelagic water. *Ceratoscopelus maderensis*
258 was the most preyed fish by juvenile hake during the summer. While, during the autumn, one of the
259 most important prey was *M. muelleri*. All the bioluminescent bathypelagic species were essential
260 for the survival and growth of Mediterranean hake. This was a clear proof of the complex flow of
261 energy and organic matter in the meso- and bati- pelagic environment that was basically for all the
262 demersal species. Our results, according to Modica et al., 2015, highlighted how the mesopelagic
263 fishes were the greater part of the energetic input in the juvenile hake diet during all the year,
264 especially in the southern Tyrrhenian sea. Since Myctophidae and Sternopychidae live in depths
265 greater than 600 m (D’Onghia et al., 2004) and hake juveniles are generally distributed above 330
266 m in the studied area (Biagi et al., 2002), we could assert than in this area there is an inverse flux of
267 energy from deeper to epipelagic water. This intake of energy could be reallocated along the neritic
268 food chain. The upward transfers may be increased by the consumption of mysids and euphausiids
269 in winter-spring and by simultaneous consumption of other fish (e.g., Clupeidae, Argentinidae,
270 Engraulidae, *Chlorophthalmus agassizi*, Bonaparte, 1840, ecc.) that may aggregate in search of the
271 same prey as captured by hake.

272 Although hakes are demersal fishes, they feed generally in the water column, ambushing the fast-
273 swimming pelagic prey (Alheit & Pitcher, 1995). Many studies evidenced that hakes undertake
274 daily vertical migrations, feeding during the night time in mid-water or near the surface (Hickling,

275 1927; ORSI RELINI et al., 1989; C. Papaconstantinou & Caragitsou, 1987). These migrations are
276 more frequent for *M. merluccius* juveniles, which migrate from near the bottom to mid-water at
277 night (Foglia, 1973; ORSI RELINI et al., 1989; C. Papaconstantinou & Caragitsou, 1987). Some
278 authors (Beamish, 1966; Bowman & Bowman, 1980) described nocturnal vertical migrations for
279 gadoids (hake and cod); the decrease of gadids trawl catches during the night seems to be correlated
280 to this behaviour. It is interesting to focus the attention on the low number of Cephalopods in the
281 hake's diet in this GSA. Probably, this trend is due to the ecological and oceanographical properties
282 of the studied area. In the studied area the most statistically relevant preys, especially in winter and
283 autumn, was *E. encrasiculus*, *B. boop* and Clupeidae family, in hake of a size range up from 17,5 to
284 33. In other Mediterranean sub are these species are preyed about smaller juvenile hakes, like in
285 strait of Sicily (Carrozzzi et al., 2019; Fanelli et al., 2018), where *E. encrasiculus* are the most
286 important prey for hake below 14 cm TL, and the bigger hake prey mainly other bony fishes (*T.*
287 *trachurus*, *Lepidopus caudatus*, Euphrasen, 1788) and decapods (Carrozzzi et al., 2019). Engraulidi
288 and Clupeidi are distributed largely on the continental coastal shelf forming schools usually deeper
289 than 25 m, this is due to ecologic reasons that occur in the Tyrrhenian sea, especially in the central
290 region (Bauchot, 1987). Especially in winter and autumn, they are the most available and
291 abundance preys. This feeding habit highlights and confirms the opportunistic ecological behaviour
292 and the generalize niche of nectobenthic top-predator that the Mediterranean hake occupies in the
293 demersal and meso- epi- pelagic food chain, occupying different trophic level during its ontogenetic
294 development (Alheit & Pitcher, 1995; Carpentieri et al., 2005; J. E. Cartes, Maynou, et al., 2009;
295 Mellon-Duval et al., 2017; Sinopoli et al., 2012). This fish, mainly in the adult age, can increase his
296 spectrum of prey, diversifying its feeding habit. *Merluccius merluccius* can prey in benthic, supra
297 benthic, and pelagic domain, doing daily vertical migration and oblique displacements (Carpentieri
298 et al., 2005), following the seasonal and daily changes in distribution and availability of necto-
299 benthic, benthopelagic, benthic and pelagic prey categories (J. E. Cartes et al., 2004; Joan E. Cartes
300 et al., 2009; Costas Papaconstantinou & Stergiou, 1995; Velasco & Olaso, 1998).

301 In the present study, we observed that in hake with size > 36 cm TL there are also decapods with
302 other fish in the stomach contents. This is probably concerning the size-depth distribution of the
303 individuals. Juveniles live mostly between 100 and 200 m depth, intermediate hakes reach the
304 highest abundance mainly on the shelf (<100 m), while large hakes (>36 cm) live in a wide depth
305 range, concentrate on the shelf break during the spawning period (Alvarez et al., 2001; F Colloca et
306 al., 2000; Recasens et al., 1998).

307 Different authors report that cannibalism plays an important role in the large hakes (>36 cm), and it
308 should be carefully assessed in stock-recruitment analyses (A. Bozzano et al., 1997; Guichet, 1995;
309 Link & Garrison, 2002; Macpherson Mayol, 1979). As reported by Link and Garrison ,2002, in
310 silver hake (*Merluccius bilinearis*, Mitchell, 1814) cannibalism not increased with ontogeny. As
311 showed by (Roel & Macpherson, 1988) the 50% of food item found in the stomachs of large cape
312 hakes, *Merluccius capensis*, Castelnau, 1861, the hakes were the dominant food items for
313 individuals larger than 60 cm; while for the congeneric sympatric species, *Merluccius paradoxus*
314 Franca, 1960, from the same area was observed a low cannibalism rate (Payne et al., 1987). Hake
315 has a high impact to the fish population including anchovy and other species as well as on
316 crustaceans in the Adriatic Sea (Foglia, 1973; Piccinetti & Piccinetti Manfrin, 1971; Riccioni et al.,
317 2018; Zupanovic, 1968) and in the Tyrrhenian Sea (Carpentieri et al., 2005). The literature under-
318 line that the hake feed is different in the Mediterranean and the Atlantic waters. In the Atlantic
319 waters, the main role in the hake's diet is played by the blue whiting, *Micromesistius poutassou*,
320 Risso, 1827; European anchovy, *E. encrasicolus*; and, in largest individuals, by Atlantic horse
321 mackerel, *T. trachurus* (A. Bozzano et al., 1997; Cabral & Murta, 2002; Guichet, 1995; Olaso,
322 1990; Velasco & Olaso, 1998). In Agreement with Sinopoli et al.,2012, our findings confirm that is
323 also important the presence of decapod crustaceans (*P. martia*, *A. foliacea*, *P. sivado*, *P. cerisii* and
324 *A. cataphractus*) in the VIII size class of the C group (hake size>36 cm TL) if we consider that our
325 study area includes important Gulf for the fishing of decapod crustacea (Giordano et al., 1999).

326 For further future analysis will be interesting to combine the traditional stomach content analysis
327 whit the relatively recent techniques of stable isotope analysis and metabarcoding method based on
328 COI PCR amplification. These innovative techniques were applied to the hake in other
329 Mediterranean areas like Adriatic and strait of Sicily (Fanelli et al., 2018; Riccioni et al., 2018).
330 The new approaches take into control the analysis of the stable isotopes C and N and it allows to
331 extrapolate essential information about the trophic position of the species, particularly information
332 about the trophic chain of the demersal environment, in the relationship from mesopelagic and
333 epipelagic water.

334 The meta-barcoding approach detects a wide spectrum of prey (Riccioni et al., 2018) than the
335 traditional morphological analysis. This approach is useful to improve the knowledge of
336 Mediterranean hake's diet and ecology, more overall improving the management actions to preserve
337 this relevant ecological and commercial fish stock.

338

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342

343 **6. Conflict of Interest**

344 The authors have no conflicts of interest to declare. All co-authors have seen and agree with the
345 contents of the manuscript and there is no financial interest to report. We certify that the submission
346 is original work and is not under review at any other publication.

347

348 **7. Data Accessibility Statement**

349 All the data are property of U.E.

350 Data accessibility:

351 <https://datacollection.jrc.ec.europa.eu>

352 <https://dcf-italia.cnr.it/web/#/pages/home>

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358 **Figure Captions:**

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360 Figure. 1 – Sampling hauls from Garigliano river to San Vito Cape GSA 10.

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362 Figure 2. Dendrogram (X) and MDS (Y) based on %IRI values of the eight classes.

363 Size classes (I: 6-10.5; II: 11 -14; III: 14.5-17.5; IV: 18-21; V: 21.5-24.5; VI: 25-28; VII: 28.5-36;

364 VIII: 36.5-73); Using group average clustering from Bray-Curtis similarity on diet data.

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Size class	Total length	Individuals sampled	Empty	Full	Everted	Stomach sampled	$VC = (Ne/N) * 100$
I	< 10 cm LT	106	42	23	41	23	6,36
II	10,5-15 cm	231	92	63	76	60	13,94
III	15,5-20 cm	115	31	49	35	50	4,85
IV	20,5-32,5 cm	208	44	105	59	110	6,67
V	> 32,5 cm	74	20	30	24	31	3,03
Σ		734	229	270	235	274	34,85

383

384 Table 1: Number of individuals of European hake sampled in the North sector of the Strait of Sicily

385 with full, empty and everted stomachs by size class. The vacuity index (%VC) is also shown.

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Taxa	%F	%W	%N	IRI	%IRI
<i>Solenocera membranacea</i>	0,67	0,16	0,53	0,47	0,00
<i>Processa acutirostris</i>	2,01	0,24	1,59	3,69	0,03
<i>Plesionika martia</i>	0,67	0,30	0,53	0,55	0,00
<i>Aristaeomorpha foliacea</i>	1,34	3,14	1,06	5,64	0,04
<i>Pasiphae sivado</i>	0,67	0,09	0,53	0,41	0,00
<i>Pseudosquillopsis cerisii</i>	0,67	0,42	0,53	0,64	0,00
<i>Aegaeon cataphracta</i>	0,67	0,23	0,53	0,51	0,00
Decapoda n.d.	2,68	0,26	2,12	6,38	0,05
Overall Decapoda	9,40	4,84	7,41	115,10	0,86
<i>Meganyctphanes norvegica</i>	1,34	0,37	1,06	1,91	0,01
<i>Stylocheiron longicorne</i>	2,68	0,13	2,12	6,04	0,05
Euphasiacea n.d.	10,07	0,21	7,94	81,99	0,62

Overall Euphausiacea	14,09	0,71	11,11	166,55	1,25
Mysidacea nd.	20,81	0,06	16,40	342,47	2,57
Overall Mysidacea	20,81	0,06	16,40	342,47	2,57
Crustacea nd.	3,36	0,24	2,65	9,67	0,07
<i>Rondeletiola minor</i>	0,67	0,23	0,53	0,51	0,00
Cephalopoda n.d.	1,34	0,01	1,06	1,43	0,01
Overall Cephalopoda	2,01	0,23	1,59	3,66	0,03
<i>Boops boops</i>	3,36	22,29	2,65	83,67	0,63
<i>Chlorophthalmus agassizi</i>	1,34	6,48	1,06	10,11	0,08
<i>Capros aper</i>	0,67	0,12	0,53	0,44	0,00
<i>Cepola macroptalma</i>	2,01	1,26	1,59	5,72	0,04
<i>Ceratoscopelus maderensis</i>	2,01	0,46	1,59	4,11	0,03
<i>Conger conger</i>	0,67	0,13	0,53	0,44	0,00
<i>Engraulis encrasicolus</i>	9,40	23,75	7,41	292,74	2,20
<i>Hymenocephalus italicus</i>	1,34	0,25	1,06	1,76	0,01
<i>Lampanyctus crocodilus</i>	0,67	0,61	0,53	0,76	0,01
<i>Trachurus trachurus</i>	1,34	4,17	1,06	7,02	0,05
<i>Macroramphosus scolopax</i>	0,67	0,38	0,53	0,61	0,00
<i>Maurolicus Muelleri</i>	0,67	0,17	0,53	0,47	0,00
Clupeidae n.d.	8,05	13,21	6,35	157,50	1,18
Osteichtyes n.d.	42,28	18,60	33,33	2195,75	16,48
Ophichthidae n.d.	0,67	0,08	0,53	0,41	0,00
Myctophidae n.d.	4,70	1,14	3,70	22,77	0,17
Sparidae Spp	0,67	1,07	0,53	1,07	0,01
Overall Osteichtyes	80,54	94,16	63,49	12696,81	95,29

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388 Table 2. Diet composition of *Merluccius merluccius* sampled in the Central and Southern
 389 Tyrrhenian Sea. Frequency of occurrence (%F); Percentage in weight (%W); Percentage in number
 390 (%N); Index of relative importance IRI and %IRI.

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Trophic group	A		B		C			
	I	II	III	IV	V	VI	VII	VIII
Crustacea								
<i>Solenocera membranacea</i>				0,003				
<i>Processa acutirostris</i>						0,028		
<i>Plesionika martia</i>							0,005	
<i>Aristaemorpha foliacea</i>							0,043	
<i>Pasiphaea sivado</i>							0,003	
<i>Pseudosquillopsis cerisii</i>							0,005	
<i>Aegaeon cataphracta</i>							0,004	
<i>Meganycophanes</i>								
<i>norvegica</i>	0,014							
<i>Stylocheiron longicorne</i>	0,045							
Unid. Euphausiacea	0,615							
Unid. Mysidacea	2,570	2,570						
Unid. Decapoda					0,048			
Unid. Crustacea						0,073		
Cephalopoda								
<i>Rondeletiola minor</i>				0,004				
Unid. Cephalopoda	0,011							
Osteichthyes								
<i>Boops boops</i>					0,628	0,628		
<i>Chlorophthalmus agassizi</i>						0,076		
<i>Capros aper</i>							0,003	
<i>Cepola macrophtalma</i>				0,043		0,043		
<i>Ceratoscopelus maderensis</i>	0,031	0,031						
<i>Conger conger</i>							0,003	
<i>Engraulis encrasicolus</i>				2,197	2,197	2,197		
<i>Hymenocephalus italicus</i>							0,013	
<i>Lampanyctus crocodilus</i>	0,006			0,006				
<i>Trachurus trachurus</i>					0,053			
<i>Macroramphosus scolopax</i>					0,005			
<i>Maurolicus Muelleri</i>	0,003							

<i>Clupeidae n.d.</i>				1,182		1,182
<i>Ophichthidae n.d.</i>	0,003					
<i>Myctophidae n.d.</i>				0,171		1,182
<i>Sparidae Spp</i>						0,008
	16,47	16,47		16,47	16,47	16,47
Unid Osteychthyes	9	9	16,479	9	16,479	9
					16,479	16,479

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404 Table 3. Diet composition of the eight size classes of hake in the Central and Southern Tyrrhenian
 405 Sea in terms of % IRI (index of relative importance). *Size class: I: 6-10,5 cm TL; II: 11 – 14 cm
 406 TL; III: 14,5 – 17,5 cm TL; IV: 18 – 21 cm TL; V: 21,5 – 24,5 cm TL; VI 25 – 28 cm TL; VII 28,5
 407 – 36 cm TL; VIII 36,5 – 73 cm TL; ** Number of individuals for size class: I:118; II: 185; III: 102;
 408 IV: 67; V: 55; VI: 69; VII: 96; VIII: 43.

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Hake size (cm)

≤ 14	Group A	Average similarity: 87,71				
	<i>Species</i>	Av. Abund Av.Sim Contrib% Cum.%				
	Unid. Osteychthyes	4,06	62,88	71,69	71,69	
	Unid. Mysidacea	1,60	24,83	28,31	100,00	
14,5 – 17,5	Group B	Less than 2 samples in group				
≥ 18	Group C	Average similarity: 73,12				
	<i>Species</i>	Av. Abund Av.Sim Contrib% Cum.%				
	Unid. Osteychthyes	4,06	64,43	88,12	88,12	
	Engraulis encrasiculus	0,89	5,66	7,74	95,85	

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413 Table 4. SIMPER similarity analysis.

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