



# 1 Mesoscale variations in the assemblage structure and trophodynamics 2 of mesozooplankton communities of the Adriatic basin (Mediterranean 3 Sea)

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11 **Abstract.** Zooplankton are critical to the functioning of ocean food webs because of their utter abundance and vital  
12 ecosystem roles. Zooplankton communities are highly diverse and thus perform a variety of ecosystem functions, thus  
13 changes in their community or food web structure may provide evidence of ecosystem alteration. Assemblage structure  
14 and trophodynamics of mesozooplankton communities were examined across the Adriatic basin, the northernmost and  
15 most productive basin of the Mediterranean Sea. Samples were collected in June-July 2019 along coast-offshore transects  
16 covering the whole western Adriatic side, consistently environmental variables were also recorded. Results showed a  
17 clear separation between samples from the northern-central Adriatic and the southern ones, with a further segregation,  
18 although less clear, of inshore vs. off-shore stations, the latter mostly dominated in the central and southern stations by  
19 gelatinous plankton. Such patterns were mainly driven by chlorophyll-*a* concentration (as a proxy of primary production)  
20 for northern-central stations, *i.e.* closer to the Po river input, and by temperature and salinity, for southern ones, with the  
21 DistLM model explaining 46% of total variance. The analysis of stable isotopes of nitrogen and carbon allowed to identify  
22 a complex food web characterized by 3 trophic levels from herbivores to carnivores, passing through the mixed feeding  
23 behavior of omnivores, shifting from phytoplankton/detritus ingestion to microzooplankton. Trophic structure also  
24 spatially varied according to sub-area, with the northern-central sub-areas differing from each other and from the southern  
25 stations. Our results highlighted the importance of environmental variables as drivers of zooplanktonic communities and  
26 the complex structure of their food webs. Disentangling and considering such complexity is crucial to generate realistic  
27 predictions on the functioning of aquatic ecosystems, especially in high productive and, at the same time, overexploited  
28 area such as the Adriatic Sea.

29 **Key-words:** mesozooplankton, community composition, environmental drivers, food webs, stable isotopes, Adriatic Sea



## 30 **1 Introduction**

31 In an oligotrophic system, such as the Mediterranean Sea, coastal productivity largely depends on inputs from rivers and  
32 areas of high productivity are mainly restricted to waters close to major freshwater inputs (D'Ortenzio and Ribera d'Alcalà,  
33 2009, Ludwig et al., 2009). Here, the Adriatic basin represent an anomaly, with the northern Adriatic being one of the  
34 most productive Mediterranean areas. While the northern part is a shallow sub-basin, characterised by inputs of several  
35 rivers, with the Po representing the major buoyancy input with an annual mean discharge rate of  $1500\sim 1700\text{ m}^3\text{s}^{-1}$ , and  
36 accounting for about one third of the total riverine freshwater input in the Adriatic (Raicich, 1996), the southern part is  
37 characterized by highly saline and oligotrophic waters (Franco and Michelato, 1992; Boicourt et al., 1999). Thus, a trophic  
38 gradient, decreasing from northwest to southeast, is typically observed in the basin, in which the nutrient-rich waters  
39 coming from the rivers are mainly spread southward and eastward from the Italian coast (Bernardi Aubry et al., 2006;  
40 Solidoro et al., 2009). Such differences may be reflected in the population dynamics of the marine biotic components  
41 (Revelante and Gilmartin 1977; Simonini et al. 2004; Hermand et al. 2008), from zooplankton (Siokou-Frangou and  
42 Papathanassiou 1991; Hwang et al. 2010) to fish (Wets et al., 2011). However, such dynamics both in terms of community  
43 composition and trophic relationships have never been investigated at the scale of the whole Adriatic basin. Zooplankton  
44 play a key role in marine ecosystems, forming the base of marine food web because of the diversity of their functions.  
45 Zooplankton is a link between primary producers of organic matter and the higher-order consumers, it provides grazing  
46 control on phytoplankton blooms (Kjørboe 1993) and helps regulating fish stocks (Beaugrand et al. 2003), being this last  
47 aspect of crucial importance in the Adriatic basin. Because of these important zooplankton functions, a better  
48 understanding of their distribution and the patterns of their response to changes in the chemical and physical properties  
49 of marine waters is essential, especially under a global warming scenario, being zooplankton sensitive beacon of climate  
50 change (Richardson, 2008). Moreover, trophic relationships in pelagic ecosystems are complex and complicated by the  
51 large degree of omnivory of most zooplanktonic species (Bode and Alvarez-Ossorio, 2003), which may feed on similar  
52 diets composed of a mixture of phytoplankton, detritus, and microplankton (e.g., Stoecker and Capuzzo, 1990; Irigoien  
53 et al., 1998; Batten et al., 2001). Several experimental studies allowed zooplankton (mostly copepods) to be categorised  
54 from pure carnivores to omnivores with a variety of mixtures of algae and animal prey up to strictly herbivore species  
55 (Irigoien et al., 1998; Batten et al., 2001; Halvorsen et al., 2001). Such variety in the diet makes the quantification of  
56 flows between compartments or trophic levels difficult. In the last decades, stable isotope analyses (SIA) have been widely  
57 used in food-web studies, different studies dealt with high taxonomical groups of zooplankton (Burd et al., 2002;  
58 Blachowiak-Samolyk et al., 2007; Tamelander et al., 2008), while few investigations were focused on low taxonomical  
59 resolution (Koppelman et al., 2003; Rumolo et al., 2017), essential to disentangle the food web structure of pelagic  
60 communities (Fanelli et al., 2011). Analysis of stable isotope composition provides indications of the origin and  
61 transformations of organic matter. Stable isotopes of carbon and nitrogen integrate short-term variations in diet and thus



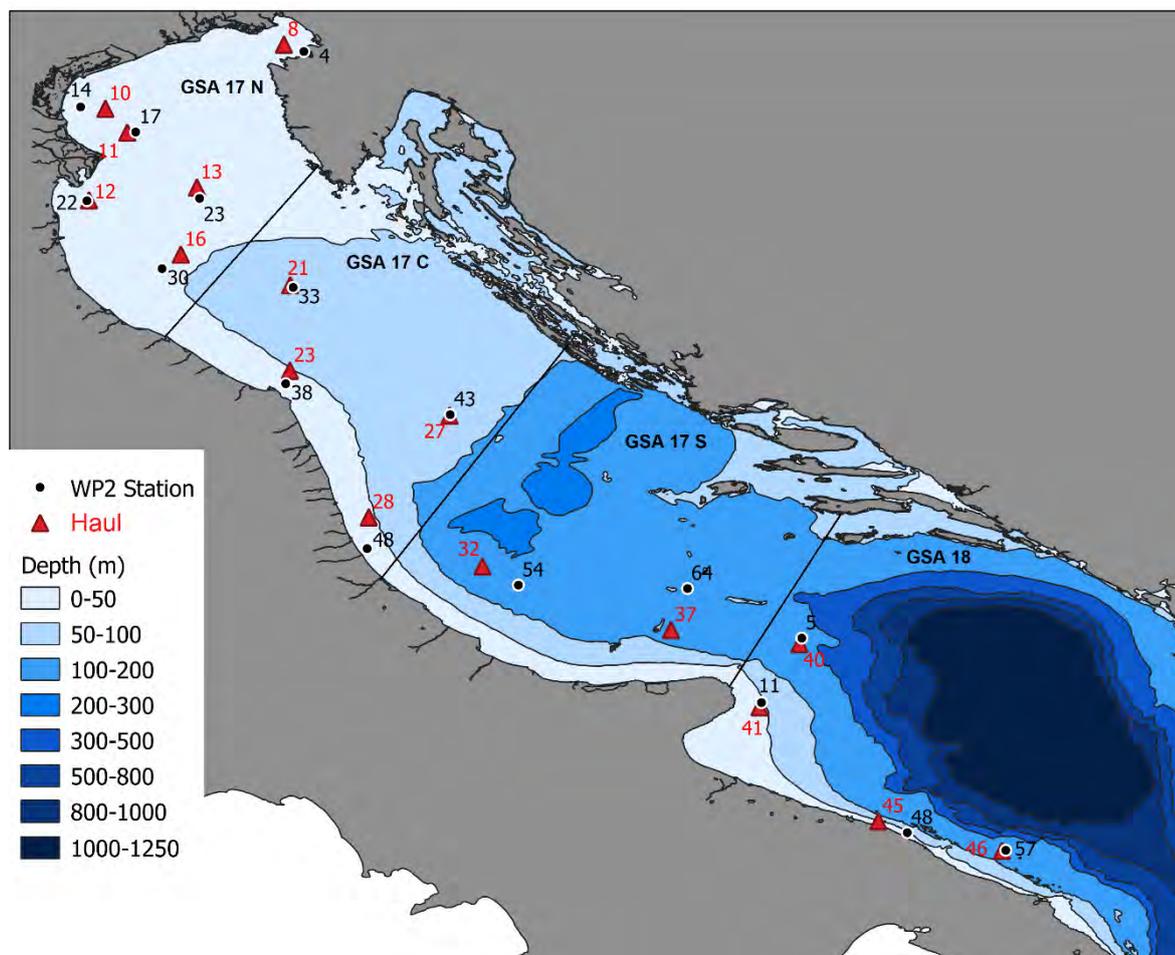
62 are less subject to temporal bias. The  $\delta^{15}\text{N}$  in tissues of consumers are typically greater by 2–3‰ relative to their prey  
63 and can be used as a proxy of the trophic level of organisms (Owens, 1987), while  $\delta^{13}\text{C}$  may act as a useful indicator of  
64 primary organic carbon sources of an animal's diet, as tissues tend to be rather weakly enriched in  $^{13}\text{C}$  at progressively  
65 higher trophic levels (1‰).

66 In this context the main aim of this study is to analyse mesoscale variations in the assemblage structure and  
67 trophodynamics of mesozooplankton communities in the whole basin. Additionally, considering the complex  
68 hydrological condition of the basin, characterised by such contrasting oceanographic settings from north to south, here  
69 we explored and identified which environmental variables best explain the observed patterns.

## 70 **2 Materials and Methods**

### 71 **2.1. Study area**

72 The Adriatic Sea is an elongated semi-enclosed basin, with its major axis in the northwest–southeast direction, located in  
73 the central Mediterranean, between the Italian peninsula and the Balkans (**Figure 1**). It is 800 km long and 150–200 km  
74 wide. It has a total volume of 35,000 km<sup>3</sup> that belongs for 5% to the Northern basin, 15% to the middle basin and 80% to  
75 the Southern basin. The Northern Adriatic is very shallow, with an average depth of 35 m with a very gradual topographic  
76 slope along its major axis and it is characterized by strong river runoff, being the Po the second main contributor (about  
77 20%) to the whole Mediterranean river runoff (Struglia et al., 2004).



78

79 **Figure 1:** Map of the study area with indication of WP2 net stations (black dots) and mid-water trawl hauls.

80

81 Due to this input, there is a positive water balance of 90-150 km<sup>3</sup> that is exported to the Mediterranean. The turnover time  
82 for the whole basin is 3-4 years (Artegiani *et al.*, 1997; Marini, Bombace and Iacobone, 2017). The middle Adriatic is a  
83 transition zone between northern and southern sub-basins, with the two Jabuka/Pomo depressions reaching 270 m depth.  
84 The southern sub-basin is characterized by a wide depression about 1200 m in depth. Water exchange with the  
85 Mediterranean takes place through the Otranto Strait, which has an 800 m deep sill (Artegiani *et al.*, 1997; Marini,  
86 Bombace and Iacobone, 2017). The Adriatic is a temperate warm sea, with surface temperature ranging from 6 °C in the  
87 northern part in winter to 29 °C, in summer. Even the temperatures of the deepest layers are, for the most part, above 10  
88 °C. The South Adriatic is warmer than its central and northern parts during winter. In other seasons, the horizontal  
89 temperature distribution is more uniform (Artegiani *et al.*, 1997; Marini, Bombace and Iacobone, 2017).



90 Water circulation in the Adriatic is mainly driven by dominant winds (Bora and Scirocco) that cause a cyclonic circulation,  
91 with three closed circulation cells (one for each sub-basin). Three different water masses dominate the basin circulation:  
92 the Adriatic Surface Water (AdSW), the Levantine Intermediate Water (LIW) and the Adriatic Deep Water (AdDW),  
93 which branches out in Northern (NAdDW), Middle (MAdDW) and Southern (SAdDW) Adriatic Deep Water. The  
94 hypersaline LIW is formed in the Levantine Basin and experiences a salinity decrease on its way to the Adriatic. The  
95 AdDW are formed in the Adriatic basin and the NAdDW in the Northern part; due to its high density, it fills up the  
96 Jabuka/Pomo Pit and only occasionally spreads to the Southern Adriatic. The MAdDW is formed in the Jabuka/Pomo Pit  
97 area, when there is no intensive north-westward flow, (*i.e.* during periods of low Mediterranean water inflow). The  
98 SAdDW originates in the South Adriatic Pit. As mentioned above, the Adriatic is a very productive basin, compared to  
99 the rest of the Mediterranean. Despite being only the 5% of the total Mediterranean surface area, the Adriatic Sea produces  
100 about 15% of total Mediterranean landings (and 53-54% of Italian landings), with a fish production density of 1.5 t/km<sup>2</sup>,  
101 which is three times the Mediterranean density (Marini, Bombace and Iacobone, 2017). This impressive feature is shaped  
102 by three main factors: river runoff, shallow depths and oceanographic structure. Rivers can indeed provide nutrients,  
103 which favour phytoplanktonic blooms, thus causing a bottom-up effect of the whole trophic chain. Rivers can also provide  
104 suspended particulate organic matter and organic detritus, that feed numerous particulate feeders and detritivores, such as  
105 bivalves (which is one of the main fisheries of the North Adriatic Sea). The wide continental shelf favours a short trophic  
106 chain that likely improve the efficiency of energy transfer from lower trophic levels to higher ones. Moreover, the structure  
107 of the basin allows water mixing during winter, especially in North and Middle Adriatic, transferring nutrients from  
108 sediments to the water column. From a fishery management point of view, the General Fishery Commission for the  
109 Mediterranean (GFCM) has divided the basin in two Geographical Sub-Areas (GSAs), the GSA 17, encompassing the  
110 northern and the middle sub-basin and the GSA 18, including the southern part.

## 111 **2.2 Zooplankton collection and analysis**

112 Samples for this study were collected on board R/V “G. Dallaporta” during the acoustic survey MEDIAS 2019 GSA 17  
113 and GSA 18, that took place in June-July 2019, in the Adriatic Sea (Leonori *et al.*, 2020), within the framework of the  
114 MEDIAS (MEDiterranean International Acoustic Surveys) project. MEDIAS coordinates the acoustic surveys performed  
115 in the Mediterranean and Black Sea to assess the biomass and spatial distribution of small pelagic fish (MEDIAS, 2019)  
116 (<http://www.medias-project.eu>).

117 Zooplankton samples were collected through 200 µm-mesh size WP2 net, with a circular mouth of 57 cm diameter and  
118 2.6 m long, equipped with a MF 315 flowmeter to estimate the volume of filtered water. Vertical tows were performed  
119 with a towing speed of 1 m/s, starting from three meters above the bottom, to the surface. Sampling stations were located  
120 along acoustic sampling transects (Figure 1).

121 Zooplankton samples near the fishing hauls were subsampled: half of each sample was frozen at -20 °C, to be used for  
122 this study, while the other half was preserved in formalin. Concurrently with each vertical plankton haul, a CTD cast was



123 performed, to acquire information on the oceanographic parameters of the chosen site. For the purpose of this study, the  
124 whole Western Adriatic (GSA 17 and GSA 18) has been divided in four different sub-areas, based mainly on  
125 oceanographic characteristics: 1. GSA 17 North (GSA17N), characterized by a low depth, up to about 60 m, and mostly  
126 influenced by the Po River input; 2. GSA 17 Central (GSA17C), with deeper bottoms, up to about 100 m; 3. GSA 17  
127 South (GSA17S), characterized by the presence of the Pomo Pit and 4. GSA 18, characterized by the presence of the  
128 South Adriatic Pit and the Otranto Channel. For each sub-area, hauls that were representative of variations in  
129 oceanographic characteristics (mainly depth, distance from the coast and latitude) were selected. Such differences were  
130 first tested by one-way PERMANOVA (Permutational Multivariate Analysis of Variance; Anderson et al., 2008) and if  
131 no significant differences were found, sub-areas were merged for the following analyses.

132 Selected zooplankton samples were analysed in the laboratory to characterize the planktonic community. First, the frozen  
133 sample was defrosted and filtered with 200  $\mu\text{m}$  sieve and the obtained mass was weighted. Then samples were quickly  
134 sorted, and larger animals isolated for first and placed in Petri dishes located on ice, in order to preserve tissue integrity.  
135 Individuals were then identified to the lowest taxonomic level possible and stored for subsequent analysis. About 10% of  
136 the sample was therefore weighted and all organisms in the sub-sample were identified to the lowest taxonomic level  
137 possible.

138 All identified taxa were then counted and weighted with an analytical weight scale, to obtain abundance and biomass  
139 estimations.

### 140 **2.3. Samples preparation for stable isotope analyses**

141 The most abundant taxa in each sample were prepared for stable isotope analyses. Selected taxa were oven-dried for 24  
142 hours at 60 °C. Dried samples were converted to a fine powder with a mortar and pestle. For each taxon, three replicates  
143 (when possible) were weighted (ca 0.3-1.3 mg) and placed into tin capsules. Since it was not possible to obtain enough  
144 material of a single taxon for stable isotope analyses from stations 22\_17 and 38\_17, a bulk of the whole mesoplankton  
145 community of the stations was prepared for the analyses. Acidification of samples prior to stable isotope analyses is  
146 usually regarded as a standard procedure, since inorganic carbon could lead to an increase of  $\delta^{13}\text{C}$ , because it is  
147 isotopically heavier than most carbon of organic origin and could reflect the isotopic signature of environmental carbon  
148 (Schlacher and Connolly, 2014). However, for this study, no acidification was carried out, as this procedure generally  
149 reduces sample biomass, leading to too little matter available for isotope analyses. Moreover, some authors revealed  
150 negligible differences between acidified and not acidified samples (Rumolo *et al.*, 2018). However, in order to have an  
151 indication of the possible bias, only one species was acidified, *Euchaeta sp.*, which is a very abundant copepod in Adriatic  
152 communities. This taxon was also chosen because it has a more calcified exoskeleton and it was abundant enough to  
153 undergo this process. Half of the sample was acidified with HCl 1M, by adding it drop by drop to the sample until bubble



154 cessation, then samples were oven-dried again at 60 °C for 24 h. The other half, for the analysis of  $\delta^{15}\text{N}$ , was not acidified,  
155 as several studies demonstrated that the acidification procedure can alter nitrogen isotopic signature (Kolasinski, Rogers  
156 and Frouin, 2008). Then, six replicates of each sub-samples were prepared for isotope analyses. Samples were analysed  
157 through an elemental analyser (Thermo Flash EA 1112) for the determination of total carbon and nitrogen, and then  
158 analysed for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in a continuous-flow isotope-ratio mass spectrometer (Thermo Delta Plus XP) at the  
159 Laboratory of XX of the University of Palermo (Italy). Stable isotope ratio was expressed, in relation to international  
160 standards (atmospheric  $\text{N}_2$  and PeeDee Belemnite for  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ , respectively), as:

$$\delta^{13}\text{C} \text{ or } \delta^{15}\text{N}: [(R_{\text{sample}}/R_{\text{standard}})-1]*10^3$$

161 where  $R = {}^{13}\text{C}/{}^{12}\text{C}$  or  ${}^{15}\text{N}/{}^{14}\text{N}$ . Analytical precision based on standard deviations of internal standards (International  
162 Atomic Energy Agency IAEA-CH-6; IAEA-NO-3; IAEA-N-2) ranged from 0.10 to 0.19‰ for  $\delta^{13}\text{C}$  and 0.02 to 0.08‰  
163 for  $\delta^{15}\text{N}$ .

#### 165 2.4. Community data analyses

166 Zooplankton abundance and biomass were standardized to a constant value. The adopted constant was the volume of  
167 water filtered by the net, calculated as follows:

$$V(\text{m}^3) = A \cdot B \cdot C$$

168 where A is the number of spins from the flowmeter, B is the number of meters travelled with each spin (given by the  
169 manufacturer) and C is the area of the net mouth ( $\text{m}^2$ ). When flowmeter data were not available (due to malfunctioning),  
170 the volume was calculated as a mean value of similar nearby stations. Zooplankton abundance was expressed as number  
171 of individuals per  $\text{m}^3$ , while zooplankton biomass was expressed as mg of wet weight (WW) per  $\text{m}^3$ .

172 First, the Shannon-Wiener diversity index of each station was calculated. Then, total biomass, total abundance and  $H'$   
173 diversity index were tested by univariate PERMANOVAs. Tests were run on Euclidean distance resemblance matrixes  
174 of untransformed data and using a two-way design with sub-area as a fixed factor with three levels (GSA17N, GSA17C-  
175 S and GSA18) and inshore-offshore location as a fixed factor with two levels (inshore vs. offshore), crossed within each  
176 other, in order to assess the presence and significance of differences between stations. As the preliminary analyses showed  
177 no significant differences between samples from GSA17C and GSA17S, these two sub-areas were merged, allowing to  
178 use a crossed design, otherwise impossible due to the absence of the “inshore” level within the sub-area GSA17S.  
179 Univariate PERMANOVA test were run under 9999 permutations, with permutation of residuals under a reduced model,  
180 as permutation method, significant p-values were set at  $p < 0.05$ .

181 In order to test for differences among areas and inshore vs. offshore communities a PERMANOVA test was performed  
182 on the Bray-Curtis resemblance matrix of 4<sup>th</sup>-root transformed abundance zooplankton data, using the same design  
183



184 described for univariate analyses. A CAP analysis (Canonical Analysis of Principal coordinates, Anderson and Willis,  
185 2003) was then run to visualize the observed pattern, on the factor found to be significant by PERMANOVA.  
186 A SIMPER analysis was carried out according to the same sampling design to identify the most typifying taxon  
187 contributing to the average similarity/dissimilarity among sub-areas and inshore vs. offshore locations. This was  
188 conducted using Bray-Curtis similarity, with a cut-off for low contribution at 60%.  
189 In order to identify the environmental drivers of zooplanktonic communities and their structure across the sampling area,  
190 biotic data were correlated to environmental variables. Environmental data considered were pressure (db), temperature  
191 (°C), fluorescence (µg/l), turbidity (NTU), dissolved oxygen (expressed as ml/l and saturation percentage), salinity (PSU)  
192 and density (km/m<sup>3</sup>). All data were collected through a CTD for each station. Environmental data were tested for  
193 collinearity among variables by using a Draftsman plot, with fluorescence, Dissolved O<sub>2</sub> concentration (DO, ml/l), % of  
194 O<sub>2</sub> saturation and turbidity data being Log (X+1)-transformed to fit a linear distribution in the Draftsman plot. Finally, a  
195 DistLM (Distance based linear models) was run with temperature, fluorescence, turbidity, oxygen and salinity as  
196 environmental variables, using step-wise as selection procedure and AIC (Akaike Information Criterion) as selection  
197 criterion.

198

## 199 **2.5. Stable isotopes data analysis**

200 Since lipids can alter the values of δ<sup>13</sup>C (Post *et al.*, 2007), samples with high lipid concentration can be defatted to avoid  
201 <sup>13</sup>C depletion. However, lipid extraction can alter δ<sup>15</sup>N values, can complicate sample preparation and reduce samples  
202 availability, a crucial point when analysing small animals. For these reasons, δ<sup>13</sup>C of samples rich in lipids was normalized  
203 according to Post equation (Post *et al.*, 2007):

$$204 \quad \delta^{13}\text{C}_{\text{normalized}} = \delta^{13}\text{C}_{\text{untreated}} - 3.32 + 0.99 \text{ C/N}_{\text{sample}}$$

205 C/N ratio was used as a proxy of lipid content, because their values are strongly related in animals (Post *et al.*, 2007). In  
206 particular, the normalization was applied to samples with a C/N ratio > 3, according to Post *et al.* (2007).

207 A hierarchical cluster analysis (Euclidean distance, average grouping methods) on the bivariate matrix of δ<sup>13</sup>C and δ<sup>15</sup>N  
208 mean values of each taxon was performed in order to elucidate the planktonic food web structure. Obtained clusters were  
209 also compared with literature data on the trophic guild of analysed taxa. Five main trophic groups were established a  
210 priori: primary consumers (PC), omnivores of type 1 (OMN1), encompassing mostly herbivore species, but that can feed  
211 also small particles and ciliates, omnivores of type 2 (OMN2), similarly to OMN1 but with greater preference for small  
212 zooplankton, carnivores (CAR) and parasite species (PAR). Differences among groups were tested by means of a one-  
213 way PERMANOVA test with “trophic group” (with four levels) as fixed factor.

214 The trophic level of the different species was estimated according to Post (2002) as:

$$215 \quad ((\delta^{15}\text{N}_i - \delta^{15}\text{N}_{\text{PC}})/\text{TEF}) + \lambda$$



216 where  $\delta^{15}\text{N}_i$  is the  $\delta^{15}\text{N}$  value of the taxon considered,  $\delta^{15}\text{N}_{\text{PC}}$  is the  $\delta^{15}\text{N}$  values of a primary consumer, *i.e.* an herbivore  
217 or a filter feeder, used as baseline of the food web, TEF is the trophic enrichment factor which is considered varying  
218 between 2.54 (Vanderklift and Ponsard, 2003) and 3.4 (Vander (*e.g.* Vander Zanden and Rasmussen, 2001; Post, 2002)  
219 and here is assumed to be 2.54 for low trophic level species, according to Fanelli et al (2009; 2011), and  $\lambda$  is the trophic  
220 position of the baseline, which is 2 in our case.

221 Then, differences in the isotopic composition of the overall communities by sub-area and inshore *vs.* offshore  
222 communities were tested by two-way PERMANOVA on the same design used for assemblage analysis. The same  
223 procedure was also used to perform univariate two-way PERMANOVA and one-way PERMANOVA with pairwise test  
224 for the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values, separately.

225 Finally, maximum likelihood standard ellipses were created for the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values following Jackson et al. (2011)  
226 to assess the community niche width in the different sub-areas. In addition to standard ellipse area (SEA; contain ca. 40%  
227 of the data and represent the core isotopic niche) and standard ellipse areas corrected for small sample size (SEAc),  
228 traditional convex hulls and four Layman metrics were also estimated (Layman et al., 2007). Specifically, we calculated  
229 TA, which is the area of convex hull containing, in the case of SIBER (Stable Isotope Bayesian Ellipses in R, Jackson et  
230 al., 2011), the means of the populations that comprise the community, d15N\_range that is the distance in units between  
231 the min and max y-axis population means, d13C\_range, *i.e.* the distance in units between the min and max x-axis  
232 population means, and CD which is the mean distance to centroid from the means. Ellipse sizes were compared between  
233 groups (*i.e.* sub-areas) using Bayesian inference techniques.

234 All analyses were run using the software PRIMER7&PERMANOVA+ (Anderson *et al.*, 2008; Clarke and Gorley, 2006)  
235 and within the jags and SIBER packages in R 4.1.0 ([www.r-project.org](http://www.r-project.org)).

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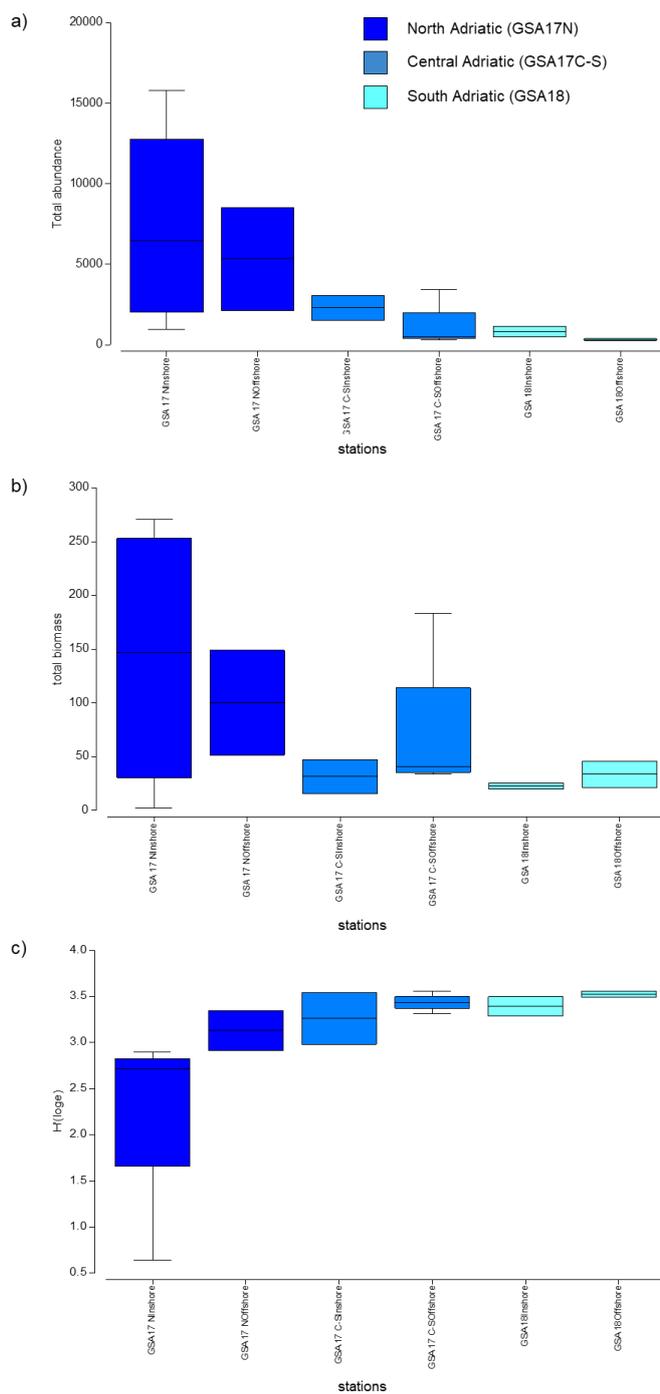
### 238 3. Results

#### 239 3.1. Zooplankton community and spatial changes

240 A total of 52,016 specimens belonging to 113 taxa were collected through the WP2 sampling (**Table S1**). Zooplanktonic  
241 communities in the whole area were dominated by small copepods of the genus *Acartia* (mostly *A. tonsa*), *Oncaea*,  
242 *Oithona* (mainly *O. similis*) and copepodites. Abundant large copepods were Calanoida belonging to the genera *Euchaeta*,  
243 *Calanus*, *Centropages* and *Temora*. Since samples were frozen on board after collection, a quite considerable number of  
244 specimens (particularly amphipods and mysids and those taxa/specimens characterized by soft carapace) were damaged  
245 and therefore hard to identify at species level. Generally, they were identified to order level or indicated as “damaged  
246 unid.” in **Table S1**. Other common crustaceans were hyperiids, such as *Lestrigonus schizogeneios* and *Phronima*



247 *atlantica*, decapod larvae (mainly zoeae and megalopae), mysids and euphausiids. Among non-crustaceans, molluscs  
248 were quite common, both as larvae of benthic organisms and adult pteropods. Chaetognatha were also locally abundant.  
249 Gelatinous zooplankton was represented mainly by thaliaceans and calycophorans, while ichthyoplankton was not very  
250 abundant, with few fish eggs and larvae found.  
251 Zooplankton abundance and biomass varied according to geographic sub-area decreasing from the Northern to the  
252 Southern Adriatic (**Figure 2a-b**), being significant only differences in abundance, while inshore-offshore differences were  
253 not, neither for abundance nor for biomass (**Table 1**).



254

255

256

**Figure 2.** Total abundance (N ind./m<sup>3</sup>, a), total biomass (mg WW/m<sup>3</sup>, b) and diversity ( $H'$ , c) of mesozooplankton at each group of stations by sub-area and distance from the coast (inshore vs. off-shore stations). Colours define the different sub-areas.



257

258 Diversity (in terms of  $H'$ ) increased southward (**Figure 2c**), although differences were not significant for any of the  
 259 investigated factors.  $H'$  values were on average  $3.25 \pm 0.31$ , with the only exception of station 22\_17, located in the  
 260 GSA17N inshore, in front of the Po delta, showing the lowest  $H'$  value (0.64).

261

262 **Table 1.** PERMANOVA results of univariate analyses carried out on zooplankton abundance, biomass and diversity (in terms of  $H'$   
 263 index).

Source	df	MS	Abundance		MS	Biomass		MS	Diversity ( $H'$ )	
			Pseudo-F	P(MC)		Pseudo-F	P(MC)		Pseudo-F	P(MC)
Sub-area	3	11.81	202.11	0.0001	0.86	39.42	0.0012	0.55	2.65	0.31
Inshore-Offshore	1	0.63	0.19	0.32	0.1	0.16	ns	0.46	1.1	0.45
Sub-area x Inshore-Offshore	2	0.02	0.01	0.86	0.01	0.02	ns	0.2	0.49	0.57
Residuals	9	3.26			0.65			0.42		
Total	15									

264 ns=not significant difference

265

266 PERMANOVA revealed that differences in zooplanktonic communities, based on geographic sub-areas and inshore-  
 267 offshore factor were significant, while any significant differences occurred for the interaction factor (**Table 2a-b**).

268 **Table 2.** PERMANOVA results of multivariate analysis on zooplankton abundance, a) main test, b) pairwise comparisons for factor  
 269 “sub-area”

270

a)

Source	df	MS	Pseudo-F	P
Sub-area	2	3495	3.01	0.002
Inshore-offshore	1	2645.8	2.28	0.02
Sub-area x Inshore-offshore	2	1011.8	0.87	0.62
Residuals	10	1159.9		
Total	15			

271

b)

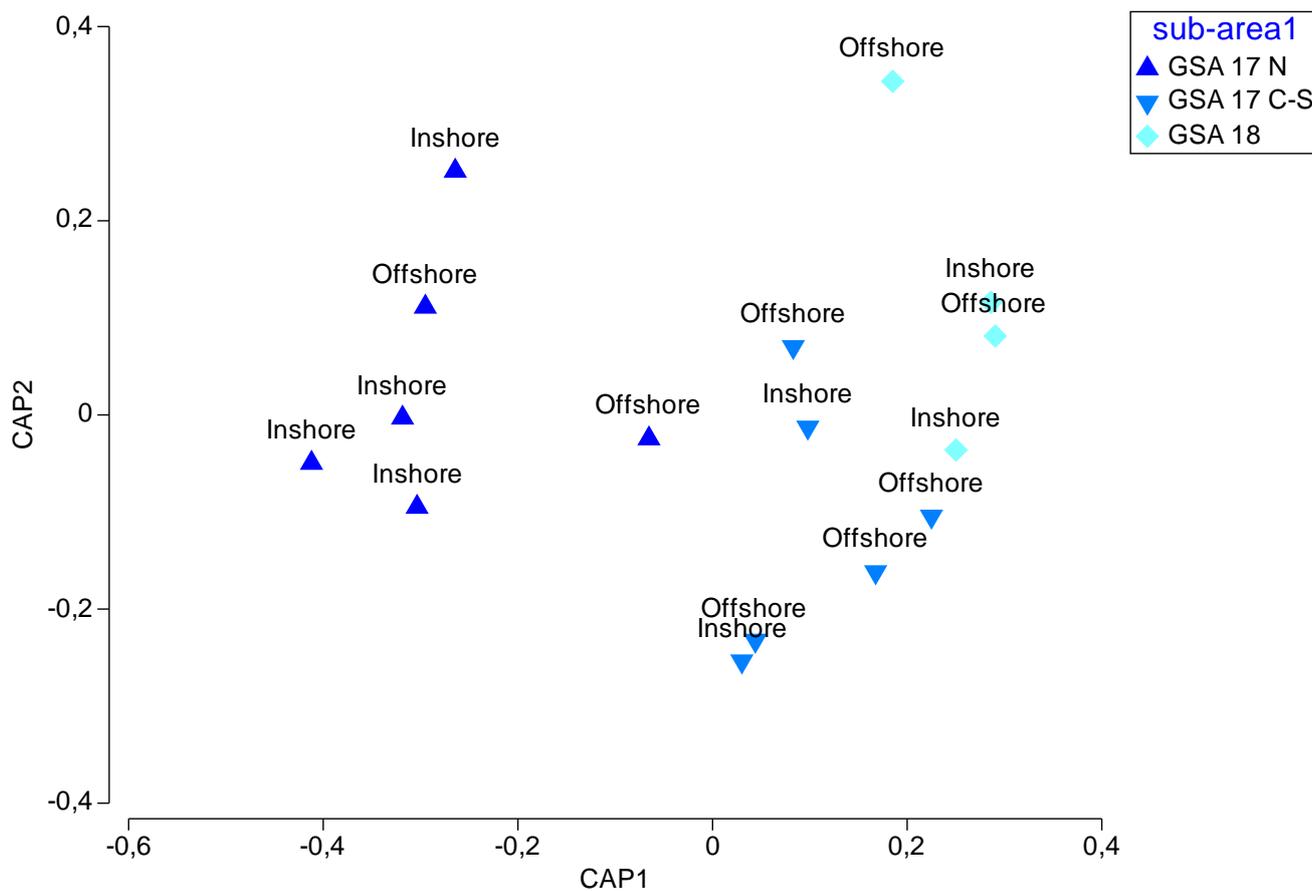
Groups	t	P
GSA17N vs. GSA17C-S	1.55	0.02
GSA17C-S vs. GSA18	1.40	0.03
GSA17N vs. GSA18	2.15	0.01

272

273

274 The CAP plot showed a clear separation between samples from the GSA17N and all the other stations, on the first axis  
 275 (**Figure 3**).

276



277

278 **Figure 3.** CAP plot of the mesozooplanktonic communities of the Adriatic basin by sub-area and inshore vs. offshore location, based  
279 on abundance data. Colours indicate the sub-areas, as described in the text.

280

281 SIMPER analysis (**Table 3a**) showed that *Acartia* sp., *Oithona* sp., unidentified Calanoida, mostly composed by  
282 copepodites, mainly contributed to dissimilarity from GSA17N vs. GSA17C-S. Bivalve and gastropod larvae, together  
283 with *Acartia* sp., *Oithona* sp. and unidentified Calanoida were the main responsible for the dissimilarity between the  
284 subareas GSA17C-S and GSA18. Overall, the inshore zooplanktonic communities were mostly typified by *Acartia* sp.,  
285 gastropod larvae, copepodites, *Podon* sp. and *Centropages typicus*, while the offshore ones were mainly characterised by  
286 Calycophorae, *Calanus helgolandicus* and *Evadne tergestina* (**Table 3b**).

287

288

289

290



291 **Table 3.** Results of SIMPER analysis examining a) dissimilarity between contiguous pair of sub-area groups across all Inshore vs.  
 292 offshore groups, and b) dissimilarity between Inshore vs. offshore groups across all sub-areas, with a 50% cut-off for low contribution.

293

a)

GSA17N vs. GSA17 C-S      Average dissimilarity = 52.29					
Taxon	GSA17N Av.Abund	GSA17C-S Av.Abund	Av.Diss	Contrib%	Cum.%
<i>Acartia</i> spp.	6.04	2.7	3.98	7.61	7.61
<i>Oithona</i> spp.	4.94	4.12	2.63	5.03	12.65
<i>Penilia avirostris</i>	1.48	1.91	2.33	4.45	17.1
Copepoda unid.	6.04	3.62	2.12	4.06	21.16
<i>Evadne spinifera</i>	2.43	0.59	2.09	4	25.16
Gasteropoda larvae	2.33	1.64	1.55	2.97	28.13
"Calanus-like" copepods	0.26	2.01	1.52	2.9	31.03
<i>Centropages typicus</i>	2.51	1.65	1.44	2.76	33.79
<i>Euterpina acutifrons</i>	1.82	0.52	1.42	2.71	36.5
<i>Euchaeta</i> sp.	0.12	1.84	1.41	2.71	39.21
<i>Podon</i> spp.	3.37	2.17	1.29	2.47	41.68
<i>Evadne tergestina</i>	0.59	0.31	1.27	2.42	44.1
<i>Temora longicornis</i>	1.12	0.35	1.17	2.24	46.34
<i>Oncaea</i> sp.	1.43	2.5	1.09	2.08	48.42
<i>Gaetanus tenuispinus</i>	0.1	0.55	0.92	1.76	50.18

GSA17C-S vs. GSA18      Average dissimilarity = 49.15					
Taxon	GSA17 C-S	GSA 18	Av.Diss	Contrib%	Cum.%
Bivalvia larvae	2.37	0.47	2.16	4.4	4.4
<i>Oithona</i> sp.	4.12	2.04	2	4.07	8.47
<i>Acartia</i> sp.	2.7	0.71	1.97	4	12.47
Copepoda unid.	3.62	4.17	1.55	3.16	15.63
Gasteropoda larvae	1.64	0.53	1.41	2.87	18.5
<i>Penilia avirostris</i>	1.91	1.34	1.33	2.71	21.21
"Calanus-like" copepods	2.01	0.97	1.23	2.5	23.71
<i>Podon</i> sp.	2.17	1.49	1.22	2.48	26.19
Appendicularia	1.04	1.2	1.19	2.41	28.6
<i>Temora stylifera</i>	0.81	0.93	1.06	2.17	30.77
<i>Coryceus</i> sp.	1.1	1.55	1.05	2.14	32.91
Chaetognatha	1.22	2.13	1	2.03	34.94
Thaliacea	0.88	1.05	0.97	1.98	36.92
<i>Creseis acicula</i>	0.93	1.27	0.95	1.93	38.84
<i>Engraulis encrasicolus</i> eggs	0.86	0.18	0.85	1.73	40.57
<i>Oncaea</i> sp.	2.5	2.45	0.81	1.66	42.23
<i>Centropages typicus</i>	1.65	1.1	0.81	1.66	43.88



<i>Lucicutia flavicornis</i>	0.12	0.62	0.78	1.58	45.46
Calycophorae	0.62	0.89	0.75	1.52	46.98
<i>Microsetella</i> sp.	0.41	0.63	0.69	1.41	48.39
<i>Calanus helgolandicus</i>	1.52	1.12	0.66	1.34	49.73
<i>Calanus minor</i>	0.56	0.94	0.66	1.34	51.06

294

295

b)

Taxon	Average dissimilarity = 49.12				
	Inshore		Offshore		Cum.%
	Av.Abund	Av.Abund	Av.Diss	Contrib%	
<i>Acartia</i> spp.	4.1	2.81	2.33	4.73	4.73
<i>Penilia avirostris</i>	1.89	1.32	1.96	3.98	8.71
<i>Oithona</i> spp.	3.92	3.89	1.94	3.95	12.67
Gasteropoda larvae	2.31	0.93	1.59	3.23	15.9
Copepoda unid.	5.43	3.89	1.57	3.19	19.09
<i>Podon</i> spp.	3.22	1.68	1.5	3.06	22.16
<i>Centropages typicus</i>	2.31	1.37	1.41	2.88	25.04
Calycophorae	0.23	1.17	1.33	2.71	27.75
<i>Evadne tergestina</i>	0.36	0.44	1.18	2.4	30.15
<i>Calanus helgolandicus</i>	0.52	1.71	1.14	2.32	32.47
<i>Oncaea</i> sp.	1.77	2.41	1.11	2.26	34.73
<i>Evadne spinifera</i>	1.35	0.92	1.1	2.25	36.98
Chaetognatha	0.65	1.66	1.05	2.14	39.12
"Calanus-like" copepods	0.59	1.6	0.93	1.89	41.01
Bivalvia larvae	1.75	1.79	0.87	1.78	42.79
Appendicularia	0.35	1.03	0.85	1.73	44.51
Thaliacea	0.33	0.96	0.83	1.69	46.2
Zoea Brachyura	1.13	1.07	0.82	1.68	47.88
<i>Temora stylifera</i>	0.53	0.65	0.82	1.66	49.54
<i>Engraulis encrasicolus</i> eggs	0.58	0.89	0.81	1.65	51.19

296

### 297 3.2. Correlation between zooplankton data and environmental variables

298 Draftsman plot allowed to assess collinearity between pair of variables at  $\rho > 0.7$ . DO concentration (ml/l) and % of oxygen  
 299 saturation covaried, as well as density and pressure, therefore, only temperature, fluorescence, turbidity, DO and salinity  
 300 were used for DistLM analysis.

301 DistLM results showed that 26.9% of the variance was explained by salinity, 11% by fluorescence and 8.6% by DO, those  
 302 three variables cumulatively accounting for 46.5% of variance (**Table 4**).



303

304 **Table 4.** Results of sequential test for DistLM model

Variable	AIC	SS(trace)	Pseudo-F	P	Prop.	Cumul.	res.df
Salinity	116.47	6643.1	5.15	0.001	0.27	0.27	14
Fluorescence	115.86	2716.6	2.30	0.032	0.11	0.38	13
DO	115.47	2126.6	1.93	0.044	0.09	0.46	12

305

### 306 3.3. Stable isotope composition of zooplankton

307 Stable isotope analyses provided  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of 26 different taxa (**Table 5**). Acidification of crustaceans was  
308 proved to be unnecessary, as the tested samples of *Euchaeta* sp. showed little and not significant differences in  $\delta^{13}\text{C}$  value  
309 ( $-21.39\pm 0.06$  for untreated samples vs.  $-21.02\pm 0.15$  for acidified samples, paired T-test,  $T = -0.34$ ,  $P = 0.74$ ).

310

311 **Table 5.** Mean values of zooplankton samples, trophic group (TG), Trophic position (TP), sub-area and number of samples analysed (N). PC = primary consumers, OMNI = omnivores of type  
312 1, OMN2 = omnivores of type 2, CAR = carnivores, question marks (?) indicate taxa with unknown trophic group

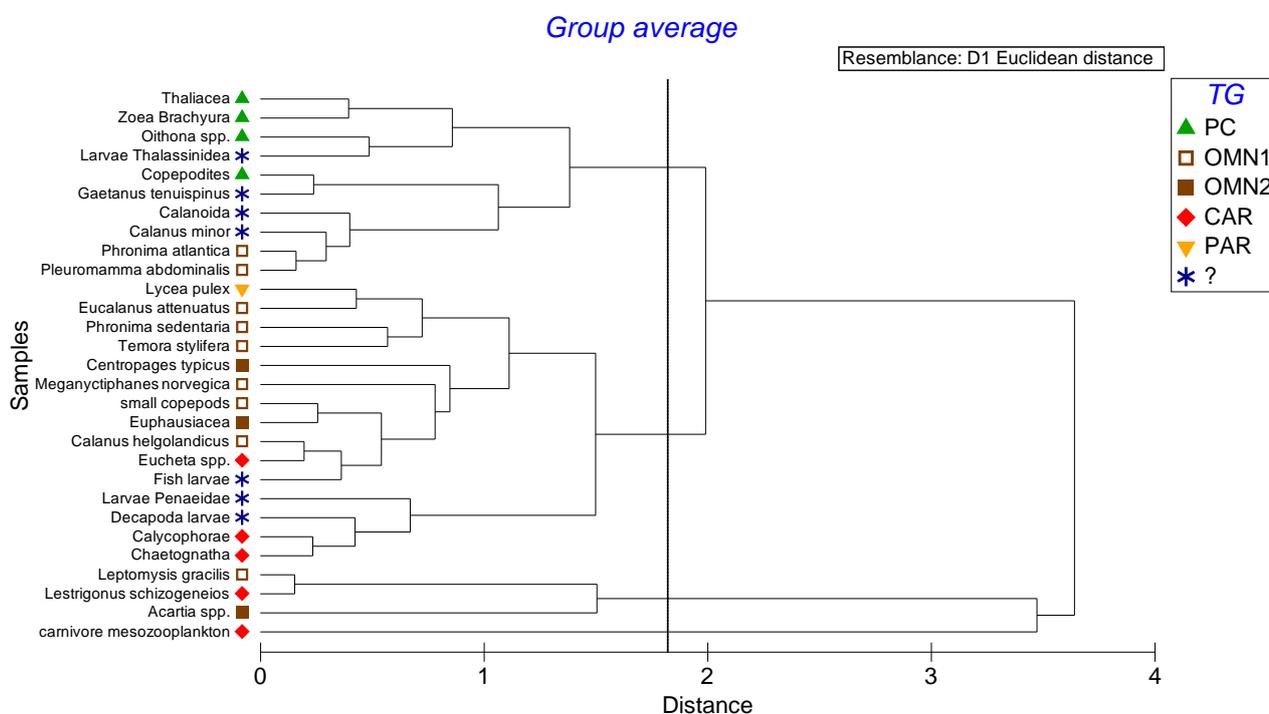
Group	Code	Taxon	$\delta^{15}\text{N}\pm\text{SD}$	$\delta^{13}\text{C}\pm\text{SD}$	TG	TP	GSA17N	GSA17C-S	GSA18	N
COPEPODA	Gie	<i>Gaetanus tenuispinus</i>	2.68	-20.44	Unk	2.0		*		1
COPEPODA	Nmi	<i>Nannocalanus minor</i>	3.53±0.37	-20.77±0.21	Unk	2.3		*		3
HYPERIIDEA	Pat	<i>Phronima atlantica</i>	3.58	-20.98	OMN1	2.4		*		1
COPEPODA	Pab	<i>Pleuronamma abdominalis</i>	3.59	-21.14	OMN1	2.4		*		1
COPEPODA	Cli	Calanus-like	3.80±0.22	-21.28±0.48	Unk	2.4		*		2
THALACEA	Tha	Thalacea	3.82±0.82	-19.56±1.16	PC	2.4		*		10
DECAPODA	Bra	Brachyura (Zoea)	3.89±0.06	-19.17±0.07	PC	2.5		*		2
DECAPODA	Thl	Thalassinidea (Zoea)	4.14	-19.92	Unk	2.6				1
COPEPODA	Cai	Calanoida (copepodites)	4.16±1.09	-20.53±0.34	PC	2.6		*		3
EUPHAUSIACEA	Meg	<i>Meganyctiphanes norvegica</i>	4.48±0.54	-21.18±0.57	OMN1	2.7		*		4
EUPHAUSIACEA	Eup	Euphausiacea	4.69	-20.39	OMN2	2.8		*		1
HYPERIIDEA	Lpu	<i>Lyca pulex</i>	4.69	-19.64	CAR	2.8		*		1
COPEPODA	Par	<i>Pareucalanus attenuatus</i>	4.92	-20.01	OMN1	2.9		*		1
OSTEYCHTHYES	Fis	Fish larvae	5.09±0.53	-20.57±0.26	CAR	2.9		*		4
COPEPODA	Che	<i>Calanus helgolandicus</i>	5.16±0.94	-20.83±0.40	OMN1	3.0		*		15
COPEPODA	Euc	<i>Euchaeta sp.</i>	5.19±0.40	-20.91±0.23	CAR	3.0		*		16
HYPERIIDEA	Pse	<i>Phronima sedentaria</i>	5.42	-19.6	OMN1	3.1		*		1
COPEPODA	Cty	<i>Centropages typicus</i>	5.42±1.43	-21.38±0.34	OMN2	3.1		*		4
COPEPODA	Tst	<i>Temora stylifera</i>	5.47±1.08	-20.17±0.56	OMN1	3.1		*		2
CHAETOGNATHA	Cha	Chaetognatha	6.19±1.40	-19.82±0.43	CAR	3.4		*		20
SIPHONOPHORA	Cal	Calycophorae	6.21±1.25	-20.05±1.28	CAR	3.4		*		9
DECAPODA	Pen	Penaeidae (Zoea)	6.34±1.12	-20.54±0.41	Unk	3.4		*		4
DECAPODA	Dec	Decapoda (Zoea)	6.59±1.59	-19.81±0.24	Unk	3.5		*		5
COPEPODA	Aca	<i>Acartia sp.</i>	7.86±0.86	-21.58±0.61	OMN2	4.0		*		3
HYPERIIDEA	Lsc	<i>Lestrigonus schizogenetos</i>	8.06±3.30	-20.16±0.65	CAR	4.1		*		2
MYSIDA	Lgr	<i>Leptomysid gracilis</i>	8.14	-20.03	OMN1	4.1		*		1

313





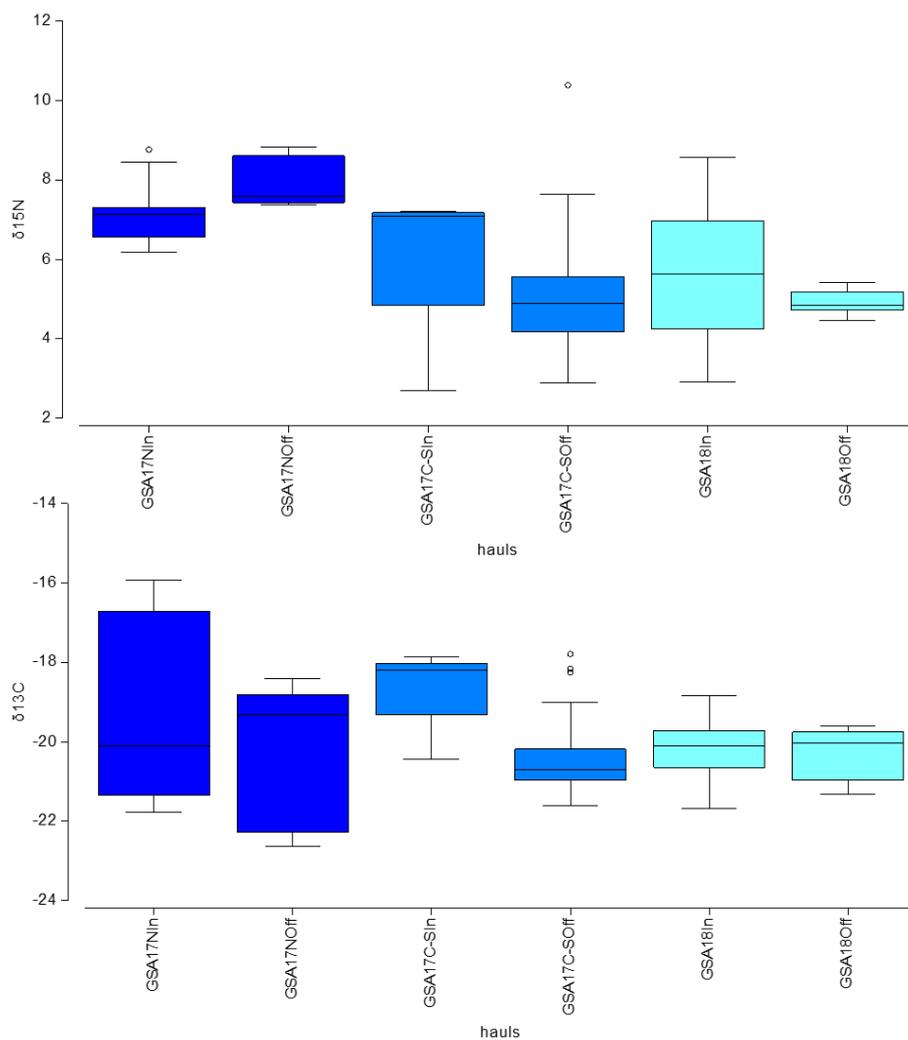
314 Cluster analysis allowed to group animals according to their  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values, and partially with the trophic groups  
 315 previously established, based on literature data when available (**Figure 4**). One-way PERMANOVA test run on factor  
 316 “trophic groups-TG” proved significant differences ( $pseudo-F_{5,28}=2.57$ ,  $p=0.02$ ), with primary consumers being  
 317 significantly different from omnivores of type 2 and carnivores (PC vs. OMN2:  $t=2.67$ ,  $p=0.03$ ; PC vs. CAR:  $t=3.01$ ,  
 318  $p=0.006$ ) and omnivores of type 1 from carnivores ( $t=1.98$ ,  $p=0.049$ ).  
 319



320  
 321 **Figure 4.** Cluster analysis on the bivariate matrix of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of dominant zooplankton taxa. Colours indicate trophic  
 322 groups: OMN1 are omnivores of type 1, OMN2 are omnivores of type 2, PC are primary consumers, CAR are carnivores, question  
 323 marks (?) indicate taxa with unknown trophic group. The dashed line is placed at 1.8 distance.  
 324

325 The estimates of Trophic Levels (TLs), considering the  $\delta^{15}\text{N}$  value of *Gaetanus spinosus* as baseline, allowed to assign  
 326 zooplanktonic taxa to 3 TLs from herbivores located at TL 2 to carnivores at TL 4 (**Table 5**).

327 Overall, the  $\delta^{15}\text{N}$  of the mesozooplanktonic community was greater in the GSA17N, both for inshore and offshore  
 328 communities (**Figure 5**). Similarly, the median  $\delta^{13}\text{C}$  value was similar among the different sub-areas, although in the  
 329 GSA17N both the greater and lower values were found in the GSA17N than in the other sub-areas, although, than for the  
 330 other communities (**Figure 5**).  
 331



332

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334

**Figure 5.** Box plot of mean  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values of zooplanktonic taxa for each sub-area at inshore vs. offshore locations.

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Two-way PERMANOVA on the multivariate matrix of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  showed a significant separation according to all factors (**Table 6a**). The pairwise comparison on sub-area factor, considering only comparisons between contiguous sub-areas showed a significant separation between the isotopic composition of zooplanktonic taxa from the GSA17N vs. GSA17C-S, but not between the GSA17C-S and GSA18. The pairwise test run on the interaction factor for pairs of level of factor “inshore vs. offshore” provided evidence for significant variations in the isotopic composition between inshore and offshore communities only for the GSA17C-S. One-way PERMANOVA tests run separately on  $\delta^{15}\text{N}$  values showed significant variation only for factor sub-area (**Table 6b**) with the isotopic composition of zooplankton from GSA17N significantly different from that of GSA18. Conversely,  $\delta^{13}\text{C}$  values significantly varied between inshore and offshore



343 communities and for the interaction factor (**Table 6c**), being differences between inshore and offshore communities  
344 significant in GSA17C-S.

345  
346

**Table 6.** Results of PERMANOVA a) main test and pairwise comparisons for b) sub-area factor and c) interaction term for pairs of levels of factor "inshore vs. offshore" run on the Euclidean resemblance matrix of untransformed  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values and for  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ , separately.

Source	df	MS	Pseudo-F	P	$\delta^{15}\text{N}$		$\delta^{13}\text{C}$			
					a)	b)	a)	b)		
Sub-area	2	27.52	10.86	0.0001	25.33	16.74	0.0001	2.18	2.14	0.12
Inshore vs. offshore	1	11.10	4.38	0.01	1.39	0.92	0.33	9.72	9.52	0.003
Sub-areax Inshore vs. offshore	2	7.20	2.84	0.03	3.77	2.49	0.09	3.43	3.36	0.04
Residuals	121	2.53			1.51			1.02		
Total	126									
b)										
Groups	t	P(perm)	Groups		t	P(perm)	Groups		t	P(perm)
GSAI17N, GSAI17C-S	3.58	0.0001	GSAI17N, GSAI17C-S	5.15	0.0001	GSAI17N, GSAI17C-S	5.15	0.0001		
GSAI17C-S, GSAI18	1.41	0.14	GSAI17C-S, GSAI18	0.50	0.62	GSAI17C-S, GSAI18	0.50	0.62		
c)										
Within level 'GSAI17N' of factor 'subarea'										
Groups	t	P(perm)	Groups		t	P(perm)	Groups		t	P(perm)
Inshore vs. offshore	0.88	0.40	In. Off	0.58	0.57	In. Off	0.58	0.57		
Within level 'GSAI17C-S' of factor 'subarea'										
Inshore vs. offshore	2.95	0.004	In. Off	4.43	0.0008	In. Off	4.43	0.0008		
Within level 'GSAI18' of factor 'subarea'										
Inshore vs. offshore	1.10	0.28	In. Off	0.50	0.62	In. Off	0.50	0.62		

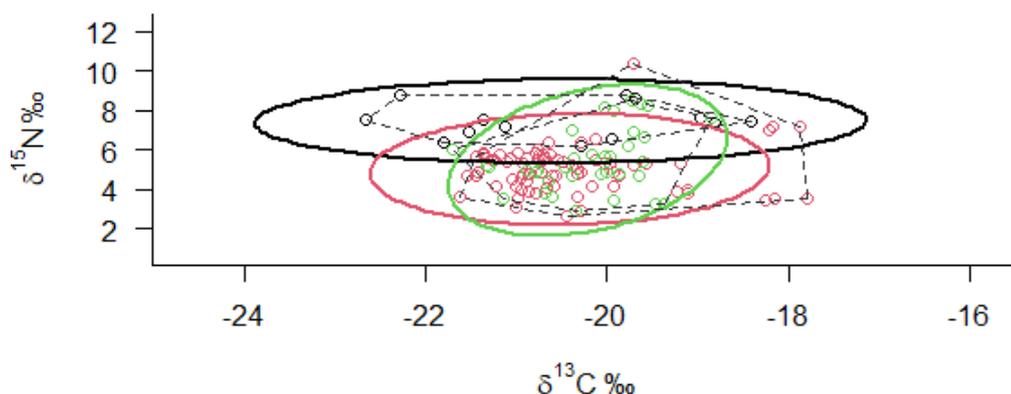
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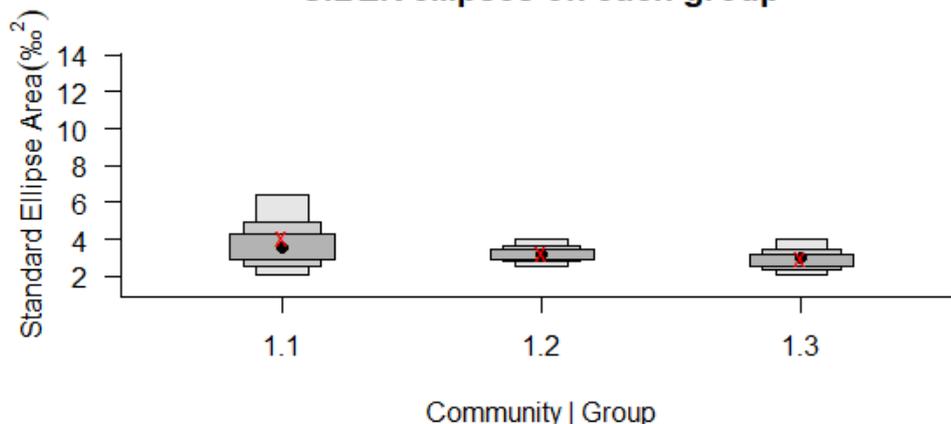




349 Finally, the SIBER method for calculating ellipse-based metrics of niche width provided evidence of larger niche width for  
350 the zooplanktonic community from GSA17N than GSA17C-S and GSA18 (**Figure 6** and **Table 7**). Estimated overlap by  
351 Bayesian inference evidenced almost null overlap between GSA17N and GSA17C-S ( $<10^{-15}$ ), while a high overlap existed  
352 between GSA17C-S and GSA18 (0.26). The greater  $d_{15N\_range}$  was observed for GSA17C-S and GSA18 communities, while  
353 the higher  $d_{13C\_range}$  occurred in GSA17N communities, where also CD value was the greatest (**Table 7**).  
354



### SIBER ellipses on each group



355 **Figure 6.** Top: Standard Ellipse Areas for the three zooplanktonic communities analysed; the black circle and symbols indicate the  
356 GSA17N community, the red ones the GSA17C-S and the green ones the GSA18. Bottom: Credible intervals for the estimated SEAc of  
357 the three communities, 1.1= GSA17N, 1.2= GSA17C-S, 1.3= GSA18.

358

359

360



361 **Table 7.** Estimates of Convex hulls (TA). Standard Ellipse Areas (SEA and SEAc. as corrected for low sample size). and Layman metrics  
362 d15N\_range. d13C\_range and Mean Distance to Centroid (CD). calculated for the three sub-areas zooplanktonic communities.

Metrics	GSA17N	GSA17C-S	GSA18
TA	7.96	19.39	10.38
SEA	3.74	3.24	2.92
SEAc	4.08	3.28	3.00
d15N_range	2.65	7.71	5.65
d13C_range	4.23	3.83	2.84
CD	1.93	1.16	1.42

363

#### 364 4. Discussion

365 These are the first results on mesozooplankton community composition and food web structure conducted at basin scale for  
366 the Adriatic Sea. Considering that the Adriatic Sea is one of the largest areas of occurrence of demersal and small pelagic  
367 shared stocks in the Mediterranean (FAO, 2020), this study may represent an important piece to reconstruct the whole pelagic  
368 food web and changes at mesoscale level across the basin. Still, considering the increasing fishing pressure in the basin together  
369 with evidence of primary production (climate-change related) decrease after the 1980s (Solidoro et al., 2009; Mozetic et al.,  
370 2010), this study may represent a valid baseline for future comparison on the synergic and cumulative effect of climate change  
371 and overfishing in one of the most impacted regions within the Mediterranean Sea (Coll et al., 2012; Micheli et al., 2013).

#### 372 4.1. Mesoscale variations in zooplankton biomass, abundance and composition

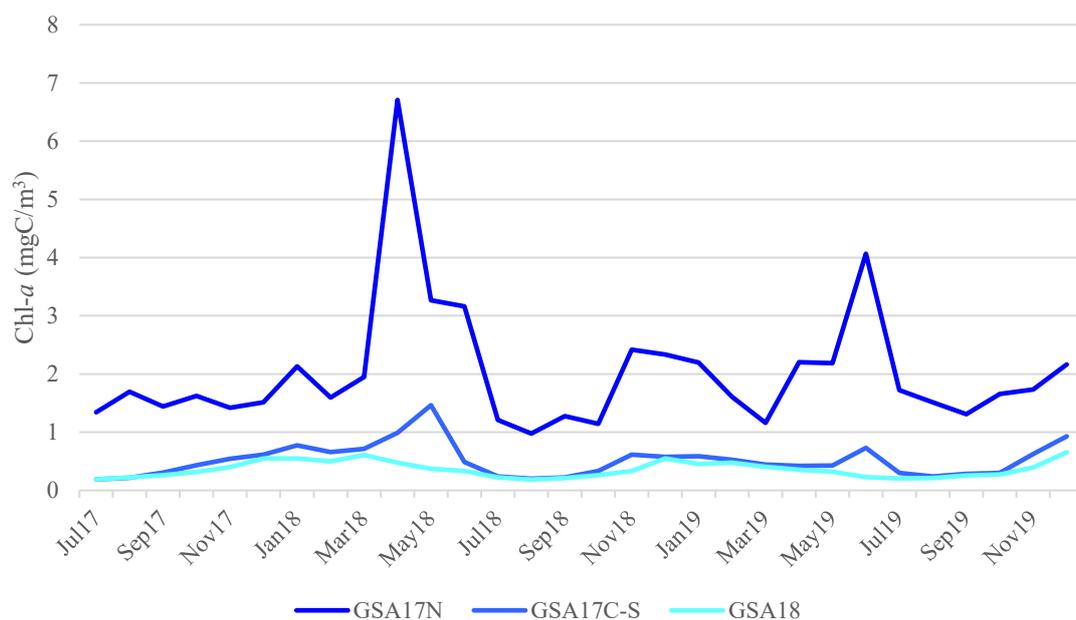
373 Overall, 113 taxa and 57 species have been identified during June-July 2019 in the Adriatic basin (**Table S1**). These values  
374 were only slightly lower than those observed for the Central Adriatic at 0-50 m depths where 150 taxa were counted (Hure et  
375 al., 2018). Such differences maybe only apparent and attributable to the storage method we used, as samples were kept frozen  
376 for subsequent stable isotope analyses, determining a damage in many organisms, which were impossible to identify to species  
377 or even genus level (Fanelli et al., 2011). In terms of species abundance, the most representative species were *Acartia tonsa*,  
378 *Oithona similis* and *Centropages typicus* among copepods, and the cladocerans *Podon intermedius*, *P. polyphemoides*, *Penilia*  
379 *avirostris*, *Evadne tergestina* and *E. spinifera*, in agreement with previous studies on the mezooplanktonic communities of the  
380 Adriatic basin (Fonda-Umani et al., 2005; Bernardi Aubry et al., 2012).

381 Zooplankton biomass and abundance were higher in the Northern Adriatic Sea and slowly decreased moving towards the  
382 Southern Adriatic. This trend was also observed by Fonda Umani (1996) and can be explained by the influence of Po River,  
383 which can determine a high nutrient input in the Northern Adriatic favouring primary production and therefore zooplankton  
384 growth. Notwithstanding the general primary production reduction observed in the last years (Mozetič et al., 2010) in the North  
385 Adriatic Sea, the area is still characterised by higher phytoplankton biomass with respect to the central and the southern basin,  
386 because of the nutrients input from the Po River. Chlorophyll-a concentration values from satellite data (**Figure 7**,  
387 <https://giovanni.gsfc.nasa.gov/giovanni>) analysed from four months before the sampling period to survey simultaneous period



388 (July 2019), revealed indeed a peak in primary production in May 2019, two months before the sampling period, in the area in  
389 front of the Po River delta, fuelling in turn zooplankton production (Bernardi Aubry et al., 2012).

390



391

392

393 **Figure 7.** Monthly time-series area-averaged map of satellite-derived (Sensor MODIS Aqua from <https://giovanni.gsfc.nasa.gov/giovanni>)  
394 Chlorophyll a concentration ( $\text{mgC}/\text{m}^3$ ) from July 2017 to December 2019 for the three sub-areas considered in this study.

395

396 Although, in the north-western Adriatic offshore waters are less productive than inshore coastal waters and productivity of the  
397 inshore zone decreases southward away from the Po Rivers' nutrient influx (Vollenweider et al., 1998), here we did not find  
398 significant differences in terms of abundance and biomass between inshore and offshore communities or for the interaction  
399 factors. Such differences were instead observed when we compared zooplanktonic communities' composition. Indeed,  
400 multivariate analyses evidenced a clear separation of samples as function of sub-area and inshore vs. offshore locations, and  
401 especially between the mesozooplanktonic community of the Northern Adriatic from the other two. This was not surprising as  
402 the northern Adriatic is characterised by shallower and colder waters than the rest of the basin and under the influence of  
403 riverine input, thus hosting a typical neritic community with coastal and estuarine elements. This area was dominated also by  
404 *Acartia clausi*, *Oithona similis*, cladocerans (mostly *Evadne spinifera*), copepodites (here comprised within the "Copepoda  
405 unid." group), gastropod larvae with some differences with respect to previous studies (Bernardi Aubry et al., 2012), in terms  
406 of temporal shift of species maximum abundance. This could be related to the peak in primary production occurring in May  
407 2019, quite delayed with respect to the usual pattern of the area (Kamburska and Fonda-Umani, 2009) (see **Figure 7**).



408 Conversely, the southern Adriatic basin, except for the Gargano promontory, being characterised by a narrow continental shelf  
409 and a steep slope, reaching high depths close to the coasts, was dominated by typical offshore species such as tunicates,  
410 chaetognaths, siphonophores and *Euchaeta* spp. These results were supported by Fonda Umani (1996), that identified a clear  
411 distinction in zooplanktonic communities collected in offshore location of Northern and Central-Southern Adriatic: the  
412 Northern Adriatic was characterized by neritic communities, with moderate biomass, while the Central and the Southern  
413 Adriatic Sea were characterized by an “oceanic” community, with a higher abundance of carnivorous zooplankton, such as  
414 *Euchaeta* sp., a more oceanic carnivorous genus (Razouls et al., 2021), and Chaetognatha, a phylum of carnivorous animals  
415 quite abundant in open waters (Terazaki, 2000). Consistently, diversity was the greatest in the southern basin, with 80 taxa  
416 (out of 113) identified, likely due to the occurrence of both neritic and oceanic species in this area and comparable to other  
417 studies (Miloslavac et al., 2012) which included also deep stations.

#### 418 4.2. Environmental drivers of zooplankton communities variability

419 Separation among samples according to sub-areas and inshore and offshore locations were consistent with the main drivers  
420 resulted by the distance-based multivariate model, *i.e.* salinity, fluorescence and DO concentration, with salinity itself  
421 explaining 27% of the variance. Salinity values were on average 36 psu in the Northern basin with the lowest value of 34.7  
422 psu recorded at station 22\_17 in front of the Po; salinity increased southward reaching a mean value of 38.7 psu in the southern  
423 basin. Fluorescence values decreased southward from 2.45  $\mu\text{g/l}$  to 0.77  $\mu\text{g/l}$ , with the highest (4.9  $\mu\text{g/l}$ ) and the lowest (0.59  
424  $\mu\text{g/l}$ ) values recorded at station 22\_17 (in front of the Po river delta) and at station 44\_18 (in the Otranto channel), respectively.  
425 These two variables were mainly linked to freshwater inputs from the Po River and were responsible of the main separation  
426 between the Northern Adriatic, more coastal-estuarine zooplanktonic communities, from the central and southern Adriatic,  
427 more oceanic zooplanktonic communities. On the other hand, changes in DO which decreased southward from a mean value  
428 of 5.32 ml/l recorded in GSA17N stations to 4.36 ml/l were observed in GSA18 CTD casts. This is in full agreement also with  
429 the decreasing trend in zooplankton biomass from the GSA17N to GSA18. Several studies indicated that oxygen concentration  
430 could be a limiting factor for zooplankton growth and survival (Olson, 1987; Moon et al., 2006), with inhibition of egg hatching  
431 in some copepod species (Roman et al., 1993). DO was found to be also the driving factor of zooplanktonic communities in  
432 the strait of Sicily (Rumolo et al., 2016)

#### 433 4.3. Food web structure of zooplankton communities

434 The trophic groups highlighted by cluster analysis fully agreed with putative trophic groups established *a priori* based on  
435 literature information and allowed to assign species with unknown feeding ecology to a trophic group. Two main groups were  
436 evident, a first one grouping primary consumers (*i.e.* herbivore/filter feeder taxa) and copepodites assigned to omnivore of  
437 level 1, *i.e.* taxa that may act both as primary consumer eating phytoplankton or detritus particles or shifting to small prey, *i.e.*  
438 microzooplankton. Taxa with unknown feeding mode such as *Gaetanus tenuispinus* and small calanoids (including



439 copepodites) were sorted together with *Nannocalanus minor*, *Phronima atlantica* and *Pleuromamma abdominalis*, because of  
440 their lowest  $\delta^{15}\text{N}$  value, similar to that of filter feeders (Rumolo *et al.*, 2016). According to literature, *Phronima atlantica*  
441 should be a carnivorous species, feeding on salp tissue (Madin and Harbison, 1977). However, Elder and Seibel (2015) also  
442 reported feeding on host mucus, which could lower their trophic position, being more similar to the basal source, *i.e.* the  
443 particulate organic matter or POM (Fanelli *et al.*, 2011). Zoeae of Thalassinidea and Brachyura were also placed in this group,  
444 close to thaliaceans, that are herbivorous filter feeders (Madin, 1974).

445 The second group encompass different taxa mostly carnivores and omnivores of both level 1 and 2, *i.e.* taxa that mostly prefer  
446 animal prey but that can shift to phytodetritus when prey was scarce or competition was high (Fanelli *et al.*, 2011). This is the  
447 case of *Meganichthyphanes norvegica* which can vary its diet regionally and with growth, showing a preference for  
448 phytoplankton in certain areas, seasons or when juveniles (Schmidt, 2010; Fanelli *et al.*, 2011), or preying exclusively on  
449 calanoids when adults or depending on energy requirements (McClatchie, 1985). Other examples of this kind are represented  
450 by the calanoid *Calanus helgolandicus* or *Centropages typicus*. *C. typicus* is an omnivorous copepod that feeds on a wide  
451 spectrum of prey, from small algae (3–4  $\mu\text{m}$  equivalent spherical diameter) to yolk-sac fish larvae (3.2–3.6 mm length). It uses  
452 both suspensivorous and ambush feeding strategies, depending on the characteristics of the prey (Calbet *et al.*, 2007) Although  
453 *C. helgolandicus* was described as an herbivore species (Paffenhoffer, 1976) some authors described density-dependent  
454 mortality through cannibalism in *Calanus* spp., as a form of population self-limitation (Ohman and Hirche, 2001), thus pointed  
455 out to an omnivorous feeding behaviour. Omnivorous copepods can display increased predatory behaviour in the absence of  
456 other food (Daan, 1988), and may actively target eggs even when phytoplankton is not limiting (Bonnet *et al.* 2004). Finally,  
457 a mixed group formed by the mysid *Leptomysis gracilis*, the copepod *Acartia tonsa* and the hyperiid *Lestrignonus schizogeneios*,  
458 clustered close to other carnivore species. Hyperiids generally use gelatinous substrate for reproduction and feeding, some of  
459 them living in symbiosis (Gasca and Haddock, 2004) other being parasite such as the genus *Hyperia* (now *Lestrignonus*). *A.*  
460 *tonsa* may display both predatory and suspension feeding behaviour (Saiz and Kiorboe, 1995), similarly to *L. gracilis* (Fanelli  
461 *et al.*, 2009) and accordingly to their isotopic composition and position in the zooplanktonic food web.

462 The average enrichment between the different plankton taxa was greater than the mean value of 2.56 expected between adjacent  
463 trophic levels (e.g., Vanderderklift and Ponsard, 2003; Fanelli *et al.*, 2011) pointing to the organization of mesozooplanktonic  
464 taxa in three trophic levels, from the copepod *Gaetanus tenuispinus*, positioned at the trophic level 2, to the highest-level  
465 species represented by *Lestrignonus schizogeneios* and *Leptomysis gracilis*, located at the trophic level 4. Such results confirmed  
466 other findings (Fanelli *et al.*, 2009, 2011) about the complexity of pelagic food webs and of their lower trophic levels, calling  
467 attention on the appropriate compartmentation of zooplankton in ecosystem modelling with the final scope of small pelagic  
468 stock management (D'Alelio *et al.*, 2016).

469

#### 470 4.4. Spatial variability in the isotopic composition of mesozooplankton from the Adriatic basin



471 Overall, stable isotope values of zooplankton differed significantly for all factors considered, with  $\delta^{15}\text{N}$  values decreasing  
472 southward, and  $\delta^{13}\text{C}$  showing more constant patterns with the exception of the GSA17N. The presence of differences in isotopic  
473 signature of zooplankton between inshore and offshore locations has already been reported by other authors (Bode et al., 2003;  
474 Chouvelon *et al.*, 2014) and it could be linked to the different contribution of terrestrial *vs.* marine sources of nitrogen and  
475 carbon moving from inshore to offshore waters, and/or to different trophic dynamics between costal and oceanic food webs.  
476 Here  $\delta^{13}\text{C}$  values were highly variable in accordance with the wide array of food sources (*i.e.*, marine and continental) available  
477 in the area due to the riverine inputs. Accordingly, the niche width of zooplanktonic community in the area is the greatest and  
478 SEAc decreased southward, where zooplanktonic community were likely sustained mostly by marine sources (Coll et al.,  
479 2007). Standard ellipses were mainly stretched along the x-axis ( $\delta^{13}\text{C}$ ) for GSA17N and GSA17C-S showing a progressive  
480 decrease of the continental influence from the Northern to the Central Adriatic basin. SEAc of GSA18 was conversely mostly  
481 extended along the y-axis ( $\delta^{15}\text{N}$ ), likely because of the occurrence of a well-structured community with all TLs represent. The  
482 low  $\delta^{15}\text{N}$  range (and the general high  $\delta^{15}\text{N}$  values) observed for GSA17N community suggest a shift to omnivory in  
483 zooplanktonic communities in this area to avoid competition (Doi et al. 2010) in high-density condition, as that generated after  
484 the phytoplankton bloom (Bernardi Aubry et al, 2012) here observed in June.

## 485 5. Conclusions

486 This study represents the first application of the stable isotope approach to the analysis of the mesozooplanktonic food web at  
487 Adriatic basin scale including both coastal and offshore communities. The results unveiled the presence of significant  
488 differences in zooplankton abundance, biomass, and community composition at mesoscale level, with the main differences  
489 observed between the Northern Adriatic and the rest of the basin, due to the peculiar oceanographic conditions (*i.e.*, cold  
490 waters) and the strong influence of the Po river. Such differences were also particularly evident in terms of isotopic  
491 composition, where a further separation between offshore and inshore communities were evident for the progressive increase  
492 of marine contribution to food sources for zooplankton in offshore communities. Such findings may represent a valuable  
493 baseline for food web studies encompassing lower to high trophic level species and against changes in oceanographic  
494 conditions under a climate change scenario, considering the rapid response of zooplankton communities to global warming.

## 495 Author contribution

496 IL, AdF and SM designed the survey and carried it out. EF conceived the experimental design. EF and SM analysed the  
497 samples. EF analysed the data and prepared the manuscript with contributions from all co-authors.

## 498 Competing interests

499 The authors declare that they have no conflict of interest.



## 500 **Data availability**

501 Data can be requested to the corresponding author upon reasonable request.

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