# Spatial changes in community composition and food web structure of mesozooplankton across the Adriatic basin (Mediterranean Sea)

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**Abstract.** Zooplankton are critical to the functioning of ocean food webs because of their utter abundance and vital ecosystem roles. Zooplankton communities are highly diverse and thus perform a variety of ecosystem functions, thus changes in their community or food web structure may provide evidence of ecosystem alteration. Assemblage structure and trophodynamics of mesozooplantkon communities were examined across the Adriatic basin, the northernmost and most productive basin of the Mediterranean Sea. Samples were collected in June-July 2019 within the framework of the MEDIAS (MEDiterranean International Acoustic Surveys) project, along coast-offshore transects and from the surface to ca. 150 m of depths, covering the whole western Adriatic side, consistently environmental variables were also recorded. Results showed a clear separation between samples from the northern-central Adriatic and the southern ones, with a further segregation, although less clear of inshore *vs*. off-shore stations, the latter mostly dominated in the central and southern stations by gelatinous plankton. Such patterns were mainly driven, based on the outputs of the Distance-based Linear model, by fluorescence (as a proxy of primary production) for northern-central stations, *i.e.*, closer to the Po River input, and by dissolved oxygen, together explaining 44% of total variance. Overall, at basin level, the analysis of stable isotopes of nitrogen and carbon allowed to identify a complex food web characterized by 3 trophic levels from filter feeders-herbivores to carnivores, passing through a general pattern of omnivory with varying preference towards herbivory or carnivory. Stable isotope signatures spatially varied between inshore vs. offshore communities and across sub-areas, with the Northern Adriatic exhibiting greater $\delta^{15}N$ and more variable $\delta^{13}C$ than the other two sub-areas, likely attributable to the occurrence in the area, of organic matter of both terrestrial and marine origin. Our results contribute to the knowledge of mesozooplankton community and trophic structure, at basin scale across a costal-offshore gradient, also providing a baseline for future assessment of pelagic food webs within the EC Marine Strategy Framework Directive.

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

## 1 Introduction

In an oligotrophic system, such as the Mediterranean Sea, coastal productivity largely depends on inputs from rivers and areas of high productivity are mainly restricted to waters close to major freshwater inputs (D'Ortenzio and Ribera d'Alcalà, 2009, Ludwig et al., 2009). Here, the Adriatic basin represent an anomaly, with the northern Adriatic being one of the most productive Mediterranean areas. While the northern part is a shallow sub-basin, characterised by inputs of several rivers, with the Po representing the major buoyancy input with an annual mean discharge rate of 1500~1700 m$^3$s$^{-1}$, and accounting for about one third of the total riverine freshwater input in the Adriatic (Raicich, 1996, Marini et al., 2008, Morello and Arneri, 2009), the southern part is characterized by highly saline and oligotrophic waters (Franco and Michelato, 1992; Boicourt et al., 1999). Thus, a trophic gradient, decreasing from northwest to southeast, is typically observed in the basin, in which the nutrient-rich waters coming from the rivers are mainly spread southward and eastward from the Italian coast (Bernardi Aubry et al., 2006; Solidoro et al., 2009). Such differences may be reflected in the population dynamics of the marine biotic components (Revelante and Gilmartin, 1977; Simonini et al., 2004; Hermand et al., 2008), from zooplankton (Siokou-Frangou and Papathanassiou, 1991; Hwang et al., 2010) to fish (Wets et al., 2011).

However, these dynamics both in terms of community composition and trophic relationships have never been investigated at the scale of the whole Adriatic basin. Zooplankton play a key role in marine ecosystems, forming the base of marine food web because of the diversity of their functions. Zooplankton is a link between primary producers of organic matter and the higher-order consumers, it provides grazing control on phytoplankton blooms (Kiørboe, 1993) and helps regulating fish stocks (Beaugrand et al., 2003), being this last aspect of crucial importance in the Adriatic basin. Because of these important zooplankton functions, a better understanding of their distribution and the patterns of their response to changes in the chemical and physical properties of marine waters is essential, especially under a global warming scenario, being zooplankton sensitive beacon of climate change (Richardson, 2008).

Moreover, trophic relationships in pelagic ecosystems are complex and complicated by the large degree of omnivory of most zooplanktonic species (Bode and Alvarez-Ossorio, 2003), which may feed on similar diets composed of a mixture of phytoplankton, detritus, and microplankton (e.g., Stoecker and Capuzzo, 1990; Irigoien et al., 1998; Batten et al., 2001). Several experimental studies allowed zooplankton (mostly copepods) to be categorised from pure carnivores to omnivores with a variety of mixtures of algae and animal prey up to strictly herbivore species (Irigoien et al., 1998; Batten et al., 2001; Halvorsen et al., 2001; see also Benedetti et al., 2016 and Hebert et al., 2016, for a review on functional traits of zooplankton). Such variety in the diet makes the quantification of flows between compartments or trophic levels difficult. In the last decades, stable isotope analyses (SIA) have been widely used in food-web studies, different studies dealt with high taxonomical groups of zooplankton (Burd et al., 2002; Blachowiak-Samolyk et al., 2007; Tamelander et al., 2008), while few investigations were focused on low taxonomical resolution (Koppelmann et al., 2003; Rumolo et al., 2017),

63 essential to disentangle the food web structure of pelagic communities (Fanelli et al., 2011). Analysis of stable isotope

64 composition provides indications of the origin and transformations of organic matter. Stable isotopes of carbon and

65 nitrogen integrate short-term variations in diet and thus are less subject to temporal bias. The $\delta^{15}N$ in tissues of consumers

66 are typically greater by 2–3‰ relative to their prey and can be used as a proxy of the trophic level of organisms (Owens,

67 1987), while $\delta^{13}C$ may act as a useful indicator of primary organic carbon sources of an animal's diet, as tissues tend to

68 be rather weakly enriched in $^{13}C$ at progressively higher trophic levels (1‰).

69 In this context, the main aim of this study is to analyse spatial variations in the assemblage structure and trophodynamics

70 of mesozooplankton communities in the whole basin. Additionally, considering the complex hydrological condition of

71 the basin, characterised by such contrasting oceanographic settings from north to south, here we explored and identified

72 which environmental variables best explain the observed patterns.

73 **2 Materials and Methods**

74 **2.1. Study area**

75 The Adriatic Sea is an elongated semi-enclosed basin, with its major axis in the northwest–southeast direction, located in

76 the central Mediterranean, between the Italian peninsula and the Balkans (**Figure 1**). It is 800 km long and 150-200 km

77 wide. It has a total volume of 35,000 km³ that belongs for 5% to the Northern basin, 15% to the middle basin and 80% to

78 the Southern basin. The Northern Adriatic is very shallow, with an average depth of 35 m with a very gradual topographic

79 slope along its major axis and it is characterized by strong river runoff, being the Po the second main contributor (about

80 20%) to the whole Mediterranean river runoff (Struglia et al., 2004).
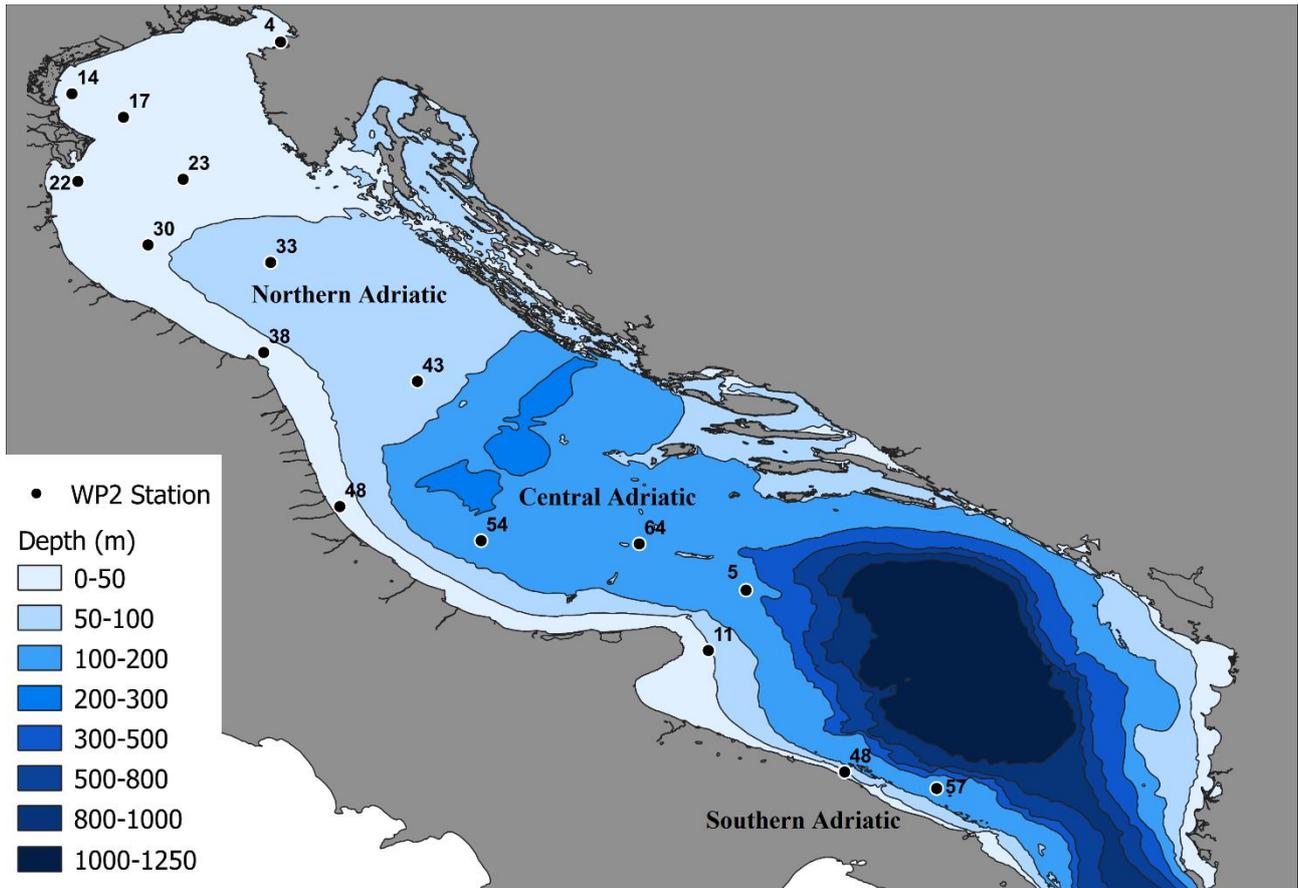
**Figure 1:** Map of the study area with indication of WP2 net stations (black dots).

Due to this input, there is a positive water balance of 90-150 km³ that is exported to the Mediterranean. The turnover time for the whole basin is 3-4 years (Artegiani *et al.*, 1997; Marini, Bombace and Iacobone, 2017). The middle Adriatic is a transition zone between northern and southern sub-basins, with the two Jabuka/Pomo depressions reaching 270 m depth. The southern sub-basin is characterized by a wide depression about 1200 m in depth. Water exchange with the Mediterranean takes place through the Otranto Strait, which has an 800 m deep sill (Artegiani *et al.*, 1997; Marini, Bombace and Iacobone, 2017). The Adriatic is a temperate warm sea, with surface temperature ranging from 6 °C in the northern part in winter to 29 °C, in summer. Even the temperatures of the deepest layers are, for the most part, above 10 °C. The South Adriatic is warmer than its central and northern parts during winter. In other seasons, the horizontal temperature distribution is more uniform (Artegiani *et al.*, 1997; Marini, Bombace and Iacobone, 2017).

Water circulation in the Adriatic is mainly driven by dominant winds (Bora and Scirocco) that cause a cyclonic circulation, with three closed circulation cells (one for each sub-basin). During the winter season, meteorological depressions pass over the Adriatic Sea, the first sector of the cyclone exposes the sea to warm Saharan air, the Scirocco. As the cyclone passes, the winds reverse and expose the Adriatic Sea to a polar continental air mass, the so-called Bora, coming from the north over central Europe and blowing the Adriatic Sea from the north and north-east. In summertime, corresponding to the time of our sampling, besides local breezes, the dominant wind, the Maestrale, comes from the northwest (Orlić et al., 1994). Climatological studies about the heat content of the water column (Artegiani *et al.*, 1997) have resulted in the following definition of the Adriatic marine seasons: winter spans from January to April, spring occurs in May-June, summer goes from July to October, and autumn occurs in November -December.

Regarding temperature and salinity during the sampling period, in summer, the bathymetric effect (i.e.temperature gradients are at the same locations of topographic gradients) is evident: higher temperatures are observed in the northern part and along the western coast and lower temperature in the southern part and along the eastern coast. For concerns spring, conditions are more like the summer ones (Russo and Artegiani, 1997). The distribution of salinity in the surface layer is strongly influenced (especially in the northern part and along the western coast) by river outflow, above all Po and other northern rivers. during summer, thermal stratification allows a wide horizontal distribution of these river waters inside the basin (vertically they are confined within the mixed layer, 10-30 m thick). The 38.0 psu isohaline spreads southward and offshore, during spring and summer (Russo and Artegiani, 1996).

Three different water masses dominate the basin circulation: the Adriatic Surface Water (AdSW), the Levantine Intermediate Water (LIW) and the Adriatic Deep Water (AdDW), which branches out in Northern (NAdDW), Middle (MAdDW) and Southern (SAdDW) Adriatic Deep Water. The hypersaline LIW is formed in the Levantine Basin and experiences a salinity decrease on its way to the Adriatic. The AdDW are formed in the Adriatic basin and the NAdDW in the Northern part; due to its high density, it fills up the Jabuka/Pomo Pit and only occasionally spreads to the Southern Adriatic. The MAdDW is formed in the Jabuka/Pomo Pit area, when there is no intensive north-westward flow, (*i.e.* during periods of low Mediterranean water inflow). The SAdDW originates in the South Adriatic Pit. During the period of the MEDIAS survey (June), wind forcing is generally weak and volume flux from the Po River low, although the Po plume remained a significant feature in the northern and western Adriatic (Marini *et al*., 2008).

As mentioned above, the Adriatic is a very productive basin, compared to the rest of the Mediterranean. Despite being only the 5% of the total Mediterranean surface area, the Adriatic Sea produces about 15% of total Mediterranean landings (and 53-54% of Italian landings), with a fish production density of 1.5 t/km², which is three times the Mediterranean density (Marini, Bombace and Iacobone, 2017). This impressive feature is shaped by three main factors: river runoff, shallow depths and oceanographic structure. River runoff is particularly strong in the northern basin and affects the circulation through buoyancy input and the ecosystem by introducing large fluxes of nutrients (Zavatarelli *et al*., 1998), which favour phytoplanktonic blooms and in turn cause a bottom-up effect of the whole trophic chain. Rivers can also

provide suspended particulate organic matter and organic detritus, that feed numerous particulate feeders and detritivores, such as bivalves (which is one of the main fisheries of the North Adriatic Sea). The wide continental shelf favours a short trophic chain that likely improve the efficiency of energy transfer from lower trophic levels to higher ones. Moreover, the structure of the basin allows water mixing during winter, especially in North and Middle Adriatic, transferring nutrients from sediments to the water column. From a fishery management point of view, the General Fishery Commission for the Mediterranean (GFCM) has divided the basin in two Geographical Sub-Areas (GSAs), the GSA 17, encompassing the northern and the middle sub-basin and the GSA 18, including the southern part.

**2.2 Zooplankton collection and analysis**

Samples for this study were collected on board R/V "G. Dallaporta" during the acoustic survey MEDIAS 2019 GSA 17 and GSA 18, that took place in June-July 2019, in the Adriatic Sea (Leonori *et al*., 2020), within the framework of the MEDIAS (MEDiterranean International Acoustic Surveys) project (Leonori *et al*., 2021). MEDIAS coordinates the acoustic surveys performed in the Mediterranean and Black Sea to assess the biomass and spatial distribution of small pelagic fish (MEDIAS, 2019) (http://www.medias-project.eu). Acoustic surveys are echo-surveys carried out by using a split beam echo–sounder set at specific frequencies which allow to discriminate between small pelagic fishes and zooplankton (see details in MEDIAS, 2019). Simultaneously to echo-sampling, traditional surveys were carried out on both the zooplanktonic and the fish fraction (this latter by using a pelagic trawl).

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

Zooplankton samples near the fishing hauls were subsampled and frozen at -20 °C, because of the requirements for SIA (see also Fanelli *et al*., 2009a-b, 2011, 2013; Rumolo *et al*., 2017, 2018). Concurrently with each vertical plankton haul, a CTD cast was performed, to acquire information on the oceanographic parameters of the chosen site. Environmental data recorded were pressure (dab), temperature (°C), fluorescence (μg/l), turbidity (NTU), dissolved oxygen (expressed as ml/l and saturation percentage), salinity and density (km/m$^3$).  To this study, the whole Western Adriatic has been divided in three different sub-basins or sub-areas, as described above (Artegiani et al., 1997): the Northern Adriatic sub-area (NA), encompassing the stations from 4 to 38, the Central Adriatic (CA) including stations 43-64 and the Southern Adriatic (SA) comprising stations 5-57 (**Figure 1**).

Selected zooplankton samples were analysed in the laboratory to characterize the planktonic community. First, frozen samples were defrosted and filtered with 200 μm sieve and the obtained mass was weighted (Wet Weight-WW in g, precision 10$^{-3}$). Then samples were quickly sorted, and larger animals isolated for first and placed in Petri dishes located on ice, to preserve tissue integrity. Individuals were than identified to the lowest taxonomic level possible and stored for

160    subsequent analysis. About 10% of the sample was therefore weighted (WW in g, precision $10^{-5}$) and all organisms in the

161    sub-sample were identified to the lowest taxonomic level possible (Cartes et al., 2011, 2013).

162    All identified taxa were then counted and weighted with an analytical weight scale, to obtain abundance and biomass

163    estimations.

164    **2.3. Samples preparation for stable isotope analyses**

165    The most abundant taxa in each sample were prepared for stable isotope analyses. Selected taxa were oven-dried for 24

166    hours at 60 °C. Dried samples were converted to a fine powder with a mortar and pestle. For each taxon, three replicates

167    (when possible) were weighted (ca 0.3-1.3 mg) and placed into tin capsules. Since it was not possible to obtain enough

168    material of a single taxon for stable isotope analyses from stations 22 and 38, a bulk of the whole mesoplankton

169    community of the stations was prepared for the analyses. Acidification of samples prior to stable isotope analyses is

170    usually regarded as a standard procedure, since inorganic carbon could lead to an increase of $\delta^{13}C$, because it is

171    isotopically heavier than most carbon of organic origin and could reflect the isotopic signature of environmental carbon

172    (Schlacher and Connolly, 2014). However, for this study, no acidification was carried out, as this procedure generally

173    reduces sample biomass, leading to too little matter available for isotope analyses. Moreover, some authors revealed

174    negligible differences between acidified and not acidified samples (Rumolo *et al.*, 2018). However, to have an indication

175    of the possible bias, only one species was acidified, *Euchaeta sp.*, which is a very abundant copepod in Adriatic

176    communities. This taxon was also chosen because it has a more calcified exoskeleton, and it was abundant enough to

177    undergo this process. Half of the sample was acidified with HCl 1M, by adding it drop by drop to the sample until bubble

178    cessation, then samples were oven-dried again at 60 °C for 24 h. The other half, for the analysis of $\delta^{15}N$, was not acidified,

179    as several studies demonstrated that the acidification procedure can alter nitrogen isotopic signature (Kolasinski, Rogers

180    and Frouin, 2008). Acidification of crustaceans was proved to be unnecessary, as the tested samples of *Euchaeta* sp.

181    showed little and not significant differences in $\delta^{13}C$ value (-21.39±0.06 for untreated samples *vs.* -21.02±0.15 for acidified

182    samples, paired T-test= -0.34, p=0.74). Then, six replicates of each sub-samples were prepared for isotope analyses.

183    Samples were analysed through an elemental analyser (Thermo Flash EA 1112) for the determination of total carbon and

184    nitrogen, and then analysed for $\delta^{13}C$ and $\delta^{15}N$ in a continuous-flow isotope-ratio mass spectrometer (Thermo Delta Plus

185    XP) at the Laboratory of Stable Isotopes Ecology of the University of Palermo (Italy). Stable isotope ratio was expressed,

186    in relation to international standards (atmospheric $N_2$ and PeeDee Belemnite for $\delta^{15}N$ and $\delta^{13}C$, respectively), as:

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

188    where R = $^{13}C/^{12}C$ or $^{15}N/^{14}N$. Analytical precision based on standard deviations of internal standards (International

189    Atomic Energy Agency IAEA-CH-6; IAEA-NO-3; IAEA-N-2) ranged from 0.10 to 0.19‰ for $\delta^{13}C$ and 0.02 to 0.08‰

190    for $\delta^{15}N$.

## 2.4. Community data analyses

Zooplankton abundance and biomass were standardized to a constant value. The adopted constant was the volume of water filtered by the net, according to Harris et al. (2000). When flowmeter data were not available (due to misfunctioning), the volume was calculated as a mean value of similar nearby stations. Zooplankton abundance was expressed as number of individuals per $m^2$, while zooplankton biomass was expressed as mg of wet weight (WW) per $m^2$. This allows to minimise the differences in the water column depths samples in the different stations, otherwise the use of data averaged in the water column (i.e., N or B / $m^3$) should have reduced the importance of offshore stations as the numbers will be "diluted" in a large volume of water.

First, the Shannon-Wiener diversity index of each station was calculated. Then, total biomass, total abundance, and *H'* diversity index were tested by univariate PERMANOVAs (Permutational Multivariate Analysis of Variance, Anderson et al., 2008). Tests were run on Euclidean distance resemblance matrixes of log(x+1)-transformed data for abundance and biomass data and untransformed *H'* values (as data were normally distributed), and using a two-way design with sub-area as a fixed factor with three levels (NA, CA and SA, as described above) and inshore-offshore location as a fixed factor with two levels (inshore *vs.* offshore), crossed within each other, in order to assess the presence and significance of differences between stations. Inshore and offshore stations were selected according to Liquete et al. (2011). Univariate PERMANOVA test were run under 9999 permutations, with permutation of residuals under a reduced model, as permutation method, significant p-values were set at p<0.05.

To test for differences among sub-areas and inshore *vs.* offshore communities a PERMANOVA test was performed on the Bray-Curtis resemblance matrix of log(x+1)-transformed abundance zooplankton data, using the same design described for univariate analyses. Data transformation is recommended for ecological data, because they are often highly skewed and/or range over several orders of magnitude (as in this case), to downweigh the contributions of quantitatively dominant species to the similarities calculated between samples. This is particularly important for the most useful, and commonly used, resemblance measures like Bray-Curtis similarity, which do not incorporate any form of scaling of each species by its total or maximum across all samples. Here we used a severe transformation, i.e., the log(x+1), that compresses large values, to take notice also of the less-abundant (Anderson et al., 2008). A CAP analysis (Canonical Analysis of Principal coordinates, Anderson and Willis, 2003) was then run to visualize the observed pattern, on the factor found to be significant by PERMANOVA.

A SIMPER analysis was carried out according to the same sampling design to identify the most typifying taxon contributing to the average similarity/dissimilarity among sub-areas and inshore *vs.* offshore locations. This was conducted using Bray-Curtis similarity, with a cut-off for low contribution at 50%.

To identify the environmental drivers of zooplanktonic communities and their structure across the sampling area, biotic data were correlated to environmental variables. Environmental data were tested for collinearity among variables by using

223    a Draftsman plot, with fluorescence, Dissolved $O_2$ concentration (DO, ml/l), % of $O_2$ saturation and turbidity data being

224    Log (X+1)-transformed to fit a linear distribution in the Draftsman plot. Finally, a DistLM (Distance based linear models,

225    Anderson et al., 2008) was run with temperature, fluorescence, turbidity, oxygen and salinity as environmental variables,

226    using "step-wise" as selection procedure and "AIC (Akaike Information Criterion)" as selection criterion.

227

228    **2.5.    Stable isotopes data analysis**

229    Since lipids can alter the values of $\delta^{13}C$ (Post *et al.*, 2007), samples with high lipid concentration can be defatted to avoid

230    $^{13}C$ depletion. However, lipid extraction can alter $\delta^{15}N$ values, can complicate sample preparation and reduce samples

231    availability, a crucial point when analysing small animals. For these reasons, $\delta^{13}C$ of samples rich in lipids was normalized

232    according to Post equation (Post *et al.*, 2007):

233    $$\delta^{13}C_{normalized} = \delta^{13}C_{untreated} - 3.32 + 0.99 \, C/N_{sample}$$

234    C/N ratio was used as a proxy of lipid content, because their values are strongly related in animals (Post *et al.*, 2007). In

235    particular, the normalization was applied to samples with a C/N ratio > 3, according to Post *et al.* (2007).

236    A hierarchical cluster analysis (Euclidean distance, average grouping methods) on the bivariate matrix of $\delta^{13}C$ and $\delta^{15}N$

237    mean values of each taxon was performed to elucidate the planktonic food web structure. Obtained clusters were also

238    compared with literature data on the trophic guild of analysed taxa. Four main trophic groups were established a priori on

239    the basis of literature data, where available, and adapting the classification suggested in Hebert et al. (2016), Benedetti et

240    al. (2016), Fanelli et al. (2011), and recent findings based on both SIA and fatty acids for some of the species here analysed

241    (Protopapa et al., 2019). Thus, trophic groups used for the following analyses were filter feeders/herbivores (FF-HERB)

242    considered as primary consumers, omnivores with a clear tendency toward herbivory (OMN-HERB), encompassing

243    mostly herbivore species, but that can feed also small particles and ciliates, small carnivores (OMN-CARN), similarly to

244    OMN-HERB but with greater preference for small zooplankton, and carnivores (CARN), including also the parasite

245    hyperiid *Lycaea pulex*. Differences among groups were tested by means of a one-way PERMANOVA test with "trophic

246    group" (with four levels, corresponding to FF-HERB, OMN-HERB, OMN-CARN and CARN) as fixed factor.

247    The trophic level of the different species was estimated according to Post (2002) as: $((\delta^{15}N_i - \delta^{15}N_{PC})/TEF) + \lambda$

248    where $\delta^{15}N_i$ is the $\delta^{15}N$ value of the taxon considered, $\delta^{15}N_{PC}$ is the $\delta^{15}N$ values of a primary consumer, *i.e.* an herbivore

249    or a filter feeder, used as baseline of the food web, TEF is the trophic enrichment factor which is considered varying

250    between 2.54 (Vanderklift and Ponsard, 2003) and 3.4 (Vander (*e.g.* Vander Zanden and Rasmussen, 2001; Post, 2002)

251    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

252    position of the baseline, which is 2 in our case. Here, we used three different values as baselines for the food web of the

253    three sub-areas, specifically the average values of FF-HERB taxa.

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

Finally, maximum likelihood standard ellipses were created for the $\delta^{13}$C and $\delta^{15}$N values following Jackson et al. (2011) to assess the community niche width in the different sub-areas. In addition to standard ellipse area (SEA; contain ca. 40% of the data and represent the core isotopic niche) and standard ellipse areas corrected for small sample size (SEAc), traditional convex hulls and four Layman metrics were also estimated (Layman et al., 2007). Specifically, we calculated TA, which is the area of convex hull containing, in the case of SIBER (Stable Isotope Bayesian Ellipses in R, Jackson et al., 2011), the means of the populations that comprise the community, d15N_range that is the distance in units between the min and max y-axis population means, d13C_range, *i.e.* the distance in units between the min and max x-axis population means, and CD which is the mean distance to centroid from the means. Ellipse sizes were compared between groups (*i.e.* sub-areas) using Bayesian inference techniques.

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

## 3.    Results

### 3.1. Zooplankton community and spatial changes

A total of 52,016 specimens belonging to 113 taxa were collected through the WP2 sampling (**Table S1**). Zooplanktonic communities in the whole area were dominated by small copepods of the genus *Acartia* (mostly *A. clausi*), *Oncaea*, *Oithona* (mainly *O. similis*) and copepodites. Abundant large copepods were Calanoida belonging to the genera *Euchaeta*, *Calanus*, *Centropages* and *Temora*. Since samples were frozen on board after collection for subsequent isotopic analyses, a quite considerable number of specimens (particularly amphipods and mysids and those taxa/specimens characterized by soft carapace) were damaged and therefore hard to identify at species level. Generally, they were identified to order level or indicated as "damaged unid." in **Table S1**. Other common crustaceans were hyperiids, such as *Lestrigonus schizogeneios* and *Phronima atlantica*, decapod larvae (mainly zoeae and megalopae), mysids and euphausiids. Among non-crustaceans, molluscs were quite common, both as larvae of benthic organisms and adult pteropods. Chaetognatha were also locally abundant. Gelatinous zooplankton was represented mainly by thaliaceans and calycophorans, while ichthyoplankton was not very abundant, with few fish eggs and larvae found.

283  Zooplankton abundance and biomass varied according to geographic sub-area decreasing from the Northern to the
284  Southern Adriatic (**Figure 2a-b**) and to the distance from the coasts. However, differences at sub-area scale were
285  significant only for abundance, while inshore-offshore differences did only for biomass (**Table S2**).

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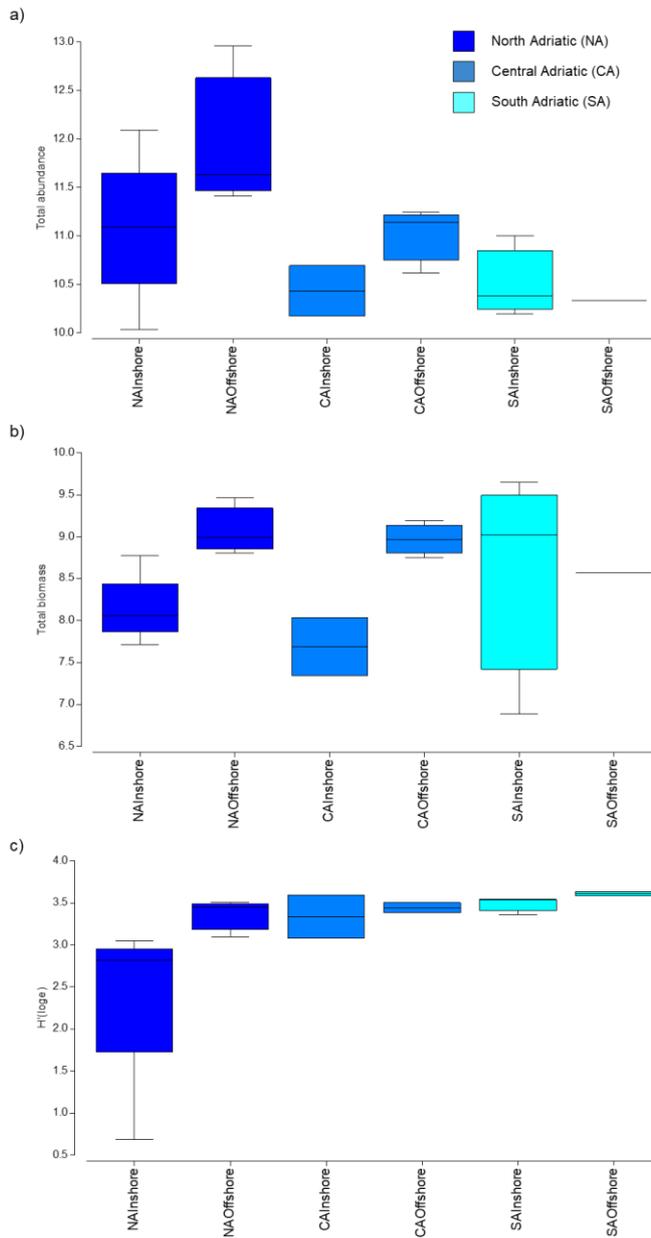
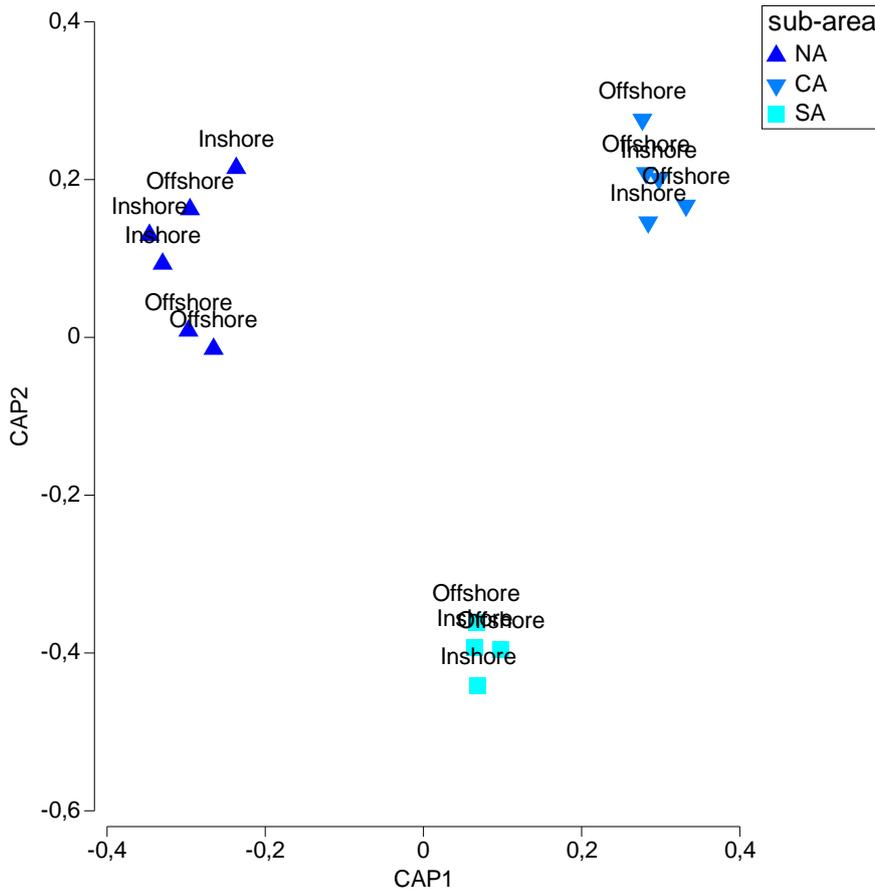


**Figure 2.** Total abundance (N ind./m$^2$), a), total biomass (mg WW/m$^2$, b) and diversity (*H'*, c) of mesozooplankton at each group of
stations by sub-area and distance from the coast (inshore *vs*. off-shore stations). Colours define the different sub-areas. Boxes are
interquartile ranges, black lines that divide the box into two parts represent the medians and the upper and lower whiskers represent
scores outside the middle 50%.

291  Diversity (in terms of *H'*) increased southward (**Figure 2c**), although differences were not significant for any of the
292  investigated factors. *H'* values were on average 3.25±0.31, with the only exception of station 22, located in the GSA17N
293  inshore, in front of the Po delta, showing the lowest *H'* value (0.64).
294  PERMANOVA revealed that differences in zooplanktonic communities, based on geographic sub-areas and inshore-
295  offshore factor were significant, while any significant differences occurred for the interaction factor (**Table S3a-b**).
296  The CAP plot showed a clear separation among samples from each sub-area, with the first axis separating samples from
297  NA from those belonging to CA and SA sub-areas (**Figure 3**).

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299  **Figure 3**. CAP plot of the mesozooplanktonic communities of the Adriatic basin by sub-area and inshore *vs.* offshore location, based
300  on abundance data. Colours indicate the sub-basins, as described in the text.
301

302  SIMPER analysis showed that *Calanus*-like copepods, *Euchaeta* sp., *Euterpina acutifrons* and *Evadne spinifera* mainly
303  contributed to dissimilarity between NA *vs.* CA (**Table S4a**). Bivalve and gastropod larvae, together with *Acartia* sp.,
304  were the main responsible for the dissimilarity between the subareas CA and SA. Within NA samples, the dissimilarity
305  between inshore vs. offshore zooplanktonic communities were mostly driven by Calycophorae, *Calanus helgolandicus*

and Chaetognatha, being more abundant at offshore stations. The cladoceran *Penilia avirostris*, thaliaceans, ostracods and *Calanus helgolandicus* were responsible for the dissimilarity between inshore *vs*. offshore stations within CA, with *P. avirostris* occurring only at inshore stations, and thaliaceans, ostracods and *Calanus helgolandicus* as dominant at offshore ones (**Table S4b**). Large calanoid copepods dominated the inshore communities within SA sub-area, while the euphausiid *Meganyctiphanes norvegica* was more abundant at offshore stations (**Table S4b**).

### 3.2. Environmental variables and correlation with zooplankton data

During the sampling period, temperature values were on average 18.5 °C (±0.88 SD), with lowest and the greatest values observed at inshore and offshore stations, respectively, in the Central and Southern sub-basins (**Table S5**). Salinity values were on average 36 in the Northern basin with the lowest value of 34.7 recorded at station 22_17 in front of the Po; salinity increased southward reaching a mean value of 38.7 in the southern basin (**Table S5**). Fluorescence values decreased southward from 2.45 µg/l to 0.77 µg/l, with the highest (4.9 µg/l) and the lowest (0.59 µg/l) values recorded at station 22_17 (in front of the Po River delta) and at station 44_18 (in the Otranto channel), respectively (**Table S5**). On the other hand, dissolved oxygen (DO) decreased southward from a mean value of 5.32 ml/l recorded in NA stations to 4.36 ml/l observed in SA CTD casts (**Table S5**). Significant variations were observed for all tested variables for sub-area and inshore *vs*. offshore factors, and for the interaction term only for temperature and dissolved oxygen (**Table S6a**). Pairwise comparisons evidenced significant differences in salinity, fluorescence and dissolved oxygen values between NA and CA (**Table S6b**). Significant differences between inshore vs. offshore stations occurred in the southern sub-basin for temperature, salinity and DO, in the central sub-basin for temperature and salinity, and in the northern sub-basin only for salinity (**Table S6b**).

According to the results of the draftsman plot, DO concentration (ml/l) and % of oxygen saturation covaried ($\rho > 0.7$), as well as density and pressure, therefore, only temperature, fluorescence, turbidity, DO, and salinity were used for DistLM analysis. DistLM results showed that 44% of the variance was explained by fluorescence (33%) and by dissolved oxygen (11%), (**Table 1, sequential test**) and provide the best model solution in terms of both AIC and $R^2$ values.

**Table 1.** Results of the marginal and the sequential test for DistLM model, with indication of the best model.

| MARGINAL TESTS | | | | | | | |
|---|---|---|---|---|---|---|---|
| Variable | SS(trace) | Pseudo-F | P | Prop. | | | |
| Temperature (C°) | 1463 | 1.16 | 0.29 | 0.08 | | | |
| Fluorescence (µg/l) | 5943.8 | 6.51 | 0.0001 | 0.33 | | | |
| Turbidity (NTU) | 1679.9 | 1.35 | 0.20 | 0.09 | | | |
| Oxygen (ml/l) | 2035.4 | 1.68 | 0.12 | 0.11 | | | |
| Salinity | 5724.8 | 6.16 | 0.0001 | 0.32 | | | |
| SEQUENTIAL TESTS | | | | | | | |
| Variable | AIC | SS(trace) | Pseudo-F | P | Prop. | Cumul. | res.df |
| Fluorescence (µg/l) | 104.1 | 5943.8 | 6.51 | 0.0002 | 0.33 | 0.33 | 13 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Oxygen (ml/l) | | | 103.52 | 1873.7 | 2.25 | 0.006 | 0.11 | 0.44 | 12 |

BEST SOLUTION

| AIC | $R^2$ | RSS | No.Vars | Selections |
|---|---|---|---|---|
| 103.52 | 0.44 | 9992.3 | 2 | 2;4 |

2=fluorescence, 4=oxygen

### 3.3. Stable isotope composition of zooplankton

Stable isotope analyses provided $\delta^{13}C$ and $\delta^{15}N$ values of 25 different taxa (**Table 2**).

**Table 2.** Mean values of zooplankton samples analysed for each sub-area (NA= Northern Adriatic, CA= Central Adriatic; SA= Southern Adriatic), trophic group (TG) and Trophic level (TL). FF-HERB = filter feeders-herbivores, OMN-HERB = omnivores with preference towards herbivory, OMN-CARN = omnivores with preference towards carnivory, CARN = carnivores. "Base" indicates the species used for the estimation of the average $\delta^{15}N$ values of the baseline for TL calculation (see text for further details).

| Group | Taxon | $\delta^{15}N$ | SD | $\delta^{13}C$ | SD | sub-area | TG | TL |
|---|---|---|---|---|---|---|---|---|
| COPEPODA | *Nannocalanus minor* | 3.12 | | -21.01 | | NA | FF-HERB | base |
| COPEPODA | *Oithona sp.* | 4.10 | | -20.41 | | NA | FF-HERB | base |
| DECAPODA | Thalassinidea (zoea) | 4.14 | | -19.92 | | NA | OMN-HERB | 2 |
| COPEPODA | *Calanus helgolandicus* | 4.55 | 0.98 | -20.59 | 0.27 | NA | OMN-HERB | 2 |
| COPEPODA | *Temora stylifera* | 4.71 | | -20.56 | | NA | OMN-HERB | 2 |
| COPEPODA | *Centropages typicus* | 5.42 | 1.43 | -21.38 | 0.61 | NA | OMN-CARN | 3 |
| COPEPODA | Unid.large copepods | 7.19 | 0.12 | -16.39 | 0.41 | NA | OMN-CARN | 3 |
| CHAETOGNATHA | Chaetognatha | 7.07 | 2.40 | -19.90 | 0.17 | NA | CARN | 3 |
| SIPHONOPHORA | Calycophorae | 7.49 | 0.11 | -19.71 | 1.97 | NA | CARN | 4 |
| DECAPODA | Decapoda (zoea) | 7.58 | 1.45 | -19.81 | 0.19 | NA | CARN | 4 |
| COPEPODA | *Euchaeta sp.* | 7.86 | 0.86 | -21.58 | 0.61 | NA | CARN | 4 |
| COPEPODA | *Gaetanus tenuispinus* | 2.68 | | -20.44 | | CA | FF-HERB | base |
| THALIACEA | Thaliacea | 3.77 | 0.67 | -20.75 | 0.41 | CA | FF-HERB | base |
| COPEPODA | *Nannocalanus minor* | 3.80 | 0.22 | -21.28 | 0.48 | CA | FF-HERB | base |
| DECAPODA | Brachyura (zoea) | 3.89 | 0.06 | -19.17 | 0.07 | CA | OMN-HERB | 2 |
| EUPHAUSIACEA | *Meganyctiphanes norvegica* | 4.48 | 0.54 | -21.18 | 0.57 | CA | OMN-HERB | 2 |
| DECAPODA | Decapoda (zoea) | 4.16 | | -20.16 | | CA | OMN-HERB | 2 |
| OSTEYCHTHYES | Fish larvae | 5.09 | 0.53 | -20.57 | 0.26 | CA | OMN-HERB | 3 |
| COPEPODA | *Calanus helgolandicus* | 5.19 | 0.52 | -20.89 | 0.35 | CA | OMN-HERB | 3 |
| DECAPODA | Penaeidae (zoea) | 5.77 | 0.07 | -20.74 | 0.04 | CA | OMN-CARN | 3 |
| HYPERIIDEA | *Lestrigonus schizogeneios* | 5.73 | | -20.62 | 0.65 | CA | OMN-CARN | 3 |
| SIPHONOPHORA | Calycophorae | 5.18 | 0.39 | -20.32 | 0.41 | CA | CARN | 3 |
| COPEPODA | *Euchaeta sp.* | 5.43 | 0.47 | -21.03 | 0.25 | CA | CARN | 3 |
| CHAETOGNATHA | Chaetognatha | 5.77 | 0.57 | -19.95 | 0.46 | CA | CARN | 3 |
| COPEPODA | Unid large copepods | 7.12 | 0.10 | -18.09 | 0.19 | CA | CARN | 3 |
| THALIACEA | *Thaliacea* | 3.35 | 0.78 | -19.59 | 0.40 | SA | FF-HERB | base |
| COPEPODA | *Nannocalanus minor* | 3.74 | 0.09 | -20.64 | 0.04 | SA | FF-HERB | base |
| COPEPODA | *Pleuromamma abdominalis* | 3.59 | | -21.14 | | SA | OMN-HERB | 2 |
| COPEPODA | *Calanus helgolandicus* | 4.48 | 1.59 | -20.89 | 0.42 | SA | OMN-HERB | 2 |
| COPEPODA | *Pareucalanus attenuatus* | 4.92 | | -20.01 | | SA | OMN-HERB | 3 |
| EUPHAUSIACEA | Euphausiacea (furcilia) | 4.69 | | -20.39 | | SA | OMN-CARN | 2 |
| HYPERIIDEA | *Lycea pulex* | 4.69 | | -19.64 | | SA | OMN-CARN | 2 |
| HYPERIIDEA | *Phronima sedentaria* | 5.42 | | -19.60 | | SA | OMN-CARN | 3 |
| COPEPODA | *Euchaeta sp.* | 5.09 | 0.12 | -20.90 | 0.36 | SA | CARN | 3 |
| COPEPODA | *Chiridius sp.* | 6.24 | | -19.77 | | SA | CARN | 3 |

| DECAPODA | Decapoda (zoea) | 6.81 | 0.17 | -19.64 | 0.08 | SA | CARN | 3 |
|---|---|---|---|---|---|---|---|---|
| CHAETOGNATHA | Chaetognatha | 7.13 | 1.36 | -19.70 | 0.44 | SA | CARN | 3 |
| DECAPODA | Penaeidae (zoea) | 8.02 | | -19.93 | | SA | CARN | 4 |
| MYSIDA | *Siriella sp.* | 8.14 | | -20.03 | | SA | CARN | 4 |

340  Cluster analysis allowed to group animals according to their $\delta^{13}C$ and $\delta^{15}N$ values, and according to the trophic groups
341  previously established, based on literature data (**Figure 4a**). Still, the nMDS analysis evidenced a gradient from strictly
342  herbivore species towards carnivore taxa (**Figure 4b**). One-way PERMANOVA test run on factor "trophic groups-TG" was
343  significant (*pseudo-F$_{3,25}$*=13.12, p=0.0001), with significant differences between each level of pairwise comparisons across
344  the herbivory-carnivory trophic gradient (FF-HERB *vs*. OMN-HERB: t=20.52, p=0.02; OMN-HERB *vs*. OMN-CARN:
345  t=22.69, p=0.005; OMN-CARN *vs*. CARN: t=22.11, p=0.007).

346

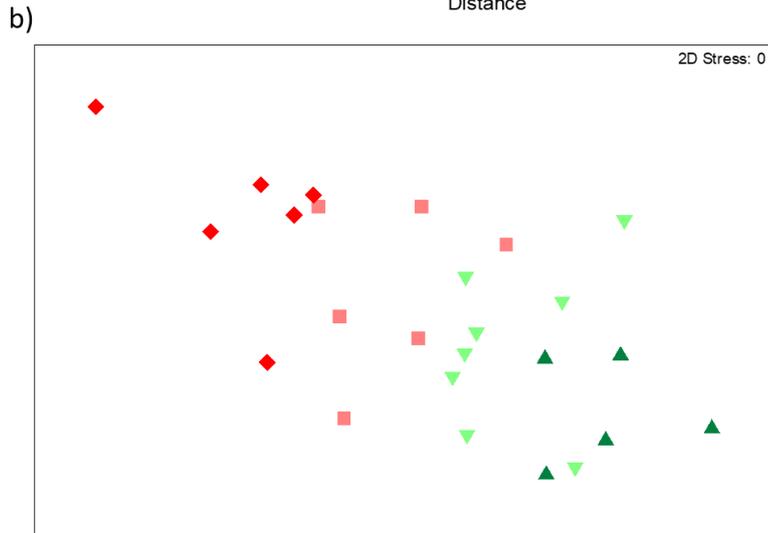
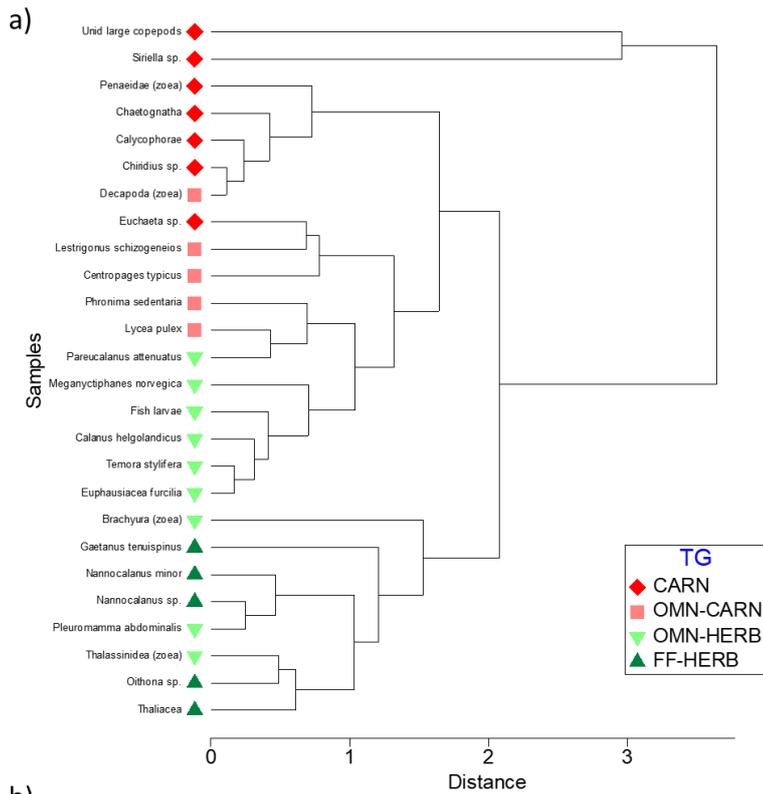



**Figure 4**. Cluster (a) and nMDS (b) analyses on the bivariate matrix of δ13C and δ15N values of dominant zooplankton taxa averaged for the whole sampling area. Colours indicate trophic groups: FF-HERB = filter feeders-herbivores (dark green), OMN-HERB = omnivores with preference towards herbivory (light green), OMN-CARN = omnivores with preference towards carnivory (orange), CARN = carnivores (red).

351 The estimates of Trophic Levels (TLs), considering the average $\delta^{15}N$ value of FF-HERB for each sub-area as baseline (from

352 **Table 2**), and specifically $\delta^{15}N =3.6$ for NA, 3.4 for CA and 3.5 for SA, allowed to assign zooplanktonic taxa to 3 TLs from

353 strictly herbivores located at TL 2 to carnivores at TL 4 (**Table 3**).

354 Overall, the $\delta^{15}N$ of the mesozooplanktonic community was greater in the NA, especially for inshore communities (**Figure 5**).

355 Conversely, the median $\delta^{13}C$ value was similar among the different sub-areas, however the larger variability was observed in

356 the inshore communities of the NA sub-area (**Figure 5**).

357

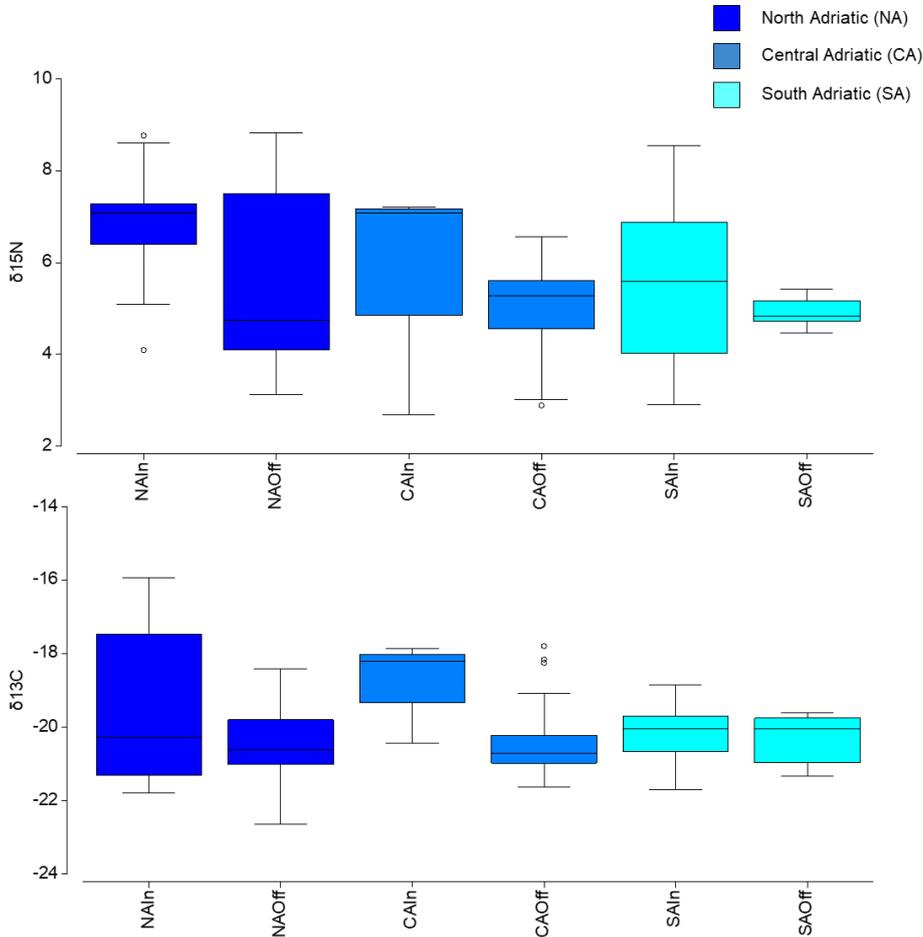



**Figure 5. Box plot of mean $\delta^{15}N$ and $\delta^{13}C$ values of zooplanktonic taxa for each sub-area at inshore *vs.* offshore locations.** Boxes are
interquartile ranges, black lines that divide the box into two parts represent the medians and the upper and lower whiskers represent scores
outside the middle 50%.

361 Two-way PERMANOVA on the multivariate matrix of $\delta^{13}C$ and $\delta^{15}N$ and one-way PERMANOVA on $\delta^{15}N$ values, showed a

362 significant separation according to sub-area and inshore vs. offshore factors, but not for the interaction (**Table S7a**). However,

363 the pairwise comparisons on sub-area factors did not show significant differences between contiguous sub-areas (but only

364 between NA and SA), while the pairwise test run on the interaction factor for pairs of level of factor "inshore *vs.* offshore"

365 provided evidence for significant variations in the overall isotopic composition ($\delta^{13}C$-$\delta^{15}N$) and in the $\delta^{15}N$ values between

366 inshore and offshore communities only for taxa from CA (**Table S7b**). One-way PERMANOVA tests run on $\delta^{13}$C values

367 showed significant variation for factor inshore *vs*. offshore and for the interaction term (**Table S7a**). $\delta^{13}$C values significantly

368 varied between CA and SA taxa and between inshore and offshore communities at CA (**Table S7b**).

369 Finally, the SIBER method for calculating ellipse-based metrics of niche width provided evidence of larger niche width for

370 the zooplanktonic community from NA than CA and SA (**Table 3** and **Figure 6**). Estimated overlap by Bayesian inference

371 evidenced low overlap among standard ellipse areas from contiguous sub-areas, being 2.71 between NA and CA and 2.07

372 between CA and SA. The greater d15N_range was observed for NA and SA communities, while the higher d13C_range

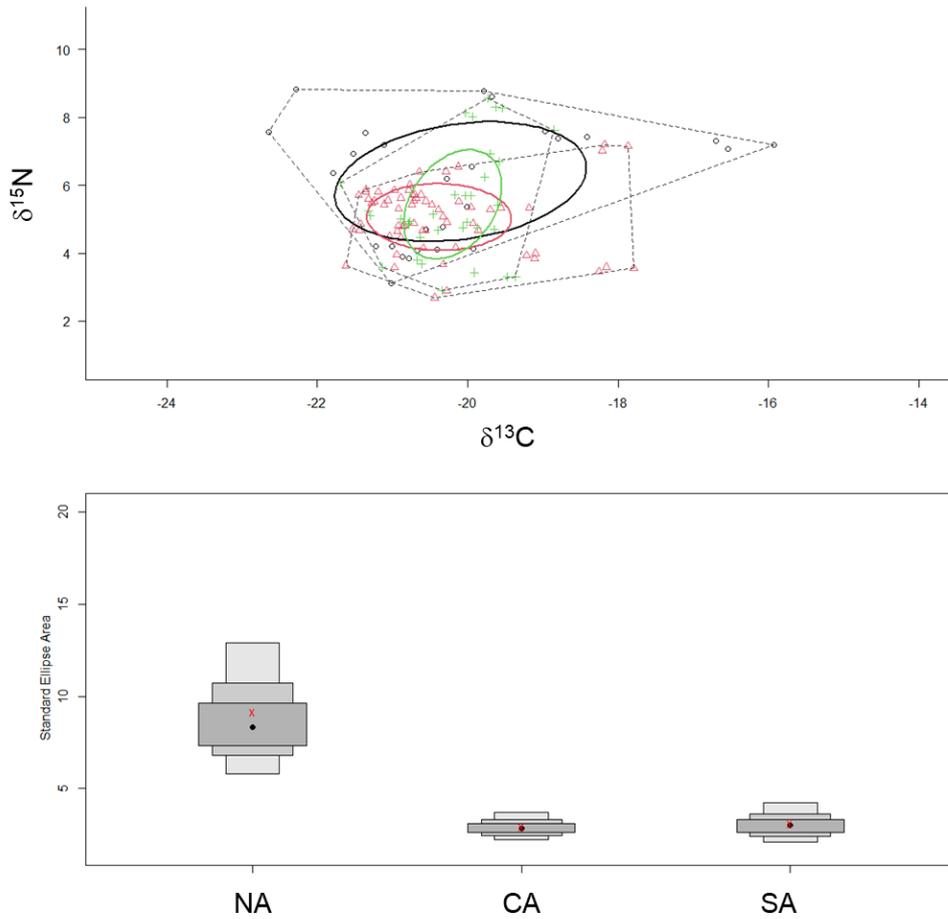
373 occurred in NA communities, where also CD value was the greatest (**Table 3**).

374

375 **Table 3.** Estimates of Convex hulls (TA), Standard Ellipse Areas (SEA and SEAc, as corrected for low sample size), and Layman metrics
376 d15N_range, d13C_range and Mean Distance to Centroid (CD), calculated for zooplanktonic communities from the three sub-areas.
377 NA=Northern Adriatic, CA= Central Adriatic, SA= Southern Adriatic. Sample size is also provided.

378

|  | NA | CA | SA |
|---|---|---|---|
| TA | 20.81 | 13.11 | 10.36 |
| SEA | 8.80 | 2.90 | 3.03 |
| SEAc | 9.15 | 2.95 | 3.12 |
| d15N_range | 5.72 | 4.51 | 5.65 |
| d13C_range | 6.72 | 3.83 | 3 |
| CD | 2.16 | 1.19 | 1.42 |
| size | 27 | 65 | 36 |

379

381 **Figure 6.** Top: Standard Ellipse Areas for the three zooplanktonic communities analysed; the black circle and symbols indicate the NA
382 community, the red ones the CA and the green ones the SA. Bottom: Credible intervals for the estimated SEAc of the three communities,
383 NA=Northern Adriatic, CA= Central Adriatic, SA= Southern Adriatic.

## 4. Discussion

386 These are the first results on mesozooplankton food web structure conducted at basin scale for the Adriatic Sea. Considering

387 that the Adriatic Sea is one of the largest areas of occurrence of demersal and small pelagic shared stocks in the Mediterranean

388 (FAO, 2020), this study may represent an important piece to reconstruct the whole pelagic food web and spatial changes across

389 the basin. Still, considering the increasing fishing pressure in the basin together with evidence of primary production (climate-

390 change related) decrease after the 1980s (Solidoro et al., 2009; Mozetic et al., 2010), this study may represent a valid baseline

391 for future comparison on the synergic and cumulative effect of climate change and overfishing in one of the most impacted

392 regions within the Mediterranean Sea (Coll et al., 2012; Micheli et al., 2013).

**4.1 Spatial variations in zooplankton biomass, abundance and community structure**

394 Overall, 113 taxa and 57 species have been identified during June-July 2019 in the Adriatic basin (**Table S1**). These values

395 were slightly lower than those observed for the Central Adriatic at 0-50 m depths where 150 taxa were counted (Hure et al.,

396 2018). Such differences maybe only apparent and attributable to the storage method we used, as samples were kept frozen for

397 subsequent stable isotope analyses, determining a damage in many organisms, which were impossible to identify to species or

398 even genus level (Fanelli et al., 2011). Although this method may represent a considerable bias in species identification and

399 biomass estimation, it allows to have indication on both community and food web structure (Fanelli *et al*., 2011, 2013; Rumolo

400 *et al*., 2018; Parapato *et al*., 2019). In terms of species abundance, the most representative species were *Acartia clausi, Oithona*

401 *similis* and *Centropages typicus* among copepods, and the cladocerans *Podon intermedius*, *P. polyphemoides*, *Penilia*

402 *avirostris*, *Evadne tergestina* and *E. spinifera*, in agreement with previous studies on the mezooplanktonic communities of the

403 Adriatic basin (Fonda-Umani *et al*., 2005; Bernardi Aubry *et al*., 2012).

404 Zooplankton abundances were higher, though very variable within sites, in the Northern Adriatic Sea and slowly decreased

405 moving towards the Southern Adriatic, while biomass showed an increasing coastal-offshore trend, except for inshore southern

406 stations, characterise by a large within samples' variability. The abundance trend here found was also observed by Fonda

407 Umani (1996) and can be explained by the influence of Po River, which can determine a high nutrient input in the Northern

408 Adriatic favouring primary production and therefore zooplankton growth. Notwithstanding the general primary production

409 reduction observed in the last years (Mozetič et al., 2010) in the North Adriatic Sea, the area is still characterised by higher

410 phytoplankton biomass with respect to the central and the southern basin, because of the nutrients input from the Po River.

411 Chlorophyll-a concentration values from satellite data (**Figure S1**, https://giovanni.gsfc.nasa.gov/giovanni) analysed from four

412 months before the sampling period to survey simultaneous period (July 2019), revealed indeed a peak in primary production

413 in May 2019, two months before the sampling period, in the area in front of the Po River delta, fuelling in turn zooplankton

414 production (Bernardi Aubry *et al*., 2012).

415 Although, in the north-western Adriatic, offshore waters are less productive than inshore coastal waters and productivity of

416 the inshore zone decreases southward away from the Po Rivers' nutrient influx (Vollenweider *et al*., 1998), here we did not

417 find significant differences in terms of abundance and biomass between inshore and offshore communities or for the interaction

418 factors. Such differences were instead observed when we compared zooplanktonic communities' composition. Indeed,

419 multivariate analyses evidenced a clear separation of samples as function of sub-area and inshore *vs*. offshore locations, and

420 especially between the mesozooplanktonic community of the Northern Adriatic from the other two. This was not surprising as

421 the northern Adriatic is characterised by shallower and colder waters than the rest of the basin and under the influence of

422 riverine input, thus hosting a typical neritic community with coastal and estuarine elements. This area was dominated also by

423 *Acartia clausi*, *Oithona similis*, cladocerans (mostly *Evadne spinifera*), copepodites (here comprised within the "Copepoda
424 unid." group), gastropod larvae with some differences with respect to previous studies (Bernardi Aubry *et al*., 2012), in terms
425 of temporal shift of species maximum abundance. This could be related to the peak in primary production occurring in May
426 2019, quite delayed with respect to the usual pattern of the area (Kamburska and Fonda-Umani, 2009) (see **Figure S1**).
427 Conversely, the southern Adriatic basin, except for the Gargano promontory, being characterised by a narrow continental shelf
428 and a steep slope, reaching high depths close to the coasts, was dominated by typical offshore species such as tunicates,
429 chaetognaths, siphonophores and *Euchaeta* spp. These results were supported by Fonda Umani (1996), that identified a clear
430 distinction in zooplanktonic communities collected in offshore location of Northern and Central-Southern Adriatic: the
431 Northern Adriatic was characterized by neritic communities, with moderate biomass, while the Central and the Southern
432 Adriatic Sea were characterized by an "oceanic" community, with a higher abundance of carnivorous zooplankton, such as
433 *Euchaeta* sp., a more oceanic carnivorous genus (Razouls *et al*., 2021), and Chaetognatha, a Phylum of carnivorous animals
434 abundant in open waters (Terazaki, 2000). Consistently, diversity was the greatest in the southern basin, with 80 taxa (out of
435 113) identified, likely due to the occurrence of both neritic and oceanic species in this area and comparable to other studies
436 (Miloslavic *et al*., 2012) which included also deep stations.

437 **4.2. Environmental drivers of zooplankton communities' variability**

438 Separation among samples according to sub-areas and inshore and offshore locations were consistent with the main drivers
439 resulted by the distance-based multivariate model*, i.e.*, fluorescence and DO concentration, with fluorescence itself explaining
440 33% of the variance. Fluorescence was strictly linked to freshwater inputs from the Po River and was likely responsible of the
441 main separation between the Northern Adriatic, more coastal-estuarine zooplanktonic communities, from the central and
442 southern Adriatic, more oceanic zooplanktonic communities. Fluorescence was also found to be the main driver of zooplankton
443 community in the North Aegean Sea (Isari *et al*., 2006), another important area for small pelagics fishery. Several studies
444 indicated that oxygen concentration could be a limiting factor for zooplankton growth and survival (Olson, 1987; Moon et al.,
445 2006), with inhibition of egg hatching in some copepod species (Roman *et al.*, 1993). DO was found to be also the driving
446 factor of zooplanktonic communities in the strait of Sicily (Rumolo *et al*., 2016)

447 **4.3.    Food web structure of zooplankton communities**

448 The trophic groups highlighted by cluster analysis fully agreed with putative trophic groups established *a priori* based on
449 literature information and previous classification on copepod functional traits (Hebert *et al*., 2016, Benedetti *et al*. 2016,
450 Protopapa *et al*., 2019, Fanelli *et al*., 2011 and references cited therein, Rumolo *et al*., 2018, Conese *et al*., 2019). Conversely
451 to similar works carried out on deep-sea zooplankton (Fanelli *et al*., 2009, 2011, 2013, Koppelmann *et al.,* 2009), our analysis
452 evidenced a trophic gradient from strictly herbivore species towards carnivory, with a general pattern of omnivory including
453 taxa that may act both as primary consumers eating phytoplankton or detritus particles or shifting to small prey, *i.e.*
454 microzooplankton.

455 Moving from herbivores-filter feeders towards carnivores, a first group of omnivores, with phytoplankton as an important

456 component of their diet occurred. This group contains both small-bodied calanoids that are numerically very important in the

457 Mediterranean epipelagic (*Temora stylifera*; Mazzocchi et al., 2014), and also larger calanoids, some of which are strong

458 vertical migrants, such as *Calanus helgolandicus*, or *Pleuromamma* spp. (Andersen et al., 2001, 2004). These exhibit mixed

459 feeding strategies, depending on the available food items. This is also the case of *Meganichtyphanes norvegica* which can vary

460 its diet regionally and with growth, showing a preference for phytoplankton in certain areas, seasons or when juveniles

461 (Schmidt, 2010; Fanelli et al., 2011), or preying exclusively on calanoids when adults or depending on energy requirements

462 (McClatchie, 1985). Concerning *C. helgolandicus,* this has described as an herbivore species (Paffenhoffer, 1976), but some

463 authors described density-dependent mortality through cannibalism in *Calanus* spp., as a form of population self-limitation

464 (Ohman and Hirche, 2001), thus pointed out to an omnivorous feeding behaviour.

465 Upscaling the pelagic food web, we found omnivore taxa that mostly prefer animal prey but that can shift to phytodetritus

466 when prey was scarce or competition was high (Fanelli et al., 2011), such as *Centropages typicus*. *C. typicus* is an omnivorous

467 copepod that feeds on a wide spectrum of prey, from small algae (3–4 μm equivalent spherical diameter) to yolk-sac fish larvae

468 (3.2–3.6 mm length). It uses both suspensivorous and ambush feeding strategies, depending on the characteristics of the prey

469 (Calbet et al., 2007). Omnivorous copepods can display increased predatory behaviour in the absence of other food (Daan,

470 1988), and may actively target eggs even when phytoplankton is not limiting (Bonnet et al. 2004). Hyperiids (*Lycaea pulex*

471 and *Lestrigonus schizogeneios*) also cluster with this group. Hyperiids generally use gelatinous substrate for reproduction and

472 feeding, some of them living in symbiosis (Gasca and Haddock, 2004) other being parasite such as the genus *Hyperia* (now

473 *Lestrigonus*). Finally, strictly carnivore species such as *Euchaeta* or chaetognats clustered together with some siphonophores

474 (Calycophorae). These species are known to prey on smaller copepods, doliolids (Takahashi et al., 2013), larvaceans (Ohtsuka

475 and Onbé, 1989) and fish larvae (Yen, 1987).

476 The average enrichment between the different plankton taxa was greater than the mean value of 2.56 expected between adjacent

477 trophic levels (e.g., Vanderderklift and Ponsard, 2003; Fanelli et al., 2011) pointing to the organization of mesozooplanktonic

478 taxa in three trophic levels, from herbivore taxa (*Nannocalanus* spp., *Gaetanus tenuispinus*, thaliaceans) positioned at the

479 trophic level 2, to the highest-level species represented by large copepods and the mysis *Siriella* sp., located at the trophic level

480 4. Such results confirmed other findings (Fanelli et al., 2009, 2011) about the complexity of pelagic food webs and of their

481 lower trophic levels, calling attention on the appropriate compartmentation of zooplankton in ecosystem modelling with the

482 final scope of small pelagic stock management (D'Alelio et al., 2016). Moreover, predation on protozoa may have been

483 overlooked by traditional stable isotope measurements, as phagotrophic protists do not necessarily follow the systematic [15]N

484 trophic enrichment that is well-established for metazoan consumers (Gutiérrez-Rodriguez et al., 2014). Thus, the uncertainties

485 associated with missing one or more trophic levels using stable isotopes or other techniques significantly challenge our

486 understanding of pelagic food-web structure.

487 Finally, based on our results, the isotopic composition of some species/taxa differed from literature, as for the hyperiid

488 *Phronima atlantica*. This species is reported as a carnivore, feeding on salp tissue (Madin and Harbison, 1977). However,

489  Elder and Seibel (2015) also reported feeding on host mucus, which could lower their trophic position, being more similar to

490  the basal source, *i.e.* the particulate organic matter or POM (Fanelli et al., 2011). Zoeae of Thalassinidea and Brachyura were

491  also placed in this group, close to thaliaceans, that are herbivorous filter feeders (Madin, 1974).

492

493  **4.4. Spatial variability in the isotopic composition of mesozooplankton from the Adriatic basin**

494  Overall, stable isotope values of zooplankton differed significantly for both sub-areas and inshore *vs.* offshore factors

495  considered, with $\delta^{15}N$ values decreasing southward, and $\delta^{13}C$ showing more constant patterns across the basin, but with large

496  variability at NA. The presence of differences in isotopic signature of zooplankton between inshore and offshore locations has

497  already been reported by other authors (Bode *et al.*, 2003; Chouvelon *et al.*, 2014; Espinosa-Leal *et al.*, 2020) and it could be

498  linked to the different contribution of terrestrial *vs.* marine sources of nitrogen and carbon moving from inshore to offshore

499  waters, and/or to different trophic dynamics between costal and oceanic food webs. Here $\delta^{13}C$ values were highly variable at

500  NA (spanning from -15.9‰ to -22.6‰) in accordance with the wide array of food sources (*i.e.*, marine and continental)

501  available in the area due to the riverine inputs. Accordingly, the niche width of zooplanktonic community in the area is the

502  greatest and SEAc decreased in CA and SA, where zooplanktonic community were likely sustained mostly by marine sources

503  (Coll et al., 2007). Standard ellipses were mainly stretched along the x-axis ($\delta^{13}C$) for NA and CA showing a progressive

504  decrease of the continental influence from the Northern to the Central Adriatic basin. SEAc of SA was conversely mostly

505  extended along the y-axis ($\delta^{15}N$), likely because of the occurrence of a well-structured community with all TLs represent. The

506  low $\delta^{15}N$ range (and the general high $\delta^{15}N$ values) observed for NA community suggest a shift to omnivory in zooplanktonic

507  communities in this area to avoid competition (Doi *et al.,* 2010) in high-density condition, as that generated after the

508  phytoplankton bloom (Bernardi Aubry *et al.*, 2012) here observed in June.

509  **5.      Conclusions**

510  This study represents the first application of the stable isotope approach to the analysis of the mesozooplanktonic food web at

511  Adriatic basin scale including both coastal and offshore communities. The results unveiled the presence of significant

512  differences in zooplankton abundance, biomass, and community composition at mid-spatial level, with the main differences

513  observed between the Northern Adriatic and the rest of the basin, due to the peculiar oceanographic conditions (i.e., cold

514  waters) and the strong influence of the Po River. Such differences were also particularly evident in terms of isotopic

515  composition, where a further separation between offshore and inshore communities were evident for the progressive increase

516  of marine contribution to food sources for zooplankton in offshore communities. Such findings may represent a valuable

517  baseline for food web studies encompassing lower to high trophic level species and against changes in oceanographic

518  conditions under a climate change scenario, considering the rapid response of zooplankton communities to global warming.

519 **Author contribution**

520 IL, AdF and SM designed the survey and carried it out, with SaM participating in sample collection. EF conceived the
521 experimental design. EF and SaM analysed the samples. EF analysed the data and prepared the manuscript with contributions
522 from all co-authors.

523 **Competing interests**

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

525 **Data availability**

526 Data can be requested to the corresponding author upon reasonable request. Isotopic data are available at PANGEA repository.

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529 GSA 17 and GSA 18.

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