Allelopathic interactions between phytobenthos and meiofaunal community in an Adriatic benthic ecosystem: Understanding the role of aldehydes and macroalgal structural complexity

Allelopathic interactions between phytobenthos and meiofaunal community in an Adriatic benthic ecosystem: understanding the role of aldehydes and macroalgal structural complexity

Denise Lenzo[1], Laura Pezzolesi[1*], Chiara Samorì[2], Fabio Rindi[3], Andrea Pasteris[1], Rossella Pistocchi[1], Marina Antonia Colangelo[1]

[1] Dipartimento di Scienze Biologiche, Geologiche e Ambientali - Università di Bologna, via Sant'Alberto 163, 48123 Ravenna (Italy)

[2] Dipartimento di Chimica "Giacomo Ciamician" - Università di Bologna, via Selmi 2, 40126 Bologna (Italy)

[3] Dipartimento di Scienze della Vita e dell'Ambiente - Università Politecnica delle Marche, via Brecce Bianche, 60131 Ancona (Italy)

*Corresponding Author

Dr. Laura Pezzolesi

Tel. +39 (0)544 937373; fax: +39 (0)544 937411

laura.pezzolesi@unibo.it

Abstract

Macroalgae produce several allelopathic substances, including polyunsaturated aldehydes (PUAs), which may inhibit photosynthesis and growth rates of other algal species, and grazing. Additionally, macroalgal structural complexity is an important factor in determining abundance patterns and size structure of epiphytic organisms.

In this study the PUAs production of two Mediterranean macroalgae, *Dictyopteris polypodioides,* (DP, Phaeophyceae, Dictyotales) and *Cystoseira compressa* (CC, Phaeophyceae, Fucales), was characterized to clarify the relationships between the meiobenthic and microphytobenthic communities. Results showed a higher PUAs production and a diverse qualitative profile for DP, which reported long-chain compounds (i.e. C14-C16) as main aldehydes, than CC, with the short-chain C6:2 as main compound, as well as variability among sampling times. A clear separation of the meiofauna and microphytobenthos assemblages was found for the macroalgae, but with different temporal trends. Dissimilarities were due to five microalgal orders, namely Naviculales, Lyrellales, Gonyaulacales (i.e. *Ostreopsis*), Bacillariales, and Licmophorales, and to the meiofaunal groups nematodes, copepods, and copepod nauplii, which were more abundant on DP than on CC. Results indicate that macroalgal complexity is a major determinant of the meiofaunal community structure (accounting to 26% of the variation), rather than PUAs production itself (17%). PUAs effects seem species-specific, thus affecting some grazers instead of the entire community. Conversely, microphytobenthos affected the meiofauna assemblages, particularly harpacticoids, confirming the role of these organisms as primary food source of all marine food chain producers. Since PUAs are produced also by several epiphytic diatoms, the understanding of their effects on the community structure and on the relationships among taxa in the field is complicated and requires further in-depth investigations in simplified systems (i.e. microcosms).

Keywords

Brown macroalgae; PUAs; microalgae; meiofauna; harpacticoid copepods; chemical ecology.

1. Introduction

Chemical compounds produced by aquatic organisms and microorganisms have received increasing attention for the important role that may have in modulating the interactions in marine ecosystems (Paffenhöfer et al., 2005; Ianora et al., 2012). Many organisms produce allelochemicals, secondary metabolites that affect survival and growth of other species and may give a competitive advantage to their producers (Legrand et al., 2003; Fistarol et al., 2004; Tillmann, 2004; Allen et al., 2016).

Several studies regarding chemical interactions between various marine organisms, including copepods, urchins, sea stars, algae, mollusks, and polychaetes, were focused on direct responses of target species to allelochemicals and have been performed mainly under laboratory conditions (e.g. Ianora et al., 2004; Adolph et al., 2004; Caldwell et al., 2004; Vardi et al., 2006 Ribalet et al., 2007; Taylor et al., 2007).

Algae produce a variety of these allelopathic substances, such as phenolic compounds, alkaloids, peptides, oxoacids, and polyketides, among which there are toxins synthesized by several species (Snyder et al., 2003; Agostini-Costa et al., 2012; Pistocchi et al., 2012), polyunsaturated fatty acid (PUFAs) (Grima et al., 1995; Tonon et al., 2002; Patil et al., 2007) and their derivatives, such as polyunsaturated aldehydes (PUAs) (Wichard et al., 2005; Ianora et al., 2012; Pezzolesi et al., 2017).

Consequences of allelopathic interactions between different algal species can be of various nature and magnitude, including loss of motility, cell deformation (Tang and Gobler, 2010; Pichierri et al., 2017), pigmentation loss, cytoplasm aggregation, formation of vesicles and cellular lysis (Fistarol et al., 2004). However, the majority of allelochemicals have milder effects, for example inhibition of photosynthesis, reduction of growth rate, and inhibition of grazing (Legrand et al., 2003).

The effect of PUAs have been mostly investigated for planktonic microalgae and only a few studies have evidenced PUAs production by benthic species, such as diatoms (Jüttner et al., 2010; Scholz and Liebezeit, 2012; Pezzolesi et al., 2017), and macroalgae (Kajiwara et al., 1996; Akakabe et al., 2003; Pezzolesi et al., 2021). Macroalgae are among the most important components of marine coastal

ecosystems because they are highly productive (Pinckney and Zingmark, 1993), have a high taxonomic diversity, and may act as foundation species providing habitat for different organisms (Cacabelos et al., 2010). The shape and structural complexity of macroalgae are important factors in determining the abundance patterns and size structure of the epiphytic organisms (McAbendroth et al., 2005). The more structurally complex macroalgal species show abundant and various populations of invertebrates because they provide greater surface area for the colonization of epifaunal assemblages and epiphytic microalgae (Chemello and Milazzo, 2002). Among epifaunal assemblages, macrofauna is the most investigated, while meiofaunal communities are overlooked. Meiofauna represent the most abundant and taxonomically diversified metazoans on Earth (Giere, 2009) and on hard substrates can overcome macrofauna in terms of abundance (Gibbons and Griffiths, 1986). Meiofauna plays a dominant role in the exchange of organic matter (Sandulli et al., 2014; Semprucci et al., 2016) as part of the "small food web" (size class 45-1000 µm). Moreover, it supports most of the higher trophic levels (Giere, 2009), being an important food resource for macrofauna, small fish, juveniles and other epibenthic predators (Chardy and Dauvin, 1992). In phytal environments, harpacticoid copepods are the dominant meiofauna group (Hicks, 1977; Coull and Wells, 1983; Hall and Bell, 1993) and show high diversity (Sarmento and Santos, 2012). They feed mainly on diatoms and, for this reason, they have a high content of fatty acids and play a key nutritional role for fish, carnivorous crustaceans (prawns and their larvae), and polychaetes (Coull, 1999; Giere, 2009).

Indeed, few studies have focused on the benthic environment and included the potential role of macroalgae both in terms of production of allelochemical compounds and regulation of interactions between various organisms (Kajiwara et al., 1996; Akakabe et al., 2003), while only one laboratory study analyzed the effects of PUAs on a species of harpacticoid (i.e. *Tisbe holothuriae*) (Taylor et al., 2007). PUAs strongly impair the reproduction of various potential grazers in *in vitro* studies (Poulet et al., 1994; Ianora et al., 2004b), while in the field the relationship between aldehyde production and reproductive failure of other higher-level organisms (i.e. copepods) remains unclear (Leflaive and

Ten-Hage, 2009). Some studies performed to investigate the allelopathic activity of fresh thalli or extracts of *Ulva* spp. reported inhibitory effects on the growth of several microalgae, including harmful species (Tang and Gobler, 2011). The production of aldehydes and their potential consequences on biotic interactions may thus be explored, especially in macroalgae. This would help to determine whether these compounds, which are present across phylogenetic and environmental barriers, may play a role in the ecology of their producers (Leflaive and Ten-Hage, 2009), perhaps affecting the development of epiphytic organisms, such as meiofauna or microalgae.

The main aims of this study were to i) analyze the qualitative and quantitative production of aldehydes by two different macroalgae over a period of some months (Mediterranean spring and summer); ii) understand the relationship between meiobenthic communities, with particular interest to harpacticoid copepods, and the microphytobenthos present on the two macroalgal species, considering the structural complexity of macroalgae and the potential role of aldehydes in regulating their interaction.

Specifically, this study was performed in a benthic environment of the North-western Adriatic Sea (Piscinetta del Passetto, Ancona, Italy), characterized by the presence of a rich phytobenthic assemblage and by annual blooms of a toxic dinoflagellate, *Ostreopsis* cf. *ovata* Fukuyo, 1981. Two macroalgal species, *Cystoseira compressa* (Esper) Gerloff & Nizamuddin and *Dictyopteris polypodioides* A.P. De Candolle J.V. Lamouroux, commonly present in this site (Rindi et al., 2020) and representative of the Mediterranean phytobenthic community were selected, based on their different complexity and PUAs composition (Pezzolesi et al., 2021). To our knowledge, this is the first study where macroalgal allelochemistry has been investigated to understand both the microphytobenthos and meiofauna community structure and their interactions, in relation to the algal complexity.

2 Material and methods

128 *2.1 Sampling area and sampling procedure*

129 The study was performed in a semi-enclosed and shallow (mean depth 1.5 m) inlet called Piscinetta
130 del Passetto (Conero Riviera, Italy, northern Adriatic Sea: 43°37'09" N, 13°31'54" E), described and
131 showed in Pezzolesi et al. (2021). Sampling was carried out at six different times from May to
132 September 2018 (see table S2), with monthly frequency except for September (when sampling was
133 performed twice). In the Mediterranean area, this period corresponds to late spring and summer.
134 Surface temperature and salinity were measured with a multiparameter water probe HQ30d (Hach-
135 Lange GmbH) and a refractometer Atago S-10, respectively.

136 Apical parts (i.e. tips) of the thalli (first 5-8 cm) of two macroalgae, i.e., *Cystoseira compressa*
137 (Phaeophyceae, Fucales) and *Dictyopteris polypodioides* (Phaeophyceae, Dictyotales), were sampled
138 at a depth of approximately 0.5 m. At the study site, during the time of the year in which sampling
139 was carried out, these species are present and mostly occur in a well-developed habit. For each
140 macroalgal species, six replicates were collected by snorkeling at each sampling time using 50 mL
141 polypropylene tubes (VWR International), avoiding the dispersion of the associated epiphytic
142 organisms.

143 Water samples for assessment of dissolved aldehydes and nutrient analysis were collected in 2 L
144 polyethylene bottles (VWR International) in the proximity of the sampled macroalgae. Water was
145 subsequently filtered using GF/F Whatman filters (0.7 µm porosity, 47 mm) and stored at -22 °C until
146 analysis. Macroalgae were treated to remove all associated benthic organisms as described in
147 Pezzolesi et al. (2021) using filtered seawater. Briefly, each tube containing the thallus and their
148 storage water was vigorously shaken to separate the macroalga from the epiphytic microalgae and the
149 meiofauna. Then the tube was rinsed with filtered seawater and vigorously washed several times until
150 epiphytic organisms were completely removed. The seaweed thalli were dried with absorbent paper,
151 then weighed to determine fresh weight (g fw) and photographed; finally, they were stored at -80 °C
152 in new tubes. The total volume of washing seawater of each sample (approximately 150 mL) was
153 measured and then divided into two aliquots, one for the microphytobenthos and the other for the

154 meiofauna analyses. Aliquots for microphytobenthos (about 75 mL) were fixed with Lugol and stored
155 in 250 ml dark glass bottles. Aliquots for meiofauna analysis were sieved through a 1000-µm mesh
156 and a 45-µm mesh; the meiobenthic organisms retained on the finer mesh sieve were fixed with 70%
157 alcohol and stored in a 50 ml falcon until subsequent analyses. Each apical part of macroalga was
158 placed on a white surface with a reference scale and then photographed using a digital camera Canon
159 EOS 750D. To take into account the different morphology and complexity of the two algal species,
160 pictures were processed using the program ImageJ v1.53u. For each image the scale was fixed and
161 transformed to binary format with a pixel width of 1 cm. Then the area, the perimeter, and the fractal
162 complexity (D) were measured based on the image and using a method analogous to the grid method
163 (boundary dimension) proposed by Sugihara and May (1990). In this method, the fractal dimension
164 is the slope of the linear fit of log N(s) versus log (1/s); where s represents the scale of analysis and
165 N(s) is the number of objects observed at that scale.
166
167 *2.2 Aldehydes*
168 *2.2.1 Aldehydes (PUAs) produced by macroalgae*
169 The extraction and quantification of PUAs produced by the different macroalgae was carried out as
170 described in Pezzolesi et al. (2021) by gas chromatography-mass spectrometry (GC-MS).
171 Specifically, a portion of the apical part of the thallus (about 0.2-0.8 g f. wt.) was shredded with
172 mortar and pestle, in liquid nitrogen. The powder thus obtained was transferred into 10 mL tubes.
173 Derivatization of the polyunsaturated aldehydes was performed with O-(2,3,4,5,6-
174 pentafluorobenzyl)hydroxylamine hydrochloride solution (PFBHA HCl) and quantification was
175 based on the internal standard (i.e. benzaldehyde). All reagents were purchased from Sigma-Aldrich
176 (Milan, Italy) and used without any further purification.
177
178 *2.2.2 Dissolved aldehydes (dPUAs) in seawater*

179 Dissolved aldehyde concentration was determined following the protocol described by Vidoudez and
180 Pohnert (2008) with slight modifications, using PFBHA HCl in Tris–HCl 100 mM pH 7.2 as
181 derivatizing reagent and benzaldehyde as internal standard, as described in Pezzolesi et al. (2021).
182
183 *2.3 Nutrients*
184 Nitrate and phosphate analyses were performed on filtered seawater aliquots (GF/F Whatman
185 filters) and analyzed spectrophotometrically according to Strickland and Parsons (1972).
186
187 *2.4 Microphytobenthic community*
188 The quali-quantitative analysis of the microphytobenthos associated with the two macroalgae was
189 performed using an inverted optical microscope (Zeiss Axiovert 100) at 320x and 200x magnification.
190 Sub-samples (3-5 ml) of epiphytic communities fixed with Lugol were settled in counting chambers
191 after homogenization, according to the Utermöhl's sedimentation method (Utermöhl, 1958; Edler and
192 Elbrächter, 2010). Counting was performed in different ways. The microphytobenthos community
193 was examined at 320x magnification on 30 random fields or 4–5 transects; then a counting at 200x
194 of the organisms present on the whole sedimentation chamber was performed to obtain a correct
195 evaluation of uncommon taxa. The microphytobenthos was identified and recognized following
196 various manuals and identification keys (e.g. Tomas, 1997; Kraberg et al., 2010). The identification
197 of individuals was based exclusively on observable morphological characters (such as shape, size,
198 number of chloroplasts); the current taxonomic status for the microalgae was confirmed following
199 AlgaeBase (Guiry and Guiry, 2021).
200
201 *2.5 Meiofauna and harpacticoid copepods*
202 Meiofaunal organisms of each sample were counted and identified at higher taxonomic levels. All
203 harpacticoids (Order Harpacticoida Sars G.O., 1903) were collected under a stereomicroscope (Nikon
204 SMZ 1500) and stored in 70% alcohol inside 1.5 mL-Eppendorf labeled for subsequent identification.

205 Harpacticoids were identified to species level using Lang (1948; 1965), and Boxshall and Halsey
206 (2004).
207
208 *2.6 Data analyses*
209 PUAs produced by the macroalgae and dissolved in the seawater were analyzed and expressed as µg
210 g^{-1} fw and µg L^{-1}, respectively. Microphytobenthos was analyzed in terms of total epiphytic cells and
211 main orders or genera; data were expressed as the number of cells per gram of fresh macroalgae
212 weight (cells g^{-1} fw). Meiofauna and harpacticoid communities were analysed according to total
213 density (N), standardized towards the fresh weight of macroalgae (ind. g^{-1} fw), the total number of
214 taxa or species (S), and Shannon diversity (H'). For each variable, the average value of the six
215 replicates ± standard error is reported. All univariate variables were analyzed by a 2-way crossed
216 ANOVA; the factors considered were the macroalgal species (fixed, two levels: *Cystoseira*
217 *compressa* (CC) and *Dictyopteris polypodioides* (DP)) and time (fixed, 6 levels). Cochran's test was
218 used to check for the homogeneity of variances and data were transformed, if necessary (Underwood,
219 1996). If even after transformation it was not possible to obtain homogeneity of the variances,
220 untransformed data were analysed and results were considered robust if significant at $p<0.01$ to
221 compensate for the increased probability of type I error (Underwood, 1996). When significant main
222 effects or interactions were detected, the Student–Newman–Keuls (SNK) test was used for pairwise
223 a posteriori comparisons.
224 The community structure of each assemblage (microphytobenthos, meiofauna and harpacticoids) was
225 analyzed by non-metric multidimensional scaling (nMDS) based on Bray-Curtis similarity of square
226 root-transformed data. Differences in community structures were assessed by permutational non-
227 parametric multivariate analysis of variance (PERMANOVA) following the same experimental
228 design adopted for ANOVA (Anderson, 2001; 2005). When significant main effects or interactions
229 were detected, the specific procedure provided within PERMANOVA was used for pairwise a

230 posteriori comparisons. The analyses were performed using unrestricted permutation of the raw data
231 and 9999 permutations.
232 Taxa that mostly contributed to the dissimilarity/similarity among/within macroalgal species and
233 times were identified using the SIMPER analysis (Clarke, 1993).
234 Relationships between macroalgae complexity, PUAs, dominant microphytobenthic taxa, and both
235 meiofauna and harpacticoid copepod assemblages were analyzed by the distance-based linear model
236 (DistLM) procedure in PERMANOVA+ (Anderson et al., 2008).
237 A total of 14 explanatory variables, grouped in three sets, were considered: macroalgae complexity
238 descriptors (perimeter/area, D, Area), PUA concentrations (C14:5, C16:4, C16:3, C6:2), and
239 microphytobenthic taxa abundances (*Navicula* spp. J.B.M. Bory de Saint-Vincent, 1822, *Lyrella* spp.
240 N.I. Karayeva, 1978, *Cocconeis* spp. C.G. Ehrenberg, 1837, *Cylindrotheca* spp. L. Rabenhorst,
241 1859/*Nitzschia* spp. A.H. Hassall, 1845, other diatoms, *Ostreopsis* cf. *ovata*, other dinoflagellates).
242 The relationships between meiobenthic taxa data and harpacticoid abundance (square root
243 transformed data) and the three groups of variables were analyzed by explicitly examining the
244 proportion of variation in the taxa data (or harpacticoid species abundance) that was explained by
245 PUAs concentrations and microphytobenthic taxa abundances over and above the amount explained
246 by macroalgae complexity descriptors.
247 The Akaike Information Criterion with correction (AICc) was used to select the model. Prior to the
248 DistLM, a draftsman plot and correlation matrices were performed to detect possible skewness of the
249 variables and/or strong correlation among pairs of variables (Anderson and Robinson, 2001).
250 Variables were not strongly correlated, so all variables were entered in the analysis. Concentrations
251 of *Lyrella* spp., and *Cocconeis* spp were square root transformed. Finally, Pearson correlation analysis
252 was used to test which macroalgal attribute (i.e. complexity descriptors such as D and macroalgal
253 surface (S), and PUAs composition) explained the variation of some representative genera of
254 microphytobenthos, of the main meiofauna taxa and of the most representative harpacticoid species.

255 Densities of microphytobenthic genera, main taxa and harpacticoid species were square root
256 transformed.
257 Significance level was set at 0.05 (5%) for all tests. ANOVA, Cochran test, SNK test, and correlations
258 were performed by R (version 3.5.3) using packages Lme4; all multivariate analyses were conducted
259 with PRIMER v7 (Clarke and Gorley, 2015) with the PERMANOVA + add-on (Anderson et al.,
260 2008).
261

262 ## *3.* Results

263 *3.1 PUAs production by macroalgae*

264 The interpretation of the chromatograms and relative mass spectra obtained by GC-MS gave the
265 quali-quantitative profile of the main aldehydes produced by *Dictyopteris polypodioides* (DP) and
266 *Cystoseira compressa* (CC).
267 The major aldehydes production was found in T1 (May) for both macroalgae, with values of 225.5 ±
268 35.6 and 17.1 ± 4.0 μg g^{-1} fw for DP and CC, respectively. Conversely, the lowest amounts were
269 measured at times T3 (July) for DP and T2 (June) for CC, with a concentration of 44.3 ± 5.3 and 2.0
270 ± 0.3 μg g^{-1} fw, respectively (Table 1). From a quantitative point of view, ANOVA results (Table S1)
271 showed a higher PUAs production in DP than in CC and variability among different sampling times
272 ($F=17.73$, $P<0.001$).
273 From a qualitative point of view, the main compounds detected were the short-chain PUA hexadienal
274 (C6:2), which was present in both algae and resulted as the main compound in CC, and some long-
275 chain PUAs, namely hexadecatrienal (C16:3), hexadecatetraenal (C16:4), and tetradecapentaenal
276 (C14:5), which were detected exclusively in DP (Fig. S1). Specifically, C14:5 was the most abundant
277 compound in DP, with average concentrations ranging from a maximum of 115.3 ± 20.8 μg g^{-1} fw at
278 time T1 to a minimum of 13.2 ± 2.4 μg g^{-1} fw at time T3, corresponding to the 50% and 32% of the
279 total aldehydes (Table 1; Fig. S1). C16:4 had the maximum average concentration at time T1 with
280 36.4 ± 8.8 μg g^{-1} fw, decresasing gradually to a minimum of 5.9 ± 1.5 μg g^{-1} (relative abundance of

281 8-29%) at time T6. C16:3 showed a similar pattern and an average maximum concentration of 30.9
282 ± 9.3 μg g^{-1} fw at T1 (relative abundance of 12%); then, its amount decreased to a minimum of 8.7 ±
283 3.1 μg g^{-1} fw at time T4, and it was not detected at all at T6. In DP the most variable compound was
284 C6:2, with a maximum average concentration of 16.1 ± 2.2 μg g^{-1} fw at time T1; this aldehyde was
285 not detected at times T2 and T5. C6:2 was the main aldehyde in CC, accounting for 64-91% of the
286 total amount, and its average concentration had the maximum and minimum values of 15.4 ± 3.5 and
287 1.0 ± 0.1 μg g^{-1} fw at time T1 and T2, respectively. This short-chain aldehyde resulted not
288 significantly different between the two algae at the different sampling times.
289
290 *3.2 PUAs (dPUAs) and nutrients in seawater*
291 The total average concentrations of dPUAs detected in the seawater in the proximity of the two
292 macroalgae (Fig. S2) highlighted high values (ranging from 99.0 to 287.8 μg L^{-1}, corresponding to
293 0.9 and 2.7 μM) and temporal changes, with concentrations significantly lower at times T2 and T3,
294 and higher (about 200 μg L^{-1}) in the subsequent times (T4-T6) (Table S1). A maximum value was
295 recorded at time T4 for DP and at time T6 for CC (287.8 ± 34.2 and 261.7 ± 33.6 μg L^{-1}, respectively).
296 ANOVA carried out on dPUAs concentrations in seawater showed highly significant differences for
297 the factor time ($F=11.8$, $P<0.001$), while no significant differences were found between the two algal
298 species (Table S1). Seawater temperatures during the sampling times ranged between 23 and 26°C,
299 with salinity values of about 34-39 which were higher at T4-T5 (August-September). Concerning
300 nutrients, the concentration of nitrates was generally low, with a peak of 3.26 μM in T2 (June), while
301 phosphates were about 0.1-0.2 μM, with a peak at T5 (0.24 μM) (Table S2).
302
303 *3.3 Macroalgal complexity*
304 Surface area (S) and fractal dimension (D) were calculated to evaluate the different morphological
305 complexity of the apical parts of the two macroalgae that were analysed in the present work (Fig. S3).
306 Overall S and D were higher in DP than in CC. In CC the surface remained relatively constant along

307 the sampling period (20-25 cm^2 g^{-1}), while in DP S was higher at T5 and T6 than in the previous times
308 (max value 127 cm^2 g^{-1}). As for D, more marked differences between the two algae were found at
309 times T5 and T6, with higher values in DP than in CC (max values 1.84 and 1.73, respectively).
310

311 *3.4 Microphytobenthic community*

312 In total 25 genera were identified, belonging to 18 orders, and mostly to diatoms and dinoflagellates.
313 Sporadically, cyanobacteria (Cyanophyceae Schaffner, 1909) and juvenile stages of green algae
314 (Chlorophyta Pascher, 1914) were also observed.

315 Total density was significantly higher in DP (2608441 ± 425527 cells g^{-1} fw) than in CC (486309 ±
316 77877 cells g^{-1} fw) (Fig. 1; Table S3). Results of ANOVA showed a significant interaction between
317 algae and times ($F=5.75$, $P<0.001$) (Table S3) due to a different temporal trend of density between
318 the two macroalgae. Generally, diatoms represented the most abundant component of the
319 microphytobenthic community, accounting for average values of 96% and 87% of the organisms in
320 DP and CC, respectively.

321 Microalgal species belonging to the diatom group were classified into 22 genera and 15 orders. Some
322 species remained unidentified, namely undetermined pennate and centric diatoms (Fig. 2). On both
323 macroalgae the most represented order was Naviculales Bessey, 1907, that in DP showed an
324 increasing trend from T1, with an average density of 198287 ± 81945 cells g^{-1} fw, to T6, with an
325 average density of 3489032 ± 1159137 cells g^{-1} fw (Table S4), which represented 58% of the
326 epiphytic diatoms (Fig. 2A). For Bacillariales Hendey, 1937, a constant amount of about 60,000 cells
327 g^{-1} fw was observed in DP from T1 to T4, and a maximum at time T5 (average density of 621096 ±
328 213575 cells g^{-1} fw) was recorded, which corresponded to 15% of the total diatoms (Fig. 2A). In
329 terms of average abundance, Lyrellales D.G. Mann and Cocconeidales E.J. Cox, 2015 represented
330 less than 10% of the diatoms (ranging from 25,8831 to 10,865 cells g^{-1} fw), while species belonging
331 to other orders (i.e. Surirellales D.G. Mann, 1990, Thalassiophysales D.G. Mann, 1990, Mastogloiales

332 D.G. Mann, Licmophorales Round, 1990, Striatellales Round, 1990, Tabellariales Round, 1990)
333 occurred with average densities below 100,000 cells g^{-1} fw.
334 On CC almost all genera identified had a peak at T5, while a constant average density was observed
335 from T1 to T4, except for Licmophorales that reached a maximum at T2 (229178 ± 91470 cells g^{-1}
336 fw), accounting for 33% of the diatoms (Fig. 2B). Specifically, Naviculales, whose concentration
337 ranged from 25 to 56% of the entire diatom community, occurred with the lowest density of 70,457
338 ± 31,328 cells g^{-1} fw at T1 and a maximum average density at T5 (27,5598 ± 89,219 cells g^{-1} fw).
339 Bacillariales were about 10% of the total diatoms and increased consistently from T1 (8,869 ± 2,935
340 cells g^{-1} fw) to T5 (79,071 ± 18,396 cells g^{-1} fw), then they decreased at T6 (13,850 ± 4,154 cells g^{-1}
341 fw). Cocconeidales had a peak at T1 (33,615 ± 16,335 cells g^{-1} fw), accounting for the 16% of the
342 total diatoms. All other orders (i.e. Surirellales, Thalassiophysales, Mastogloiales, Licmophorales,
343 Striatellales, Tabellariales) represented less than 5% of the entire diatom assemblage.
344 The average density of dinoflagellates was significantly higher on DP than on CC. This group was
345 recorded especially from T4 to T6 (Table S4) due to the presence of a bloom of the toxic dinoflagellate
346 *Ostreopsis* cf. *ovata*, whose concentration reached a maximum average density at T6 (364,167 ±
347 77,126 cells g^{-1} fw).
348 The nMDS plot carried out on the microphytobenthic communities showed a clear separation of the
349 assemblages associated with the two macroalgae (Fig. 3A), but with a different temporal trend.
350 PERMANOVA supported this pattern resulting in a significant interaction between algae and times
351 (pseudo-F= 2,2779, $P \leq 0.001$; Table S5), and the post-hoc analysis carried out between the two algae
352 at the various sampling times confirmed the different structure of the two communities at each time.
353 SIMPER analysis revealed that average similarities for DP was 36% and for CC was 40%, whereas
354 dissimilarities between the two macroalgae at each time ranged from 66% and 82% (Table S6).
355 Results showed that the average dissimilarities between the two algae at each time were largely due
356 to the higher abundances of five orders, namely Naviculales, Lyrellales, Gonyaulacales Taylor, 1980

357 (i.e. *Ostreopsis* cf. *ovata*), Bacillariales, and Licmophorales, that were always more abundant on DP
358 than on CC.
359
360 *3.5 Meiobenthic community*
361 A total of 12 taxa belonging to the meiobenthos were identified, one represented by larval stage
362 Copepoda nauplii (referred from now on as copepod nauplii) defined as the larvae of the meiobenthic
363 copepod species (mainly harpacticoids). Copepods and their nauplii were counted separately in view
364 of their different ecology (Hicks and Coull 1983). Harpacticoida (33.1%) and Nematoda (31.2%)
365 were the dominant taxa, followed by copepod nauplii (25.2%), Gastropoda Cuvier, 1795 (5.4%),
366 Isopoda Latreille, 1817 (1.6%), Polychaeta Grube, 1850 (1.6%), Amphipoda Latreille, 1816 (0.7%)
367 Halacaroidea Cunliffe, 1954 (0.5%), Ostracoda Latreille, 1802 (0.3%), Kinorhyncha Reinhard, 1885
368 (0.3%), Chironomidae Newman, 1834 (0.2%) and Bivalvia Linnaeus, 1758 (0.1%). Overall, total
369 density (N) and number of taxa (S) at high taxonomic resolution resulted higher on DP than on CC
370 (Figs 4 and S4). Total density was in mean 371 ± 46 and 90 ± 15 N g^{-1} fw in DP and CC, respectively
371 (Fig. 4), while the number of taxa was on average 5.6 ± 0.2 in DP and 4.4 ± 0.2 in CC (Fig. S4).
372 ANOVA results showed a significant interaction between the factors alga and time ($F=7.06$, $P< 0.001$;
373 $F=5.9$, $P< 0.001$) for both total density and number of taxa (Table S7), suggesting that the differences
374 between macroalgae were not consistent over time. For total density (Fig. 5), significantly higher
375 values occurred on DP than on CC at corresponding times, except for T1 (Table S7). Moreover, for
376 DP densities increased with a peak at T6 (828 ± 126 N g^{-1} fw), while for CC the highest density
377 occurred at T5 (217 ± 63 N g^{-1} fw) and then decreased at T6.
378 The number of taxa showed the highest value on DP at T2 (7 ± 1) and the lowest on CC at T6 (3 ±
379 0). It resulted significantly higher on DP than on CC at times T2, T4, and T6, while was lower at T5
380 (5 ± 1) (Fig. S4; Table S7).
381 The nMDS analysis of meiofauna communities showed a clear separation between samples belonging
382 to the two macroalgae but, for each alga, the composition of the meiofaunal assemblage changed

following a different temporal pattern (Fig. 3B). PERMANOVA supported this pattern with a significant interaction between algae and times (pseudo-F= 2,606, P≤0.001; Table S8). The assemblages on the two algae resulted significantly different at corresponding sampling times, with the exception of T5 and, within each alga community, the structure changed among times as shown by post hoc comparisons (Table S8).

SIMPER analysis revealed that average similarities for each macroalga were 42.8% for DP and 43.7% for CC. Moreover, the dissimilarities between macroalgae at each time ranged from 52% to 82%. Both similarities and dissimilarities were largely due to the variations in abundance of the three dominant taxa: nematodes, copepods, and copepod nauplii (Table S9).

In DP average densities of nematodes (Fig. 5A; Table S10) increased from T1 (57 ± 22 N g^{-1} fw) to a maximum in T6 (287 ± 46 N g^{-1} fw), as well as the average values of the density of the copepods (Fig. 5B) that showed a similar trend to that of nematodes, with a pick at T6 (285 ± 69 N g^{-1} fw). The densities of copepod nauplii (Fig. 5C), defined as the larvae of the meiobenthic Copepod species (harpacticoids), showed two peaks at times T2 (215 ± 52 N g^{-1} fw) and T6 (217 ± 34 N g^{-1} fw).

Conversely, in CC nematodes, copepods and copepod nauplii had lower mean densities at each time when compared with those found in DP (Fig. 5). Both nematodes and copepods densities were very low from time T1 (14 ± 6 and 8 ± 2 N g^{-1} fw) to T4 (10 ± 2 and 14 ± 3 N g^{-1} fw); only at T5 an increase occurred (105 ± 32 and 77 ± 27 N g^{-1} fw) and then a decrease took place at T6 (19 ± 4 and 15 ± 7 N g^{-1} fw). Instead, the mean abundance of copepod nauplii slowly increased until T6 (43 ± 15 N g^{-1} fw).

3.6 Harpacticoid community

Twelve harpacticoid species were identified from the two macroalgae; they belonged to 11 genera and 11 families. The dominant species was *Heterolaophonte minuta* Boeck, 1873 (32.5%), followed by *Amphiascus parvulus* Claus, 1866 (11.9%), *Harpacticus gracilis* Claus, 1863 (11.6%), *Paradactylopodia brevicornis* Claus, 1866 (10.8%), *Parastenhelia spinosa* Fischer, 1860 (10.2%),

409 and *Ectinosoma melaniceps* Boeck, 1865 (9.6%). The other species ranged from 6.8% (*Porcellidium*
410 *viride* Philippi, 1840) to 0.2% (*Scutelledium longicaudum longicaudum* Philippi, 1840). Results of
411 ANOVA for total density (N), and number of species (S) are shown in table S11. Generally, total
412 densities (N) were significantly higher in DP than in CC at T2, T3, T4, and T6 (Fig. 5B), while the
413 number of harpacticoid species (S) was higher on DP compared to CC at T2 (7 *vs* 3) and T6 (7 *vs* 3)
414 (Table S12).
415 The nMDS plot of harpacticoid species showed a clear separation between algae and a different
416 temporal pattern of assemblages on each macroalga (Fig. 3C). These results were supported by the
417 significant interaction between the factors alga and time (PERMANOVA pseudo-F=19.084; P<0.01).
418 In particular, harpacticoid assemblages resulted significantly different between the two algae at each
419 time, with the exception of T1 and T5 (Table S13). A significant gradual change in the community
420 structure took place in DP, whereas on CC significant community structure changes were evident at
421 T5. The details of pairwise comparisons among times for each alga are shown in Table S13.
422 SIMPER results revealed that average dissimilarity between the two algae at each time was largely
423 due to the high abundances of five species, namely *Heterolaophonte minuta, Parastenhelia spinosa,*
424 *Paradactylopodia brevicornis, Harpacticus gracilis,* and *Porcellidium viride,* that resulted more
425 abundant in DP than in CC (Table S14).
426 The mean abundance over time of these six species is shown in figure 6. In DP, the densities of
427 *Heterolaophonte minuta* showed two peaks at times T4 (68 ± 17 N g^{-1} fw) and T6 (82 ± 20 N g^{-1} fw),
428 whereas on CC the densities were always low, with a peak at T3 (17 ± 7 N g^{-1} fw). In DP the density
429 of *Parasthenelia spinosa* increased at T5 (17 ± 4 N g^{-1} fw) and T6 (27 ± 9 N g^{-1} fw), while in CC
430 there was an increase at T5 (12 ± 5 N g^{-1} fw), then a decrease at T6 (4 ± 1 N g^{-1} fw). *Paradactylopodia*
431 *brevicornis* showed higher densities at times T3 (11 ± 6 N g^{-1} fw), T5 (16 ± 6 N g^{-1} fw), and T6 (31
432 ± 11 N g^{-1} fw) in DP, while on CC this species was almost always absent, except at T5 (16 ± 6 N g^{-1}
433 fw). The average densities of *Harpacticus gracilis* resulted always higher on DP. The densities of
434 *Porcellidium viride* showed the highest values on DP at T3 (31 ± 11 N g^{-1} fw), then decreased and

finally disappeared at T6; conversely, the same pattern occurred for CC, but with lower densities. On DP the density of *Ectinosoma melaniceps* increased at T6 (19 ± 6 N g^{-1} fw), while on CC this species was almost always absent, except at T5 (7 ± 3 N g^{-1} fw).

3.7 Relationship between the meiofauna and harpacticoid assemblages with macroalgae complexity, PUAs, and microphytobenthos

The results of DistLM (Table S15) carried out to analyze the relationships between all the three sets of variables and the meiofauna assemblage showed that for the marginal test the three macroalgae complexity descriptors alone accounted for 26% of the variation in the meiofauna abundance, PUAs for 17% and microphytobenthic taxa for 38%.

After fitting complexity descriptors, PUAs and microphytobenthos taxa explained an additional 7% and 13% respectively, resulting in a total variation of 47%. These additional amounts were significant according to the sequential test.

The same analysis carried out on the harpacticoid assemblages showed that the three complexity descriptors alone accounted for 19% of the variation in the harpacticoid abundance, PUAs for 13%, and microphytobenthic taxa for 23%.

After fitting complexity, PUAs and microphytobenthos taxa explained an additional 9% and 10% respectively, resulting in a total variation of 38%. Only the additional amount of PUAs resulted significant according to the sequential test.

4. Discussion

Qualitative and quantitative differences were observed in the PUAs produced by the two macroalgae considered (*Dictyopteris polypodioides*, DP, and *Cystoseira compressa*, CC), in agreement with previous results obtained for the same species (Pezzolesi et al., 2021). DP produced, in fact, higher average concentrations than CC, but also a variety of long-chain compounds, such as hexadecatrienal (C16:3), hexadecatetraenal (C16:4) and tetradecapentaenal (C14:5), while *C. compressa* produced

461 the short-chain hexadienal (C6:2) as main compound, demonstrating the ability of macroalgae to
462 produce short, middle and/or long-chain aldehydes (Kajiwara et al., 1996; Akakabe et al., 2003;
463 Pezzolesi et al., 2021). These results highlighted that PUAs profile could be a fingerprint for each
464 algal species, since the same compounds were consistently detected regardless of the sampling period,
465 while their relative and total amount may vary depending on environmental conditions or
466 morphotypes (Alsufyani et al., 2014; Pezzolesi et al., 2021). In addition, since the apical parts of both
467 algae are those in which growth actively takes place, they are the most metabolically active parts, and
468 therefore presumably also those in which the greatest production of PUAs takes place. Dissolved
469 PUAs (dPUAs) concentrations in proximity of the macroalgae was high (in the order of μM),
470 especially when compared with results of previous studies carried out in an Adriatic planktonic
471 community (Ribalet et al., 2014), but are in accordance with values previously recorded in the same
472 site in other studies (Bartual et al., 2020; Pezzolesi et al., 2021) or hypothesized to occur in proximity
473 of the PUA producers (Ribalet et al., 2008; Bartual et al., 2018). These high concentrations are
474 reasonable considering that the sampling area is colonized by a well structured phytobenthic
475 community (macro- and microalgal), thus dPUAs derive from the contribution of the different algal
476 species. Additionally, the reduced hydrodynamism of this benthic site causes a low dispersion of any
477 secondary metabolites produced by the various organisms.
478 Seawater parameters (temperature, salinity and nutrients) were within the range previously reported
479 for the Piscinetta site (e.g. Accoroni et al., 2012), and confirmed the seasonal variability associated
480 to this shallow inlet, that is subjected to a moderate anthropic impact (mainly in the form of summer
481 tourism, as it is a popular site for swimming in the summer months). Results showed a predominace
482 of diatoms in the microphytobenthic community, as usually reported in the Adriatic Sea, even in
483 planktonic communities (e.g. Accoroni et al., 2016). Dinoflagellates were present at low densities,
484 except during the bloom of *Ostreopsis* cf. *ovata*, which was the main dinoflagellate and showed its
485 typical blooming trend, with maximum abundances recorded in late summer (September) and within
486 ranges previously observed (e.g. Gémin et al., 2020).

487 The main diatom genera found in the present study (e.g. *Navicula, Cylindrotheca, Lyrella, Cocconeis,*
488 *Gyrosigma* A.H. Hassall, 1845*, Licmophora* C. Agardh, 1827*, Nitzschia, Mastogloia* Thwaites ex
489 W.Smith, 1856*, Striatella* C. Agardh, 1832*, Coscinodiscus* Ehrenberg, 1839) are among the most
490 common on Mediterranean macroalgae and in the microphytobenthos, particularly in the Adriatic Sea
491 (Carnicer et al., 2015; Accoroni et al., 2016; Rogelja et al., 2016; Pennesi and Danovaro, 2017;
492 Ternon et al., 2020). Species belonging to the orders Naviculales and Lyrellales on DP, to the
493 Licmophorales on CC and, generally, to centric diatoms on both algae, showed an inverse trend (low
494 density at T1 and high at subsequent times) in relation to the production of PUAs by macroalgae.
495 These results could be ascribed to a negative effect of these compounds, as also demonstrated by
496 Ribalet et al. (2007) for some planktonic species. Long-chain aldehydes, such as C14-C16, can induce
497 a stronger growth inhibition than short-chain PUAs, probably due to longer alkyl chains that increase
498 the reactivity of the molecules (Adolph et al., 2003).
499 Since many diatom species recorded in the present study, as those belonging to the genus
500 *Cylindrotheca* spp. and *Nitzschia* spp., are themselves among the main PUAs producers (Wichard et
501 al., 2005a; Lavrentyev et al., 2015; Pezzolesi et al., 2017; Cózar et al., 2018), they may have
502 developed different sensitivities and/or tolerances to these compounds in a species-specific way.
503 Studies have also shown a different susceptibility based on the life cycle, with more resistant juvenile
504 cells and more sensitive stationary phase cells (Ribalet et al., 2007; Leflaive and Ten-Hage, 2009)
505 and based also on other factors, such as cell size, wall properties and lipid content. In particular,
506 species with a well-structured and mineralized cell wall, a low surface-volume ratio and a certain
507 lipid content can limit the ability of these compounds to penetrate the cell. Centric diatoms could
508 potentially be more sensitive to PUAs, as those found in the present study (*Chaetoceros* spp.
509 Ehrenberg, 1844, *Coscinodiscus* spp., *Guinardia* spp. H. Peragallo, 1892 and *Rhizosolenia* spp.
510 Brightwell, 1858) are not listed among the species able to produce these compounds (Wichard et al.,
511 2005).

512 *Ostreopsis* cf. *ovata* does not seem to be negatively influenced, in terms of abundance, by the
513 production of PUAs by macroalgae. Conversely, macroalgal compexity seems to explain the different
514 abundances found on CC and DP. The presence of a rigid cell wall and the high biovolume could
515 partially explain the apparent lower sensitivity of *Ostreopsis* cells to PUAs compared to other species,
516 in addition to the protective role that can be offered by the mucilaginous layer produced by *O.* cf.
517 *ovata*, which provides an additional barrier against the substances dissolved in the water column
518 (Allen et al., 2016). Similarly, benthic dinoflagellates have shown a higher resistance than planktonic
519 ones to potential allelochemicals (Ben Gharbia et al., 2017).
520 Regarding the meiofauna, 12 major taxa associated with the two macroalgae were found, with a total
521 density recorded in some samples of over 1300 individuals per gram of alga and within the range
522 found in previous studies (Jarvis and Seed, 1996). Harpacticoid copepods, together with their nauplii,
523 were the most abundant taxon, comprising 58% of the total meiofauna, followed by Nematodes that
524 were 33%, as reported in previous studies (Carlo Heip, Magda Vincx, 1985; De Troch et al., 2005).
525 By comparing the results obtained for the two macroalgae, the average abundances of total meiofauna
526 and the number of taxa resulted higher on DP than CC, as well as the abundances of copepods and
527 nematodes. As a result, the community structure resulted different between the two macroalgae and
528 showed a different temporal pattern, although they were subjected to the same environmental
529 characteristics in terms, for instance, of hydrodynamism and tides. DP and CC are different for life
530 cycle, thallus structure and production of compounds. In particular, DP is formed by ribbon-like
531 fronds, with very irregular and proliferating edges, on which numerous meiobenthic organisms can
532 settle. In the area where sampling was conducted, the species persists in its fully developed habit for
533 most of spring and summer. CC is a highly branched, leathery macrophyte; when fully grown, in the
534 study area it may reach 1-1.5 m in height. For a large-sized, habitat-forming species, its growth is
535 relatively fast, especially if compared with other Mediterranean fucalean brown algae. However, in
536 the area of the Passetto its full development is limited to a quite restricted period of the year (from
537 May to mid July); by mid summer this species loses most of its branches and persists in a more

538 reduced habit, with a few short branches. This means that most of its fronds occur in the field for a
539 shorter time and are not available long enough to allow the settlement of a very diverse epiphytic
540 community. The shorter temporal availability of this substrate also means that there will be a lower
541 accumulation of sediment and detritus, a potential source of food for meiofaunal organisms (Hicks,
542 1980; Gibbons, 1988; Frame et al., 2007). Both species have apical growth, determined by divisions
543 of a group of meristematic cells in DP and a single apical cell in CC. Therefore, the apical parts are
544 the youngest parts of the thallus, on which sediment and epiphytes had less time to settle.
545 Taking into consideration all these aspects, our results suggest that the morphological complexity of
546 DP may affect total number of individuals, but also the associated species that have evolved
547 morphological adaptations necessary for the adhesion to a thallus of this type (Taylor and Cole, 1994;
548 Chemello and Milazzo, 2002). Although macrophytes are not true fractal objects, estimates of
549 complexity using tools of fractal geometry have proved to be a useful approach for quantifying and
550 separating effects of habitat architecture from those of habitat quantity (Gee and Warwick, 1994;
551 McAbendroth et al., 2005; Hooper and Davenport, 2006). However, fractal measures performed for
552 not truly fractal objects are just an estimate of complexity for a given scale.
553 The putative role of PUAs on structuring meiofauna may be reflected by the low density of nauplii,
554 copepods, and nematodes at time T1 recorded on both macroalgae, which could be related to the high
555 concentration of PUAs in this period, as observed also for microphytobenthos. Moreover, host
556 specificity may be supposed, according to what determined by Bates and DeWreede (2007), that
557 found specific chemical, structural and morphological characteristics of the algal species.
558 Harpacticoids have a number of features that make them an attractive group of benthic organisms in
559 which PUA toxicity responses could be investigated, such as abundance, ecological importance, and
560 short generation cycles (Raisuddin et al., 2007). In this study, harpacticoids associated with the two
561 macroalgae consisted of 12 species. Even if the number of species was relatively low compared to
562 results of previous studies (Hicks, 1977b; Arroyo et al., 2006), it is interesting to note that the 12
563 species belonged to 12 genera and 11 families, so showing a high taxonomic distinctness. Moreover,

564 no species was present exclusively on a single macroalga; only the relative abundance of individual
565 species was different between the two macroalgae, with *Heterolaophonte minuta* as the dominant
566 species on both. Almost all species showed low abundance at T1, potentially suggesting a role of
567 PUAs, as previously postulated for meiofauna and microphytobethos. To our knowledge, only one
568 study was carried out in laboratory to analyze the effects of these compounds on the harpacticoid
569 *Tisbe holothuriae* (Taylor et al., 2007); while, other studies carried out on planktonic ecosystem have
570 shown deleterious effects of PUAs produced by diatoms on the reproduction of calanoid copepods
571 (e.g. *Temora stylifera, Calanus helgolandicus*), which feed on them (Ianora et al., 2003, 2012; Miralto
572 et al., 1999), as well as apoptosis in maturing oocytes (Poulet et al., 2007a) during embryo
573 development (Romano et al., 2003) and in newly hatched nauplii (Ianora et al., 2004b).
574 It has to be considered that the present study was carried out in the field, so the link between PUAs
575 effects on the community structure is more difficult to evaluate, due to the variability of PUAs
576 production both by diatoms (Wichard et al., 2005b) and macroalgae (Pezzolesi et al., 2021), of the
577 copepod sensitivity (Ianora et al., 2003; Sommer, 2009) and to the detoxification ability developed
578 by certain species of copepods (e.g. Taylor et al., 2007; Wichard et al., 2008). As attested by Taylor
579 et al. (2007), the benthos tends to be a more stressful environment compared to planktonic, where
580 rapid fluctuations in physical conditions occur and both natural and anthropogenic toxins can
581 accumulate at high levels within the sediments between the algal fronds. Since benthic organisms
582 must be highly adapted to survive in such a harsh environment, it is not unreasonable to speculate
583 that harpacticoids may have a more developed detoxification system than planktonic calanoid
584 copepods, thus being better equipped to resist to the toxic effect of oxylipins. Indeed, a number of
585 candidate detox genes were found in an analysis of 686 sequence tags expressed by *Tigriopus*
586 *japonicus* (Lee et al., 2005, 2008). Therefore, it is possible that the species found during the present
587 study have developed effective and efficient detoxification strategies to survive in an environment
588 such as the Passetto area. The different community structure between the two macroalgae and the
589 temporal changes could be also explained by the ecological and trophic role of the various species,

590 and by the different morphological evolution of the two macroalgae during the sampling time. This
591 is an important aspect to consider in the case of this study, as we sampled apical parts of thalli of CC
592 and DP, that may be not representative of the entire thalli, as are the youngest parts on which epiphytes
593 had less time to settle and also those most exposed to light. In particular, four species (*Porcellidium*
594 *viride, Parastenhelia spinosa, Heterolaophonte minuta,* and *Harpacticus gracilis*) belonged to the
595 phytal group sensu strictu, and two (*Paradactylopodia brevicornis,* and *Ectinosoma melaniceps*)
596 belonged to migrator and cosmopolitan group (Mascart et al., 2015). *E. melaniceps* is a tolerant
597 eurytopic species, which presumably is not affected by the biochemical compounds produced by
598 macroalgae, as for example reported for the green alga *Ulva lactuca* (Hicks, 1980). *P. brevicornis* is
599 cosmopolitan species that have a wide distribution range and was found in different habitats, therefore
600 able to adapt to a large number of different environmental conditions (Hicks, 1980). Conversely, *P.*
601 *viride, H. minuta* and *H. gracilis* are endemic species of the Mediterranean Sea with certain
602 morphological characteristics that allow them to live adhering to macroalgal surfaces; therefore, they
603 could either be affected by the effect of the various compounds produced by both macroalgae and
604 microphytobenthos, or adapt to the various environmental conditions.
605 The DistLm procedure highlighted that the observed differences in the meiofauna and harpacticoid
606 community structure between the two macroalgal species could be mainly explained by
607 microphytobenthic main taxa, after fitting macroalgal complexity.
608 Microalgae, in fact, are at the base of the food web and provide energy for all the trophic levels above
609 them; thus, these results confirm their important role as primary food source of essentially all marine
610 food chains producers. In fact, most of the epifaunal taxa directly graze upon the macroalgae or the
611 epiphytes for their food source, thus variations in the epiphytic algal density and composition between
612 two macroalgal species may influence the abundance of associated epifauna (Gestoso et al., 2010).
613 Additionally, the macroalgal complexity, rather than PUAs production alone, resulted an important
614 component influencing the community structure. The role of the shape and structural complexity of
615 macroalgae in determining the abundance patterns and size structure of the epiphytic organisms is

supported in literature, either for epifaunal and epiphytic assemblages (e.g. Chemello and Milazzo, 2002; McAbendroth et al., 2005; Cacabelos et al., 2010). To better investigate the effect of macroalgal complexity and PUAs composition on variations of the microphytobenthos at some representative genera levels, on main meiofauna taxa, and on harpacticoid species Pearson correlation analysis was used (Fig. 7). Among PUAs, long-chain compounds (i.e. C14-C16), when compared with the short one (i.e. C6:2), showed higher effects on the abundances of some representative microalgae (i.e. *Navicula* spp., *Lyrella* spp., *Cocconeis* spp., *Cylindrotheca* spp./*Nitzschia* spp., and *Ostreopsis* cf. *ovata*), on the main meiobenthos taxa (i.e. Nematoda, Copepoda, copepod nauplii) and on harpacticoid species (i.e. *H. minuta, H. gracilis, P. viride*). Results are in agreement with laboratory studies performed to investigate the responses of allelochemicals, such as PUAs, on target organisms, including copepods and algae (Ianora et al., 2004a; Taylor et al., 2007; Adolph et al., 2004; Caldwell et al., 2004; Ribalet et al., 2007; Pichierri et al., 2016). Macroalgal complexity, either in terms of fractal dimension and area, is highlty correlated and showed significant relationships with almost all the taxa considered, except with *P. viride,* protentially due to its ecological role, being a phytal species able to colonize a variety of macroalgae thanks to morphological adaptations that has evolved to attach to morphologically diverse thalli (Hicks, 1980).

It is important to point out that several metabolites, as well as PUAs, are known to be produced by macroalgae, including some (e.g. diterpenoids, polyphenols) that inhibit the growth of microalgae, bryozoan or other benthic species (Ternon et al., 2020), thus their contribution to the observed dynamics could not be excluded. Additionally, the epiphytic community that colonize the macroalgal surface may contribute either to the surface metabolome, adding inhibitory effects on the co-occurring species (Monti and Cecchin, 2012) or to the higher trophic levels, and directly interacting with the epifaunal, thus adding complexity to the understanding of the relationships among these organisms and to the role of PUAs. Finally, toxic microalgae such as *O*. cf. *ovata,* that was found to bloom during the sampling period, have been reported to affect copepods, and particularly nauplii (Guidi-Guilvard et al., 2012), with different sensitivities among species (Pavaux et al., 2019).

642 Taking into consideration all these aspects, PUAs production by macroalgae, together with their
643 complexity, resulted one of the main factors involved in the benthic community structure dynamics,
644 but it is not enough to explain the differences in the microphytobenthos and meiofana assemblages.
645

646 **5. Conclusion**

647 Epiphytic communities on the two macroalgae highlighted a clear separation of the meiofauna and
648 microphytobenthos assemblages with different temporal trends. The average dissimilarities were due
649 to several microalgal orders, namely Naviculales, Lyrellales, Gonyaulacales (i.e. *Ostreopsis* cf.
650 *ovata*), Bacillariales, and Licmophorales, and to the three meiofauna dominant taxa (nematodes,
651 copepods, and copepod nauplii) that were always more abundant on DP than on CC. Particularly,
652 average dissimilarities of harpacticoid copepods were largely due to the abundances of five species,
653 namely *Heterolaophonte minuta, Parastenhelia spinosa, Paradactylopodia brevicornis, Harpacticus*
654 *gracilis, Porcellidium viride,* and *Ectinosoma melaniceps*. Generally, variations in the meiofauna and
655 harpacticoid abundances were mainly due to macroalgal complexity variables and
656 microphytobenthos, while a minor contribution was due to PUAs. Results documented that i)
657 microphytobenthos resulted to affect the meiofauna population dynamics, in particular the
658 harpacticoid assemblages, attesting the role of these organisms as primary food source of essentially
659 all marine food chains producers, being at the base of the food web and providing energy for all the
660 trophic levels above them, ii) the macroalgal complexity rather than PUAs production alone could be
661 a major trigger of the community structure. PUAs effects, in fact, resulted species-specific, thus
662 affecting some grazers instead of the entire community structure, as demonstrated also by Pearson's
663 ρ correlations between taxa abundances and several macroalgal parameters. Among PUAs, long-
664 chain compounds (i.e. C14-C16), with respect to the short one (i.e. C6:2), showed higher effects on
665 the abundances of some representative microalgal genera, harpacticoid species and on the main
666 meiobenthos taxa.

Since several of the epiphytic diatom species found, in addition to macroalgae, can produce PUAs, the understanding of the effects of these compounds on the community structure and on the relationships among taxa in field studies are complicated, thus opening to further in-depth investigations in simplified systems (i.e. microcosms).

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CRediT author statement

Denise Lenzo: Data curation, Investigation, Formal analysis, Writing- Original draft preparation. Laura Pezzolesi: Conceptualization, Methodology, Data curation, Resources, Writing- Original draft preparation, Funding acquisition. Chiara Samorì: Data curation, Writing - Review & Editing. Fabio Rindi: Resources, Reviewing and Editing. Andrea Pasteris: Supervision, Reviewing and Editing. Rossella Pistocchi: Funding acquisition, Reviewing and Editing. Marina Antonia Colangelo: Conceptualization, Methodology, Formal analysis, Software, Writing- Original draft preparation, Supervision.

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1018 **Figure captions**
1019 **Figure 1** – Total density of microphytobenthos community (cells g-1 fw) in *Dictyopteris*
1020 *polypodioides* (DP) and *Cystoseira compressa* (CC) at the different sampling times (T1-T6).
1021 **Figure 2** – Relative abundance (%) of species belonging to the Bacillariophyceae orders in A)
1022 *Dictyopteris polypodioides* (DP) and B) *Cystoseira compressa* (CC) at the different sampling times
1023 (T1-T6).
1024 **Figure 3** - Two-dimensional nMDS of centroids for A) microphytobenthos orders, B) meiofauna,
1025 and C) harpacticoid community on the two algae *Dictyopteris polypodioides* (DP) and *Cystoseira*
1026 *compressa* (CC) at the different sampling times (T1-T6).
1027 **Figure 4** – Total density (N g-1 fw) of meiobenthos in *Dictyopteris polypodioides* (DP) and
1028 *Cystoseira compressa* (CC) at the different sampling times (T1-T6).

1029 **Figure 5** – Total density (n° organisms g-1 fw) of A) nematodes, B) copepods and C) copepod
1030 nauplii in *Dictyopteris polypodioides* (DP) and *Cystoseira compressa* (CC) at the different sampling
1031 times (T1-T6).

1032 **Figure 6** – Total density (N g-1 fw) of harpacticoids in A) *Dictyopteris polypodioides* (DP) and B)
1033 *Cystoseira compressa* (CC) at the different sampling times (T1-T6).

1034 **Figure 7** – Results of Pearson correlation analysis between substrate attribution (D and S) and PUA
1035 composition and densities of more representative genera of microphytobenthos, main meiofauna
1036 taxa and harpacticoid species, after square root transformation. Non-significant relationships are
1037 indicated by white squares. Signif. codes: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$. D, fractal
1038 dimension; S, macroalgal surface; tot, total PUA; C14:5, tetradecapentaenal; C16:4,
1039 hexadecatetraenal; C16:3, hexadecatrienal; C6:2, hexadienal.

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1044 Table 1: Relative abundance (%) of identified and unknown polyunsaturated aldehydes (PUAs) and
1045 total concentration of PUAs (µg g-1 fw) in *Dictyopteris polypodioides* (DP) and *Cystoseira*
1046 *compressa* (CC) at the different sampling times (T1-T6).

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Time	Alga	C6:2	C16:3	C16:4	C14:5	Unknown	Tot (µg g^{-1} fw)
T1	DP	10%	12%	15%	50%	13%	225.5
T2	DP	0%	39%	20%	29%	14%	73.9
T3	DP	7%	21%	29%	32%	11%	44.3
T4	DP	7%	8%	14%	60%	11%	100.3
T5	DP	0%	12%	19%	61%	7%	82.1
T6	DP	6%	0%	8%	79%	7%	87.4
T1	CC	91%	0%	0%	0%	9%	17.1
T2	CC	64%	0%	0%	0%	36%	2.0
T3	CC	81%	0%	0%	0%	19%	2.3
T4	CC	88%	0%	0%	0%	12%	3.4
T5	CC	83%	0%	0%	0%	17%	5.3
T6	CC	68%	0%	0%	0%	32%	4.8

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1068 Figure 1

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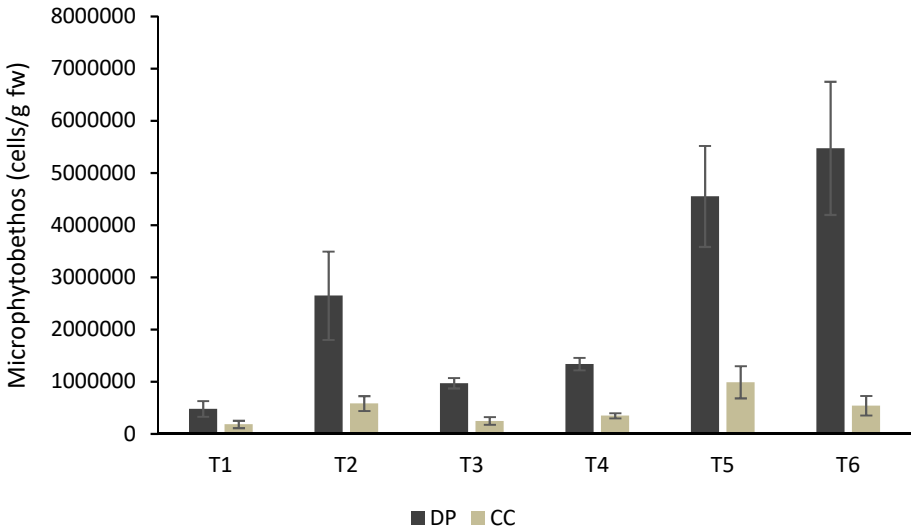

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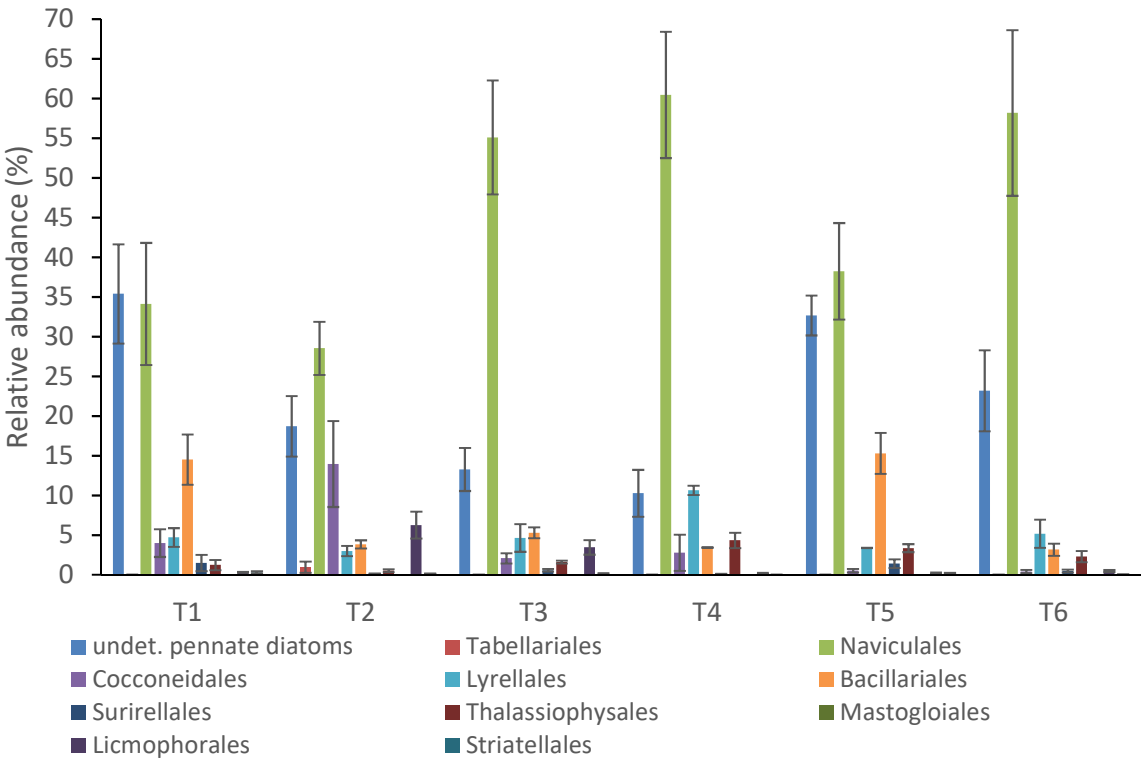
1090 Figure 2

1091 A)


Relative abundance (%)
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
T1
T2
T3
T4
T5
T6
undet. pennate diatoms
Tabellariales
Naviculales
Cocconeidales
Lyrellales
Bacillariales
Surirellales
Thalassiophysales
Mastogloiales
Licmophorales
Striatellales

B)

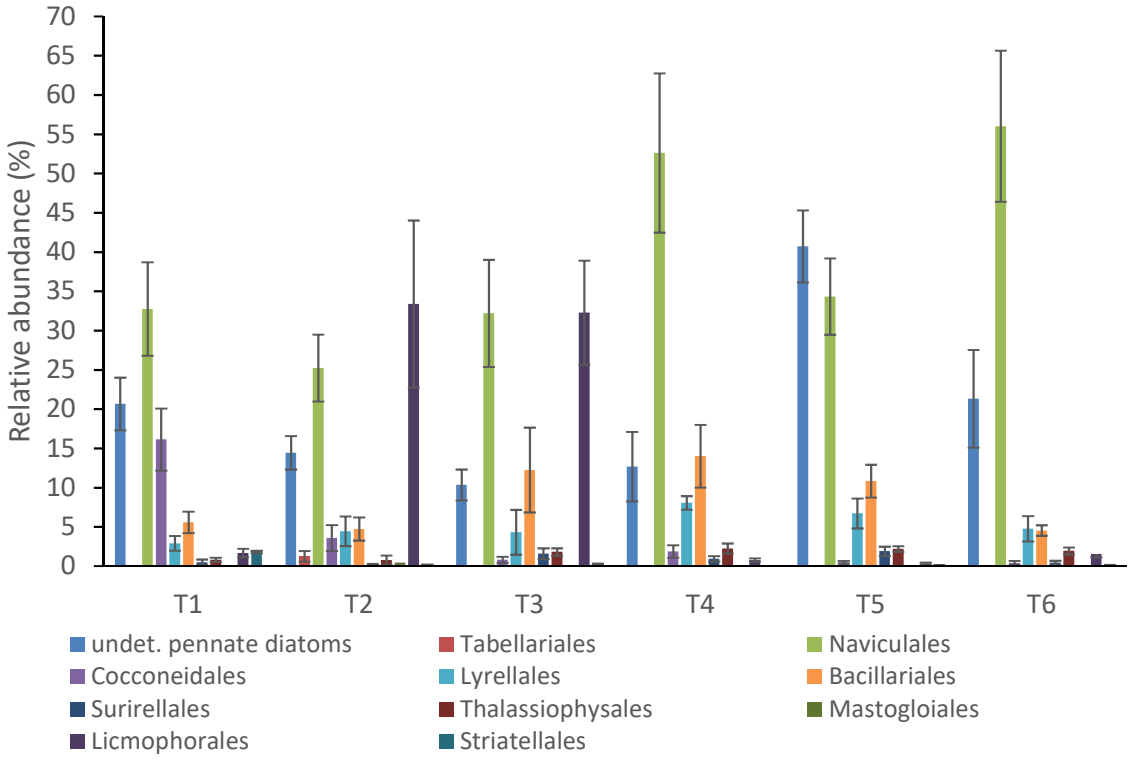
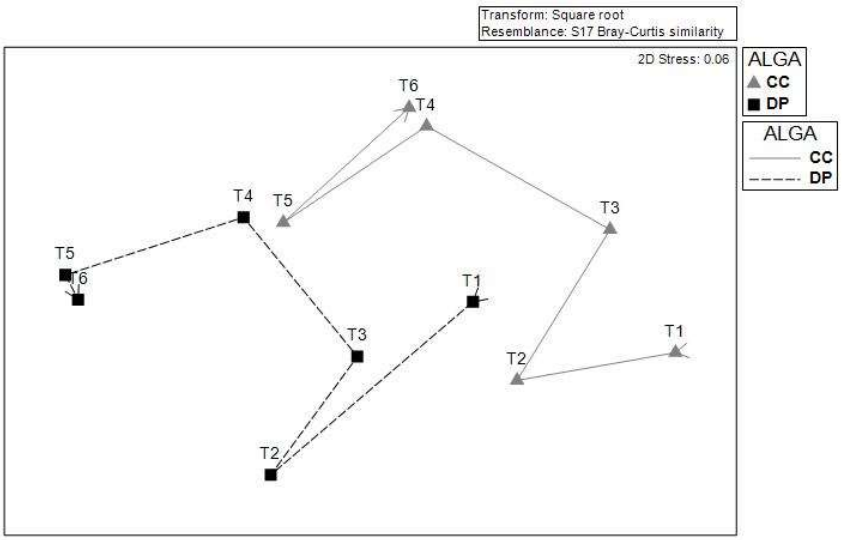

Relative abundance (%)
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
T1
T2
T3
T4
T5
T6
undet. pennate diatoms
Tabellariales
Naviculales
Cocconeidales
Lyrellales
Bacillariales
Surirellales
Thalassiophysales
Mastogloiales
Licmophorales
Striatellales

Figure 3

A)



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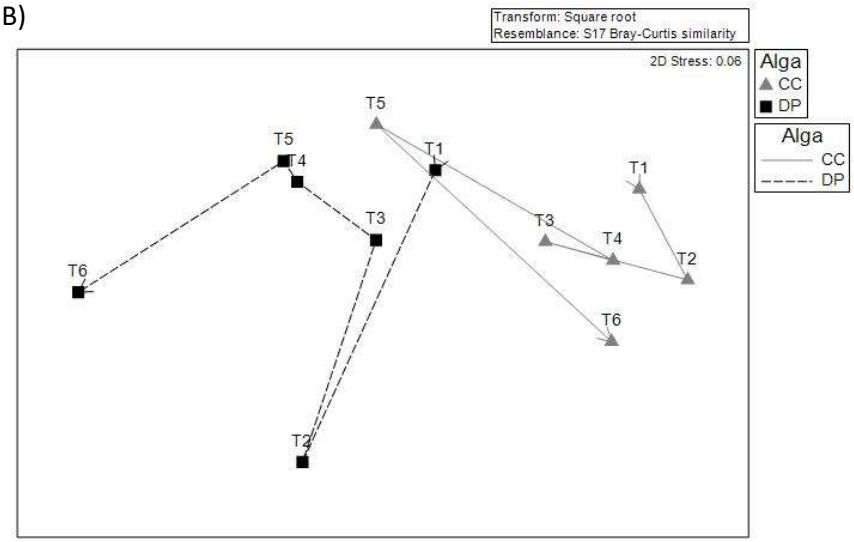
B)



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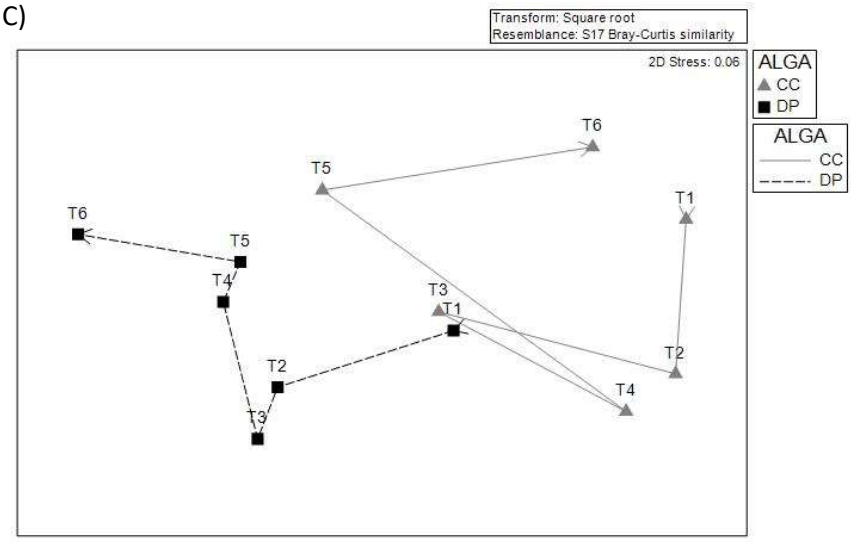
C)



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1102 Figure 4

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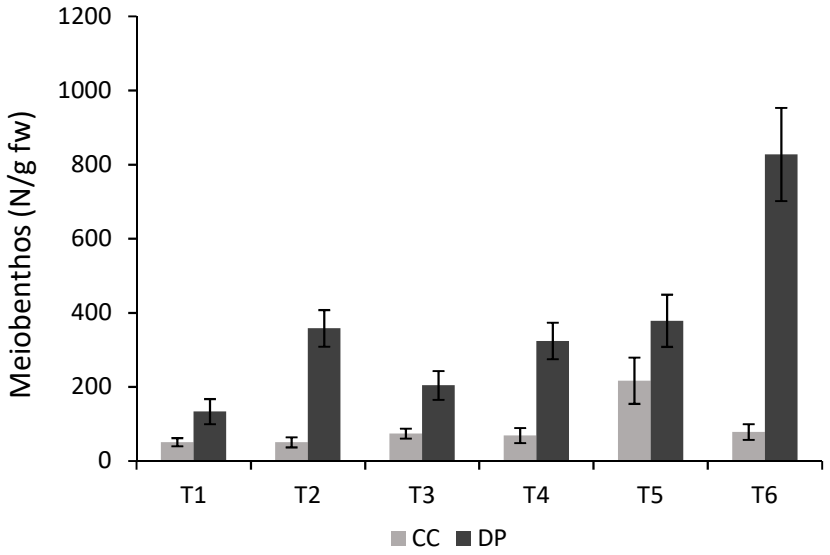
Meiobenthos (N/g fw)
1200
1000
800
600
400
200
0
T1
T2
T3
T4
T5
T6
CC
DP

Figure 5

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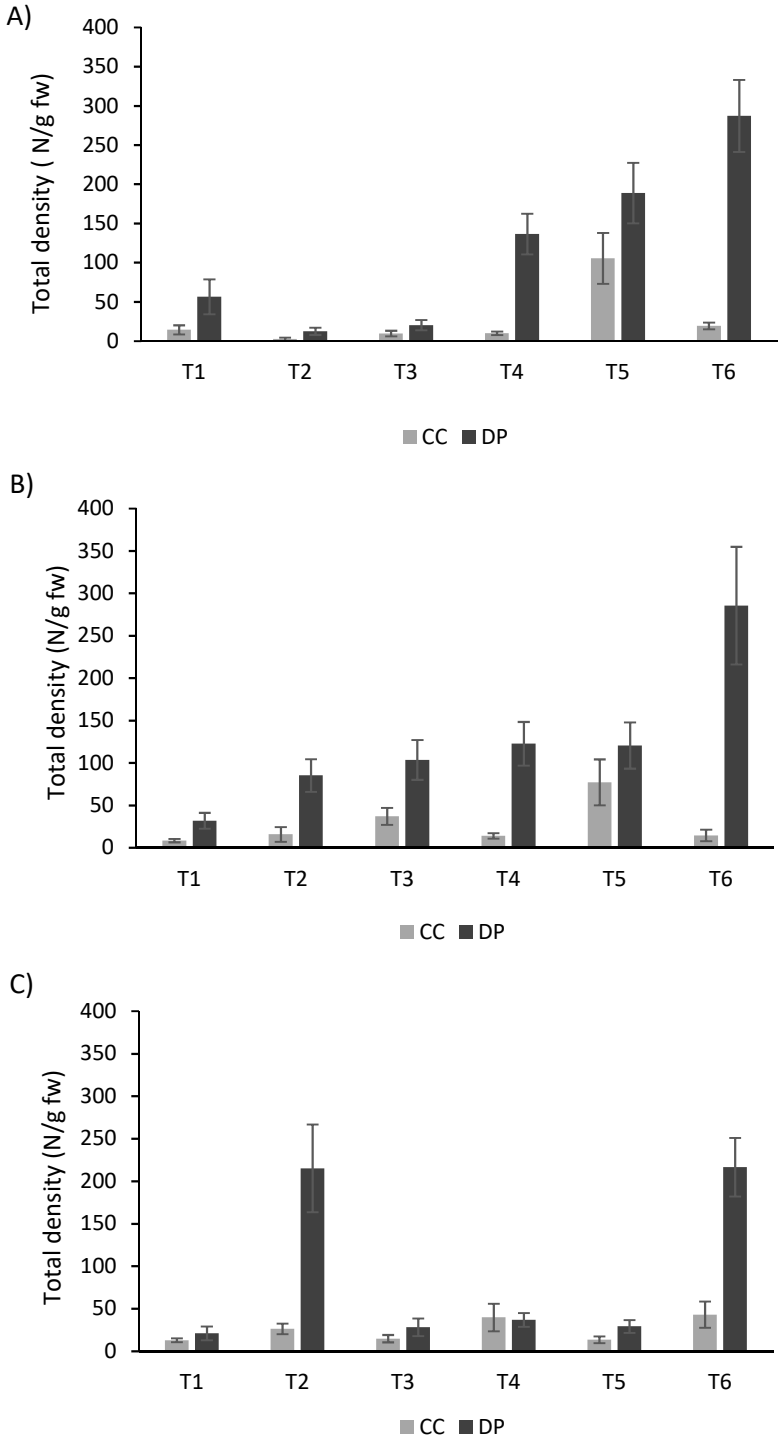

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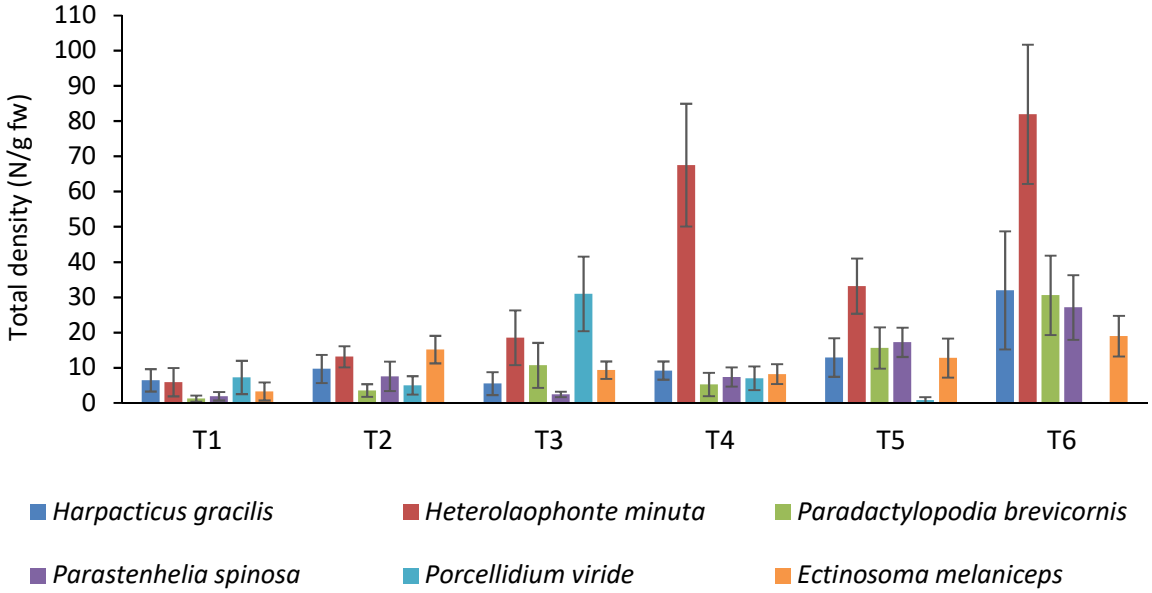
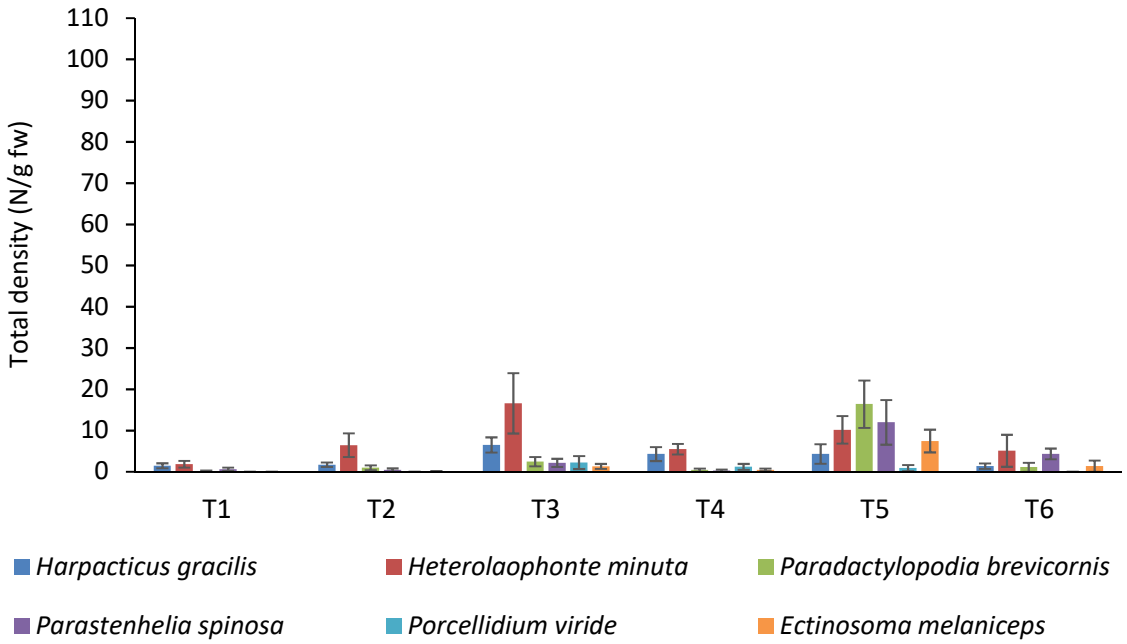
1134 Figure 6

A)


Total density (N/g fw)
110
100
90
80
70
60
50
40
30
20
10
0
T1
T2
T3
T4
T5
T6
Harpacticus gracilis
Heterolaophonte minuta
Paradactylopodia brevicornis
Parastenhelia spinosa
Porcellidium viride
Ectinosoma melaniceps

B)


Total density (N/g fw)
110
100
90
80
70
60
50
40
30
20
10
0
T1
T2
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T4
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T6
Harpacticus gracilis
Heterolaophonte minuta
Paradactylopodia brevicornis
Parastenhelia spinosa
Porcellidium viride
Ectinosoma melaniceps

Figure 7

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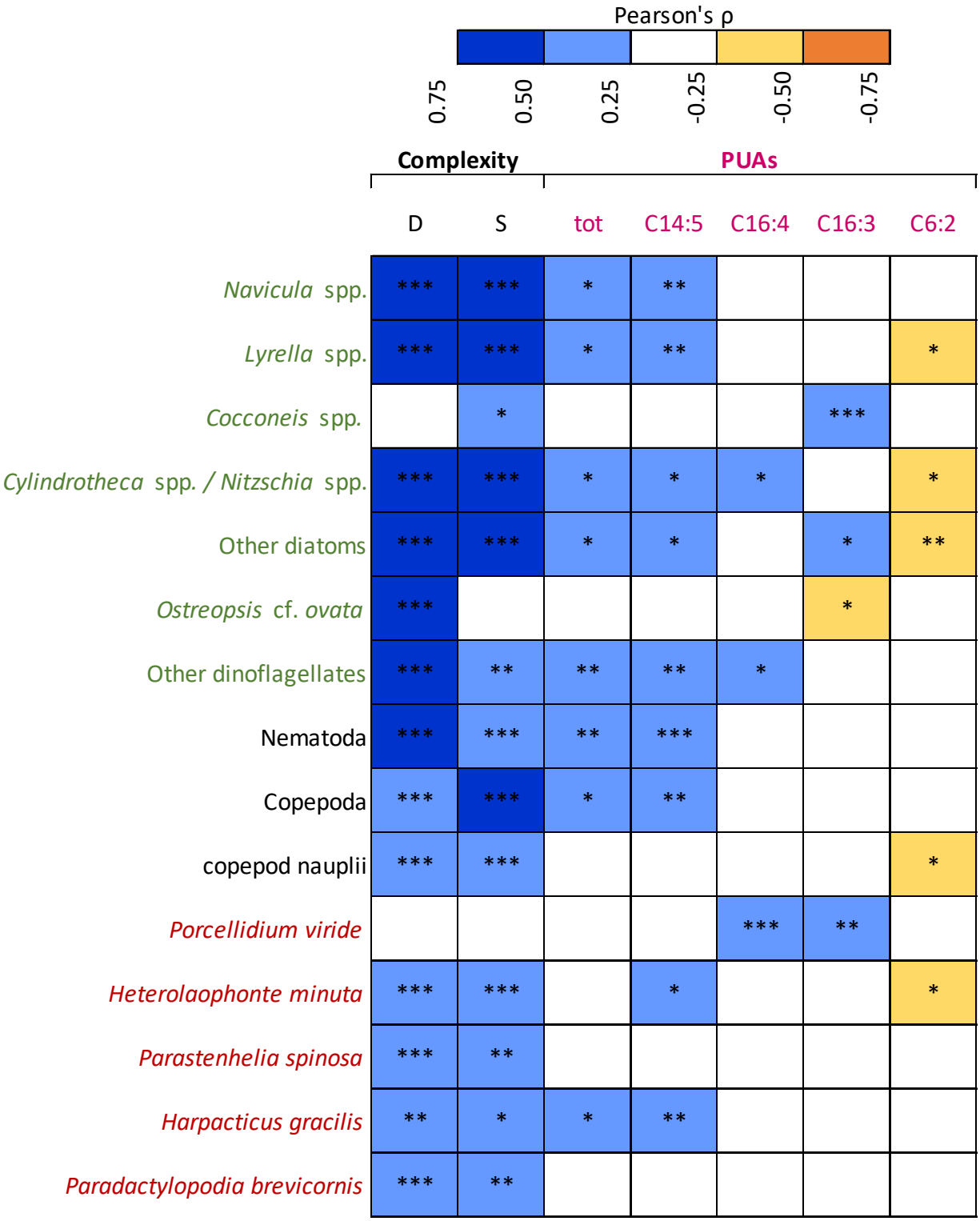
Pearson's ρ
0.75
0.50
0.25
-0.25
-0.50
-0.75
Complexity
PUAs
D
S
tot
C14:5
C16:4
C16:3
C6:2
Navicula spp.
Lyrella spp.
Cocconeis spp.
Cylindrotheca spp. / Nitzschia spp.
Other diatoms
Ostreopsis cf. ovata
Other dinoflagellates
Nematoda
Copepoda
copepod nauplii
Porcellidium viride
Heterolaophonte minuta
Parastenhelia spinosa
Harpacticus gracilis
Paradactylopodia brevicornis

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