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1 **Trophic status and meiofauna biodiversity in the Northern Adriatic Sea:**
2 **insights for the assessment of good environmental status**

3
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26 **Abstract**

27 The Descriptor 5 (Eutrophication) of the EU Marine Strategy Framework Directive aims at
28 preventing the negative effects of eutrophication. However, in coastal systems all indicators based
29 on water column parameters fail in identifying the trophic status and its effects on biodiversity and
30 ecosystem functioning. We investigated benthic trophic status, in terms of sedimentary organic
31 matter quantity, composition and quality, along with meiofaunal abundance, richness of taxa and
32 community composition in three coastal sites (N Adriatic Sea) affected by different levels of
33 anthropogenic stressors. We show that, on the basis of organic matter quantity and composition, the
34 investigated areas can be classified from oligo- to mesotrophic, whereas meiofauna as a descriptor,
35 their environmental quality ranged from sufficient to moderately impacted. Our results show that
36 the benthic trophic status based on organic matter variables, is not sufficient to provide a sound
37 assessment of the environmental quality in marine coastal ecosystems. However, data reported here
38 indicate that the integration of the meiofaunal variable allow providing robust assessments of the
39 marine environmental status.

40 **Highlights**

- 41 • Organic matter quantity and quality and meiofaunal diversity were investigated in the N
- 42 Adriatic Sea
- 43 • Investigated sediments were found to be from oligo- to mesotrophic
- 44 • Environmental quality, based on meiofauna attributes, ranged from sufficient to moderately
- 45 impacted
- 46 • Environmental quality evaluated through the analysis of meiofaunal assemblages is not
- 47 closely linked to benthic trophic status
- 48 • Benthic trophic status cannot be used alone to assess good environmental status (GES)

49

50 **Keywords:** sedimentary organic matter, meiofauna, benthic trophic status, eutrophication, good

51 environmental status

52

1. Introduction

Marine ecosystems worldwide are experiencing impacts of unprecedented intensity and frequency generated by the synergistic effects of multiple stressors (Claudet and Fraschetti, 2010): these are causing severe changes in oceans' biodiversity, assemblage structure, organization and functioning (Worm et al., 2006). Marine ecosystems are characterized by complex internal dynamics and can show rapid and non-linear responses to multiple stressors (DeYoung et al., 2008). These features limit our capability to predict the trajectories of change of marine ecosystems in different scenarios of socio-economic development and climate change (Pastres and Solidoro, 2012).

Eutrophication, i.e., the enhanced primary production stimulated by large inputs of nutrients, is almost ubiquitous in oceans worldwide, and in the last 50 years has caused large and severe alterations of many coastal regions (Cloern, 2001; Pinckney et al., 2001). As a consequence, during the last decades, ecologists have developed tools and indicators to assess background levels of trophic status of marine coastal environments (Dell'Anno et al., 2002; Pusceddu et al., 2009). In this regard, the assessment of the trophic status of marine coastal ecosystems has been historically based on measurements of the main elements (e.g., inorganic and organic N and P), molecules (organic matter, chlorophyll-a concentrations) used as surrogates of biomass in the water column (Coelho et al., 2007) and combinations of biotic and abiotic variables (e.g. Vollenweider et al., 1998). However, changes in the trophic status of a given marine ecosystem are not only associated with increases in primary production levels, and can exert consequences at different hierarchical levels of ecosystem organization (Lotze et al., 2006). For example, increased levels of primary production can also be associated with the accumulation of large amounts of detrital (i.e. non-living) organic material, with associated changes in the biochemical composition and bioavailability of food for heterotrophs (Pusceddu et al., 2009). Moreover, in shallow marine ecosystems, where hydrodynamic forcing can modify the structure and biology of the water column at very short-time intervals (e.g. from minutes to hours), the assessment of trophic status using only variables measured in the water column can lead to misleading classifications (Izzo et al., 1997; Dell'Anno et

79 al., 2002). In this regard, marine sediments underlying shallow waters can be considered a sort of
80 "recorder" of the biological processes that occur in the overlying water column (Dell'Anno et al.,
81 2002). Accordingly, the trophic status of marine coastal environments has been recently assessed
82 using the quantity and biochemical composition of sedimentary organic matter (Pusceddu et al.,
83 2007a, 2009, 2011). This approach can be even profitably applied also to the deep sea, lacking
84 primary production (Bianchelli et al., 2008; Pusceddu et al., 2010).

85 Natural and anthropogenic changes in the benthic trophic status, in terms of organic matter
86 quantity and biochemical composition, can in turn exert consequences on the composition and
87 structure of the benthic communities (Pusceddu et al., 2011; Foti et al., 2014). In this regard,
88 meiofauna, due to their strong sensitivity to environmental disturbances, high abundance, lack of
89 pelagic larval dispersion and the short life cycles, have recently acquired the rank of a "wide
90 spectrum" tool in marine environmental monitoring (Heip et al., 1985; Semprucci et al., 2015a).
91 Indeed, meiofauna have become a common tool to assess the effects of various sources of
92 disturbance on the marine environment (Danovaro et al., 2000; Austen and Widdicombe, 2006; De
93 Troch et al., 2006; Semprucci et al., 2015b,c), and, in particular, to assess the impacts of benthic
94 eutrophication (Pusceddu et al., 2007b; Mirto et al., 2014).

95 The Northern Adriatic Sea has been for a long time considered among the most productive and,
96 at the same time, most environmentally compromised basins of the Mediterranean Sea (Micheli et
97 al., 2013). In the last 30 years, the Adriatic Sea has experienced large changes in the trophic regime,
98 structure and organization of pelagic and benthic communities also in response to current climate
99 shifts (Kamburska and Fonda Umani, 2006; Danovaro et al., 2009; Conversi et al., 2010; Mozetič et
100 al., 2010; Giani et al., 2012; Di Camillo and Cerrano, 2015). Nevertheless, within this basin there
101 are still areas characterized by relatively low levels of anthropogenic impact, deserving attention for
102 their conservation (Micheli et al., 2013).

103 In this framework, our aim was to investigate the reliability of previously tested variables in
104 describing the benthic biodiversity response to variations in sedimentary organic matter quantity

105 and biochemical composition under different levels of environmental impairment, in the Adriatic
106 Sea coastal ecosystems. To cope with this aim, we analyzed temporal variations in the quantity and
107 biochemical composition of sedimentary organic matter (OM) and the abundance and community
108 structure of meiofauna under three different putative levels of anthropogenic impact along the
109 Italian coasts of the Northern Adriatic Sea. More specifically, we tested the null hypothesis by
110 which benthic trophic status and meiofaunal communities (in terms of abundance and taxonomic
111 composition) do not vary among sampling times and sites characterized by the presence of different
112 levels of environmental impairment.

113

114 **2. Materials and methods**

115 ***2.1 Study areas and sampling***

116 Samples were collected in the Northern Adriatic Sea, from three coastal sites: Senigallia, Falconara
117 and Portonovo, located at 4-12 m water depths (Figure 1) and selected as representative of three
118 different levels and types of environmental impairment. Senigallia, located 3 km from the coast
119 (43°45'30"N, 13°13'00"E) is subjected to commercial and touristic maritime traffic throughout the
120 year and receives seasonally riverine inputs from the nearby Misa river. Falconara, located at about
121 0.6 km from the coast (43°39'00"N, 13°22'00"E) receives the inputs from the Esino river estuary
122 and show the presence of a petrochemical industry (refinery) located ca 1 km apart. According to
123 the report on the quality status of coastal marine water bodies for the period 2010-2012, drawn up
124 by the Regional Agency for the Environmental Protection of Marche region (ARPAM), the
125 ecological status for all the investigated sites was classified as "Sufficient" (see for details the report
126 available at: [http://www.arpa.marche.it/images/PUBBLICAZIONI/marino_costieri_2010-](http://www.arpa.marche.it/images/PUBBLICAZIONI/marino_costieri_2010-2012.pdf)
127 2012.pdf).

128 Portonovo, located ca 4.5 km apart from the coast (43°36'12"N, 13°36'42"E), is affected during the
129 summer season by tourism and maritime traffic, and, because of its ecological peculiarity, is
130 included within a Site of Community Importance (Natura 2000, site code IT5320006).

131 Sediment samples were collected by means of a Van Veen grab (sampling surface 0.15 m²), on
132 monthly basis from October 2011 to September 2012 (except for April and August 2012), on board
133 of the R/V Actea. To cope with the possible bias raised by using the Van Veen grab, which may
134 produce leaking of interstitial water during recovery, we collected samples only from deployments
135 in which the grab resulted completely watertight. Sediment sub-samples for the subsequent analyses
136 of organic matter (OM) and meiofauna were collected from three independent deployments of the
137 grab by means of plexiglass corers (internal diameter 3.6 cm). Once on board, the top first 2 cm
138 from each sediment core (three for OM and three for meiofauna) were sliced and kept at *in situ*
139 temperature until brought to the laboratory. Analyses were carried out on the top 2-cm sediment
140 layer since the concentration of sedimentary OM compounds as well as organisms belonging to
141 meiofauna are typically higher in the first top centimeters of the sediments. In the laboratory,
142 sediment samples dedicated to the analysis of organic matter were stored at -20°C until analysis
143 (usually within two weeks), whereas samples for meiofauna analyses were preserved with formalin
144 (final concentration 4% in sea water filtered on a 20 µm mesh) and stained with Rose Bengal (0.5 g
145 L⁻¹) until further treatment as described below, according to Danovaro (2010).

146

147 **2.2 Grain size**

148 Aliquots of sediment were treated with 10% hydrogenperoxide in a large beaker for 24–48 h and
149 dried in the oven at 60°C for an additional 24 h. The sediment was then sieved through a 63-µm
150 sieve and the two fractions (sands >63 µm and mud <63 µm) weighed (±0.1 mg) and expressed as
151 percentage of the initial total dry weight (Pusceddu et al., 2010).

152

153 **2.3 Biochemical composition of sediment organic matter**

154 Sediments were analysed for total phytopigment, protein, carbohydrate and lipid contents according
155 to Danovaro (2010), modified for coastal sediments.

156 Briefly, chlorophyll-a and phaeopigments (used as proxies of primary organic material
157 associated with primary producers) were analysed fluorometrically (Lorenzen and Jeffrey, 1980)
158 and total phytopigment contents defined as the sum of chlorophyll-a and phaeopigment
159 concentrations. Total phytopigment contents were utilized as an estimate of the organic material of
160 algal origin, including the living (chlorophyll-a) and senescent/detrital (i.e., phaeopigments)
161 fractions (Pusceddu et al., 2009). Sediment phytopigment concentrations were converted into
162 carbon equivalents using a mean value of $40 \mu\text{gC } \mu\text{g phytopigment}^{-1}$ (Pusceddu et al., 2009).

163 Protein, carbohydrate and lipid contents were determined spectrophotometrically (Danovaro,
164 2010) and their sum, once converted into C equivalents (using 0.49, 0.40 and $0.75 \text{ mg C mg}^{-1}$,
165 respectively, as conversion factors), referred as the biopolymeric C (BPC) (Pusceddu et al., 2000).

166 The percentage contribution of phytopigments (converted into C equivalents) to biopolymeric C
167 content was referred as the algal fraction of the biopolymeric C pools (Pusceddu et al., 2009). The
168 percentage contributions of phytopigment and protein C to biopolymeric C concentrations and the
169 values of the protein to carbohydrate ratio were then used as descriptors of ageing and nutritional
170 quality of organic matter in the sediment (Pusceddu et al., 2010).

171

172 ***2.4 Meiofaunal variables***

173 Once in the laboratory, sediment samples were sieved through a 1000- μm mesh, and a 20- μm mesh
174 was used to retain the smallest metazoan organisms. The fraction remaining on the latter sieve was
175 re-suspended and centrifuged three times with Ludox HS40 (diluted with water to a final density of
176 1.18 g cm^{-3}), according to Danovaro (2010). All animals remaining in the supernatant were passed
177 again through a 20 μm mesh net, washed with tap water and, after staining with Rose Bengal, sorted
178 under a stereomicroscope at $\times 40$ magnification (Heip et al., 1985; Danovaro, 2010).

179 According to the literature and, therefore, for comparison purposes, meiofaunal abundance was
180 expressed as number of individuals 10 cm^{-2} , and their diversity expressed as richness of taxa (i.e.,
181 number of taxa \pm standard deviation from the three true and independent replicates). Overall, total

182 richness of taxa retrieved at each site and at each time was also expressed as the number of taxa
183 retrieved from all samples or all times, irrespectively of the replicates.

184

185 ***2.5 Indicators of benthic trophic status and environmental quality***

186 For the analysis of the benthic trophic status, we used a biochemical approach based on the
187 analysis of the quantity and nutritional quality of sedimentary organic matter (Dell'Anno et al.,
188 2002; Pusceddu et al., 2009). More in details, we chose the concentration of the main biochemical
189 compounds of sedimentary organic matter (phytopigments, protein, carbohydrate, lipid and BPC),
190 as indicators of OM quantity. Moreover, we used the contributions of phytopigment and protein to
191 biopolymeric C concentrations and the values of the protein to carbohydrate ratio as descriptors of
192 the aging and nutritional quality of sediment organic matter (Pusceddu et al., 2009). The percentage
193 contribution of total phytopigments to biopolymeric C is an estimate of the freshness of the organic
194 material deposited in the sediment: since photosynthetic pigments and their degradation products
195 are assumed to be labile compounds in a trophodynamic perspective, the lower their contribution to
196 sediment organic C the more aged the organic material. Moreover, since the percentage fraction of
197 organic C associated with phytopigments is also typically associated with a higher fraction of
198 enzymatically digestible (i.e. promptly available for heterotrophs) compounds (Pusceddu et al.,
199 2003), higher values of this percentage will also be indicative of a comparatively higher nutritional
200 quality (Dell'Anno et al., 2002). Since N is the most limiting factor for heterotrophic nutrition and
201 proteins, which are degraded at faster rates than carbohydrates, and are N-rich products, the protein
202 to biopolymeric C and the protein to carbohydrate ratios are indicative of both the aging and the
203 nutritional value of the organic matter (Dell'Anno et al., 2002; Pusceddu et al., 2009).

204 According to Dell'Anno et al. (2002), hyper-trophic systems are characterized by protein and
205 carbohydrate concentrations > 4 and $> 7 \text{ mg g}^{-1}$, respectively. Eutrophic systems are characterised
206 by protein and carbohydrate concentrations comprise from 1.5 to 4 mg g^{-1} and from 5 to 7 mg g^{-1} ,
207 respectively. Finally, meso-oligotrophic systems display protein concentrations $< 1.5 \text{ mg g}^{-1}$ and

208 carbohydrate concentrations 5 mg g^{-1} . Pusceddu et al. (2009; 2011) identified eutrophic systems as
209 those characterized by BPC concentration $>3 \text{ mg g}^{-1}$ and its algal fraction $<12\%$; mesotrophic
210 systems characterized by BPC concentration $1\text{-}3 \text{ mg g}^{-1}$ and its algal fraction $12\text{-}25\%$ and
211 oligotrophic systems as those characterized by BPC concentration $<1 \text{ mg g}^{-1}$ and its algal fraction
212 $>25\%$.

213 In this study, the environmental quality of the investigated sediments was assessed using the
214 richness of higher meiofaunal taxa according to Danovaro et al. (2004). Following this
215 classification (though not responding to the terminology requested by the WFD), the sediments are
216 ranked as heavily impacted (number of taxa ≤ 4), moderately impacted (number of taxa = 4-7), with
217 sufficient (number of taxa = 8-11), good (number of taxa = 12-16) or excellent (number of taxa
218 ≥ 16) quality.

219

220 **2.6 Statistical analyses**

221 Differences among sampling sites and times, were analyzed in either the uni- and multivariate
222 contexts, following the same sampling design, with sampling site (3 levels: Senigallia, Falconara,
223 Portonovo), and sampling time (10 levels) as fixed and orthogonal factors. All the analyses were
224 carried out using the distance-based permutational analysis of variance (PERMANOVA; Anderson,
225 2001; McArdle and Anderson, 2001) and the tests were based on matrixes of Euclidean distance
226 after normalization of the data (for OM), on Bray Curtis similarity matrixes (for meiofaunal
227 abundance) and on Jaccard resemblance measure after presence/absence transformation (for
228 meiofaunal taxonomic composition) (Anderson, 2001; McArdle and Anderson, 2001).

229 In the univariate context, the analysis were carried out separately for each variable, while in the
230 multivariate context, we analyzed the OM biochemical composition (using the concentration of
231 phytopigment, protein, carbohydrate and lipid), the OM nutritional quality (using the percentage
232 contributions of phytopigment and protein to biopolymeric C concentrations and the values of the
233 protein to carbohydrate ratio) and the meiofaunal taxonomic composition. For both uni- and

234 multivariate analyses, when significant effects of the considered factors were observed, pair wise
235 tests were also carried out, to ascertain in which sampling sites and/or times the significant
236 differences were observed. To discriminate significant peaks pair-wise tests were also carried out
237 after PERMANOVA for the significant terms.

238 To visualize differences in the sedimentary OM biochemical composition and nutritional quality
239 as well as meiofaunal assemblage composition among sampling sites and/or times, bi-plots after a
240 Canonical Analysis of Principal Coordinates (CAP) were also prepared (Anderson and Willis,
241 2003). To assess the percentage dissimilarity (Gray, 2000) in the meiofaunal taxonomic
242 composition among sampling sites and/or times and to identify the meiofaunal taxa most
243 responsible for the observed differences, SIMPER analyses were carried out.

244 To determine whether the meiofaunal communities are influenced by the different biochemical
245 compounds of organic matter or sediment characteristics (i.e., sediment grain size), we carried out
246 multivariate multiple regression analyses (DistLM forward, McArdle and Anderson, 2001). P
247 values were obtained with 4,999 permutations of residuals under the reduced model. Linear
248 relationships between quantity and quality of sediment organic matter and between the richness of
249 taxa (used here as the descriptor of environmental quality) and the variables found to explain
250 significant proportions of its variations in the DistLM analyses were also investigated using linear
251 regressions, in order to evaluate the direction and scale of variations. Uni- and multivariate
252 PERMANOVA, pair wise, CAP, SIMPER and DistLM forward tests were carried out using the
253 routines included in the software PRIMER 6+ (Clarke and Gorley, 2006). Linear regressions were
254 carried out using the Excel software.

255

256

257 **3. Results**

258 The chlorophyll-a, phaeopigment, total phytopigments, protein, carbohydrate, lipid and
259 biopolymeric C contents, as well as the chlorophyll-a and protein contributions to biopolymeric C
260 and the protein to carbohydrate content ratio in the sediments, as well as total meiofaunal
261 abundance and richness of taxa are given in Table 1 and Figures 2 and 3.

262 The results of the PERMANOVA tests revealed significant effects of the interaction Site \times Time on
263 contents, biochemical composition and nutritional quality of OM (Table 2), as well as on total
264 meiofaunal abundance, richness of taxa and taxonomic composition (Table 3).

265

266 ***3.1 Biochemical composition and nutritional quality of sedimentary organic matter***

267 Organic matter content, algal and protein fractions of biopolymeric C (BPC) and values of the
268 protein to carbohydrate ratio in the sediment were significantly higher at Portonovo and Senigallia
269 than at Falconara in all sampling times, with few exceptions (i.e., May, July and September; Table
270 1, Supplementary Table S1). At all sites, all OM variables displayed significant temporal variations,
271 though with different patterns for the different variables (Supplementary Table S1).

272 In almost all samplings the OM biochemical composition and its nutritional quality varied
273 significantly among sampling sites (Supplementary Table S1). The differences in the biochemical
274 composition among sites were mostly due to highest phytopigment, protein, carbohydrate and lipid
275 contents at Portonovo in almost all times (with exception of September, when the differences were
276 due to highest protein and lipid contents at Portonovo and highest phytopigment and carbohydrate
277 contents at Falconara). OM composition varied significantly also among sampling times at each
278 site, and were mostly driven by changes in phytopigment sedimentary contents (which peaked in
279 December at Senigallia, in May at Falconara, and in March at Portonovo; Figure 4A; Table 1).

280 The OM nutritional quality varied among sites due to the highest values of the protein fraction of
281 BPC and protein to carbohydrate ratio at Portonovo and Senigallia at all times (Table 1). Such
282 variability was also due to the highest algal fraction of BPC, reported in different sampling times

283 depending on the site (at Senigallia and Portonovo in January-February, at Falconara in November,
284 May, July and September and at Portonovo in March and June; Figure 2B). Temporal variations in
285 the OM nutritional quality were driven by different combinations of variables at the three sampling
286 sites: in particular, at Senigallia and Falconara by variations in the algal fraction of BPC, and at
287 Portonovo by changes in the protein fraction of BPC and the values of the protein to carbohydrate
288 ratio (Figure 4B). Peaks in the algal fraction of BPC occurred in different sampling times depending
289 on the site (i.e. December in Senigallia, May at Falconara, March at Portonovo; Figure 2B),
290 whereas a significant peak in the protein to carbohydrate ratio occurred in July at Portonovo (Figure
291 4B, Table 1).

292

293 ***3.2 Meiofaunal abundance and diversity***

294 Total meiofaunal abundance varied significantly among sampling sites in almost all sampling
295 times, and generally showed the highest values at Portonovo or Senigallia, with few exceptions,
296 when the highest values were observed at Falconara (Figure 3A; Supplementary Table S2). Total
297 meiofaunal abundance varied significantly also among sampling times at each sampling site. At
298 Senigallia, a significant lowest peak in meiofaunal abundance was observed in March, whereas
299 values remained almost invariant during all other sampling times. At Falconara the significantly
300 lowest value was observed in February, whereas significantly increasing values were observed from
301 February to May and from June to September. At Portonovo, the total meiofaunal abundance
302 showed a significantly lowest value in October and a significant highest peak in November-
303 December (Figure 3A).

304 The richness of higher taxa showed significant differences among sampling sites in 3 sampling
305 times, with highest values at Portonovo or Senigallia (Figure 3B; Supplementary Table S2). The
306 richness of higher taxa varied significantly also among sampling times at each sampling site. At
307 Senigallia and Portonovo the number of meiofaunal higher taxa showed significantly highest values
308 in November and/or December and at Falconara peaked up significantly in June (Figure 3B). At all

309 sampling sites and times nematodes were the dominant taxon, representing 80-90% of the total
310 abundance (with exception at Falconara in June), followed by copepods (2.2-6.5%), bivalves (0.3-
311 2%), polychaetes (0.4-0.9%) and 12 other taxa (cumulatively 0.7-1.9%). Among the other taxa
312 (accounting each <1%), ostracods, kinorhynchs, oligochaetes, cumaceans, amphipods, isopods,
313 halacarids, priapulids larvae, holoturians, gnatostomulids, juveniles of ophiuroids and of hydrozoas
314 were retrieved.

315 The total richness of higher taxa varied from 5 to 11, from 5 to 9 and from 5 to 10 at Senigallia,
316 Falconara and Portonovo, respectively. Accordingly, following the classification proposed by
317 Danovaro et al. (2004), sediments at Senigallia, with sufficient quality in 70% of the sampling
318 times, good conditions in December 2011 and moderately impacted conditions during winter, were
319 characterized by generally better environmental quality scores than those observed in the two other
320 sampling sites. At Falconara and Portonovo, the sediments were ranked as moderately impacted in
321 40% of the sampling times (September-October and February-March) and showed a sufficient
322 environmental quality in all other sampling times.

323 The taxonomic composition of the meiofaunal community varied significantly among sampling
324 sites in all sampling times (with exception in May; Supplementary Table S2). Significant
325 differences were also observed among times at each site, and, in particular: i) at Senigallia between
326 November and December, between January-February and March, and between March and June; ii)
327 at Falconara among almost all sampling times and iii) at Portonovo between December-January and
328 February and among May, June, July and September (Figure 5; Supplementary Table S2).

329 The SIMPER analysis (Supplementary Table S3) revealed that the highest dissimilarity among
330 sampling sites occurred in October, November, February and June (on average 48, 43, 61 and 43%,
331 respectively). In these times, the highest dissimilarity was consistently observed between Falconara
332 and the other two sampling sites. The highest dissimilarity among sampling times occurred at
333 Falconara (on average 36%), followed by Portonovo and Senigallia (on average 24%, for both
334 sites).

335 Variations in nematode and copepod abundance were responsible for the observed percentage
336 dissimilarity, both comparing different sites at each time and sampling times at each site (SIMPER,
337 Supplementary Table S3).

338

339 ***3.3 Relationships between meiofauna and organic matter quantity and quality***

340 The results of the DistLM forward analyses revealed that the variability in the meiofaunal
341 abundance were significantly explained only by the concentration of chlorophyll-a, explaining ca.
342 3% of the observed variance (Table 6). Copepods abundance variability was significantly explained
343 by sediment grain size, OM contents and nutritional quality, whereas all the explored independent
344 variables had no effects on nematode abundance and the overall meiofaunal taxonomic
345 composition. The variability in the richness of meiofaunal higher taxa was explained for ca, 43% by
346 OM contents (in terms of phytopigment, protein, carbohydrate and lipid), for ca. 14% by OM
347 nutritional quality (i.e., protein contribution to BPC and protein to carbohydrate ratio) and for ca.
348 13% by sediment features (i.e., grain size).

349 The regression analysis indicated a significant and positive relationship between chlorophyll-a
350 and BPC sedimentary contents ($p < 0.05$, R^2 0.330; Figure 6A), and between BPC contents and
351 protein to carbohydrate ratio ($p < 0.05$, R^2 0.304; Fig. 6B). The regression analyses between
352 meiofaunal diversity and benthic trophic status revealed a significant and positive relationship
353 between BPC contents and richness of meiofaunal higher taxa ($p < 0.05$, R^2 0.282; Figure 7A). A
354 significant and positive relationship was also observed between protein to carbohydrate ratio and
355 the richness of meiofaunal higher taxa ($p < 0.05$, R^2 0.248; Figure 7B).

356

357

358 **4. Discussion**

359 ***4.1 Trophic status of coastal sediments in the Northern Adriatic Sea***

360 The protein, carbohydrate, lipid and biopolymeric C contents in the sediments have been proposed
361 and utilized to assess the benthic trophic status of marine coastal environments, including the
362 Adriatic Sea (Dell'Anno et al., 2002; 2003; Vezzulli and Fabiano, 2006). In particular, Dell'Anno et
363 al. (2002), using the protein and carbohydrate sedimentary contents as proxies, identified thresholds
364 for ranking the trophic status and the environmental quality of coastal marine ecosystems along the
365 Apulian region. Applying those thresholds to the investigated sediments, the trophic status of
366 Senigallia, Falconara and Portonovo can be overall ranked as meso-oligotrophic in terms of
367 carbohydrate contents ($<5 \text{ mg g}^{-1}$ at all sites in all times), meso-oligotrophic at Falconara, and from
368 meso-oligotrophic to eutrophic at Senigallia and Portonovo in terms of protein contents. Pusceddu
369 et al. (2009) proposed an additional classification of the benthic trophic status based on a
370 combination of biopolymeric C content and algal contribution to the biopolymeric carbon, which
371 has proven to be effective in comparing the benthic trophic status of different sites from the Adriatic
372 and Tyrrhenian Seas (Pusceddu et al., 2011). Based on the BPC content thresholds identified by
373 Pusceddu et al. (2009), the sediments of the three sites analyzed in the present study can be
374 classified as: i) meso-oligotrophic for Portonovo ($\text{BPC} = 1\text{-}3 \text{ mg C g}^{-1}$) and ii) oligotrophic for
375 Senigallia and Falconara ($\text{BPC} < 1.0 \text{ mg C g}^{-1}$). Finally, in terms of algal contribution to
376 biopolymeric C, all sampling sites would be classified as oligotrophic (algal fraction $>25\%$ at all
377 sites, in almost all periods).

378 Whatever the thresholds considered, the results of this study are apparently in contrast to the
379 common ranking of the Adriatic Sea as a eutrophic basin, due to the large input of nutrients from
380 the Po river delta (Cozzi and Giani, 2011). On the one hand, we must stress here that such a
381 discrepancy could be due to the different approaches for classifying the trophic status used here or
382 in previous studies (based on sedimentary features) and those used in other investigations (with
383 inorganic nutrients and/or chlorophyll-a in the sea water as synthetic descriptors). Indeed, such a

384 discrepancy has been already highlighted in previous studies (Dell'Anno et al., 2002). Moreover, it
385 is worth mentioning that the classification of the benthic trophic status of the Adriatic Sea as "oligo-
386 mesotrophic" finds confirmation in recent studies that have shown that this basin has undergone, in
387 the last 10-20 years, a "regime shift" that have profoundly modified its ecological features,
388 including the decreasing levels of productivity during the period 1985-2010 (Conversi et al., 2010;
389 Gasparovic, 2012; Giani et al., 2012). As a confirmation of this trend, we report here that
390 chlorophyll-a and biopolymeric C contents in the sediments of the coastal NW Adriatic Sea in this
391 study are up to 5 and 3 folds (for chlorophyll-a and biopolymeric C) lower than those reported 15
392 years ago (Danovaro et al., 2000). The apparent progressive "oligotrophication" of the NW Adriatic
393 Sea has been recently linked to the strong reduction in river nutrient inputs from main tributaries of
394 the basin, and, in particular, of the Po and Adige rivers (Cozzi and Giani, 2011; Cozzi et al., 2012).
395 The consistency of the sedimentary trophic conditions changes to those observed in the water
396 column productivity further confirms the robustness of the concentration of chlorophyll-a and
397 biopolymeric C as reliable benthic trophic status descriptors, and suggests their feasible use also for
398 long-term studies of variations in the energy regime of an ever complex basin like the NW Adriatic
399 Sea.

400 The criteria and indicators identified in Italy to achieve the objectives of Descriptor 5
401 (Eutrophication) of the EU Marine Strategy Framework Directive (2008/56/EC) for the Adriatic Sea
402 sub-region (<http://www.strategiamarina.isprambiente.it/descrittore-5-2013-eutrofizzazione>) do not
403 include yet the assessment of environmental status based on sediment organic matter enrichment.
404 Our results indicate that both the quantity and the biochemical composition of sedimentary organic
405 matter, being characterized by large spatial-temporal variability, can be used as combined
406 descriptors of the benthic trophic status (and eutrophication) of marine coastal sediments
407 (Dell'Anno et al., 2002; Pusceddu et al., 2009). As such, we postulate that our approach to assess
408 the trophic status of marine coastal sediments, providing a contextual and complementary
409 assessment of eutrophication effects on the most sensible portion of marine coastal ecosystems (i.e.

410 the benthos) should become part of the assessment for MSFD Descriptor 5. In this regard, however,
411 we must acknowledge here that our classification does not precisely reflects the quality
412 classification proposed by the EU directives. Indeed, the five canonical categories of the WFD
413 (namely, High, Good, Moderate, Poor and Bad) cannot be reliably applied to the trophic status of
414 marine ecosystems, which, per se, cannot be ranked in qualitative terms but only in quantitative
415 terms (i.e., from oligotrophic to hypertrophic or dystrophic; Nixon 1995; Cloern, 2001; Pusceddu et
416 al., 2009).

417 At the same time, we also pinpoint that the trophic status defined trough descriptors related to
418 productivity of the water column or even OM accumulation in marine sediments cannot be alone
419 considered as general descriptors of environmental quality. Indeed, highly productive systems such
420 as coastal lagoons are naturally hypertrophic systems but, unless impacted by human disturbance
421 and over-threshold levels of OM accumulation, they should be considered as characterized by good
422 environmental status (Pusceddu et al., 2003; 2011). On the other hand, highly oligotrophic systems
423 can be at times affected by detrimental agents, such as pollution, potentially able not only to further
424 reduce their low productivity levels, but also to reduce their ability to re-cycling organic detritus
425 (Pusceddu et al., 2003). In this regard, we infer that the synoptic analysis of benthic trophic status
426 and the response of meiofaunal communities (in terms of abundance, diversity and composition) to
427 changes in quantity and biochemical composition of sedimentary OM could provide a reliable
428 information about the environmental quality of marine coastal ecosystems.

429

430 ***4.2 Relationships between meiofauna biodiversity and trophic status***

431 We report here that the quantity of sedimentary organic matter, expressed as BPC
432 concentration, increased significantly with the increasing of chlorophyll-a concentration (Figure
433 6A), indicating that, under the prevalent oligo-mesotrophic conditions observed in the study area,
434 the organic enrichment of the sediment was associated with an increase of primary organic material.
435 We show here also that the increased contribution of primary organic matter to the organic pools

(i.e. high phytopigment concentrations) enhances the nutritional quality of sedimentary organic matter. This is confirmed by the significant relationship between the sedimentary BPC contents and the protein to carbohydrate ratio (used as a proxy of higher nutritional quality; Pusceddu et al., 2000; Figure 6B). In the investigated sediments, characterized by oligo- and mesotrophic conditions, the richness of meiofaunal taxa was linked to the increase in BPC sedimentary contents and the protein to carbohydrate content ratio (Figure 7). Previous studies highlighted the presence of tight relationships between changes in the trophic status of marine sediments and the biodiversity of meiofauna under different environmental conditions and ecological alteration (e.g., Pusceddu et al., 2007a; 2011; Cerrano et al., 2010; Bianchelli et al., 2013). Nevertheless, such a relationship is not consistently positive and it has been shown that the pattern of meiofaunal biodiversity responses can vary among systems characterized by different levels of initial benthic trophic status (Danovaro et al., 2004). For instance, it has been shown that the impact of aquaculture biodeposition and consequent eutrophication due to the accumulation of fish feces and uneaten food (Pusceddu et al., 2007b) can be easily detected and have a clear impact on meiofauna, especially in the initial conditions stages, when the system shifted from oligo- to mesotrophic conditions (Mirto et al., 2010; 2014; Pusceddu et al., 2011). Nevertheless, it has been repeatedly demonstrated that a strong accumulation of biopolymeric organic C, mostly accounted for by material of detrital/heterotrophic origin, may anyway lead to profound modifications of sediment distinctive features (e.g., oxygen availability; Pusceddu et al., 2009), which could in turn affect negatively the meiofaunal assemblages (Gambi et al., 2009).

Previous studies conducted in the same regional area (Frontalini et al., 2011; Semprucci et al., 2013) reported similar taxonomic composition of meiofaunal communities, with nematodes as dominant taxon, followed by polychaetes, copepods, oligochaetes and platyhelminthes. The results of the present study indicate that the composition of meiofaunal assemblages changed significantly among sites and among sampling times at each site. In particular, we report here that the highest dissimilarity in the structure of meiofaunal assemblages occurs between the putatively most

462 impacted site (i.e., Falconara) and the two less impaired ones. In that site we observed also the
463 highest taxonomic variability among sampling times. Such variability between sites and times was
464 mostly explained by changes in the relative abundance of the most abundance taxa (i.e., nematodes
465 and copepods) (Supplementary Table S3). However, the results of the multiple multivariate
466 regression analyses highlighted that OM variables explained only a negligible proportion of the
467 variance in meiofaunal variables, leading to hypothesize that other stressors, not considered in this
468 study, could be responsible for the observed changes.

469 The assessment of environmental status based on the meiofaunal taxa richness (Danovaro et al.,
470 2004) allowed us to identify a prevalence of sufficient or moderately impacted conditions. This
471 means that while the trophic status of the investigated area appears to be well far from being
472 affected by eutrophication, and besides the potential discrepancies between the classification used
473 here and the one proposed by the WFD, the overall environmental quality of the coastal sediments
474 investigated is far from a Good Environmental Status (GES). On the one hand, our results suggest
475 that the relatively low level of environmental quality of N Adriatic Sea sediments cannot be
476 uniquely the result of benthic eutrophication. On the other hand, this result confirms that changes in
477 the trophic status alone cannot represent a synthetic descriptor of environmental quality. This
478 becomes even more evident when the presence of multiples stressors contributes to impair the
479 environmental quality of benthic coastal ecosystems. Our data suggest that the analysis of benthic
480 trophic status based on organic matter variables can be influenced by different co-occurring
481 stressors. We suggest that the contextual analysis of benthic trophic status based on organic matter
482 variables and the analysis of meiofaunal response to changes in organic loads, could represent a
483 reliable tool to assess the environmental status in coastal marine sediments.

484

485

486

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491

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642

643 **Caption of Figures**

644 **Figure 1.** Location of the sampling sites (Senigallia, Falconara and Portonovo) in the Northern
645 Adriatic Sea. (The red dot indicates the study area).

646 **Figure 2.** Concentration of biopolymeric C (A) and its algal contribution (B) in sediments of
647 Senigallia, Falconara and Portonovo in all investigated sampling times.

648 **Figure 3.** Total meiofaunal abundance (A) and richness of higher taxa (B) at Senigallia, Falconara
649 and Portonovo in all investigated sampling times. Reported are also the total number of taxa (B)
650 retrieved at each sampling site and time.

651 **Figure 4.** Output of canonical analysis of principal coordinates (CAP) on sedimentary organic
652 matter biochemical composition (A) and nutritional quality (B) at Senigallia, Falconara and
653 Portonovo. (Chla-BPC = algal fraction of BPC, PRT:BPC = protein fraction of BPC, PRT:CHO
654 = protein to carbohydrate ratio).

655 **Figure 5.** Output of canonical analysis of principal coordinates (CAP) on meiofaunal taxonomic
656 composition at Senigallia, Falconara and Portonovo.

657 **Figure 6.** Relationships between chlorophyll-a and biopolymeric C contents (A) and between
658 biopolymeric C contents and the values of the protein to carbohydrate ratio (B) in the sediment.

659 **Figure 7.** Relationships between meiofaunal taxa richness and biopolymeric C contents (A) and
660 values of the protein to carbohydrate ratio (B) in the sediment.

661

Table 1. OM sedimentary contents and nutritional quality: concentration of phytopigments (in terms of chlorophyll-a, phaeopigments and total phytopigments), protein, carbohydrate, lipid, protein to BPC and protein to carbohydrate ratios are reported.

		Chlorophyll-a ($\mu\text{g g}^{-1}$)		Phaeopigment ($\mu\text{g g}^{-1}$)		Total Phytopigment ($\mu\text{g g}^{-1}$)		Protein (mg g^{-1})		Carbohydrate (mg g^{-1})		Lipid (mg g^{-1})		Protein: BPC %		Protein: carbohydrate	
		Avg	Sd	Avg	sd	Avg	sd	avg	sd	avg	sd	avg	sd	avg	sd	avg	sd
Senigallia	October 2011	0.77	0.28	3.81	1.02	4.58	1.31	0.86	0.08	0.27	0.03	0.03	0.01	75.97	0.49	3.20	0.02
	November 2011	0.42	0.10	3.61	1.04	4.03	1.13	0.60	0.11	0.14	0.02	0.03	0.00	78.37	1.09	4.21	0.07
	December 2011	1.50	0.34	12.81	2.25	14.31	2.57	2.65	1.96	0.31	0.04	0.15	0.05	79.50	4.19	8.12	1.80
	January 2012	0.22	0.05	3.51	0.26	3.73	0.21	0.59	0.03	0.15	0.02	0.04	0.02	77.36	3.78	4.07	0.31
	February 2012	0.20	0.09	2.48	0.91	2.68	1.00	0.67	0.09	0.15	0.02	0.11	0.09	70.64	8.18	4.35	0.14
	March 2012	0.03	0.01	1.45	0.34	1.48	0.33	0.41	0.17	0.10	0.03	0.02	0.00	77.31	2.90	4.08	0.27
	May 2012	0.61	0.53	1.50	0.87	1.52	0.74	1.10	0.32	0.50	0.43	0.04	0.04	74.58	4.69	3.67	2.88
	June 2012	0.32	0.08	1.62	0.41	1.94	0.49	0.63	0.08	0.13	0.05	0.02	0.01	81.87	4.42	5.13	1.32
	July 2012	0.81	0.20	3.59	0.39	4.41	0.59	1.17	0.08	0.15	0.04	0.03	0.00	87.39	1.60	8.29	1.79
	September 2012	0.34	0.11	2.70	0.68	3.04	0.57	0.91	0.04	0.15	0.05	0.03	0.01	84.49	3.32	6.25	1.67
Falconara	October 2011	0.68	0.09	1.58	0.12	2.25	0.21	0.46	0.11	0.17	0.01	0.03	0.01	71.75	2.69	2.75	0.51
	November 2011	1.06	0.27	2.58	0.88	3.64	1.15	0.41	0.03	0.19	0.02	0.03	0.00	67.97	0.67	2.21	0.05
	December 2011	0.63	0.13	1.71	0.35	2.35	0.47	0.30	0.02	0.13	0.02	0.02	0.00	69.29	1.61	2.31	0.21
	January 2012	0.30	0.06	1.29	0.13	1.59	0.19	0.74	0.57	0.14	0.03	0.01	0.00	78.15	4.67	4.69	2.90
	February 2012	0.11	0.00	0.40	0.03	0.51	0.03	0.29	0.04	0.11	0.03	0.01	0.00	73.78	1.69	2.68	0.29
	March 2012	0.21	0.02	2.20	0.51	2.42	0.52	0.35	0.03	0.18	0.01	0.03	0.01	65.81	0.80	1.99	0.03
	May 2012	0.77	0.26	7.85	0.89	8.62	1.15	0.91	0.26	0.29	0.06	0.04	0.02	75.10	0.52	3.14	0.20
	June 2012	0.17	0.01	1.22	0.19	1.39	0.18	0.53	0.05	0.14	0.00	0.01	0.00	80.27	0.57	3.86	0.32
	July 2012	0.94	0.20	2.44	0.45	3.38	0.56	0.69	0.14	0.18	0.02	0.03	0.00	78.57	1.75	3.82	0.36
	September 2012	1.88	0.19	3.66	0.94	5.54	1.14	0.94	0.15	0.27	0.09	0.03	0.01	78.62	3.80	3.67	0.74
Portonovo	October 2011	1.28	0.27	9.55	0.43	10.83	0.16	2.15	0.36	0.51	0.05	0.12	0.02	78.16	0.72	4.15	0.27
	November 2011	0.90	0.14	6.39	0.93	7.30	1.07	1.54	0.30	0.49	0.10	0.09	0.01	74.33	0.13	3.18	0.07
	December 2011	1.26	0.31	14.19	2.77	15.45	3.08	2.33	0.29	0.50	0.10	0.14	0.06	79.39	2.60	4.74	0.38
	January 2012	0.40	0.06	13.05	1.00	13.45	1.05	1.84	0.31	0.40	0.05	0.12	0.07	78.60	2.42	4.63	0.16
	February 2012	0.23	0.08	2.49	0.23	2.72	0.30	0.73	0.07	0.16	0.03	0.02	0.00	81.12	1.53	4.52	0.54
	March 2012	2.30	0.35	16.24	3.82	18.54	4.01	1.20	0.31	0.48	0.11	0.17	0.05	64.73	0.07	2.51	0.10
	May 2012	0.58	0.09	2.74	0.05	3.32	0.15	1.19	0.23	0.26	0.06	0.07	0.03	78.99	1.67	4.62	0.23
	June 2012	0.35	0.06	3.54	0.15	3.89	0.09	1.01	0.21	0.19	0.01	0.03	0.00	83.44	1.80	5.28	0.76
	July 2012	1.24	0.09	3.56	0.42	4.80	0.33	2.58	1.16	0.22	0.05	0.09	0.03	88.23	2.42	11.18	2.97
	September 2012	0.70	0.12	4.81	1.06	5.51	1.17	1.79	0.52	0.29	0.05	0.07	0.01	83.52	1.89	6.01	0.77

664 **Table 2.** Results of PERMANOVA testing variations in the sedimentary OM contents (A)
665 indicators of nutritional quality (B), biochemical composition (C) and whole nutritional quality (D).
666 dF=degree of freedom; MS=mean square; F=F statistic; ***=P<0.001; **=P<0.01; *=P<0.05; ns=
667 not significant.

	Source	dF	MS	F	P	% explained variance
A) Chlorophyll-a	Site	2	3.9	32.2	***	9.2
	Time	9	3.3	26.8	***	25.4
	Site x Time	18	2.5	20.2	***	56.6
	Residual	60	0.1			8.9
	Total	89				
Phaeopigment	Site	2	11.7	170.1	***	27.3
	Time	9	2.6	37.4	***	19.6
	Site x Time	18	21.3	30.9	***	48.2
	Residual	60	689.0			4.8
	Total	89				
Total phytopigment	Site	2	11.2	160.4	***	26.1
	Time	9	25.7	36.8	***	19.5
	Site x Time	18	21.8	31.2	***	49.4
	Residual	60	699.0			4.9
	Total	89				
Protein	Site	2	14.7	39.7	***	37.9
	Time	9	21.1	57.0	***	15.3
	Site x Time	18	10.3	27.8	***	17.4
	Residual	60	0.4			29.4
	Total	89				
Carbohydrate	Site	2	11.1	29.7	***	28.1
	Time	9	19.5	51.9	***	13.7
	Site x Time	18	14.8	39.5	***	28.9
	Residual	60	0.4			29.4
	Total	89				
Lipid	Site	2	13.4	38.5	***	33.1
	Time	9	14.3	4.1	***	9.2
	Site x Time	18	15.8	45.4	***	31.3
	Residual	60	0.3			26.5
	Total	89				
Biopolymeric C	Site	2	16.0	46.6	***	40.8
	Time	9	1.9	55.7	***	13.6
	Site x Time	18	10.7	31.0	***	18.8
	Residual	60	0.3			26.8
	Total	89				
B) Chlorophyll-a to biopolymeric C	Site	2	0.8	34.0	*	1.4
	Time	9	31.4	13.4	***	24.2
	Site x Time	18	25.0	10.6	***	56.7
	Residual	60	0.2			17.7
	Total	89				
Protein to biopolymeric C ratio	Site	2	50.4	10.0	***	13.5
	Time	9	38.7	77.1	***	33.4
	Site x Time	18	0.8	15.5	ns	8.2
	Residual	60	0.5			44.8
	Total	89				
Protein to carbohydrate ratio	Site	2	75.5	18.3	***	20.1
	Time	9	34.4	83.4	***	28.4
	Site x Time	18	10.1	24.4	**	16.8
	Residual	60	0.4			34.8
	Total	89				
C) Biochemical composition	Site	2	54.8	42.7	***	26.8
	Time	9	11.3	88.3	***	16.8
	Site x Time	18	86.8	67.6	***	37.1
	Residual	60	12.8			19.3
	Total	89				
D) Nutritional quality	Site	2	13.4	11.6	***	11.2
	Time	9	10.5	90.9	***	28.4
	Site x Time	18	42.9	37.3	***	28.8
	Residual	60	11.5			31.6
	Total	89				

668

669 **Table 3.** Results of PERMANOVA testing differences in meiofaunal abundance (A), richness of
670 taxa (B) and taxonomic composition (C). dF=degree of freedom; MS=mean square; F=F statistic;
671 ***=P<0.001; *=P<0.05; ns= not significant.

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	Source	dF	MS	F	P	% explained variance
A) Total meiofaunal abundance	Site	2	300.5	20.8	ns	1.2
	Time	9	1281.4	88.8	***	29.6
	Site x Time	18	597.8	41.4	***	35.4
	Residual	60	144.4			33.8
	Total	89				
B) Richness of taxa	Site	2	213.9	88.2	***	10.5
	Time	9	224.6	92.5	***	37
	Site x Time	18	46.3	19.1	*	12.2
	Residual	60	24.3			40.3
	Total	89				
C) Meiofaunal taxonomic composition	Site	2	1519.2	64.216	***	5.81
	Time	9	2260	95.528	***	30.56
	Site x Time	18	930.96	39.351	***	31.46
	Residual	60	236.58			32.16
	Total	89				

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Table 4. Results of DistLM forward carried out to ascertain the role of different environmental variables on total meiofaunal, nematode, and copepods abundance, richness of meiofaunal taxa and meiofaunal taxonomic composition. SS=mean square; F=F statistic; ***=P<0.001; **=P<0.01; *=P<0.05; ns= not significant.

	Variable	SS	F	P	Prop %	Cumulative prop %
Meiofaunal total abundance	Chlorophyll-a	1153.4	3.3	*	3.7	3.7
	Chl-a to BPC%	848.1	2.4	ns	2.7	6.3
	Carbohydrate	725.4	2.1	ns	2.3	8.6
	Protein	404.0	1.1	ns	1.3	9.9
	PRT to CHO ratio	300.7	0.8	ns	1.0	10.9
	% mud	194.0	0.5	ns	0.6	11.5
	% sand	194.0	0.5	ns	0.6	12.1
	Phaeopigment	156.2	0.4	ns	0.5	12.6
	Lipid	144.4	0.4	ns	0.5	13.1
	PRT to BPC%	103.3	0.3	ns	0.3	13.4
Nematodes	Chlorophyll-a	1944.3	2.0	ns	2.2	2.2
	PRT to CHO ratio	1907.4	1.9	ns	2.2	4.3
	Chl-a to BPC%	1599.1	1.6	ns	1.8	6.2
	% mud	1175.6	1.2	ns	1.3	7.5
	% sand	1175.6	1.2	ns	1.3	8.8
	Carbohydrate	1134.4	1.1	ns	1.3	10.1
	Lipid	834.9	0.8	ns	0.9	11.0
	Phaeopigment	373.3	0.4	ns	0.4	11.4
	Protein	746.8	0.7	ns	0.8	12.3
Copepods	PRT to BPC%	619.1	0.6	ns	0.7	13.0
	Protein	24559.0	11.8	***	11.8	11.8
	% mud	19269.0	9.0	***	9.3	21.1
	% sand	19269.0	9.0	***	9.3	30.3
	PRT to BPC%	17557.0	8.1	***	8.4	38.8
	PRT to CHO ratio	15825.0	7.3	**	7.6	46.4
	Phaeopigment	12211.0	5.5	**	5.9	52.3
	Chlorophyll-a	10388.0	4.6	**	5.0	57.3
	Lipid	10452.0	4.7	**	5.0	62.3
	Carbohydrate	7655.0	3.4	*	3.7	66.0
Richness of meiofaunal taxa	Chl-a to BPC%	2805.7	1.2	ns	1.3	67.3
	Protein	2315.3	13.3	***	13.1	13.1
	Chlorophyll-a	1793.9	10.0	**	10.2	23.3
	PRT to CHO ratio	1554.8	8.5	**	8.8	32.1
	Phaeopigment	1231.8	6.6	**	7.0	39.1
	Lipid	1177.2	6.3	*	6.7	45.7
	% mud	1169.3	6.2	*	6.6	52.4
	% sand	1169.3	6.2	**	6.6	59.0
	Carbohydrate	1079.2	5.7	*	6.1	65.1
	PRT to BPC%	843.0	4.4	*	4.8	69.9
Taxonomic composition	Chl-a to BPC%	224.6	1.1	ns	1.3	71.1
	Chlorophyll-a	2285.9	2.2	ns	2.4	2.4
	PRT to CHO ratio	2152.5	2.1	ns	2.3	4.7
	Chl-a to BPC%	1617.1	1.5	ns	1.7	6.4
	% mud	1516.7	1.4	ns	1.6	8.1
	% sand	1516.7	1.4	ns	1.6	9.7
	Protein	1275.7	1.2	ns	1.4	11.0
	Carbohydrate	1350.9	1.3	ns	1.4	12.5
	Lipid	1257.1	1.2	ns	1.3	13.8
	PRT to BPC%	1092.4	1.0	ns	1.2	15.0
Taxonomic composition	Phaeopigment	770.9	0.7	ns	0.8	15.8

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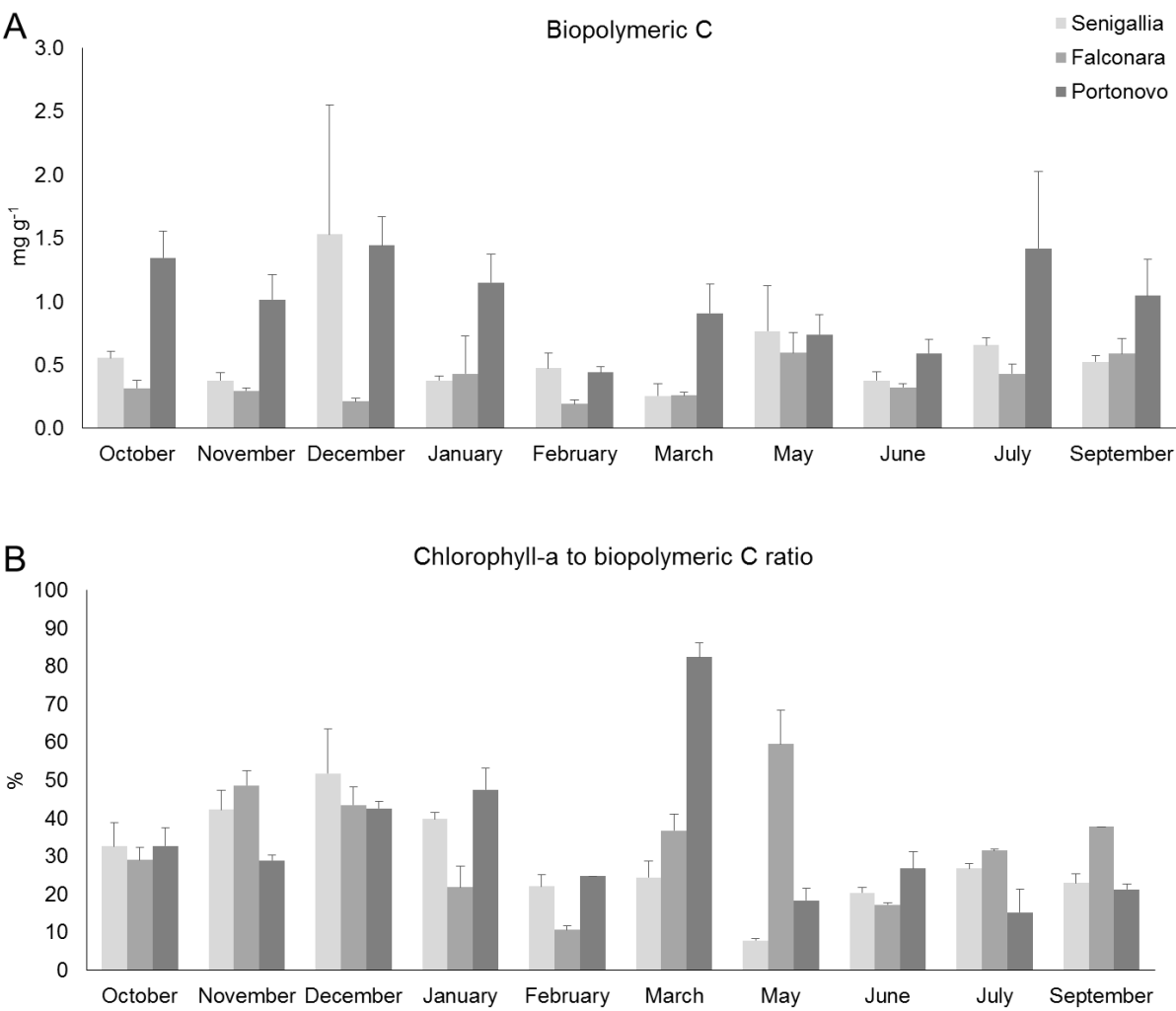
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685 **Figure 1**

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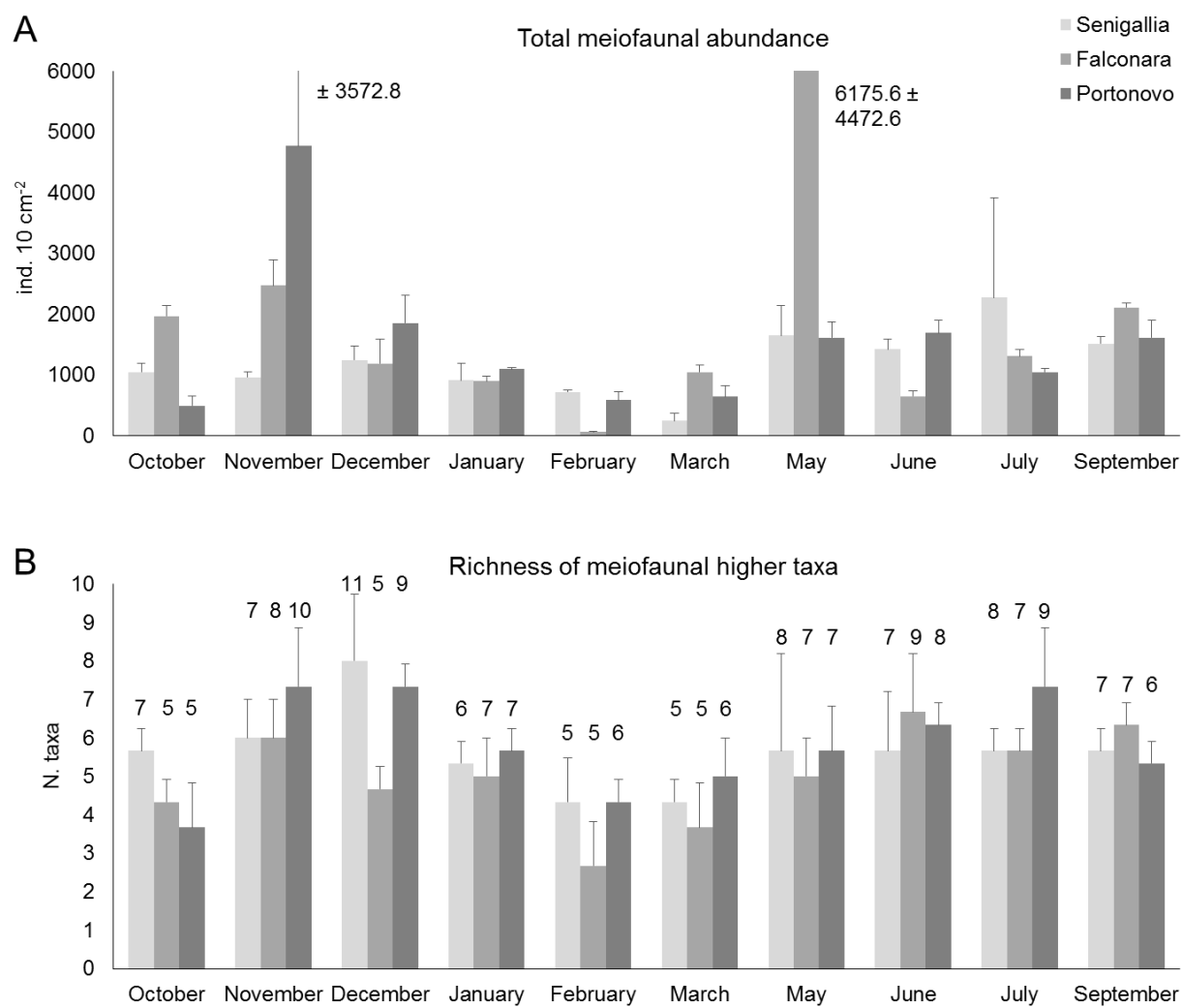


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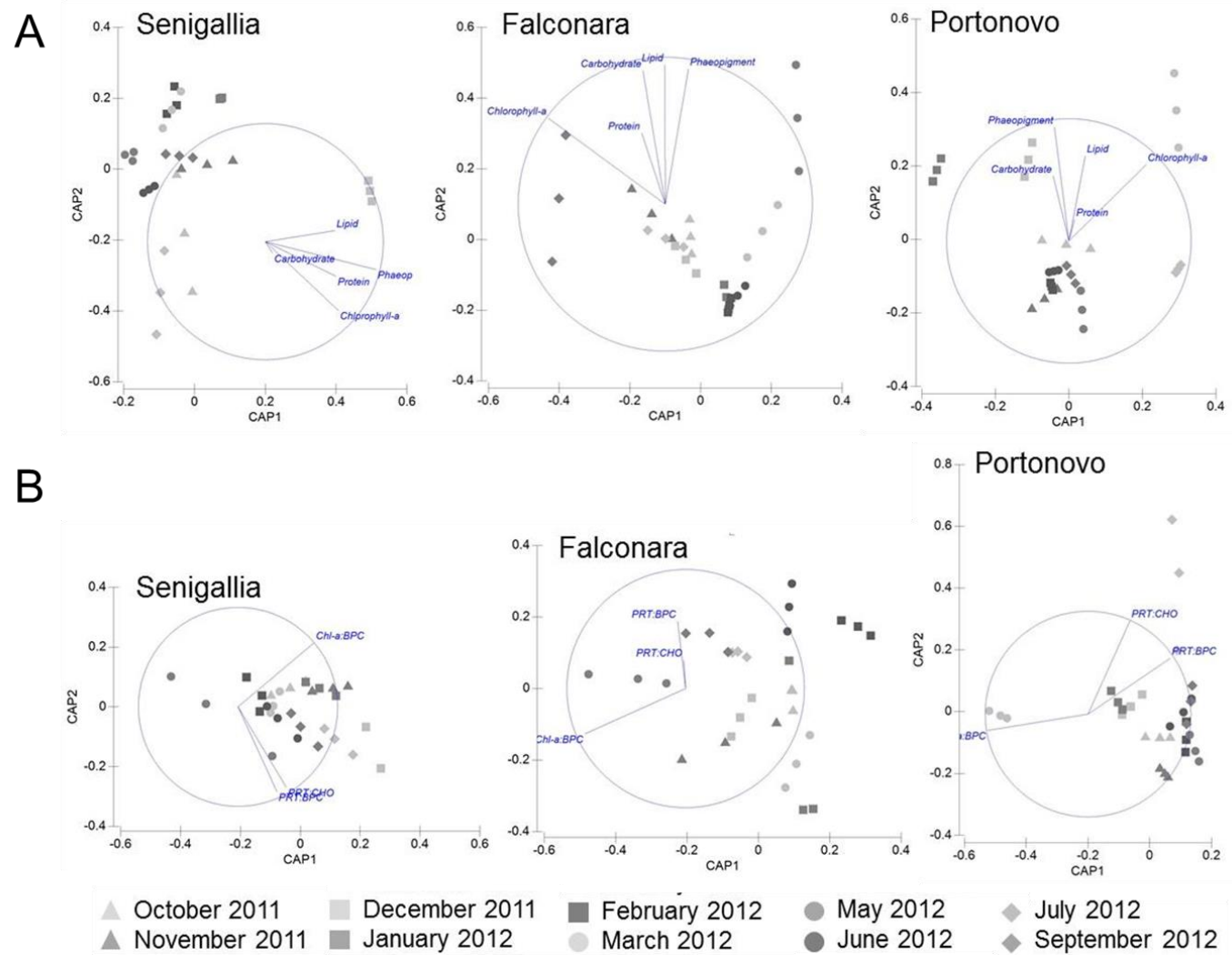
Figure 2

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692 **Figure 3**



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694 **Figure 4**

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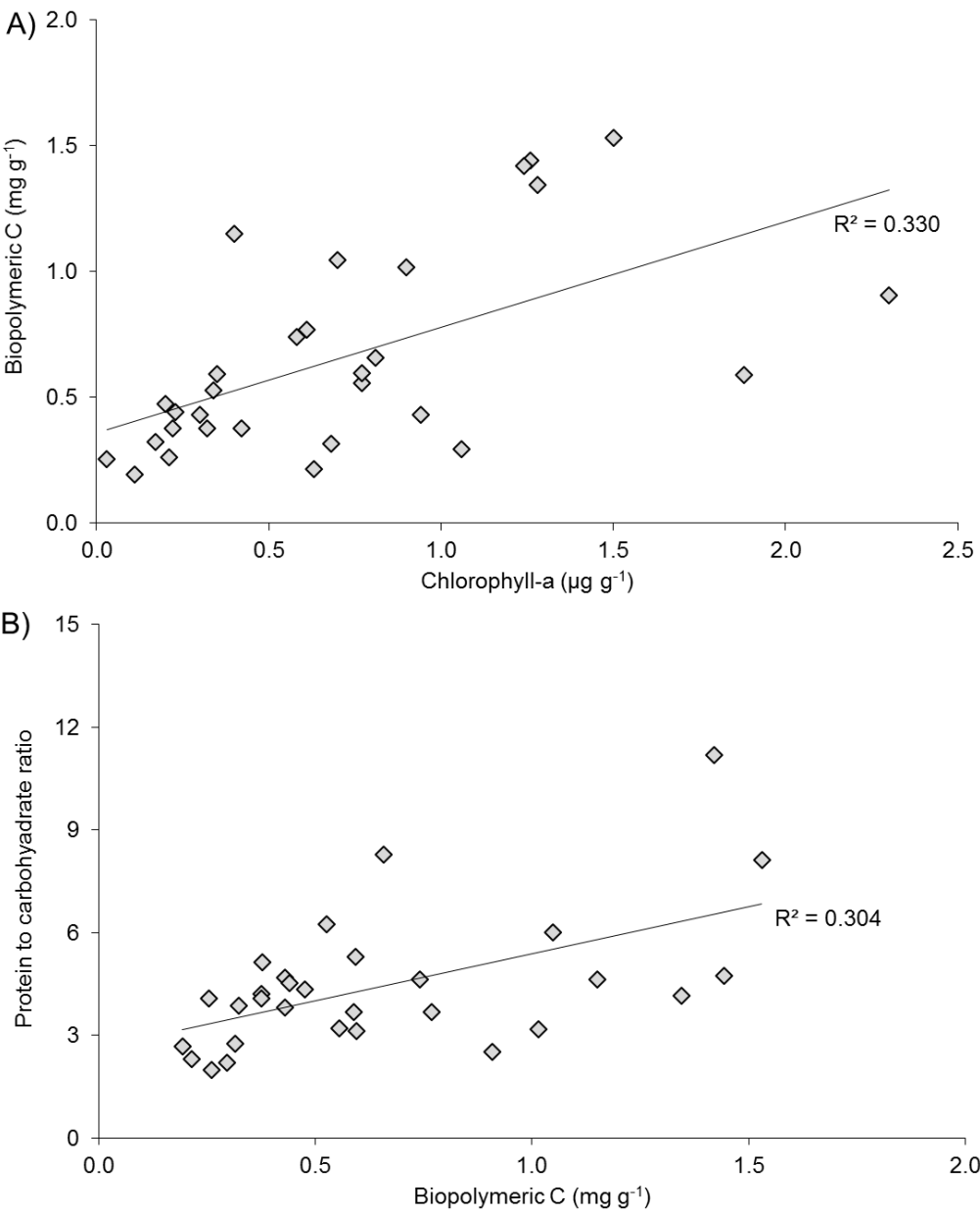


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Figure 5

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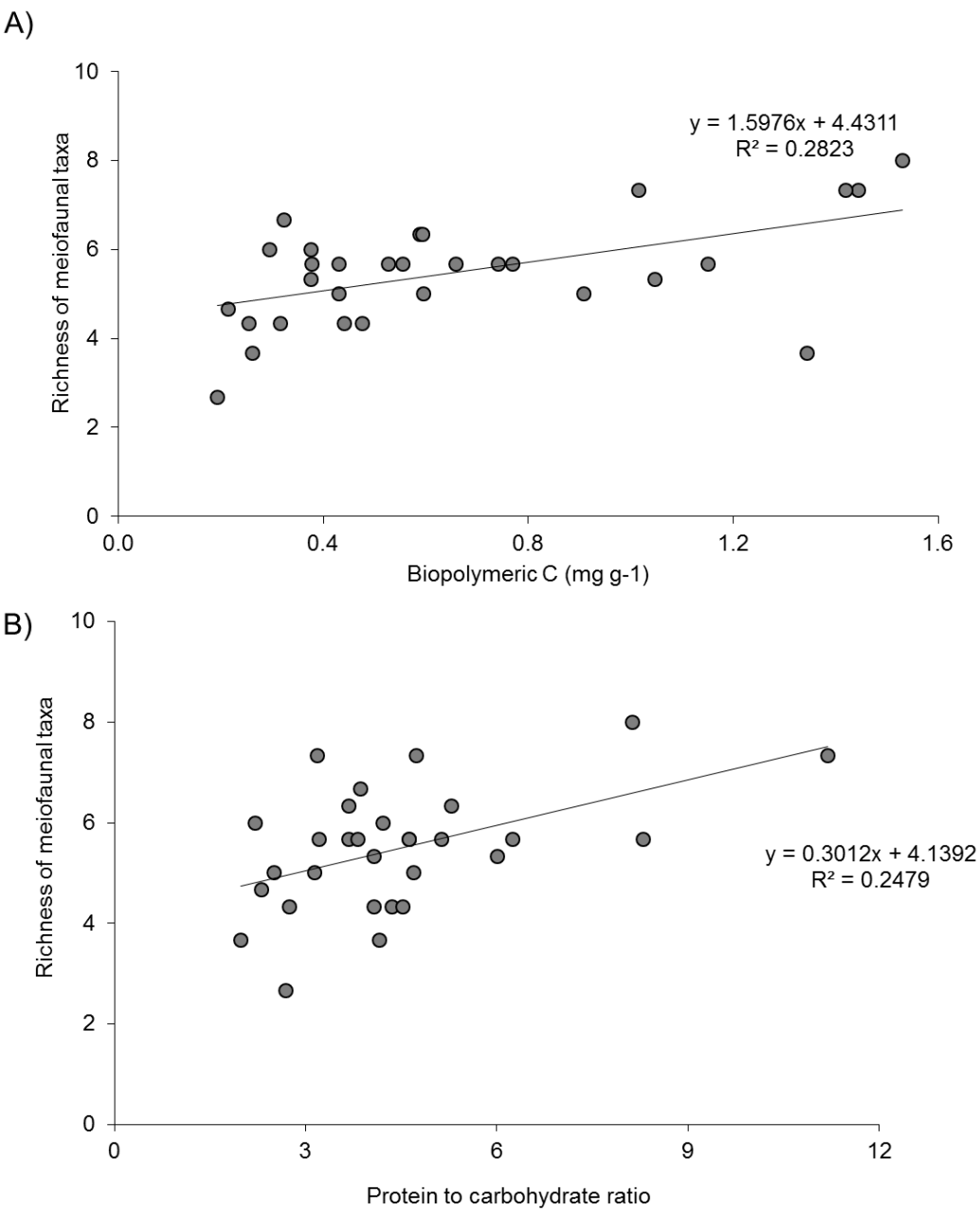
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704 **Figure 6**

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710 **Figure 7**

711 **Supplementary Table S1.** *Pair wise* tests conducted to test differences in the concentration and nutritional of OM among sites and times. Sen =
712 Senigallia, Fal = Falconara, Por = Portonovo, oct = October 2011, nov = November 2011, dec = December 2011, jan = January 2012, feb =
713 February 2012, mar = March 2012, may = May 2012, jun = June 2012, jul = July 2012, sep = September 2012, ns = not significant.

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Differences among sites	October 2011	November 2011	December 2011	January 2012	February 2012	March 2012	May 2012	June 2012	July 2012	September 2012
Chlorophyll-a	por>fal	por>sen	sen,por>fal	por>sen	sen,por>fal	por>fal>sen	ns	por>fal	por>sen	fal>por>sen
Phaeopigment	por>sen>fal	por>sen,fal	sen,por>fal	sen>fal>por	sen,por>fal	por>sen,fal	fal>sen,port	port>senig,fal	ns	ns
Total phytopigment	por>sen,fal	por>fal	sen,por>fal	por>sen>fal	sen,por>fal	por>sen	fal>sen,port	port>senig,fal	por>fal	ns
Protein	por>sen>fal	por>sen,fal	por>fal	por>sen	sen,por>fal	por>fal	ns	ns	sen,por>fal	ns
Carbohydrate	port>sen>fal	port>fal>sen	port>sen>fal	por>sen,fal	ns	por>sen,fal	ns	por>fal	ns	por,fal>sen
Lipid	por>sen,fal	por>sen,fal	sen>fal	por>sen,fal	sen>por>fal	por>sen,fal	ns	por>fal	por>sen,fal	por>sen
Biopolymeric C	por>sen>fal	por>sen>fal	por>fal	por>sen	sen,por>fal	por>sen,fal	ns	por>fal	sen>fal	ns
Chlorophyll-a to biopolymeric C	ns	sen>port	ns	ns	sen,por>fal	por>sen,fal	fal>port>sen	ns	fal>sen,por	fal>sen,port
Protein to biopolymeric C	por>sen,fal	sen>por>fal	por>fal	ns	por>fal	sen>fal,por	ns	ns	sen,por>fal	ns
Protein : carbohydrate ratio	por>sen,fal	sen>por>fal	por>fal	ns	sen,por>fal	sen>por>fal	por>fal	ns	sen,por>fal	por>fal
Biochemical composition (multivariate analysis)	por≠sen,fal	sen≠fal#por	fal≠port	sen≠fal#por	fal#por	port≠sen,fal	ns	fal≠port	ns	sen≠fal#por
Nutritional quality (multivariate analysis)	fal#por	sen≠fal#por	fal≠port	ns	fal#por	sen≠fal#por	fal≠port	fal≠port	sen,por≠fal	ns
Differences among times	Senigallia			Falconara			Portonovo			
Chlorophyll-a	dec>nov,jan,feb,mar; jan,jun,jul,sep>mar; jul>jun			oct,nov,dec>jan,feb,mar; jan,mar>feb; may,jul>jun; sep>jul			oct,nov,dec>jan,feb; mar>jan,feb,may,jun,jul,sep; may>jul; jul>jun,sep			
Phaeopigment	dec>oct,nov; may,jul>jun			oct,nov,dec,jan,mar>feb; may>mar; may,jul,sep>jun			oct,dec>nov; jan,mar>feb; jun,jul,sept>may			
Total phytopigment	dec>oct,nov,jan>feb; jul>jan,feb,mar,may,jun			oct,nov,dec>jan; may,jul,sep>jun; oct,nov,dec,jan,mar,may,jun,jul,sep>feb			oct,dec>nov; feb>dec,jan,mar; mar>may; jul,sep>jun			
Protein	oct,dec>jan>mar; jul>jan,feb,mar,may,jun; jul>sep			nov>dec; may>feb,mar; jul,sept>jun			oct,nov,dec,jan>feb; mar,may,jun,jul,sep>feb			
Carbohydrate	oct>nov,dec,jan,feb,mar,may,jun,jul,sep; dec>nov,jan,feb,mar			mar>feb; may,sep>jun,jul			oct,nov,dec,jan,mar>feb; mar,sep>jun,jul			
Lipid	nov,jul>mar; dec>oct,nov,jun,jul,sep			nov,dec,jan,mar>feb; may,jul>jun; jul,sep>jan,feb			oct,nov,dec,jan>feb; mar>feb,may,jun; jul,sept>jun			
Biopolymeric C	oct>nov; jul>jun			nov>dec; may>feb,mar; jul,sep>jun			oct,nov,dec,jan,mar>feb			
Chlorophyll-a to biopolymeric C	jan>feb,mar>may; jul>jun>may			oct,nov,dec,jan,mar,may,jun,jul,sep>feb; may,jul,sep>jun			dec,jan,mar>oct,nov,feb; mar>may; jun>jul,sep			
Protein to biopolymeric C	oct,nov,jan,mar>feb; may,jun,jul>mar			feb>mar; jun>may			jan,feb,mar,may,jun,jul,sep>nov>mar; jun,jul,sept>may			
Protein : carbohydrate ratio	nov,jan,feb,mar>oct; jul>jan,feb,mar			feb>mar; may,jun,jul>mar,nov,dec			oct,dec,jan,feb,may,jun,jul,sep>nov>mar			
Biochemical composition (multivariate analysis)	nov,dec,jan≠feb,mar; jun≠jul			oct,nov,dec≠feb; feb≠mar≠may≠jun≠jul≠sep			oct,nov,dec,jan≠feb,mar,may,jun,jul; feb≠mar; mar≠may,jun,jul,sep			
Nutritional quality (multivariate analysis)	jul≠oct,nov,jan,feb,mar			jan,feb≠oct,nov,dec,mar,may,jun,jul,sep; mar≠may≠jun≠jul≠sep			oct,dec≠nov; jan≠feb≠mar≠may≠jun≠jul≠sep			

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716 **Supplementary Table S2.** Pair wise tests on the factor Site \times Time, conducted to test differences in total meiofaunal abundance, richness of taxa
717 and community composition among sites and times. Sen = Senigallia, Fal = Falconara, Por = Portonovo, oct = October 2011, nov = November
718 2011, dec = December 2011, jan = January 2012, feb = February 2012, mar = March 2012, may = May 2012, jun = June 2012, jul = July 2012, sep
719 = September 2012, ns = not significant.

Differences among sites	October 2011	November 2011	December 2011	January 2012	February 2012	March 2012	May 2012	June 2012	July 2012	September 2012
Total meiofaunal abundance	por>fal>sen	fal>sen	ns	ns	sen,por>fal	fal>sen	ns	sen,por>fal	ns	fal>sen
Richness of taxa	sen>fal	ns	sen,por>fal	ns	por>fal	ns	ns	ns	ns	ns
Richness of taxa (rare taxa)	ns	ns	por>fal	ns	sen,por>fal	ns	ns	ns	ns	ns
Taxonomic composition (multivariate analysis)	sen,por#fal	sen#fal	sen#fal#por	fal#por	sen,por#fal	sen#fal	ns	sen#fal#por	fal#por	sen#fal#por

Differences among times	Senigallia	Falconara	Portonovo
Total meiofaunal abundance	oct,nov,dec,feb,may,jun,jul>mar	oct,nov,dec,jan,mar,may,jun,jul,sep>feb; sep>jul>jun	dec>feb,mar; jan>feb; jun>feb,mar,jul
Richness of taxa	dec>jan,feb,mar	jul>feb,mar	nov,dec>oct; dec>jan,feb,mar
Richness of taxa (rare taxa)	dec>jan,feb; feb>mar	oct,nov,dec,jan,mar,may,jun,jul,sep>feb	nov,dec>oct
Taxonomic composition (multivariate analysis)	nov#dec; jan,feb#mar; mar#jun	oct,nov#dec; dec#jan#feb; feb#mar,may; may#jun#jul#sep	dec,jan#feb; may#jun#jul#sep

720 **Supplementary Table S3.** Results of the SIMPER analysis assessing dissimilarity levels in the
721 meiofaunal communities among sampling sites and times.

	Contrast	Dissimilarity %	Avg dissimilarity %	Responsible Taxa
October	Senigallia vs Falconara	31.8	47.8	nematodes
	Senigallia vs Portonovo	45.4		nematodes, copepods
	Falconara vs Portonovo	66.3		nematodes
November	Senigallia vs Falconara	43.2	42.9	nematodes
	Senigallia vs Portonovo	37.7		nematodes
	Falconara vs Portonovo	47.7		nematodes
December	Senigallia vs Falconara	24.9	27.5	nematodes, copepods, bivalves
	Senigallia vs Portonovo	25.5		nematodes, copepods
	Falconara vs Portonovo	32.2		nematodes, copepods
January	Senigallia vs Falconara	21.8	18.8	nematodes
	Senigallia vs Portonovo	22.7		nematodes
	Falconara vs Portonovo	12.0		nematodes, copepods
February	Senigallia vs Falconara	84.7	61.3	nematodes
	Senigallia vs Portonovo	19.4		nematodes
	Falconara vs Portonovo	80.0		nematodes
March	Senigallia vs Falconara	47.8	36.9	nematodes
	Senigallia vs Portonovo	31.6		nematodes, copepods
	Falconara vs Portonovo	31.3		nematodes
May	Senigallia vs Falconara	41.9	35.7	nematodes
	Senigallia vs Portonovo	25.0		nematodes, copepods
	Falconara vs Portonovo	40.2		nematodes
June	Senigallia vs Falconara	59.3	42.7	nematodes, copepods
	Senigallia vs Portonovo	18.0		nematodes, copepods
	Falconara vs Portonovo	50.8		nematodes, copepods
July	Senigallia vs Falconara	45.0	35.2	nematodes, copepods
	Senigallia vs Portonovo	40.5		nematodes, copepods
	Falconara vs Portonovo	20.1		nematodes, ostracods, copepods
September	Senigallia vs Falconara	18.2	16.7	nematodes, bivalves
	Senigallia vs Portonovo	14.7		nematodes, copepods, ostracods
	Falconara vs Portonovo	17.1		nematodes, bivalves
	Falconara vs Portonovo	100.0		ostracods, oligochaetes
	Contrast	Dissimilarity %	Avg dissimilarity %	Responsible Taxa
Senigallia	October vs November	15.8	23.9	nematodes, polychaetes, bivalves
	November vs December	19.8		copepods, nematodes, bivalves
	December vs January	24.8		nematodes, bivalves, copepods
	January vs February	16.7		nematodes, ostracods, polychaetes
	February vs March	21.9		nematodes, copepods, ostracods
	March vs May	39.0		nematodes
	May vs June	19.7		nematodes, copepods, polychaetes
	June vs July	28.5		nematodes, copepods, ostracods
	July vs September	28.4		nematodes, copepods, bivalves
Falconara	October vs November	13.3	36.1	nematodes, bivalves, polychaetes, ostracods
	November vs December	26.6		nematodes, ostracods, bivalves
	December vs January	16.7		nematodes, ostracods, polychaetes
	January vs February	60.6		nematodes
	February vs March	63.2		nematodes
	March vs May	31.9		nematodes
	May vs June	53.5		nematodes, copepods
	June vs July	37.0		nematodes, ostracods, copepods
Portonovo	July vs September	22.4	23.8	nematodes, ostracods, bivalves
	October vs November	42.4		nematodes, copepods
	November vs December	23.1		nematodes, copepods
	December vs January	18.8		nematodes, copepods
	January vs February	22.8		nematodes, copepods, polychaetes
	February vs March	18.6		nematodes, copepods, polychaetes
	March vs May	28.6		nematodes, copepods, oligochaetes

May vs June	17.1	nematodes, copepods, ostracods, polychaetes
June vs July	19.3	nematodes, copepods, bivalves
July vs September	23.3	nematodes, copepods
