Trophic status and meiofauna biodiversity in the Northern Adriatic Sea: Insights for the assessment of good environmental status

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Submitted to *Marine Environmental Research*

Abstract

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

Highlights

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

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

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 asses 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 at 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

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

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

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

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

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

2. Materials and methods

2.1 Study areas and sampling

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

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

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

2.2 Grain size

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

2.3 Biochemical composition of sediment organic matter

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

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

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

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

2.4 Meiofaunal variables

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

According to the literature and, therefore, for comparison purposes, meiofaunal abundance was expressed as number of individuals 10 cm^{-2}, and their diversity expressed as richness of taxa (i.e., number of taxa ± 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 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 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 mg g^{-1} and its algal fraction <12%; mesotrophic
210 systems characterized by BPC concentration 1-3 mg g^{-1} and its algal fraction 12-25% and
211 oligotrophic systems as those characterized by BPC concentration <1 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

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

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

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

3. Results

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

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

3.1 Biochemical composition and nutritional quality of sedimentary organic matter

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

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

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

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

3.2 Meiofaunal abundance and diversity

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

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

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

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

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

The SIMPER analysis (Supplementary Table S3) revealed that the highest dissimilarity among sampling sites occurred in October, November, February and June (on average 48, 43, 61 and 43%, respectively). In these times, the highest dissimilarity was consistently observed between Falconara and the other two sampling sites. The highest dissimilarity among sampling times occurred at Falconara (on average 36%), followed by Portonovo and Senigallia (on average 24%, for both 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 ***3.3Relationships 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

4. Discussion

4.1 Trophic status of coastal sediments in the Northern Adriatic Sea

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

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

436 (i.e. high phytopigment concentrations) enhances the nutritional quality of sedimentary organic
437 matter. This is confirmed by the significant relationship between the sedimentary BPC contents and
438 the protein to carbohydrate ratio (used as a proxy of higher nutritional quality; Pusceddu et al.,
439 2000; Figure 6B). In the investigated sediments, characterized by oligo- and mesotrophic
440 conditions, the richness of meiofaunal taxa was linked to the increase in BPC sedimentary contents
441 and the protein to carbohydrate content ratio (Figure 7). Previous studies highlighted the presence
442 of tight relationships between changes in the trophic status of marine sediments and the biodiversity
443 of meiofauna under different environmental conditions and ecological alteration (e.g., Pusceddu et
444 al., 2007a; 2011; Cerrano et al., 2010; Bianchelli et al., 2013). Nevertheless, such a relationship is
445 not consistently positive and it has been shown that the pattern of meiofaunal biodiversity responses
446 can vary among systems characterized by different levels of initial benthic trophic status (Danovaro
447 et al., 2004). For instance, it has been shown that the impact of aquaculture biodeposition and
448 consequent eutrophication due to the accumulation of fish feces and uneaten food (Pusceddu et al.,
449 2007b) can be easily detected and have a clear impact on meiofauna, especially in the initial
450 conditions stages, when the system shifted from oligo- to mesotrophic conditions (Mirto et al.,
451 2010; 2014; Pusceddu et al., 2011). Nevertheless, it has been repeatedly demonstrated that a strong
452 accumulation of biopolymeric organic C, mostly accounted for by material of detrital/heterotrophic
453 origin, may anyway lead to profound modifications of sediment distinctive features (e.g., oxygen
454 availability; Pusceddu et al., 2009), which could in turn affect negatively the meiofaunal
455 assemblages (Gambi et al., 2009).

456 Previous studies conducted in the same regional area (Frontalini et al., 2011; Semprucci et al.,
457 2013) reported similar taxonomic composition of meiofaunal communities, with nematodes as
458 dominant taxon, followed by polychaetes, copepods, oligochaetes and platyhelminthes. The results
459 of the present study indicate that the composition of meiofaunal assemblages changed significantly
460 among sites and among sampling times at each site. In particular, we report here that the highest
461 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.

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Acknowledgements

AP had the idea. SB and EB performed the sampling and the laboratory analyses. SB, AP, RD and EB wrote the manuscript. This study is part of the project DEVOTES (Grant Agreement n. 308392), funded by the European Union's Seventh Framework Programme.

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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 ($\mu g\ g^{-1}$)		Phaeopigment ($\mu g\ g^{-1}$)		Total Phytopigment ($\mu g\ g^{-1}$)		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

Table 2. Results of PERMANOVA testing variations in the sedimentary OM contents (A) indicators of nutritional quality (B), biochemical composition (C) and whole nutritional quality (D). dF=degree of freedom; MS=mean square; F=F statistic; ***=P<0.001; **=P<0.01; **=P<0.05; ns= 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				

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.

672

		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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674

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

679

	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
	PRT to BPC%	619.1	0.6	ns	0.7	13.0
Copepods	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
	Chl-a to BPC%	2805.7	1.2	ns	1.3	67.3
Richness of meiofaunal taxa	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
	Chl-a to BPC%	224.6	1.1	ns	1.3	71.1
Taxonomic composition	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
	Phaeopigment	770.9	0.7	ns	0.8	15.8

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685 **Figure 1**

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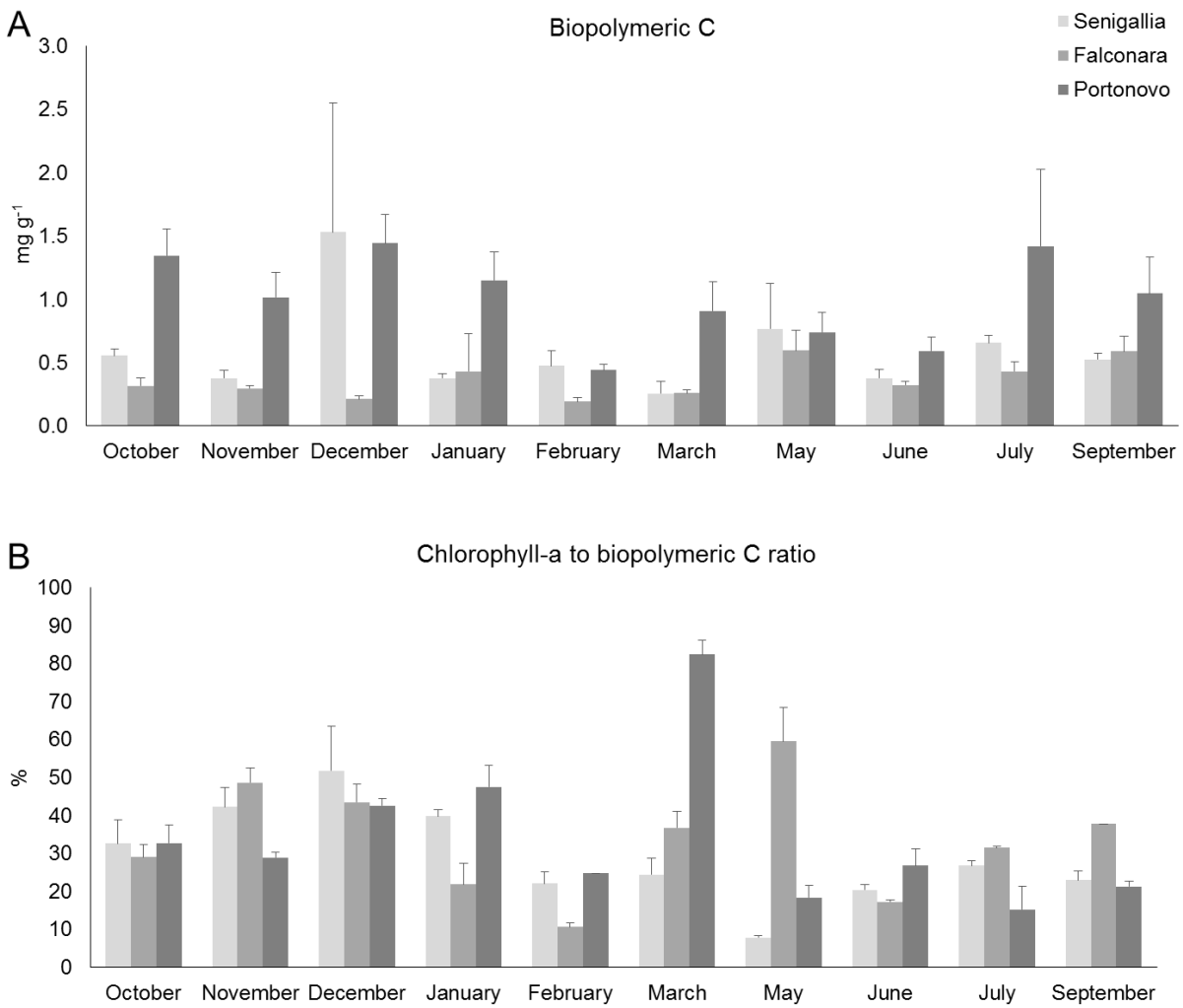
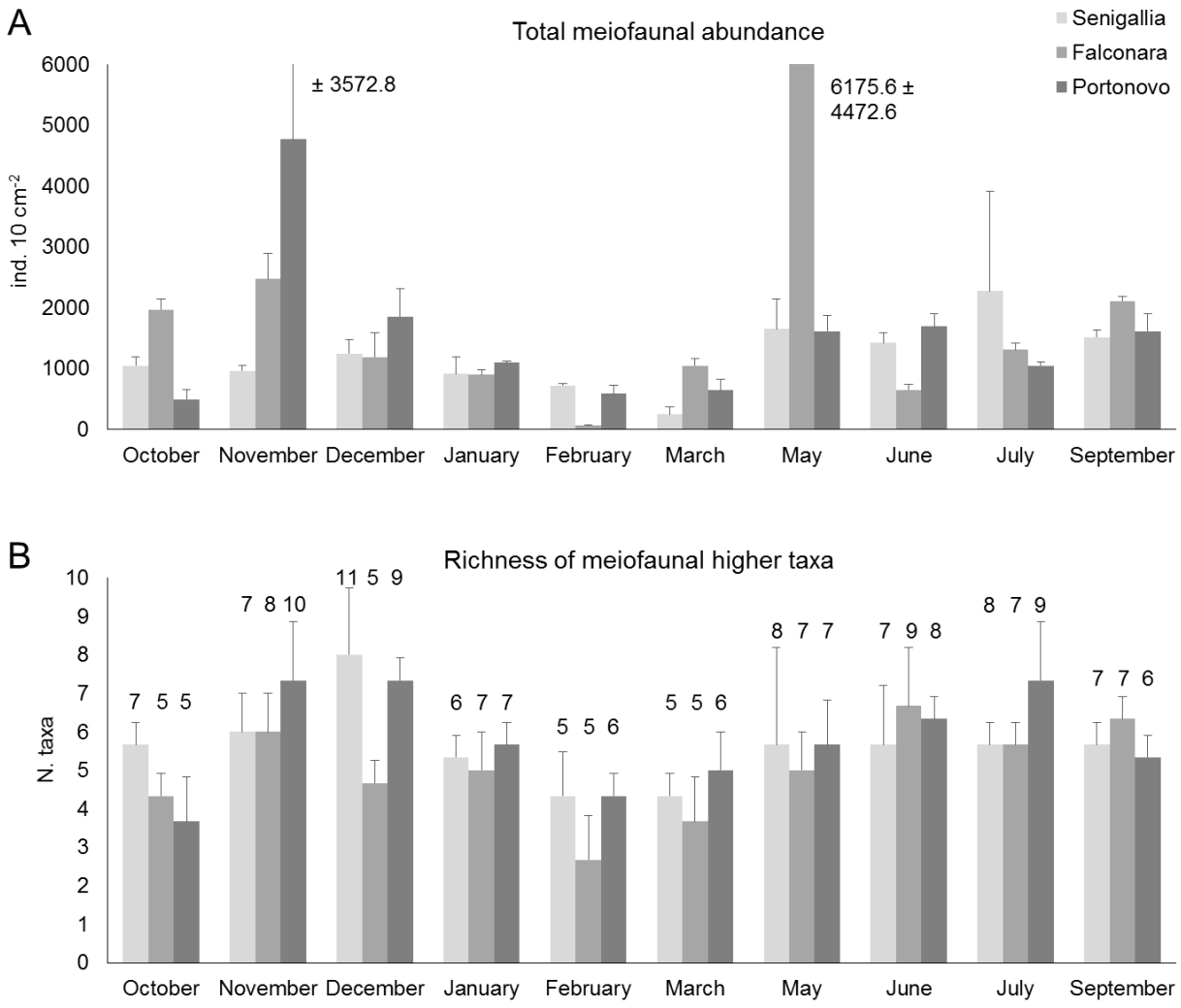


688

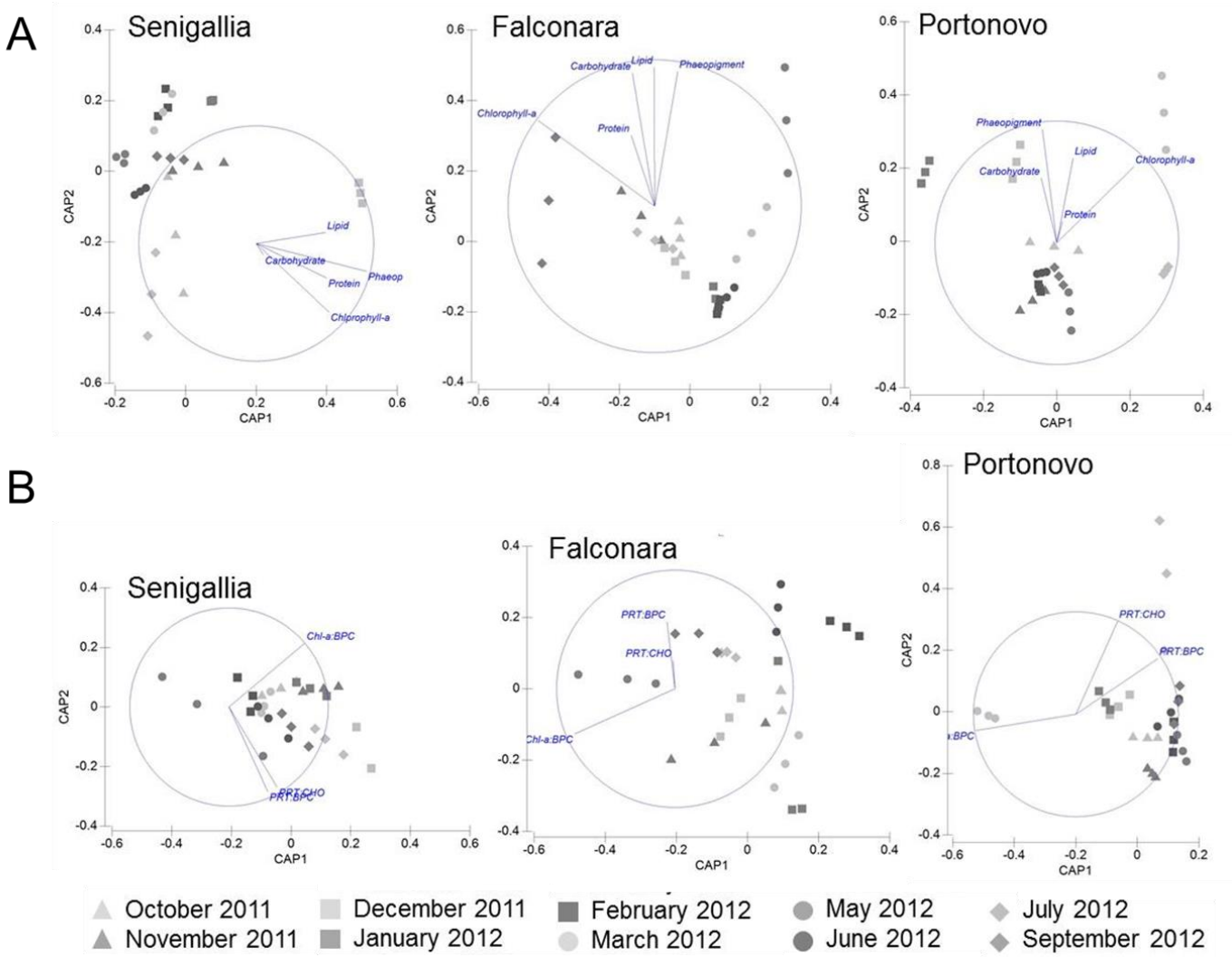
689 **Figure 2**

690



691

692 **Figure 3**



693

694 **Figure 4**

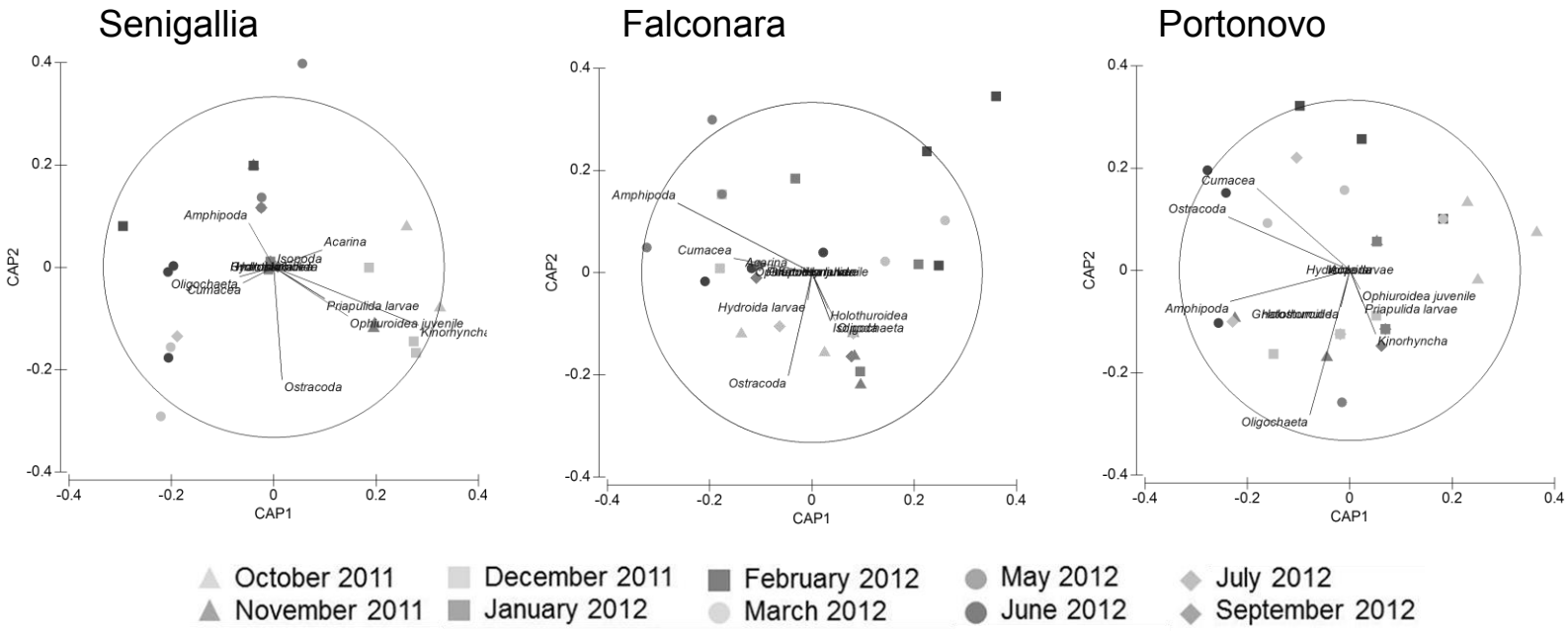

Senigallia
Falconara
Portonovo
CAP1
CAP2
Amphipoda
Acarina
Isopoda
Oligochaeta
Cumacea
Priapulida larvae
Ophiuroidea juvenile
Ostracoda
Hydroida larvae
Holothuroidea
Kinorhyncha
October 2011
November 2011
December 2011
January 2012
February 2012
March 2012
May 2012
June 2012
July 2012
September 2012

Figure 5

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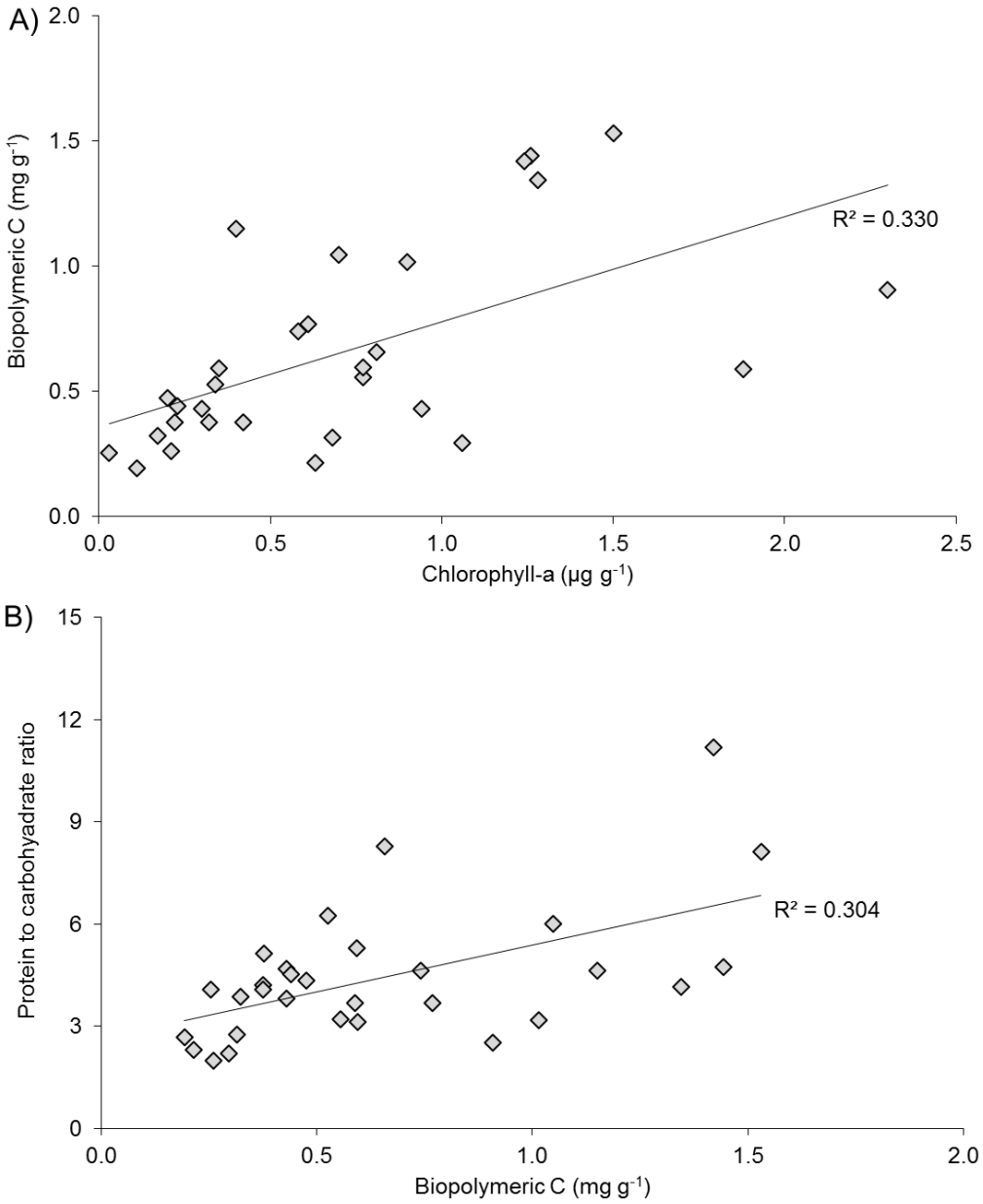

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703

704 **Figure 6**

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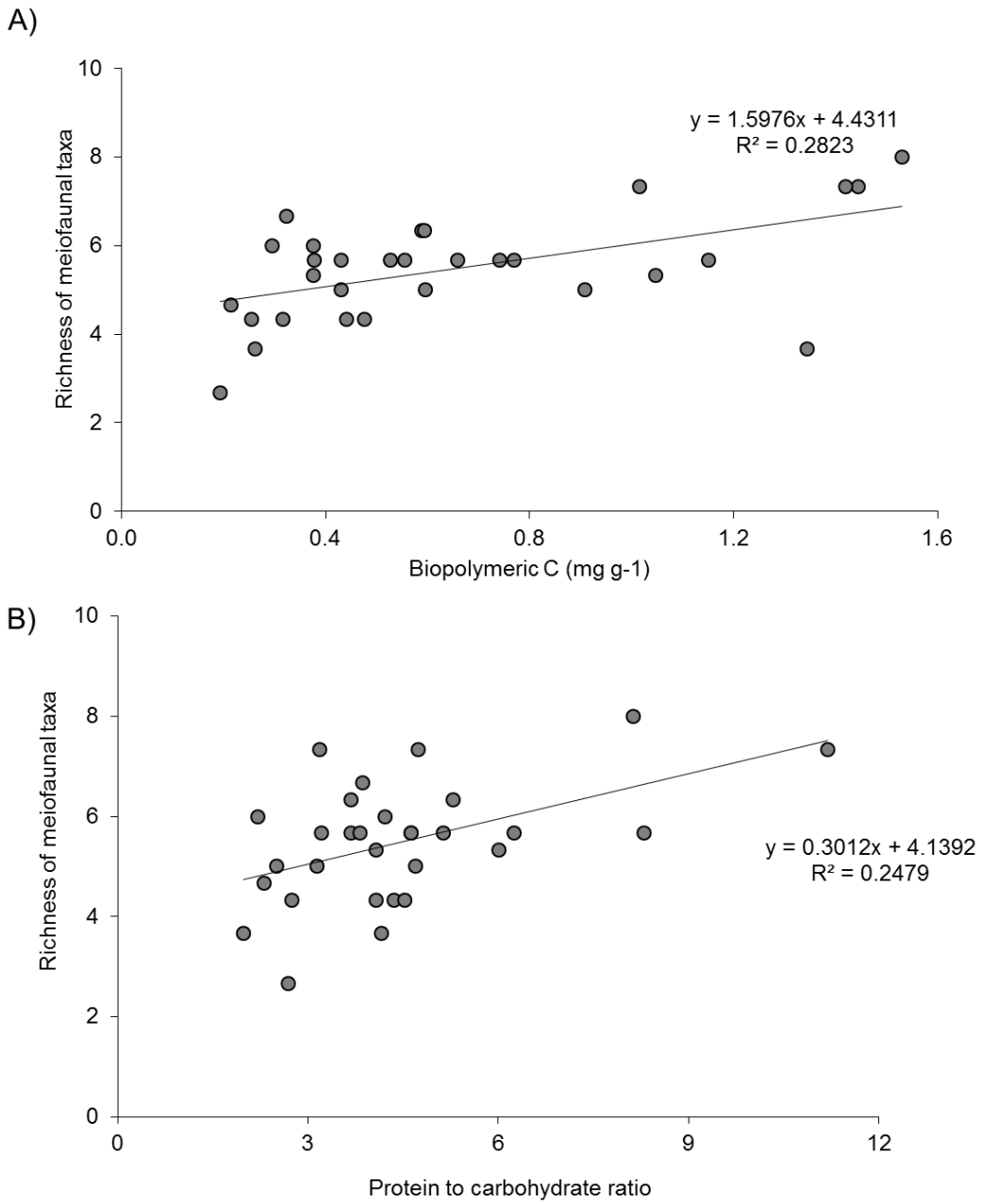

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710 **Figure 7**

Supplementary Table S1. *Pair wise* tests conducted to test differences in the concentration and nutritional of OM among sites and times. Sen = Senigallia, Fal = Falconara, Por = Portonovo, oct = October 2011, nov = November 2011, dec = December 2011, jan = January 2012, feb = February 2012, mar = March 2012, may = May 2012, jun = June 2012, jul = July 2012, sep = 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**
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≠marc≠may≠jun≠jul≠sep

Supplementary Table S2. Pair wise tests on the factor Site × Time, conducted to test differences in total meiofaunal abundance, richness of taxa and community composition among sites and times. Sen = Senigallia, Fal = Falconara, Por = Portonovo, oct = October 2011, nov = November 2011, dec = December 2011, jan = January 2012, feb = February 2012, mar = March 2012, may = May 2012, jun = June 2012, jul = July 2012, sep = 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	jun>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
	July vs September	22.4		nematodes, ostracods, bivalves
Portonovo	October vs November	42.4	23.8	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

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