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1 **Nematode biodiversity and benthic trophic state are simple tools for the**
2 **assessment of the environmental quality in coastal marine ecosystems**

3
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24 **ABSTRACT**

25 A high biodiversity is essential to guarantee the stability and functioning of coastal marine
26 ecosystems. In this perspective, the Marine Strategy Framework Directive provides prescriptions to
27 maintain (or restore) marine biodiversity in order to achieve a Good Environmental Status (GES).
28 Eutrophic conditions - as determined by the accumulation of sedimentary organic matter (OM) - are
29 often associated with biodiversity loss, so that eutrophic conditions are often considered a pre-
30 requisite or a proxy for degraded ecological conditions. The aim of this study was to investigate the
31 feasibility of the combined use of benthic trophic status and nematode biodiversity as integrated
32 indicators of the environmental status of marine coastal ecosystems. To achieve this objective, we
33 investigated nematode species diversity and assemblage composition in three areas of the Adriatic
34 Sea, characterised by different OM quantity and biochemical composition (as proxy of sedimentary
35 trophic status) and affected by different levels of anthropogenic impact. We show that, on the basis
36 of OM quantity and biochemical composition, the investigated sites can be classified from oligo- to
37 meso-trophic, whereas the analysis of nematode biodiversity indicates that the ecological quality
38 status (EQS) ranged from bad to moderately impacted. This result provides evidence that trophic
39 status and environmental quality assessments are not interchangeable tools for the assessment of
40 marine ecosystems EQS. Rather they should be considered as complementary proxies for the overall
41 assessment of the (good) ecological status. Data reported here also indicate that the loss of benthic
42 biodiversity, whatever the source of disturbance, may be associated to a decrease of the functional
43 diversity (either as feeding and life strategies traits), which might have important consequences on
44 ecosystems functioning. Our results suggest that the GES cannot be defined uniquely in terms of
45 sedimentary trophic status, especially when many other multiples stressors can contribute to
46 determine the overall environmental quality of the investigated ecosystems. Nematode biodiversity
47 is highly sensitive to differences in ecological conditions at different spatial and temporal scales and
48 it can provide reliable and complementary information for the assessment of the environmental status
49 in marine coastal sediments.

51 **Keywords:** sedimentary organic matter biochemical composition, ecological quality status (EQS),
52 Marine Strategy Framework Directive

1. Introduction

Oceans represent a major source of goods and services for the human wellbeing (Costanza et al. 1997; 2014) and have long been considered a limitless source of food, energy and benefits (Costanza, 1999). Nevertheless, although the role of the oceans in sustaining human life is widely accepted, the human exploitation of the oceans' resources is increasingly rising beyond acceptable limits, causing a loss in biodiversity, altering ecosystems characteristics and functioning (Halpern et al., 2008; 2015; Worm et al., 2006; Rockström et al., 2009).

To limit biodiversity loss and preserve ecosystem goods and services in coastal areas (or to identify priorities for their ecological restoration), several directives and legislations have recently focused on the analysis of the ecological quality status of estuarine, coastal or off-shore environments. Among these, after the Water Framework Directive (WFD, 2000/60/EC) in 2000, the European Parliament and the European Union Council enacted in 2008 the Marine Strategy Framework Directive (MSFD, 2008/56/EC), as part of the Integrated Maritime Policy (IMP) adopted by the European Commission in 2007. Through implementing environmental Directives, the European Union has moved towards coordinated and integrated catchment-to-coast management, following the most recent legislation calling for the worldwide application of ecosystem-based approaches to the management and conservation of nature and its resources. The MSFD establishes a framework for the development of strategies designed to achieve the Good Environmental Status (GES) in the marine environment, by the year 2020, using 11 qualitative descriptors (biodiversity, non-indigenous species, exploited fish and shellfish, food webs, human-induced eutrophication, sea-floor integrity, hydrographical conditions, contaminants, contaminants in fish, marine litter and introduction of energy/noise; (MSFD, 2008/56/EC)). The MSFD directive is based upon an ecosystem-based approach, with a holistic view on the management and protection of marine ecosystems (Nicholson and Jennings, 2004; Apitz et al., 2006; Borja et al., 2008), focusing on ensuring sustainable use of the seas, and providing safe, clean, healthy and productive marine waters.

78 The concept of GES, as defined by the MSFD, takes into account the structures, functions and
79 processes of marine ecosystems, bringing together physical, chemical, physiographic, geographic,
80 climatic and biological factors, and integrating these with anthropogenic impacts and activities carried
81 out in the areas of concern (European Parliament and Council, 2008; Borja et al., 2013).

82 The implementation of these descriptors requires either a refinement of the biological models
83 and indicators used (benthic vs plankton components, small vs large body size etc) and an
84 implementation of the tools and technologies enabling the best possible data quality and resolution
85 (Danovaro et al., 2016).

86 Among the European Seas, the Northern Adriatic is among the most productive and, at the same
87 time, one of the most environmentally threatened and compromised basins of the Mediterranean Sea
88 (Coll et al., 2010; 2012; Micheli et al., 2013). In the last 30 years, the Adriatic Sea has experienced
89 large changes in the trophic status, structure and organization of pelagic and benthic communities
90 also in response to current climate shifts (Kamburska and Fonda Umani, 2006; Danovaro et al.,
91 2009a; Mozetič et al., 2012; Giani et al., 2012; Di Camillo and Cerrano, 2015; Piroddi et al., 2017).
92 Due to the continental inputs entering the basin mainly through the Po river, the sediments of the
93 Adriatic Sea are characterized by the accumulation of large organic loads (Dell'Anno et al., 2008)
94 and locally experienced hypoxic crises (Alvisi et al., 2013), increased frequency of red tides,
95 intensification of mucilage formation, possibly enhancing the spread of pathogens (Danovaro et al.,
96 2009a). Recently, eutrophication phenomena have been significantly decreased, associated to the
97 decreasing nutrients input from land (Cozzi and Giani 2011; Uusitalo et al., 2016). Despite this, the
98 overall ecological conditions of the NW Adriatic Sea are still worst than those reported from other
99 Mediterranean and European regional seas (Uusitalo et al., 2016).

100 At the same time, the assessment of the environmental quality status in the Adriatic Sea still
101 largely depends upon the indicators and tools (e.g., biotic component) utilized, so that it requires the
102 simultaneous use of a wide range of ecological indicators (Uusitalo et al., 2016). Macrofaunal

103 biodiversity, for instance, whose ecological traits have been widely associated to environmental
104 alteration, is commonly utilized for the classification of the ecological status of marine benthic
105 ecosystems (Borja et al., 2008). Nevertheless, more recently, meiofauna, due to their high diversity
106 and standing stocks, high turnover rates and lack of larval pelagic dispersal, have attracted increasing
107 attention as a tool for detecting anthropogenic impact and for ranking the environmental quality status
108 of different marine ecosystems (Danovaro et al., 1995; 2000; 2009b; Mazzola et al., 1999; 2000; La
109 Rosa et al., 2001; Mirto et al., 2002; 2010; 2014; Frascchetti et al., 2006; 2016; Pusceddu et al., 2007;
110 2011; 2014a; 2016; Gambi et al., 2009; Moreno et al., 2011; Alves et al., 2013; 2015; Bianchelli et
111 al., 2010; 2016a; 2016b). Meiofauna, in fact, are very sensitive to environmental disturbances,
112 particularly to organic enrichment and eutrophication (Bianchelli et al., 2016a), at temporal scales
113 much narrower than those generally exhibited by macrofauna. Previous studies, indeed, highlighted
114 the influence of changes in the trophic status of marine sediments on the meiofaunal biodiversity
115 under different environmental conditions and ecological alteration (Pusceddu et al., 2007, 2011;
116 Bianchelli et al., 2010; 2013; 2016a). Such a relationship is not consistently positive, as the pattern
117 of meiofaunal biodiversity responses varies depending upon the levels of the benthic trophic status
118 (Pusceddu et al., 2007; Bianchelli et al., 2016a).

119 Among meiofauna, nematodes typically represent from 50 to over 90% of the total meiofaunal
120 abundance; they are cosmopolitan and their distribution, especially in coastal environments, is
121 strongly influenced by the local environmental characteristics (Mercx et al., 2009). Nematodes are
122 characterized by high levels of structural (i.e. species richness) and functional (trophic) diversity
123 (Balsamo et al., 2010; Moreno et al., 2011; Semprucci and Balsamo, 2014). Due to these
124 characteristics, they have been utilised as indicators of a plethora of different environmental
125 disturbances (Danovaro and Gambi, 2002; Steyaert et al., 2007; Moreno et al., 2008, 2011; Neher and
126 Darby, 2009; Mirto et al., 2014; Pusceddu et al., 2014a; Hannachi et al., 2016): they, for example, are
127 sensitive to hydrocarbon contamination (Danovaro et al., 1995; Mahmoudi et al., 2005; Losi et al.,

2013) and organic enrichment (Essink and Keidel, 1998; Fraschetti et al., 2006; Moreno et al., 2008; Gambi et al., 2009), including biodeposition from aquaculture activities (Duplisea and Hargrave, 1996; Mazzola et al., 2000; Mirto et al., 2002; Vezzulli et al., 2008). In particular, previous studies have reported that the amount and the nutritional quality of sedimentary organic matter may affect nematodes biodiversity, and more specifically their taxonomic composition (Moreno et al., 2008; Semprucci et al., 2014; 2015a; 2015b; Bianchelli et al., 2016b).

The aim of this study was to investigate the possibly to use nematode biodiversity and benthic trophic status as simple and reliable indicators of the environmental quality of marine coastal ecosystems. In order to achieve this objective, this study was carried out to analyse the spatial-temporal variations in structural and functional biodiversity of free-living nematodes in the coastal North-Western Adriatic Sea in relation with benthic trophic status (in terms of organic matter sedimentary contents and biochemical composition) and several environmental stressors (seasonal tourism, maritime transport associated with the presence of an oil refinery and river discharges). More specifically, we tested the null hypothesis that nematode assemblages (in terms of structural and functional biodiversity) do not vary among sampling times and sites characterized by the presence of different levels of environmental impacts and sedimentary trophic status.

144

145 **2. Materials and methods**

146 ***2.1 Study areas and sampling***

The study area is located in the North-Western sector of the Adriatic Sea, where we considered three coastal sites along the Marche Region coastline (Figure 1A), at ca. 6 m water depth, subjected to different natural and anthropogenic stressors: Senigallia (maritime traffic and riverine inputs), Falconara (riverine inputs and the presence of a petrochemical industry) and Portonovo (tourism and maritime traffic, Site of Community Importance). Detailed descriptions of the 3 investigated sites are given elsewhere (Bianchelli et al., 2016a) and reported in Table 1.

153 According to the reports on the quality status of coastal marine waters during 2010-2014, the
154 ecological status is “Sufficient” for all of the investigated sites (ARPAM, 2014; 2015). Overall, the
155 study area has been categorized as affected by a “low-medium” level of cumulative impacts (Figure
156 1B; Micheli et al., 2013).

157 For the purpose of this study, sediment samples were collected over >20 months (from January
158 2011 to September 2012) with ca. bi-monthly sampling intervals (i.e., January, May, June, September,
159 November, December 2011, January, May, June and September 2012), by means of a Van Veen grab
160 (sampling surface 0.15 m²), on board of the R/V Actea. Only deployments in which the sediments
161 resulted undisturbed were utilized for sampling. Sediment samples collection and storage were carried
162 out following the procedures reported in Danovaro (2010) and detailed in Bianchelli et al. (2016). At
163 each site and time, sediment samples were collected from three true and independent replicates, for
164 all the investigated variables.

165

166 ***2.2 Biochemical composition of sediment organic matter***

167 The sedimentary contents of total phytopigment, protein, carbohydrate and lipid were determined
168 according to Danovaro (2010). Phytopigments (chlorophyll-a and phaeopigments) were assessed
169 fluorometrically (Lorenzen and Jeffrey, 1980) and their sum (total phytopigment), once converted
170 into C equivalents using 40µgC µg phytopigment⁻¹ as conversion factor, utilized as proxy of organic
171 matter deriving from primary producers (Pusceddu et al., 2009).

172 Protein, carbohydrate and lipid concentrations, were determined spectrophotometrically
173 (Danovaro, 2010), converted into C equivalents (using 0.49, 0.40 and 0.75 mgCmg⁻¹, respectively, as
174 conversion factors) and their sum referred as biopolymeric C content (BPC; Pusceddu et al., 2000).

175 The algal fraction of the BPC pools was estimated as percentage contribution of total
176 phytopigment (expressed as C equivalents) to BPC (Pusceddu et al., 2009). The percentage
177 contributions of protein (expressed as C equivalents) to BPC and the values of the protein to

178 carbohydrate ratio were used as indicators of sedimentary organic matter nutritional quality
179 (Pusceddu et al., 2009).

180

181 **2.3 Nematode biodiversity**

182 In the laboratory, sediment samples were processed to retain meiobenthic organisms within
183 1000 and 20 μm meshes, after centrifugation-resuspension in water solutions of Ludox HS40 (density
184 1.18 g cm^{-3}) (Heip et al., 1985; Danovaro, 2010). From each replicate, 100 nematodes were then
185 randomly picked and mounted on permanent slides (Seinhorst, 1959). All nematodes were identified
186 to putative species level (Platt and Warwick, 1983; 1988; Warwick et al., 1998) and species were
187 indicated by the genus name followed by sp.1, sp.2, etc.

188 At each sampling site and time, nematode diversity was assessed in terms of species richness
189 (SR), defined as the total number of species retrieved in each sample. The expected number of species
190 for a theoretical sample of 100 specimens (ES100) was also calculated, to standardise the SR values
191 to the sample size. The Margalef diversity index (D; Margalef, 1958), Shannon-Wiener information
192 function (H' , using log-base 2) and the evenness (as Pielou's index, J; Pielou, 1975) were also
193 measured. All indices were calculated both for each replicate and for each sampling site, cumulatively
194 for the three replicates, at each time, using PRIMER v6.0+ (Plymouth Marine Laboratory, UK; Clarke
195 and Gorley, 2006).

196

197

198 **2.4 Nematode functional traits**

199 The Index of Trophic Diversity and the Maturity Index were used as indicators of functional diversity
200 and life strategies, respectively.

201 The trophic habits of the nematode assemblages were defined according to the individual stoma
202 morphology (Wieser, 1953). According to this approach, nematodes were divided into four groups:

selective (bacterial) feeders (1A, with no buccal cavity or a fine tubular); non-selective deposit feeders (1B, with large but unarmed buccal cavity); epistrate or epigrowth feeders (i.e. diatom feeders; 2A, with buccal cavity with scraping tooth or teeth), predators/omnivores (2B, with buccal cavity with large jaws). The Index of Trophic Diversity (ITD) was then calculated as $1 - ITD$, where $ITD = \frac{g_1^2 + g_2^2 + g_3^2 + \dots + g_n^2}{n}$, g is the relative contribution of each trophic group to the total number of individuals and n is the number of trophic groups (Heip et al., 1985).

The maturity index (MI) was calculated according to the weighted mean of the individual genus scores, as $\sum v(i) f(i)$, where v is the colonisers-persisters (c-p) value of the genus i and $f(i)$ is the frequency of that genus (Bongers et al., 1991).

2.5 Indicators of benthic trophic status

Benthic trophic status was assessed using the approach based on the analysis of the quantity and nutritional quality of sedimentary organic matter (Dell'Anno et al., 2002; Pusceddu et al., 2009; Bianchelli et al. 2016a). The indicators are based on the concentration of the sedimentary organic matter main biochemical compounds (phytopigments, protein, carbohydrate, lipid and biopolymeric C) for the standing stocks, and the sedimentary organic matter aging and nutritional quality (Pusceddu et al., 2009). The contribution of total phytopigments to BPC is utilized as a proxy of the freshness of the sedimentary organic material (Pusceddu et al., 2001). Moreover, since the organic C deriving from primary producers is also labile (and rapidly available for heterotrophs) (Pusceddu et al., 2003), higher values of this percentage will also be indicative of a comparatively higher nutritional quality (Dell'Anno et al., 2002). Protein to BPC and protein to carbohydrate ratios have been used as indicative of ageing and the nutritional value of the sedimentary organic matter, since N is the most limiting factors for heterotrophs and proteins are more labile than carbohydrates (Dell'Anno et al., 2002; Pusceddu et al., 2009).

227 For the purpose of this study, we compared our results with two different classification
228 schemes proposed for the assessment of the benthic trophic status:

229 1) the classification proposed by Dell'Anno et al. (2002): protein and carbohydrate
230 concentrations are >4 and $>7 \text{ mg g}^{-1}$, respectively, in hyper-trophic systems; $1.5\text{--}4 \text{ mg g}^{-1}$ and $5\text{--}7 \text{ mg}$
231 g^{-1} , respectively, in eutrophic systems; $<1.5 \text{ mg g}^{-1}$ and $<5 \text{ mg g}^{-1}$, respectively, in meso-oligotrophic
232 systems;

233 2) the classification proposed by Pusceddu et al. (2009; 2011): sedimentary contents of BPC
234 and its algal fraction are $>3 \text{ mg g}^{-1}$ and $<12\%$, respectively, in eutrophic systems; $1\text{--}3 \text{ mg g}^{-1}$ and 12--
235 25% , respectively, in mesotrophic systems; $<1 \text{ mg g}^{-1}$ and $>25\%$, respectively, in oligotrophic
236 systems.

237

238 ***2.6 Indicators of ecological quality based on nematode biodiversity***

239 First, the ecological quality of the investigated sites was evaluated qualitatively using the sensitivity
240 of the different nematode species to environmental disturbance (including OM enrichment) as a
241 proxy; information about the nematode species sensitivity was obtained from the scientific literature
242 (e.g., Giere, 1979; Frithsen et al., 1985; Danovaro et al., 1995; 2009b; Essink and Keidel, 1998;
243 Gyedu-Ababio et al., 1999; Mirto et al., 2002; 2014; Mahmoudi et al., 2005; Frascchetti et al., 2006;
244 2016; Vezzulli et al., 2008; Gambi et al., 2009; Losi et al., 2013; Alves et al., 2013). Then, the
245 ecological quality status (EQS) of the investigated sites was quantitatively assessed using nematode
246 MI, c-p, H', ITD at putative-species taxonomical level and the presence of sensitive/tolerant genera
247 as proposed by Moreno et al. (2011). The ITD index was included in our analysis, since it still results
248 a controversial indicator for EQS assessment. Indeed, ITD was first proposed as possible indicator
249 and criticized after some years (Moreno et al., 2011; Semprucci et al., 2015a), since recent studies
250 reported ambiguous influence of various stressors on ITD (Semprucci et al., 2015a; b). In this context,
251 the ITD was included here in order to provide more information on its reliability, particularly for sites
252 subjected to environmental multiple-stressors.

253

254

255 **2.7 Statistical analyses**

256 Uni- and multivariate analyses were carried out in order to ascertain differences among sampling sites
257 and periods. The sampling design included 2 fixed and orthogonal factors: site (3 levels: Senigallia,
258 Falconara, Portonovo) and time (10 levels: January, May, June, September, November, December
259 2011, January, May, June and September 2012). Despite time should be treated as a random factor
260 (Anderson et al., 2008), we used it as a factor with fixed levels to carry out pairwise tests, to verify
261 the significance and consistency of the eventual differences among sites in different times (Anderson
262 et al., 2008; Bianchelli et al., 2016a).

263 Environmental data (including the biochemical composition of OM) were normalized prior to
264 the analyses and analysed using tests based on matrixes of Euclidean distances, whereas faunal data
265 were first square-root transformed and then analysed using tests based on Bray-Curtis similarity
266 matrixes. All data were analysed using the distance-based permutational analysis of variance
267 (PERMANOVA; Anderson 2001; McArdle and Anderson, 2001) in either univariate (separately for
268 each OM biochemical compound, each indicator of nutritional quality and each nematode diversity
269 index) or multivariate contexts (for OM biochemical composition and nematode species
270 composition). Since PERMANOVA is sensitive to differences in multivariate dispersion among
271 groups, we used also a test of homogeneity of dispersion (PERMDISP) to test the null hypothesis of
272 equal dispersions among sites and/or times as either an analogous to a uni-variate test for homogeneity
273 prior to identify differences in the distribution among groups.

274 Canonical analyses of principal coordinates (CAP) were carried out to evaluate the reliability
275 of the *a priori* assignment of the multivariate data to the different sampling sites and display in a two-
276 dimensional space the spatial and temporal variations. Vectors illustrating correlation of the different
277 variables to the main axes of CAP were used to identify the variables best explaining the observed
278 patterns.

279 SIMPER test (using 90% as cutoff) was also performed to estimate the percentage of
280 dissimilarity in the species composition of nematodes assemblages between sites and/or sampling
281 times and identify the species most responsible for the observed dissimilarity, whenever significant.

282 Multivariate multiple regression analyses (DistLM forward, Anderson et al., 2008) were also
283 performed to determine if variations in the nematode species composition, trophic diversity and life
284 strategies were driven by the variations in the organic matter biochemical composition and nutritional
285 quality. This routine is used for analyzing and modeling the relationship between a multivariate
286 dataset and predictor variables (Alves et al., 2015). DistLM procedure was performed by forward
287 selection of the organic matter variables, using the R^2 as the selection criterion for fitting the best
288 explanatory variables in the model, and 4999 permutations. This allowed also for the performance of
289 marginal tests (individual variable relation with genera-derived multivariate data and significance
290 level) (Anderson et al., 2001; 2003; 2008). Plots using a principal coordinate (PCO) analysis were
291 produced to identify sedimentary OM variables mostly responsible for the differences in the
292 composition of nematode assemblages.

293 PERMANOVA, pair wise tests, PERMDISP, CAP, PCO, SIMPER and DistLM forward tests
294 were carried out by means of the software PRIMER 6+ (Clarke and Gorley, 2006).

295

296

297 **3. Results**

298 The chlorophyll-a, phaeopigment, total phytopigment, protein, carbohydrate, lipid and biopolymeric
299 C sedimentary contents, the chlorophyll-a and protein percentage contributions to biopolymeric C
300 and the values of the protein to carbohydrate content ratio in the sediments, as well as nematodes
301 diversity indexes are given in Table 2.

302 The results of the PERMANOVA tests revealed significant effects of the interaction Site $\times$
303 Time on contents, biochemical composition and nutritional quality of OM (except for the protein to
304 carbohydrate ratio), as well as on nematode diversity indexes (Table 3). Details of post-hoc tests are
305 given in the following paragraphs.

307 ***3.1 Biochemical composition and nutritional quality of sedimentary organic matter***

308 Organic matter content, algal and protein fractions of biopolymeric C (BPC) and values of the protein
309 to carbohydrate ratio in the sediment were significantly higher at Portonovo and/or Senigallia than at
310 Falconara in all sampling times, with few exceptions. At all sites, all biochemical variables displayed
311 significant temporal variations, though with varying patterns for the different variables
312 (Supplementary Table S1). At most sampling times the biochemical composition of sediments varied
313 significantly among sampling sites (Supplementary Table S1). The differences in the biochemical
314 composition among sites were mostly due to OM contents in the sediments at Portonovo, which were
315 higher than those in all other sites in almost all sampling times (Supplementary Figure S1A). At each
316 site, the biochemical composition of sediments varied significantly also among sampling times, with
317 variables responsible for the observed temporal changes varying among the three sampling sites
318 (Supplementary Figure S1B-D). At most sampling times, the OM nutritional quality varied among
319 sites mostly because of the very high values of the algal contribution to BPC at Falconara and the
320 highest values of the protein fraction of BPC and protein to carbohydrate ratio at Senigallia and
321 Portonovo (Supplementary Table S1). Differences in the nutritional quality of sedimentary OM were
322 also associated with values of the algal fraction of BPC, which peaked up in different sampling times

323 at each site (in November 2011-January 2012 at Senigallia and Portonovo, in May at Falconara). The
324 lowest values of the protein fraction of BPC and protein to carbohydrate ratio occurred in January
325 and September 2011 at all sites, whereas significant peaks occurred in different times at the three sites
326 (Supplementary Table S1). Temporal variations in the OM nutritional quality were driven by different
327 combinations of variables at the three sampling sites (Supplementary Figure S2B-D).

328

329 **3.2 Indicators of benthic trophic status**

330 Using protein and carbohydrate sedimentary contents as indicators (*sensu* Dell'Anno et al., 2002)
331 Senigallia and Portonovo can be ranked as from meso-oligotrophic to eutrophic, whereas Falconara
332 as meso-oligotrophic (Table 4A). On the basis of the biopolymeric C contents (*sensu* Pusceddu et al.
333 2009, 2011) Senigallia and Portonovo can be ranked as from oligo- to meso-oligotrophic, and
334 Falconara as oligotrophic. Using the algal fraction of biopolymeric C as an additional indicator (*sensu*
335 Pusceddu et al. 2009, 2011), all sites can be ranked as from oligo- to eutrophic (Table 4A).

336 The temporal variability of the trophic status ranking at each site is reported in Table 4B. At
337 Senigallia, the indicators based on sedimentary OM contents (with the unique exception of protein)
338 and the BPC algal fraction varied with time. At Falconara, only the algal fraction of BPC varied
339 throughout the investigated period. At Portonovo, all indicators except for carbohydrate contents
340 varied throughout sampling times.

341

342 **3.3 Nematode biodiversity**

343 A total of 9000 nematodes, belonging to 45 putative species, 35 genera and 17 families have been
344 identified. All diversity indices (SR, D, H, J and ES100) varied among sites in almost all sampling
345 times (Supplementary Table S2), with highest values consistently observed at Portonovo, with only
346 few exceptions (highest J values at Falconara in December 2011 and January 2012). The lowest
347 values of the diversity indices were observed between November 2011 and January 2012 at
348 Senigallia, in June 2011 at Falconara, and in September 2012 at Portonovo (Figure 2).

349 Species retrieved from each site/time and their relative (percentage) abundance are reported in
350 Supplementary Table S3. The results of the PERMANOVA tests revealed significant effects of the
351 interaction Site $\times$ Time on the nematode species composition (Table 3). More specifically, the
352 pairwise tests revealed that significant differences in the assemblage composition were observed
353 among the three sites in all sampling times (but January 2011) and among almost all sampling times
354 at each site (Supplementary Table S2; Figure 3A).

355 The results of the SIMPER analyses (Table 5) show that the overall dissimilarity in the
356 composition of nematode assemblages among sampling sites ranged from 44 to 64% (in May 2012
357 and January 2012, respectively). In all sampling periods, differences among sites were most
358 frequently explained by *Paramonohystera* sp1 (more abundant at Senigallia in almost all sampling
359 times), *Sabatieria* sp 1 (more abundant at Portonovo and/or Senigallia in all sampling times) and
360 *Hopperia* sp1 (more abundant at Portonovo in all sampling times).

361 Among sampling times, the overall dissimilarity in the composition of nematode assemblages
362 was 31%, 48%, and 34% at Senigallia, Falconara, and Portonovo, respectively. At Senigallia, the
363 dissimilarity in the composition of nematode assemblages among sampling times was mostly due to
364 *Sabatieria* sp1, *Metalinhomoeus* sp3 and *Paramonohystera* sp1. At Falconara, the dissimilarity in the
365 composition of nematode assemblages among sampling periods was mostly due to *Diodontolaimus*
366 sp1, *Paramonohystera* sp1, *Oncholaimellus* sp1 and *Sabatieria* sp1. At Portonovo, the species mostly
367 responsible for variations in the composition of nematode assemblages among sampling times were
368 *Paramesonchium* sp1, *Metalinhomoeus* sp3, *Paralongicyatholaimus* sp5 and *Thalassomonhystera*
369 sp1.

370 Overall, a total of 16 exclusive species were encountered in this study: three at Senigallia
371 (*Dorylaimopsis* sp1, *Neotonchus* sp1, *Paramonohystera* sp2), 7 at Falconara (*Ammothenstus* sp1,
372 *Belbolla* sp1, *Eleutherolaimus* sp1, *Mesacanthoides* sp1, *Synonchiella* sp1, *Synonchiella* sp2,

373 *Theristus* sp1) and 6 at Portonovo (*Chaetonema* sp2, *Marylynnia* sp3, *Marylynnia* sp5, *Pierrickia*
374 sp1, *Sphaerolaimus* sp4, *Subsphaerolaimus* sp2).

375 The results of the PERMDISP analysis revealed that temporal variations in the composition of
376 nematode assemblages in Falconara were significantly wider than those in the two other sites (Figure
377 3B). The analysis of principal coordinates (PCO; Figure 4) showed that differences in the assemblage
378 composition between Falconara and Portonovo were best explained by the quantity of sedimentary
379 OM (higher in Portonovo), whereas differences between Falconara and Senigallia were best explained
380 by the nutritional quality of sedimentary OM (higher in Senigallia).

381 The results of the DistLM forward analyses revealed that the variability in the nematode
382 assemblages composition were significantly explained by the concentration of phaeopigment,
383 carbohydrate, protein, algal fraction of BPC and protein to carbohydrate ratio values, cumulatively
384 explaining ca. 27% of the observed variance (Table 6).

385

386 **3.4 Nematode functional (trophic) diversity and life strategy**

387 At all sites and in all sampling times, non-selective deposit feeders (1B) were the dominant trophic
388 group (45-100%), followed by selective-bacterial feeders (1A; 0-12%) or epistrate/epigrowth feeders
389 (0-8%; 2A) at Senigallia, and by epistrate/epigrowth feeders (2A; 0-38%) and predators/omnivores
390 (2B; 0-24%) at Falconara and Portonovo (Supplementary Figure S3).

391 The results of the PERMANOVA tests show a significant effect of the interaction between Site
392 × Period on either the trophic diversity or the maturity index (Table 3). Values of the 1-ITD and MI
393 indexes differed among sampling sites in almost all sampling times (Supplementary Table S2). The
394 highest 1-ITD values were consistently observed at Portonovo in all sampling times, whereas at all
395 sites temporal variations of 1-ITD values were generally weak (Supplementary Table S2, Figure 5A).

396 The highest MI values were observed at Portonovo or Falconara; the highest MI values occurred
397 in May-June 2011 and May-June 2012 at Senigallia, in late autumn-winter months 2011 at Falconara,
398 and in September 2012 at Portonovo (Supplementary Table S2, Figure 5B).

399 The results of the DistLM forward analyses revealed that the variability in the 1-ITD was
400 significantly explained by the protein to carbohydrate ratio, explaining ca. 9% of the observed
401 variance, whereas the variability in the MI was significantly explained by protein, phaeopigment,
402 chlorophyll-a contents and the algal fraction of BPC (Table 6).

403

404 **3.5 Indicators of ecological quality status (EQS)**

405 According to the available scientific literature (Table 7), several genera (e.g., *Sabatieria*,
406 *Paramonohystera*, *Metalinhomoeus*, *Theristus*, *Odontophora*) retrieved in this study have already
407 been identified as indicators of organic enrichment from different sources, whereas others genera
408 (e.g., *Setosabatieria*, *Halalaimus*) have been previously described as sensitive or indicators of
409 moderate conditions (e.g., *Desmodora*).

410 On the basis on the values of MI, c-p, H' and ITD (*sensu* Moreno et al., 2011), the EQS at
411 Senigallia can be ranked from bad to poor, whereas at Falconara from bad to good and at Portonovo
412 from bad to good (Table 8A). Considering the species belonging to sensitive/tolerant genera (Table
413 8A), all sites can be ranked as bad, since the species *Sabatieria* sp1 ranged up to 54% (i.e. >10%,
414 indicated as threshold by Moreno et al., 2011). Moreover, at all sites, species indicating a poor to
415 moderate status were also observed, even if with relative abundances <10% (*Theristus* sp1 0-3%,
416 *Paralongicyatholaimus* sp5 0-22%, *Odontophora* sp1 0-4%, *Marylinnia* spp 0-7% and *Desmodora*
417 sp1 0-7%). At all sites, species indicating a good status (*Halalaimus* sp.1 and *Setosabatieria* sp1, both
418 0-11%) were also occasionally observed.

419 The temporal variability of the EQS ranking at each site is reported in Table 8B. At Senigallia,
420 the indicators H' and ITD change with sampling times, consistently. At Falconara, all indicators
421 change with sampling time. At Portonovo, MI, c-p and ITD change with sampling times. In some

422 sampling times (e.g., September 2011 at Falconara, June 2011 at Portonovo), very different ranking
423 (e.g., from bad to good in January and June at Portonovo) is reported, depending on the indicator
424 used.

425

426 **4. Discussion**

427 One of the initial challenges of the procedures to comply with the MSFD was the overarching need
428 to conduct a harmonized environmental assessment of marine ecosystems, despite the diverging
429 indicators and data availability across the highly variable characteristics and conditions of the
430 European Regional seas (Hummel et al., 2015; Uusitalo et al., 2016). European marine ecosystems,
431 spanning from semi- to fully enclosed basins as the Mediterranean and the Black Sea, respectively,
432 to brackish waters of the Baltic Sea and open water systems as the Atlantic Ocean and the Norwegian
433 and Barents seas, are highly heterogeneous, and characterized by large spatial and temporal
434 variabilities (Uusitalo et al., 2016). The levels of available knowledge and data within these systems
435 vary in quantity and reliability, as well as the number and typology of indicators utilized by the
436 different EU Member States to assess the ecosystem response to human pressures and their
437 environmental status (Hummel et al., 2015).

438 The Adriatic Sea represents one of the most complex basins along European seas. A recent
439 integrated assessment of the Adriatic Sea status, based on marine biodiversity indicators, revealed
440 poor conditions for this basin (Uusitalo et al., 2016). According to the sampling strategy applied in
441 the present study, our attempt was to utilize a combined approach to assess the environmental status
442 of sites affected by different anthropogenic activities, from the putatively most impacted (Falconara)
443 to the less impacted one (Portonovo). Pelagic-benthic coupling is a key process in determining the
444 trophic condition of benthic systems (Giordani et al., 2002), and the accumulation of organic matter
445 in surface sediments is important in determining the environmental conditions in which the meiofauna
446 live. Our results indicate that several indicators of trophic status and ecological status used in this

study were unable to give a consistent assessment of the investigated sites. For example, at Falconara, characterized by the lowest levels of trophic status and of biodiversity, some indicators of ecological quality status (c-p and ITD) give “moderate” or even “good” assessment in few sampling times (Table 8B). Such discrepancies suggest the need of combined simultaneous use of a wide range of ecological indicators, coupled with indicators of trophic status, in order to achieve a reliable environmental assessment (Semprucci et al., 2015a; 2015b; Chen et al., 2018).

4.1 Analysis of the environmental stressors and benthic trophic status

The Descriptor 5 (Eutrophication) of the MSFD, can be based on either water column or benthic variables; the indicators based on the benthic trophic status is effective and has been previously utilised in the Adriatic Sea (Bianchelli et al., 2016a). The same approach has been repeatedly utilized to assess the benthic trophic status of several from coastal to off-shore marine ecosystems in the Mediterranean basin (Dell'Anno et al., 2002, 2008; Vezzulli and Fabiano, 2006; Pusceddu et al., 2009; 2011; Bianchelli et al., 2016a). Applying this approach and using the thresholds proposed by Dell'Anno et al. (2002), the benthic trophic status of the investigated sites results meso-oligotrophic in terms of carbohydrate contents (all sites) and from meso-oligotrophic (Falconara) to eutrophic (Senigallia and Portonovo, respectively, only in 1-2 sampling times) in terms of proteins. Using the thresholds of BPC contents proposed by Pusceddu et al. (2009), the benthic trophic status of the investigated sites results slightly lower, ranging from oligotrophic at Falconara to meso-oligotrophic at Senigallia and Portonovo (see also Bianchelli et al., 2016a). On the other hand, when using the algal fraction of BPC as an indicator, the benthic trophic status of the three investigated sites varies widely from oligotrophic to eutrophic. Comparatively, the three indicators considered here (i.e. protein and carbohydrate contents, BPC contents and its algal fraction) provide slightly different assessments. These results pinpoint that the different indicators of benthic trophic status can provide different results for their intrinsic ability to indicate qualitative (algal fraction) vs. quantitative

472 (protein, carbohydrate and BPC contents) aspects of OM enrichment processes (Pusceddu et al.,
473 2009).

474 Whatever the indicator considered, the results of our assessment provide evidence that the
475 benthic trophic status of the Adriatic Sea has lowered in the last decade, passing from being meso-
476 eutrophic in late 90ies (Dell'Anno et al., 2008), to meso-oligotrophic in more recent years (this study).
477 This trend is in accordance with the decreasing levels of productivity documented in recent years
478 (Gasparovic, 2012; Giani et al., 2012) and linked to the strong reduction in river nutrient inputs from
479 main tributaries of the basin (Cozzi and Giani, 2011; Cozzi et al., 2012), resulting in documented
480 recovery of benthic communities (Giani et al., 2012).

481

482 ***4.2 Nematode biodiversity as indicator of Ecological Quality Status (EQS)***

483 Biodiversity is widely recognized as one of the indicators of healthy ecosystems (Worm et al., 2006),
484 and indeed, the need to maintain high levels of biological diversity is confirmed by international
485 legislation and conventions (Convention of Biological Diversity; UNEP, 1992). In this regard, the
486 European Union, through the MSFD Descriptor 1, requires member states to assess the status of
487 marine biodiversity and to take action to guarantee that it remains at or is restored to high levels, in
488 order to achieve a Good Environmental Status (GES). Previous studies showed that many
489 anthropogenic impacts have detectable effects on meiofauna (Danovaro et al., 2009b; Zeppilli et al.,
490 2015) and that, among these, nematode assemblages, because of their ubiquity, high abundance and
491 taxonomic diversity, are particularly responsive to a variety of environmental disturbances (Bongers
492 and Ferris 1999; Schratzberger et al., 2004; Steyaert et al., 2007; Moreno et al., 2008; 2011; Neher
493 and Darby, 2009). Strong modifications in nematode structural and functional diversity, assemblage
494 composition and trophic structure occur also under various scenarios of organic enrichment (Duplisea
495 and Hargrave, 1996; Essink and Keidel, 1998; Mazzola et al., 2000; Mirto et al. 2002; 2014; Fraschetti

et al., 2006; 2016; Mahmoudi et al. 2008; Moreno et al., 2008; Vezzulli et al., 2008; Gambi et al., 2009; Semprucci et al., 2014).

For these reasons, and due to their ecological characteristics, nematode diversity has been recently proposed as a possible indicator of Ecological Quality Status (EQS) of marine coastal ecosystems (Moreno et al., 2011; Semprucci et al., 2015a; b; c). In particular, Moreno et al. (2011) proposed an EQS classification based not only on nematode diversity levels (H' index), but also on their trophic diversity (ITD index) and life strategies traits (MI and c-p). In this regard, the ITD index still results a controversial indicator for the EQS assessment, for this reason it was included in our analysis in order to provide additional information on its use (Semprucci et al., 2015a).

Using this approach and applying the thresholds of H' proposed by Moreno et al. (2011), the sites under scrutiny in this study can be ranked as bad-poor (Senigallia and Falconara) and poor (Portonovo, where the highest values of all diversity indexes were observed). Using the indicators based on nematode life strategies, Senigallia can be ranked as “bad”, Falconara and Portonovo as “bad to moderate”. These results are in good agreement with those obtained by previous studies conducted in the same area but based on other indicators (Bianchelli et al., 2016a; Uusitalo et al., 2016). Deviation from the above EQS ranking emerges only using the indicator ITD (here expressed as 1-ITD), as Senigallia ES was classified as “bad to poor”, Falconara as “bad to good”, and Portonovo as “moderate”. The different rankings obtained using ITD can be due to its controversial response to different anthropogenic stressors, already reported for oil spill, biodeposition from fish farms, physical disturbance, commercial harbours, touristic marinas, eutrophicated areas, hydraulically dredged sediments and fish farming impacted sediments (Danovaro et al., 1995; Mirto et al. 2002; Moreno et al., 2011; Alves et al., 2013). Also this study suggests that the ITD is not able to give a reliable assessment of the anthropogenic disturbance and indeed too few investigations so far have shown its good performance in ecological assessments (Semprucci et al., 2015a). Furthermore, the ITD has been criticized since it confines nematode species to a single trophic group

521 (Heip et al., 1985), thus not representing the real complexity of feeding habitats of nematodes, with
522 trophic plasticity being described for most feeding types (Moens and Vincx, 1997; Moens et al., 2005;
523 Schratzberger et al., 2008; Alves et al., 2013; Semprucci et al., 2015a).

524 Conversely, the overall assessment of EQS was consistent using either nematode H' or MI or
525 c-p, with results resembling also those obtained using the richness of meiofaunal taxa as indicator.

526 Our results indicate that the composition of nematode assemblages changed significantly
527 among sites and among sampling times at each site. In particular, the highest dissimilarity in the
528 nematode assemblages' composition occurs between the most impacted sites and those apparently
529 less impacted and with the highest levels of biodiversity.

530 We notice that the genera *Sabatieria* and *Metalinhomoeus* were present in high abundances
531 in the present study. These genera are tolerant to either organic enrichment and low oxygen contents
532 or heavy metals (Heip et al., 1985; Gyedu-Ababio et al., 1999; Danovaro et al., 2009b; Armenteros
533 et al., 2010; Sandulli et al., 2014; Semprucci et al., 2014; Boufahja et al., 2016). In this regard, several
534 studies reported consistently specific nematodes genera as tolerant to different typologies of
535 anthropogenic impacts (Table 7). For instance, *Sabatieria*, *Daptonema*, *Terschellingia*, *Marylynnia* are
536 consistently reported as tolerant genera to sedimentary organic enrichment due to deposition from fish
537 farms plants, highly-contaminated harbours, and hydrocarbon impact (see Table 7 for the literature
538 review).

539 We also recall here that the observed patterns of spatial-temporal variability in nematode
540 assemblages' composition is mostly explained by changes in the relative abundance of the most
541 abundant species (*Paramonohystera* sp1 and *Sabatieria* sp1, representing cumulatively from 52 to
542 81% of assemblages). The genera *Paramonohystera* and *Sabatieria* occur at all investigated sites and
543 are considered indicators of bad EQS (Gyedu-Ababio et al 1999; Danovaro et al., 2009b; Moreno et
544 al., 2011). These results suggest that the ecological status of a certain system can be identified not
545 only by the presence/absence of some specific nematode genera (e.g. *Sabatieria* and

546 *Metalinhomoeus*) but also considering the relative abundance of highly tolerant nematode species (as
547 in the case of *Paramonohystera sp1* and *Sabatieria sp1*) (Alves et al., 2015).

548

549 **4.3 Relationships between nematode biodiversity and benthic trophic status**

550 Previous studies indicated that the biodiversity of meiofauna (analysed at higher taxonomic level) is
551 sensitive to changes in benthic trophic status and environmental stressors (Pusceddu et al., 2011;
552 Bianchelli et al., 2016a; 2016b). However, the observed responses can vary among systems
553 characterized by different levels of initial benthic trophic status (Mirto et al., 2010; Pusceddu et al.,
554 2007). For instance, it has been shown that in oligo-mesotrophic ecosystems the relationships between
555 changes in the benthic trophic status and meiofaunal biodiversity are positive (Bianchelli et al.,
556 2016a), whereas major organic loads, for instance in the case of aquaculture biodeposition, can have
557 a significant and negative impact on meiofauna, especially when a shift from oligo- to mesotrophic
558 conditions is observed (Mirto et al., 2010; 2014; Pusceddu et al., 2007; 2011). Similarly, the results
559 of the present study on nematode species indicate that the highest biodiversity levels are coupled with
560 the highest sedimentary organic matter contents.

561 Previous studies repeatedly demonstrated that a large accumulation of organic C, mostly
562 accounted for by material of detrital/heterotrophic origin, may cause profound modifications of
563 sediment distinctive features (particularly oxygen availability; Pusceddu et al., 2009), which can
564 affect also nematode functional diversity (e.g., life strategies traits; Gambi et al., 2009). The results
565 of this study confirm that organic enrichment can result in altered trophic/functional biodiversity and
566 life strategy traits, especially when comparing oligo- vs. mesotrophic systems. Such differences might
567 be associated with alterations of the ecosystem processes, such as the ability to perform the key
568 biological and biogeochemical processes (Danovaro et al., 2008; Pusceddu et al., 2014b).

569 Overall, the assessment of ecological quality status based on the nematode biodiversity allowed
570 us to identify a prevalence of “bad to moderate” conditions. This means that while the trophic status

571 of the investigated area did not identify severely harmful conditions (e.g., the presence of eutrophic
572 or dystrophic conditions), the overall environmental quality in terms of biodiversity (Descriptor 1 of
573 the MSFD) appears worst than expected from the trophic status only. In this regard, the results of the
574 multiple multivariate regression analyses indicate that changes in the variables used for determining
575 the benthic trophic status explained only up to 27% of the variance in nematode diversity. This
576 suggest that the trophic status alone is not the unique factor shaping nematode assemblages, and that
577 many other environmental parameters could have a significant influence. In this regard, we notice
578 that previous studies reported oxygen availability, which could be also linked with organic
579 enrichment, is an environmental driver of nematode biodiversity variability (Gambi et al., 2009;
580 Alves et al., 2015).

581 Overall, our results suggest that the environmental status cannot be defined uniquely in terms
582 of sedimentary OM enrichment (benthic eutrophication; sensu Pusceddu et al. 2009), especially when
583 many other multiples stressors can contribute to determine the overall environmental quality of the
584 investigated ecosystem.

585 We conclude that the analysis of nematode species, as sensitive in spatial and temporal terms
586 to changes in trophic conditions as well as cumulatively to many other anthropogenic stressors, can
587 represent a reliable tool to contribute to the assessment the environmental status in coastal marine
588 sediments.

589

590

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927 **Caption of Figures**

928 **Figure 1.** A) Location of the sampling sites (Senigallia, Falconara and Portonovo) in the Northern
929 Adriatic Sea. (the red dot indicates the study area). B) Spatial distribution of cumulative impacts
930 to the territorial waters of Italian seas, modified from Micheli et al. (2013) [Impacts considered:
931 artisanal fishing, fishing (demersal, pelagic, destructive, non-destructive, high bycatch, low
932 bycatch), benthic structures (oil rigs), coastal aggradation (coastal renourishment), coastal
933 engineering (coastal defense and harbors), coastal erosion, coastal population density, commercial
934 shipping, invasive species, nutrient input (fertilizers), ocean acidification, oil spills, organic
935 pollution (pesticides), risk of hypoxia, sea surface temperature change, urban runoff (nonpoint
936 inorganic pollution), urbanization trends, UV radiation; Micheli et al., 2013].

937 **Figure 2.** Temporal variation of nematode species richness (SR) at Senigallia (A), Falconara (B) and
938 Portonovo (C). (SR values calculated cumulatively from the 3 replicates are reported. Average
939 values $\pm$ sd are reported in Table 2).

940 **Figure 3.** Output of CAP (A) and PERMDISP on temporal variations (B) in the nematode species
941 composition.

942 **Figure 4.** Output of PCO, to identify sedimentary OM variables mostly responsible for the differences
943 in the composition of nematode assemblages.

944 **Figure 5.** Distribution of 1-ITD and MI values at Senigallia, Falconara and Portonovo. Reported are
945 minimum, maximum, median values and standard error bars.

946

947

948 **Table 1.** Characteristics of the investigated sites. Reported are location, distance from the coast and
 949 main anthropogenic pressure affecting each site.

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Station	Latitude (N)	Longitude (E)	Distance from the coast	Pressures/characteristics
Senigallia	43°45'30"	13°13'00"	3 km	commercial and touristic maritime traffic throughout the year, receives seasonally riverine inputs from the nearby Misa river
Falconara	43°39'00"	13°22'00"	0.6 km	receives inputs from the Esino river estuary and shows the presence of a petrochemical industry (refinery) located ca 1 km apart.
Portonovo	43°36'12"	13°36'42"	4.5 km	tourism and maritime traffic during the summer season, because of its ecological peculiarity, is included within a Site of Community Importance (Natura 2000, site code IT5320006).

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953 **Table 2.** OM sedimentary contents and nutritional quality (A): concentration of phytopigments (chlorophyll-a, phaeopigments and total
954 phytopigments), protein, carbohydrate, lipid, BPC, chlorophyll-a and protein to BPC and protein to carbohydrate ratios. Nematode diversity indexes
955 (B): species richness (SR), index of Margalef (D), equitability (Pielou's index, J), Shannon-Wiener information function (H'), expected species number
956 for 100 individuals (ES100), index of trophic diversity (1-ITD) and maturity index (MI).

A)		Chlorophyll-a µg g ⁻¹		Phaeopigment µg g ⁻¹		Total phytopigment µg g ⁻¹		Protein mg g ⁻¹		Carbohydrate mg g ⁻¹		Lipid mg g ⁻¹		Biopolymeric C mg g ⁻¹		Chlorophyll-a to BPC %		Protein to BPC %		Protein to carbohydrate ratio	
Site	Time	avg	sd	avg	sd	avg	sd	avg	sd	avg	sd	avg	sd	avg	sd	avg	sd	avg	sd	avg	sd
Senigallia	January 2011	0.8	0.1	10.3	0.9	11.1	1.0	1.5	0.2	0.4	0.0	0.19	0.0	1.0	0.1	3.1	0.1	71.4	1.5	3.9	0.2
	May 2011	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na
	June 2011	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na
	September 2011	0.4	0.1	4.3	0.5	4.7	0.5	0.5	0.0	0.4	0.1	0.15	0.0	0.5	0.1	3.3	0.2	46.9	1.6	1.2	0.1
	November 2011	0.4	0.1	3.6	1.0	4.0	1.1	0.6	0.1	0.1	0.0	0.03	0.0	0.4	0.1	42.3	5.1	78.4	1.1	4.2	0.1
	December 2011	1.5	0.3	12.8	2.3	14.3	2.6	2.6	2.0	0.3	0.0	0.15	0.1	1.5	1.0	51.8	34.8	79.5	12.6	8.1	5.4
	January 2012	0.2	0.0	3.5	0.3	3.7	0.2	0.6	0.0	0.1	0.0	0.04	0.0	0.4	0.0	39.8	1.6	77.4	3.8	4.1	0.3
	May 2012	0.6	0.5	1.5	0.9	1.5	0.7	1.1	0.3	0.5	0.4	0.04	0.0	0.8	0.4	7.8	0.5	74.6	14.1	3.7	2.9
	June 2012	0.3	0.1	1.6	0.4	1.9	0.5	0.6	0.1	0.1	0.0	0.02	0.0	0.4	0.1	20.3	1.5	81.9	4.4	5.1	1.3
	September 2012	0.3	0.1	2.7	0.7	3.0	0.6	0.9	0.0	0.2	0.0	0.03	0.0	0.5	0.0	23.0	2.4	84.5	3.3	6.2	1.7
Falconara	January 2011	0.2	0.0	1.4	0.1	1.6	0.1	0.2	0.0	0.2	0.0	0.06	0.0	0.2	0.0	3.9	0.1	51.5	1.9	1.4	0.1
	May 2011	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na
	June 2011	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na
	September 2011	0.6	0.1	8.0	3.6	10.4	4.1	0.5	0.0	0.5	0.1	0.08	0.0	0.5	0.1	4.8	0.6	50.4	2.6	1.1	0.1
	November 2011	1.1	0.3	2.6	0.9	3.6	1.1	0.4	0.0	0.2	0.0	0.03	0.0	0.3	0.0	48.6	11.6	68.0	0.7	2.2	0.1
	December 2011	0.6	0.1	1.7	0.3	2.3	0.5	0.3	0.0	0.1	0.0	0.02	0.0	0.2	0.0	43.5	4.6	69.3	1.6	2.3	0.2
	January 2012	0.3	0.1	1.3	0.1	1.6	0.2	0.7	0.6	0.1	0.0	0.01	0.0	0.4	0.3	21.9	16.8	78.2	14.0	4.7	2.9
	May 2012	0.8	0.3	7.8	0.9	8.6	1.1	0.9	0.3	0.3	0.1	0.04	0.0	0.6	0.2	59.5	8.9	75.1	0.5	3.1	0.2
	June 2012	0.2	0.0	1.2	0.2	1.4	0.2	0.5	0.1	0.1	0.0	0.01	0.0	0.3	0.0	17.2	0.6	80.3	0.6	3.9	0.3
	September 2012	1.9	0.1	3.7	0.9	5.5	1.1	0.9	0.1	0.3	0.1	0.03	0.0	0.6	0.1	37.7	0.0	78.6	3.8	3.7	0.7
Portonovo	January 2011	1.1	0.0	20.1	1.9	21.2	2.0	1.7	0.1	0.5	0.1	0.38	0.1	1.3	0.1	3.4	0.3	62.3	2.9	3.2	0.5
	May 2011	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na
	June 2011	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na
	September 2011	0.6	0.1	7.8	1.0	8.4	0.9	0.8	0.0	0.7	0.2	0.24	0.0	0.8	0.1	3.0	0.1	45.0	3.4	1.1	0.2
	November 2011	0.9	0.1	6.4	0.9	7.3	1.1	1.5	0.3	0.5	0.1	0.09	0.0	1.0	0.2	28.9	1.5	74.3	0.1	3.2	0.1
	December 2011	1.3	0.3	14.2	2.8	15.4	3.1	2.3	0.3	0.5	0.1	0.14	0.1	1.4	0.2	42.6	1.8	79.4	2.6	4.7	0.4
	January 2012	0.4	0.1	13.1	1.0	13.5	1.1	1.8	0.3	0.4	0.1	0.12	0.1	1.2	0.2	47.5	5.8	78.6	2.4	4.6	0.2
	May 2012	0.6	0.1	2.7	0.1	3.3	0.1	1.2	0.2	0.3	0.1	0.07	0.0	0.7	0.2	18.3	3.1	79.0	1.7	4.6	0.2
	June 2012	0.4	0.1	3.5	0.1	3.9	0.1	1.0	0.2	0.2	0.0	0.03	0.0	0.6	0.1	26.8	4.4	83.4	1.8	5.3	0.8
	September 2012	0.7	0.1	4.8	1.1	5.5	1.2	1.8	0.5	0.3	0.0	0.10	0.0	1.0	0.3	21.3	1.3	83.5	1.9	6.0	0.8

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B)		SR			D			H (log _e)			J			ES (100)			1-ITD			MI		
Site	Time	avg	sd	SR (cum)	avg	sd	D (cum)	avg	sd	H (cum)	avg	sd	J (cum)	avg	sd	ES (cum)	avg	sd	1-ITD (cum)	avg	sd	MI (cum)
Senigallia	January 2011	10.0	2.0	15	2.0	0.4	2.5	1.6	0.3	1.8	0.7	0.1	0.6	10.0	2.0	11.8	0.2	0.1	0.2	2.0	0.0	2.0
	May 2011	8.7	2.9	13	1.7	0.6	2.1	1.5	0.2	1.6	0.7	0.1	0.6	8.7	2.9	9.4	0.2	0.1	0.2	2.1	0.1	2.1
	June 2011	9.7	0.6	12	1.9	0.1	1.9	1.5	0.1	1.5	0.7	0.0	0.6	9.7	0.6	9.7	0.3	0.0	0.3	2.2	0.0	2.2
	September 2011	8.7	1.2	14	1.7	0.3	2.3	1.3	0.0	1.3	0.6	0.0	0.5	8.7	1.2	8.6	0.2	0.1	0.2	2.2	0.1	2.2
	November 2011	3.7	0.6	5	0.6	0.1	0.7	0.5	0.0	0.5	0.4	0.1	0.3	3.7	0.6	3.6	0.0	0.0	0.0	2.0	0.0	2.0
	December 2011	5.3	1.2	8	0.9	0.3	1.2	1.0	0.1	1.0	0.6	0.1	0.5	5.3	1.2	5.7	0.1	0.0	0.1	2.0	0.0	2.0
	January 2012	5.3	1.2	8	0.9	0.3	1.2	0.9	0.2	1.0	0.6	0.1	0.5	5.3	1.2	5.7	0.1	0.0	0.1	2.0	0.0	2.0
	May 2012	9.3	1.2	13	1.8	0.3	2.1	1.3	0.2	1.5	0.6	0.1	0.6	9.3	1.2	9.9	0.2	0.0	0.2	2.1	0.1	2.1
	June 2012	6.0	2.0	10	1.1	0.4	1.6	1.0	0.1	1.1	0.6	0.1	0.5	6.0	2.0	6.7	0.2	0.1	0.2	2.1	0.1	2.1
	September 2012	10.3	2.5	17	2.0	0.5	2.8	1.4	0.3	1.5	0.6	0.1	0.5	10.3	2.5	11.4	0.2	0.1	0.2	2.1	0.1	2.1
Falconara	January 2011	10.3	3.2	18	2.0	0.7	3.0	1.5	0.5	1.9	0.6	0.1	0.7	10.3	3.2	13.4	0.4	0.2	0.5	2.2	0.0	2.2
	May 2011	8.7	1.5	12	1.7	0.3	1.9	1.0	0.3	1.1	0.5	0.1	0.4	8.7	1.5	10.1	0.2	0.1	0.2	2.1	0.1	2.1
	June 2011	1.3	0.6	2	0.1	0.1	0.2	0.1	0.1	0.1	0.2	na	0.1	1.3	0.6	1.8	0.0	0.0	0.0	2.0	0.0	2.0
	September 2011	10.0	0.0	12	2.0	0.0	1.9	1.9	0.1	2.0	0.8	0.1	0.8	10.0	0.0	11.2	0.6	0.1	0.7	2.6	0.1	2.6
	November 2011	7.0	2.0	9	1.3	0.4	1.4	1.2	0.2	1.3	0.6	0.0	0.6	7.0	2.0	7.4	0.4	0.0	0.4	2.2	0.1	2.2
	December 2011	11.0	2.0	15	2.2	0.4	2.5	2.0	0.1	2.1	0.8	0.1	0.8	11.0	2.0	12.5	0.6	0.0	0.6	2.4	0.1	2.4
	January 2012	11.0	2.0	15	2.2	0.4	2.5	2.0	0.1	2.1	0.8	0.1	0.8	11.0	2.0	12.3	0.6	0.0	0.6	2.4	0.0	2.4
	May 2012	11.3	4.0	19	2.2	0.9	3.2	1.7	0.4	2.0	0.7	0.1	0.7	11.3	4.0	14.2	0.4	0.1	0.4	2.1	0.1	2.1
	June 2012	8.3	1.2	12	1.6	0.3	1.9	1.5	0.4	1.8	0.7	0.2	0.7	8.3	1.2	9.6	0.5	0.2	0.5	2.3	0.2	2.3
	September 2012	11.3	2.9	14	2.2	0.6	2.3	1.4	0.3	1.5	0.6	0.1	0.6	11.3	2.9	12.0	0.4	0.0	0.4	2.1	0.0	2.1
Portonovo	January 2011	11.0	2.0	18	2.2	0.4	3.0	1.8	0.4	2.1	0.8	0.2	0.7	11.0	2.0	13.5	0.5	0.1	0.6	2.2	0.0	2.2
	May 2011	11.3	4.2	18	2.2	0.9	3.0	1.9	0.3	2.0	0.8	0.0	0.7	11.3	4.2	13.3	0.5	0.1	0.5	2.1	0.0	2.1
	June 2011	13.3	1.2	17	2.7	0.3	2.8	2.0	0.1	2.1	0.8	0.0	0.7	13.3	1.2	13.3	0.6	0.0	0.6	2.1	0.1	2.1
	September 2011	12.7	0.6	15	2.5	0.1	2.5	2.0	0.1	2.0	0.8	0.0	0.8	12.7	0.6	12.9	0.5	0.0	0.5	2.2	0.0	2.2
	November 2011	11.7	3.1	16	2.3	0.7	2.6	1.8	0.6	2.0	0.7	0.2	0.7	11.7	3.1	13.1	0.4	0.2	0.4	2.1	0.1	2.1
	December 2011	12.7	2.1	19	2.5	0.5	3.2	1.6	0.1	1.8	0.6	0.0	0.6	12.7	2.1	13.7	0.4	0.1	0.4	2.0	0.1	2.0
	January 2012	12.7	2.1	19	2.5	0.5	3.2	1.6	0.1	1.8	0.6	0.0	0.6	12.7	2.1	13.9	0.4	0.1	0.4	2.1	0.1	2.1
	May 2012	12.7	0.6	16	2.5	0.1	2.6	1.7	0.2	1.8	0.7	0.1	0.6	12.7	0.6	12.0	0.4	0.0	0.4	2.1	0.1	2.1
	June 2012	11.7	0.6	16	2.3	0.1	2.6	1.7	0.1	1.8	0.7	0.0	0.7	11.7	0.6	11.8	0.5	0.1	0.5	2.2	0.0	2.2
	September 2012	11.3	0.6	14	2.2	0.1	2.3	2.0	0.0	2.1	0.8	0.0	0.8	11.3	0.6	12.0	0.6	0.0	0.6	2.4	0.1	2.4

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Table 3. Results of PERMANOVA testing variations in the sedimentary OM biochemical compounds contents, indicators of nutritional quality, biochemical composition (A), nematode diversity indices and species composition (B). dF=degree of freedom; MS=mean square; F=F statistic; ***=P < 0.001; **=P < 0.01; *=P < 0.05; ns=not significant.

A)	Source	df	MS	F	P	% explained variance	B)		DF	MS	F	P	% explained variance
Chlorophyll-a	Site	2	0.8	5.1	*	2.1	SR	Site	2	4232.0	36.0	***	23.0
	Time	7	3.9	23.3	***	30.5		Time	9	822.7	7.0	***	13.1
	Site × Time	14	2.4	14.4	***	55.1		Site × Time	18	910.2	7.7	***	44.2
	Residual	48	0.2			12.3		Residual	60	117.6			19.7
Phaeopigment	Site	2	7.9	122.2	***	22.8	D	Site	2	4923.1	34.4	***	29.7
	Time	7	3.1	48.0	***	23.6		Time	9	808.2	5.6	***	13.4
	Site × Time	14	2.2	33.5	***	49.0		Site × Time	18	679.7	4.7	***	32.2
	Residual	48	0.1			4.5		Residual	57	143.2			24.7
Total phytotpigment	Site	2	6.9	96.0	***	19.7	H	Site	2	3506.7	48.3	***	25.4
	Time	7	3.1	43.4	***	23.5		Time	9	634.6	8.7	***	13.5
	Site × Time	14	2.3	32.2	***	51.8		Site × Time	18	694.9	9.6	***	44.4
	Residual	48	0.1			5.0		Residual	57	72.6			14.9
Protein	Site	2	9.8	25.2	***	31.0	J	Site	2	868.9	16.8	***	15.9
	Time	7	2.1	5.4	***	15.0		Time	9	179.5	34.6	**	8.0
	Site × Time	14	1.3	3.3	**	23.4		Site × Time	18	308.4	59.5	***	48.1
	Residual	48	0.4			30.7		Residual	57	51.8			28.0
Carbohydrate	Site	2	7.3	20.9	***	23.5	ES100	Site	2	3979.6	36.0	***	31.8
	Time	7	3.6	10.2	***	29.1		Time	9	571.4	5.2	***	12.3
	Site × Time	14	1.1	3.0	**	19.1		Site × Time	18	496.1	44.9	***	30.6
	Residual	48	0.3			28.3		Residual	57	110.5			25.3
Lipid	Site	2	8.7	82.7	***	27.4	1-ITD	Site	2	17198.0	53.3	***	51.0
	Time	7	4.8	45.2	***	39.6		Time	9	897.3	2.8	**	5.6
	Site × Time	14	1.1	10.3	***	25.0		Site × Time	16	947.4	2.9	**	17.0
	Residual	48	0.1			8.1		Residual	56	322.6			26.3
Biopolymeric C	Site	2	12.1	34.4	***	37.7	MI	Site	2	718.5	77.2	***	43.4
	Time	7	1.7	4.9	***	11.7		Time	9	39.8	4.3	***	6.8
	Site × Time	14	1.3	3.6	***	23.6		Site × Time	16	54.5	5.9	***	27.7
	Residual	48	0.4			27.1		Residual	56	9.3			17.1
Biochemical camposition	Site	2	34.6	32.1	***	21.1	Species composition	Site	2	20169.0	49.6	***	2.0
	Time	7	17.4	16.2	***	27.6		Time	9	2323.3	5.7	***	12.2
	Site × Time	14	8.0	7.4	***	35.0		Site × Time	18	1812.4	4.5	***	26.8
	Residual	48	1.1			16.3		Residual	60	407.0			23.3
Chlorophyll-a to biopolymeric C ratio	Site	2	258.3	3.4	*	1.7							
	Time	7	2228.9	29.1	***	53.2							
	Site × Time	14	456.6	6.0	***	28.2							
	Residual	48	76.5			17.0							
Protein to biopolymeric C ratio	Site	2	195.0	6.9	**	3.9							
	Time	7	1264.2	45.0	***	76.1							
	Site × Time	14	52.7	1.9	*	4.5							
	Residual	48	28.1			15.6							
Protein to carbohydrate	Site	2	20.4	9.3	***	15.0							
	Time	7	17.5	7.9	***	33.7							
	Site × Time	14	3.3	1.5	ns	7.5							
	Residual	48	2.2			43.7							

Table 4. Benthic trophic status ranking of the investigated sites and comparison with thresholds proposed by Dell'Anno et al. (2002) and Pusceddu et al. (2009, 2011) (A) and temporal variation of the ranking at each site. In A) reported are the range of values for each indicator observed at each site along the whole study period. Ranking of the three sites is highlighted in light grey. In B) oligotr = oligotrophic; meso-oligotr = meso-oligotrophic, eutr = eutrophic.

		Benthic trophic status							
A)	Indicator	oligotrophic	meso-oligotrophic	eutrophic	hypertrophic	Source			
Thresholds	Protein		<1.5 mg g ⁻¹	1.5-4 mg g ⁻¹	> 4 mg g ⁻¹	Dell'Anno et al., 2002			
	Carbohydrate		<5 mg g ⁻¹	5-7 mg g ⁻¹	> 7 mg g ⁻¹	Dell'Anno et al., 2002			
	biopolymeric C	<1 mg g ⁻¹	1-3 mg g ⁻¹	>3 mg g ⁻¹		Pusceddu et al., 2009; 2011			
	algal fraction of biopolymeric C	>25%	12-25%	<12%		Pusceddu et al., 2009; 2011			
Senigallia	Protein		0.5- 2.6 mg g ⁻¹			present study			
	Carbohydrate		0.1- 0.5 mg g ⁻¹			present study			
	biopolymeric C		0.4- 1.5 mg g ⁻¹			present study			
	algal fraction of biopolymeric C		3.1-51.8%			present study			
Falconara	Protein		0.2-0.9 mg g ⁻¹			present study			
	Carbohydrate		0.1-0.5 mg g ⁻¹			present study			
	biopolymeric C	0.2-0.6 mg g ⁻¹				present study			
	algal fraction of biopolymeric C		3.9-59.5 %			present study			
Portonovo	Protein		0.8-2.3 mg g ⁻¹			present study			
	Carbohydrate		0.2-0.7 mg g ⁻¹			present study			
	biopolymeric C		0.6-1.4 mg g ⁻¹			present study			
	algal fraction of biopolymeric C		3.0-47.5 %			present study			
B)		January 2011	September 2011	November 2011	December 2011	January 2012	May 2012	June 2012	September 2012
Senigallia	Protein	meso-oligotr	meso-oligotr	meso-oligotr	eutr	meso-oligotr	meso-oligotr	meso-oligotr	meso-oligotr
	carbohydrate	meso-oligotr	meso-oligotr	meso-oligotr	meso-oligotr	meso-oligotr	meso-oligotr	meso-oligotr	meso-oligotr
	biopolymeric C	meso-oligotr	meso-oligotr	oligotr	meso-oligotr	oligotr	oligotr	oligotr	oligotr
	algal fraction of biopolymeric C	eutr	eutr	oligotr	oligotr	oligotr	eutr	meso-oligotr	meso-oligotr
Falconara	Protein	meso-oligotr	meso-oligotr	meso-oligotr	meso-oligotr	meso-oligotr	meso-oligotr	meso-oligotr	meso-oligotr
	carbohydrate	meso-oligotr	meso-oligotr	meso-oligotr	meso-oligotr	meso-oligotr	meso-oligotr	meso-oligotr	meso-oligotr
	biopolymeric C	oligotr	oligotr	oligotr	oligotr	oligotr	oligotr	oligotr	oligotr
	algal fraction of biopolymeric C	eutr	eutr	oligotr	oligotr	meso-oligotr	oligotr	meso-oligotr	oligotr
Portonovo	Protein	eutr	meso-oligotr	meso-oligotr	eutr	eutr	meso-oligotr	meso-oligotr	eutr
	carbohydrate	meso-oligotr	meso-oligotr	meso-oligotr	meso-oligotr	meso-oligotr	meso-oligotr	meso-oligotr	meso-oligotr
	biopolymeric C	meso-oligotr	oligotr	meso-oligotr	meso-oligotr	meso-oligotr	oligotr	oligotr	meso-oligotr
	algal fraction of biopolymeric C	eutr	eutr	oligotr	oligotr	oligotr	meso-oligotr	oligotr	meso-oligotr

977 **Table 5.** Results of SIMPER tests assessing dissimilarity levels in the species composition of
 978 nematodes assemblages among sampling sites and times.

979

Among Sites	Contrast	Dissimilarity %	Average dissimilarity %	Responsible species
January 2011	Senigallia vs Falconara	46.2	46.6	<i>Metalinhomoeus</i> sp3, <i>Paramonohystera</i> sp1, <i>Hopperia</i> sp1
	Senigallia vs Portonovo	48.4		<i>Sabatieria</i> sp1, <i>Metalinhomoeus</i> sp3, <i>Subsphaerolaimus</i> sp1
	Falconara vs Portonovo	45.1		<i>Metalinhomoeus</i> sp3, <i>Paramonohystera</i> sp1, <i>Hopperia</i> sp1
May 2011	Senigallia vs Falconara	51.6	54.6	<i>Setosabatieria</i> sp1, <i>Metalinhomoeus</i> sp3, <i>Paramonohystera</i> sp1
	Senigallia vs Portonovo	47.4		<i>Hopperia</i> sp1, <i>Setosabatieria</i> sp1, <i>Metalinhomoeus</i> sp3
	Falconara vs Portonovo	64.9		<i>Metalinhomoeus</i> sp3, <i>Paramonohystera</i> sp1, <i>Hopperia</i> sp1
June 2011	Senigallia vs Falconara	59.9	63.2	<i>Sabatieria</i> sp1, <i>Halalaimus</i> sp1, <i>Paramonohystera</i> sp1
	Senigallia vs Portonovo	50.6		<i>Paramonohystera</i> sp1, <i>Halalaimus</i> sp1, <i>Hopperia</i> sp1
	Falconara vs Portonovo	79.2		<i>Paramonohystera</i> sp1, <i>Sabatieria</i> sp1, <i>Enoploides</i> sp1
September 2011	Senigallia vs Falconara	50.4	51.0	<i>Paralongicyatholaimus</i> sp5, <i>Sabatieria</i> sp1, <i>Chaetonema</i> sp1
	Senigallia vs Portonovo	44.7		<i>Hopperia</i> sp1, <i>Paramonohystera</i> sp1, <i>Halalaimus</i> sp1
	Falconara vs Portonovo	57.8		<i>Hopperia</i> sp1, <i>Sabatieria</i> sp1, <i>Halalaimus</i> sp1
November 2011	Senigallia vs Falconara	39.9	49.1	<i>Maryllynia</i> sp1, <i>Enoploides</i> sp1, <i>Sabatieria</i> sp1
	Senigallia vs Portonovo	57.9		<i>Paramonohystera</i> sp1, <i>Metalinhomoeus</i> sp3, <i>Sabatieria</i> sp1
	Falconara vs Portonovo	49.5		<i>Paramonohystera</i> sp1, <i>Metalinhomoeus</i> sp3, <i>Sabatieria</i> sp1
December 2011	Senigallia vs Falconara	66.5	63.5	<i>Diodontolaimus</i> sp1, <i>Enoploides</i> sp1, <i>Sabatieria</i> sp1
	Senigallia vs Portonovo	53.7		<i>Paramonohystera</i> sp1, <i>Hopperia</i> sp1, <i>Metalinhomoeus</i> sp3
	Falconara vs Portonovo	70.2		<i>Sabatieria</i> sp1, <i>Diodontolaimus</i> sp1, <i>Hopperia</i> sp1
January 2012	Senigallia vs Falconara	66.7	64.0	<i>Diodontolaimus</i> sp1, <i>Sabatieria</i> sp1, <i>Enoploides</i> sp1
	Senigallia vs Portonovo	54.8		<i>Paramonohystera</i> sp1, <i>Hopperia</i> sp1, <i>Metalinhomoeus</i> sp3
	Falconara vs Portonovo	70.6		<i>Sabatieria</i> sp1, <i>Diodontolaimus</i> sp1, <i>Hopperia</i> sp1
May 2012	Senigallia vs Falconara	45.3	44.3	<i>Paramesonchium</i> sp1, <i>Metalinhomoeus</i> sp3, <i>Paramonohystera</i> sp1
	Senigallia vs Portonovo	39.9		<i>Enoploides</i> sp1, <i>Thalassomonhystera</i> sp1, <i>Hopperia</i> sp1
	Falconara vs Portonovo	47.6		<i>Paramesonchium</i> sp1, <i>Enoploides</i> sp1, <i>Hopperia</i> sp1
June 2012	Senigallia vs Falconara	42.8	44.7	<i>Oncholaimellus</i> sp1, <i>Sabatieria</i> sp1, <i>Paramesonchium</i> sp1
	Senigallia vs Portonovo	40.2		<i>Enoploides</i> sp1, <i>Paralongicyatholaimus</i> sp5, <i>Sabatieria</i> sp1
	Falconara vs Portonovo	51.0		<i>Oncholaimellus</i> sp1, <i>Enoploides</i> sp1, <i>Paramesonchium</i> sp1
September 2012	Senigallia vs Falconara	47.4	51.0	<i>Enoploides</i> sp1, <i>Sabatieria</i> sp1, <i>Metalinhomoeus</i> sp3
	Senigallia vs Portonovo	45.7		<i>Paralongicyatholaimus</i> sp5, <i>Paramonohystera</i> sp1, <i>Sphaerolaimus</i> sp1
	Falconara vs Portonovo	60.0		<i>Paralongicyatholaimus</i> sp5, <i>Sphaerolaimus</i> sp1, <i>Paramonohystera</i> sp1
Between Times	Contrast	Dissimilarity %	Avg dissimilarity %	Responsible species
Senigallia	January 2011 vs May 2011	36.9	31.2	<i>Hopperia</i> sp1, <i>Odontophora</i> sp1, <i>Metalinhomoeus</i> sp3
	May 2011 vs June 2011	26.4		<i>Halalaimus</i> sp1, <i>Metalinhomoeus</i> sp3, <i>Sabatieria</i> sp1
	June 2011 vs September 2011	28.7		<i>Sabatieria</i> sp1, <i>Nemanema</i> sp1, <i>Setosabatieria</i> sp1
	September 2011 vs November 2011	37.0		<i>Sabatieria</i> sp1, <i>Paramonohystera</i> sp1, <i>Halalaimus</i> sp1
	November 2011 vs December 2011	24.0		<i>Sabatieria</i> sp1, <i>Paramonohystera</i> sp1, <i>Setosabatieria</i> sp1
	December 2011 vs January 2012	15.4		<i>Sabatieria</i> sp1, <i>Paramonohystera</i> sp1, <i>Paralongicyatholaimus</i> sp5
	January 2012 vs May 2012	40.0		<i>Paramonohystera</i> sp1, <i>Metalinhomoeus</i> sp3, <i>Sabatieria</i> sp1
	May 2012 vs June 2012	39.6		<i>Paramonohystera</i> sp1, <i>Metalinhomoeus</i> sp3, <i>Sabatieria</i> sp1
	June 2012 vs September 2012	33.2		<i>Metalinhomoeus</i> sp3, <i>Setosabatieria</i> sp1, <i>Diodontolaimus</i> sp1
Falconara	January 2011 vs May 2011	42.5	48.1	<i>Paramonohystera</i> sp1, <i>Hopperia</i> sp1, <i>Halalaimus</i> sp1
	May 2011 vs June 2011	45.3		<i>Sabatieria</i> sp1, <i>Paramesonchium</i> sp1, <i>Chaetonema</i> sp1
	June 2011 vs September 2011	71.4		<i>Paralongicyatholaimus</i> sp5, <i>Paramonohystera</i> sp1, <i>Halalaimus</i> sp1
	September 2011 vs November 2011	52.1		<i>Paralongicyatholaimus</i> sp5, <i>Maryllynia</i> sp1, <i>Paramonohystera</i> sp1
	November 2011 vs December 2011	59.5		<i>Diodontolaimus</i> sp1, <i>Chaetonema</i> sp1, <i>Oncholaimellus</i> sp1
	December 2011 vs January 2012	17.6		<i>Metalinhomoeus</i> sp1, <i>Oncholaimellus</i> sp1, <i>Diodontolaimus</i> sp1
	January 2012 vs May 2012	56.1		<i>Paramesonchium</i> sp1, <i>Diodontolaimus</i> sp1, <i>Sabatieria</i> sp1
	May 2012 vs June 2012	44.3		<i>Diodontolaimus</i> sp1, <i>Sabatieria</i> sp1, <i>Oncholaimellus</i> sp1
	June 2012 vs September 2012	43.8		<i>Enoploides</i> sp1, <i>Diodontolaimus</i> sp1, <i>Paramonohystera</i> sp1
Portonovo	January 2011 vs May 2011	47.1	34.3	<i>Metalinhomoeus</i> sp3, <i>Nemanema</i> sp1, <i>Paramonohystera</i> sp1
	May 2011 vs June 2011	37.5		<i>Metalinhomoeus</i> sp3, <i>Paralongicyatholaimus</i> sp5, <i>Enoploides</i> sp1
	June 2011 vs September 2011	31.6		<i>Enoploides</i> sp1, <i>Sphaerolaimus</i> sp1, <i>Paramesonchium</i> sp1
	September 2011 vs November 2011	35.8		<i>Metalinhomoeus</i> sp3, <i>Paralongicyatholaimus</i> sp5, <i>Diodontolaimus</i> sp1
	November 2011 vs December 2011	35.0		<i>Metalinhomoeus</i> sp3, <i>Thalassomonhystera</i> sp1, <i>Sabatieria</i> sp1
	December 2011 vs January 2012	21.5		<i>Thalassomonhystera</i> sp1, <i>Sphaerolaimus</i> sp1, <i>Paramonohystera</i> sp1
	January 2012 vs May 2012	32.9		<i>Paramonohystera</i> sp1, <i>Enoploides</i> sp1, <i>Metalinhomoeus</i> sp3
	May 2012 vs June 2012	31.7		<i>Sabatieria</i> sp1, <i>Thalassomonhystera</i> sp1, <i>Paralongicyatholaimus</i> sp5
	June 2012 vs September 2012	36.0		<i>Diodontolaimus</i> sp1, <i>Paralongicyatholaimus</i> sp5, <i>Paramesonchium</i> sp1

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981 **Table 6.** Results of DistLM forward carried out to ascertain the role of different environmental
982 variables on nematode species composition, index of trophic diversity (1-ITD) and maturity index
983 (MI). 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 %
Nematode species composition	Phaeopigment	7612.10	6.42	***	8.4	8.4
	Carbohydrate	5427.60	5.04	***	6.0	14.4
	Protein	4308.30	3.78	**	4.7	19.1
	Chl-a to BPC%	3679.10	3.58	**	4.1	23.2
	PRT to CHO ratio	3114.50	3.15	**	3.4	26.6
	Lipid	1597.80	1.50	ns	1.8	28.4
	PRT to BPC%	1508.70	1.48	ns	1.7	30.0
	Chlorophyll-a	1273.20	1.00	ns	1.4	31.4
1-ITD	PRT to CHO ratio	5639.00	6.55	**	8.9	8.9
	Chl-a to BPC%	2008.70	2.43	ns	3.2	12.1
	PRT to BPC%	1882.30	2.23	ns	3.0	15.0
	Phaeopigment	1840.90	2.29	ns	2.9	17.9
	Total phytopigment	1326.40	1.62	ns	2.1	20.0
	Lipid	1159.30	1.46	ns	1.8	21.9
	C biopolimerico	888.54	1.11	ns	1.4	23.3
	Chlorophyll-a	804.66	1.00	ns	1.3	24.5
	Protein	492.74	0.61	ns	0.8	25.3
MI	Protein	88.99	8.63	**	11.4	11.4
	Phaeopigment	86.48	10.02	**	11.1	22.5
	Chlorophyll-a	48.14	6.01	*	6.2	28.7
	Chl-a to BPC%	47.74	4.90	*	6.1	34.8
	PRT to CHO ratio	7.78	0.97	ns	1.0	35.8
	Pigmenti totali	4.29	0.44	ns	0.5	36.3
	Lipid	2.13	0.26	ns	0.3	36.6
	PRT to BPC%	0.63	0.08	ns	0.1	36.7
	C biopolimerico	0.12	0.01	ns	0.0	36.7
	Carbohydrate	0.00	0.00	ns	0.0	36.7

988 **Table 7.** Review of nematode species/genera sensitive/tolerant to different anthropogenic impacts.

Impact typology	Tolerant genera/species	Sensitive genera/specie	Effects on the overall assemblages	Reference
Hypoxic-anoxic conditions due to organic enrichment	<i>Chromadorella</i> , <i>Sabatiera</i> and <i>Polysigma</i> (more tolerant to extreme conditions).	<i>Desmoscolex</i> and <i>Bolbolaimus</i> (replaced by more tolerant genera).	Selective deposit feeders and predators decreased significantly, being replaced by non-selective deposit feeders and epistrate feeders	Gambi et al., 2009
Fish farm biodeposition	Monhysterids, <i>Pontonema vulgare</i> , <i>Pierrickia</i> , <i>Dorylaimopsis</i> , <i>Sabatiera</i> , <i>Oncholaimellus</i> , <i>Oxystomina</i> , <i>Ptycholaimellus</i> , <i>Comesomoides</i> , <i>Daptonema</i> , <i>Setosabatiera</i> , <i>Polysigma</i> .	Enoploids, <i>Latronema</i> , <i>Elzalia</i> .	Species richness declines, trophic diversity increases.	Review in Danovaro et al., 2009b
	<i>Sabatiera</i> , <i>Dorylaimopsis</i> and <i>Oxystomina</i> (increase dominance). <i>Pierrickia</i> and <i>Ptycholaimellus</i> (no differences).	<i>Setosabatiera</i> , <i>Latronema</i> and <i>Elzalia</i> .	Reduced densities, diversity and richness in sediments beneath fish farms, increased individual biomass. MI indicator of nematode resilience. No changes in the trophic diversity.	Mirto et al., 2002
	<i>Daptonema</i> and <i>Prochromadorella</i> (increase dominance). <i>Microlaimus</i> (indicator of stress conditions).	<i>Richtersia</i> , <i>Desmoscolex</i> and <i>Halalaimus</i> (highly sensitive to biodeposition). <i>Desmodora</i> (indicator of pristine conditions).	Reduced biodiversity.	Mirto et al., 2014
Organic pollution	<i>Eudiplogaster pararmatus</i> , <i>Dichromadora geophila</i> (diatom feeders, increased abundance and dominance).	<i>Sabatiera ssp.</i> (sensitive to extreme decrease of oxygen availability). <i>Viscosia</i> and <i>Halichoanalaimus</i> (predators). <i>Leptolaimus papilliger</i> , <i>Daptonema sp.</i> (indicators of change in food conditions). <i>Halalaimus sp.</i> (indicator of less stressed environment). <i>Innocuonema tentabundum</i> , <i>Halalaimus gracilis</i> , <i>Hypodontolaimus balticus</i> and <i>Ptycholaimellus ponticus</i> (indicator of less stressed, more stable environment). <i>Enoplus littoralis</i> (persister).	Decrease of nematode abundance, increase in species diversity, increase in MI.	Essink and Keidel, 1998
Sewage discharge and organic enrichment			Response not predictable or unequivocal. Abundance decrease, MI and trophic diversity do not vary.	Review in Danovaro et al., 2009b; Frascchetti et al., 2006
Harbour area - low contaminants and organic matter content	<i>Chromadorita</i> , <i>Chaetonema</i> , <i>Maryllynna</i> , <i>Belbolla</i> , <i>Enoplolaimus</i>		Low diversity and high dominance found, despite the relatively low levels of contamination (probably due to the food limitation). Highest and lowest percentages of c-p 3 and c-p 2 types, respectively, reflecting the low levels of contamination.	Losi et al., 2013
Harbour area - proximity to the harbour with high levels of contamination	Dominated by <i>Sabatiera</i> , <i>Daptonema</i> , <i>Comesa</i> and <i>Terschellingia</i> . Other genera: <i>Oncholaimellus</i> , <i>Thalassoalaimus</i> , <i>Spirinia</i> , <i>Neotonchus</i> , <i>Microlaimus</i> , <i>Ptycholaimellus</i> , <i>Eleutherolaimus</i> , <i>Molgolaimus</i> .		Lower abundance, diversity indexes and MI values, low number of genera, highest percentage of opportunistic genera.	Losi et al., 2013
Harbour area - deepest stations, intermediate contaminant concentrations, high quantities of organic matter	<i>Dorylaimopsis</i> , <i>Metacyatholaimus</i> , <i>Pierrickia</i> , <i>Diplopeltoides</i> , <i>Leptolaimus</i> , <i>Halalaimus</i> , <i>Pselionema</i> , <i>Desmoscolex</i> , <i>Sphaerolaimus</i> , <i>Rhps</i> , <i>Gnomoxyalia</i> , and <i>Tricoma</i> .		Higher diversity and persister nematodes (c-p 4) %. Different trophic strategy, dominance of selective deposit feeders and the highest % of predators/omnivores.	Losi et al., 2013
Organic waste from mariculture	<i>Daptonema spp.</i> , <i>Maryllynna spp.</i> , <i>Sabatiera spp.</i> and <i>Terschellingia spp.</i>	<i>Tricoma spp.</i> , <i>Desmoscolex spp.</i> , <i>Quadricoma spp.</i> , <i>Halalaimus spp.</i>		Vezzulli et al., 2008
Hydrocarbon impact	<i>Daptonema</i> , <i>Viscosia</i> (less sensitive or even tolerant to oil hydrocarbon stress).	<i>Chromaspirina</i> , <i>Hypodontolaimus</i> , <i>Oncholaimellus</i> , <i>Paracanthonchus</i> , <i>Setosabatiera</i> , <i>Xyala</i> (immediately disappeared after oil spill. Recovered rapidly and appeared to be opportunist).	No effect on trophic diversity (non selective impact).	Danovaro et al., 1995
	<i>Enoplolaimus litoralis</i> (Became extremely abundant)			Giere, 1979
		<i>Setosabatiera</i> , <i>Sabatiera</i>	Higher trophic diversity, increase of persisters	Frascchetti et al., 2016
			Late response of community structure and trophic diversity	Frithsen et al., 1985
Diesel impact	<i>Hypodontolaimus colesi</i> , <i>Daptonema trabeculosum</i> , <i>Daptonema fallx</i> , <i>Maryllynna stekhoveni</i> (opportunistic or diesel-resistant).	<i>Chaetonema</i> , <i>Pomponema</i> , <i>Oncholaimus campylocercoides</i> .		Mahmoudi et al., 2005
Heavy metals	<i>Axonolaimus</i> , <i>Sabatiera</i> , <i>Monhystera</i> , <i>Theristus</i> (indicators of stress conditions).			Gyedu-Ababio et al., 1999
Physical disturbance	<i>Sabatiera pulchra</i> , <i>Sabatiera punctata</i> , <i>Daptonema tenuispiculum</i> , <i>Enoplolaimus spp.</i> , <i>Theristus spp.</i>		Diversity declines.	Review in Danovaro et al., 2009b
Anthropogenic pressures (population density, harbors, dredging activities) on estuaries	<i>Sabatiera</i> , <i>Daptonema</i> , <i>Terschellingia</i> , <i>Paracomesoma</i> . <i>Daptonema</i> , <i>Sabatiera</i> and <i>Dichromadora</i> tolerant to wide salinity range.			Alves et al., 2013

990 **Table 8.** Ranking of the ecological quality status (EQS) of the investigated sites according to Moreno et al. (2011) (A) and temporal variation of the
991 ranking at each site (B). Reported are the ranges of values of each indicator observed at each sampling site along the whole study period. The ranking of
992 the three sites is highlighted in light grey.

993

A)	Indicator	Ecological quality status					Source
		Bad	Poor	Moderate	Good	High	
Proposed thresholds	MI	≤2.2	2.2≤MI<2.4	2.4≤MI<2.6	2.6≤MI<2.8	>2.8	Moreno et al., 2011
	c-p	c-p 2>80%	c-p 2>60% and c-p 4<3%	c-p 2≥50% and 3<c-p 4<10%	c-p 2≥50% and c-p 4>10%	c-p 2≤50% and c-p 4>10%	Moreno et al., 2011
	H'	0<H'≤1	1<H'≤2.5	2.5<H'<3.5	3.5<H'<4.5	>4.5	Moreno et al., 2011
	ITD	1	0.6<ITD≤0.8	0.4<ITD≤0.6	0.25<ITD≤0.4	0.25	Moreno et al., 2011
	Sensitive/ Tollerant genera (>10%)	<i>Paracomesoma</i> , <i>Terschellingia</i> , <i>Sabatieria</i> group	<i>Daptonema</i> / <i>Theristus</i> , <i>Paralongicyatholaimus</i> , <i>Parodontophora</i> , <i>Odontophora</i>	<i>Anticoma</i> , <i>Desmodora</i> , <i>Spirinia</i> , <i>Marylynnia</i> , <i>Prochromadorella</i>	<i>Halalaimus</i> , <i>Setosabatieria</i> , <i>Ptycholaimellus</i>	<i>Desmoscolecidae</i> , <i>Microilaimus</i> , <i>Richtersia</i> , <i>Oncholaimus</i> , <i>Pomponema</i> , <i>Epacanthion</i>	Moreno et al., 2011
Senigallia	MI	2.0-2.2					present study
	c-p	86<c-p 2<100%					present study
	H'		0.5-1.8				present study
	ITD		0.7-1				present study
	Sensitive/ Tollerant genera (>10%)	<i>Sabatieria</i> sp1 (13-50%)	<i>Theristus</i> sp1 (0-3%), <i>Paralongicyatholaimus</i> sp5 (0-1%), <i>Odontophora</i> sp1 (0-4%)	<i>Desmodora</i> sp1 (0-1%)	<i>Halalaimus</i> sp1 (0-10%), <i>Setosabatieria</i> sp1(1-11%)		present study
Falconara	MI		2.0-2.6				present study
	c-p		55<c-p 2<100% and 0<c-p 4<11%				present study
	H'		0.1-2.1				present study
	ITD			0.3-1			present study
	Sensitive/ Tollerant genera (>10%)	<i>Sabatieria</i> sp1 (0-31%)	<i>Paralongicyatholaimus</i> sp1 (0-1%), <i>Odontophora</i> sp1 (0-4%)	<i>Desmodora</i> sp1 (0-1%), <i>Marylynnia</i> spp (0-7%)	<i>Halalaimus</i> sp.1 (0-11%), <i>Setosabatieria</i> sp1(0-1%)		present study
Portonovo	MI		2.0-2.4				present study
	c-p		64<c-p 2<92% and 0<c-p 4<6%				present study
	H'		1.8-2.1				present study
	ITD				0.4-0.6		present study
	Sensitive/Tollera nt genera (>10%)	<i>Sabatieria</i> sp1 (9-54%)	<i>Odontophora</i> sp1 (0-0.3%), <i>Paralongicyatholaimus</i> sp5 (0-22%)	<i>Desmodora</i> sp1 (1-7%), <i>Marylynnia</i> spp (0-1%)	<i>Halalaimus</i> sp.1 (0-1%), <i>Setosabatieria</i> sp1(0-3%)		present study

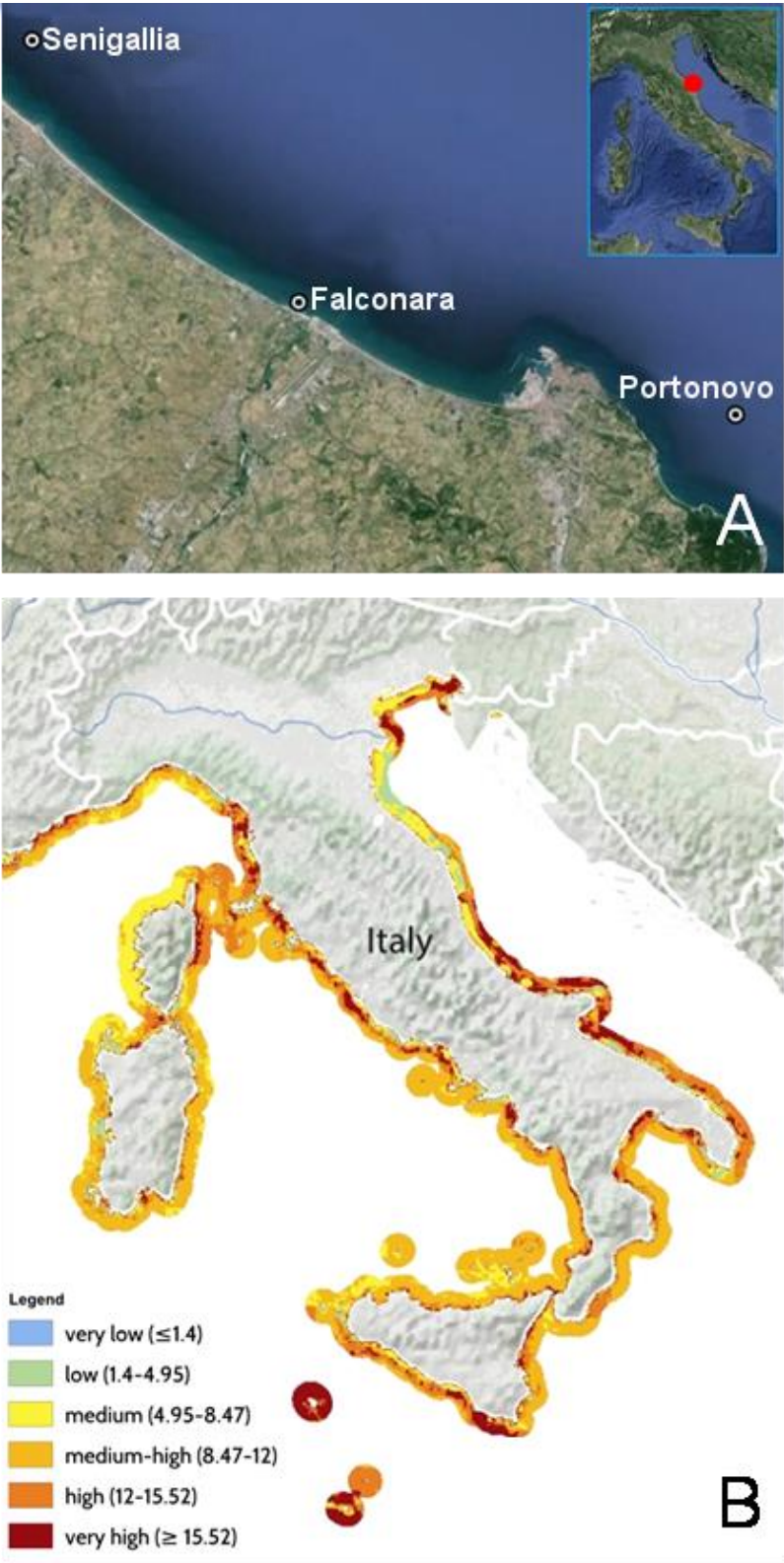
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B)	Indicator	January 2011	May 2011	June 2011	September 2011	November 2011	December 2011	January 2012	May 2012	June 2012	September 2012
Senigallia	MI	bad	bad	bad	bad	bad	bad	bad	bad	bad	bad
	c-p	bad	bad	bad	bad	bad	bad	bad	bad	bad	bad
	H'	poor	poor	poor	poor	bad	bad	bad	poor	poor	poor
	ITD	poor	poor	poor	poor	bad	bad	bad	poor	poor	poor
	Sensitive/Tollerant genera	bad	bad	bad	bad	bad	bad	bad	bad	bad	bad
Falconara	MI	bad	bad	bad	moderate	bad	poor	poor	bad	poor	bad
	c-p	bad	bad	bad	good	bad	poor	poor	bad	poor	bad
	H'	poor	poor	bad	poor	poor	poor	poor	poor	poor	poor
	ITD	moderate	poor	bad	good	moderate	good	good	moderate	moderate	moderate
	Sensitive/Tollerant genera	bad	na	na	na	bad	na	na	bad	bad	na
Portonovo	MI	poor	bad	bad	bad	bad	bad	bad	bad	bad	poor
	c-p	bad	bad	bad	bad	bad	bad	bad	bad	bad	poor
	H'	poor	poor	poor	poor	poor	poor	poor	poor	poor	poor
	ITD	good	moderate	good	moderate	moderate	moderate	moderate	moderate	moderate	good
	Sensitive/Tollerant genera	na	bad	bad	bad	bad	bad	bad	bad	bad	bad/poor

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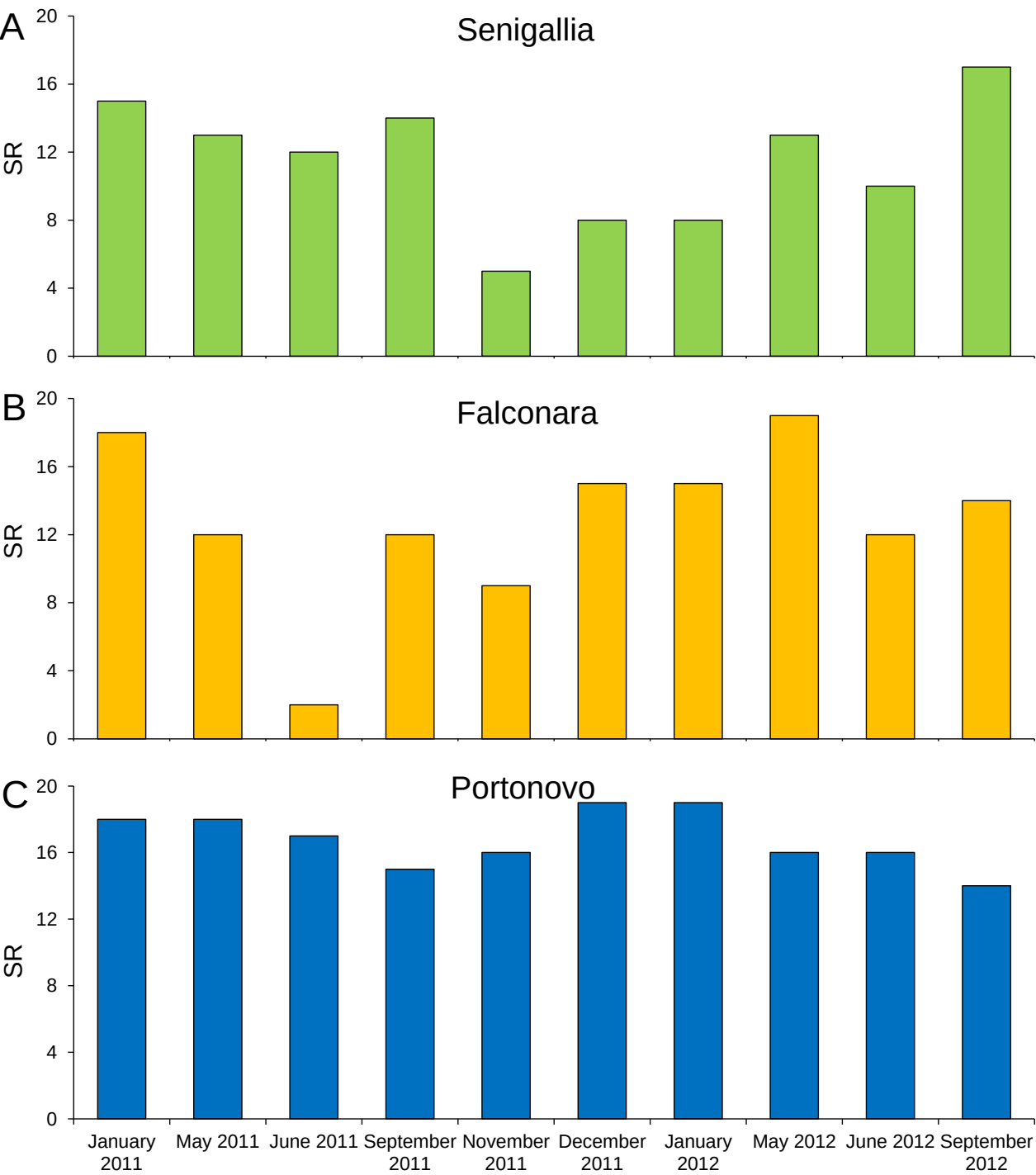
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1000 **Figure 1.**

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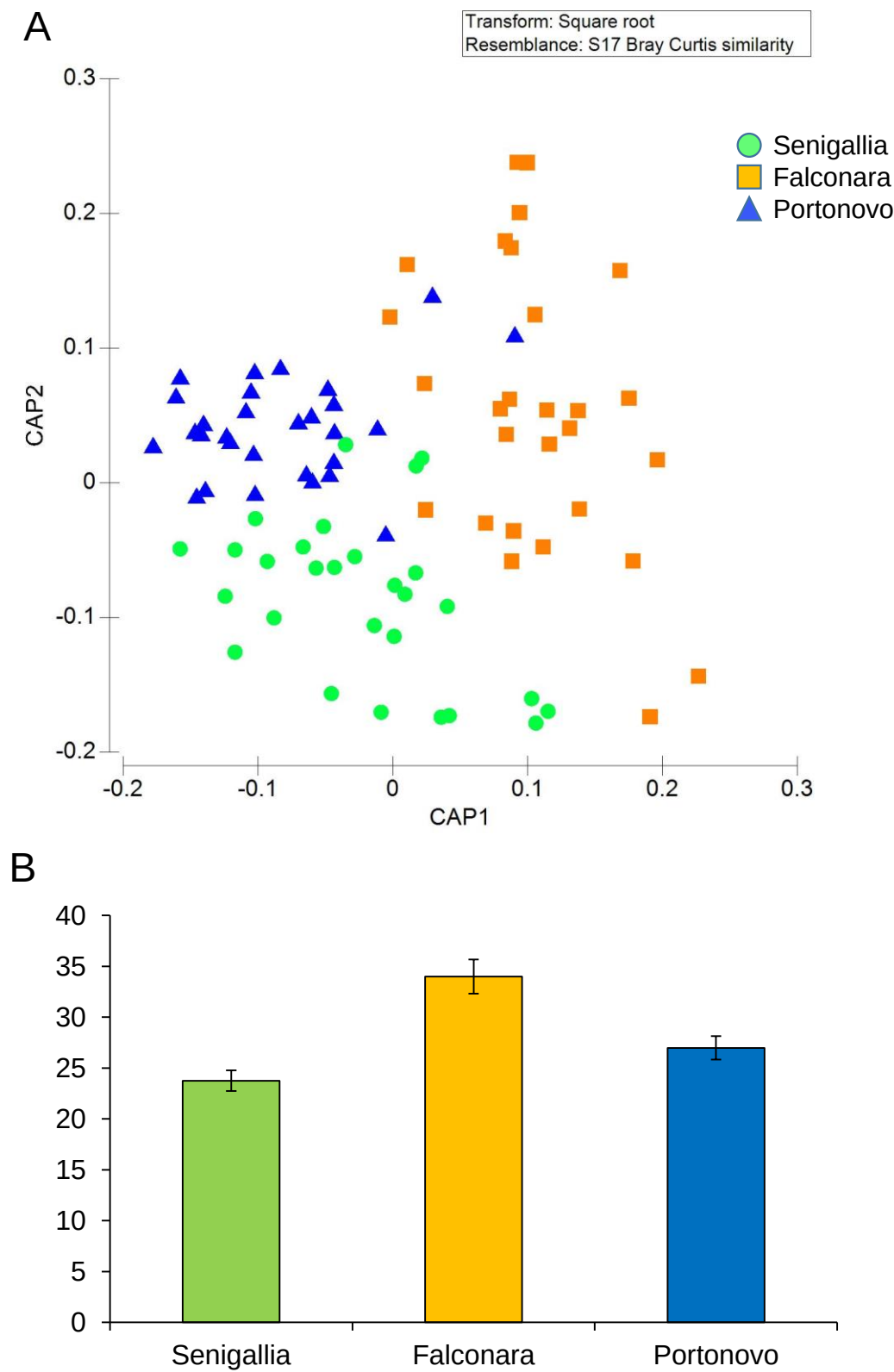
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Figure 2.

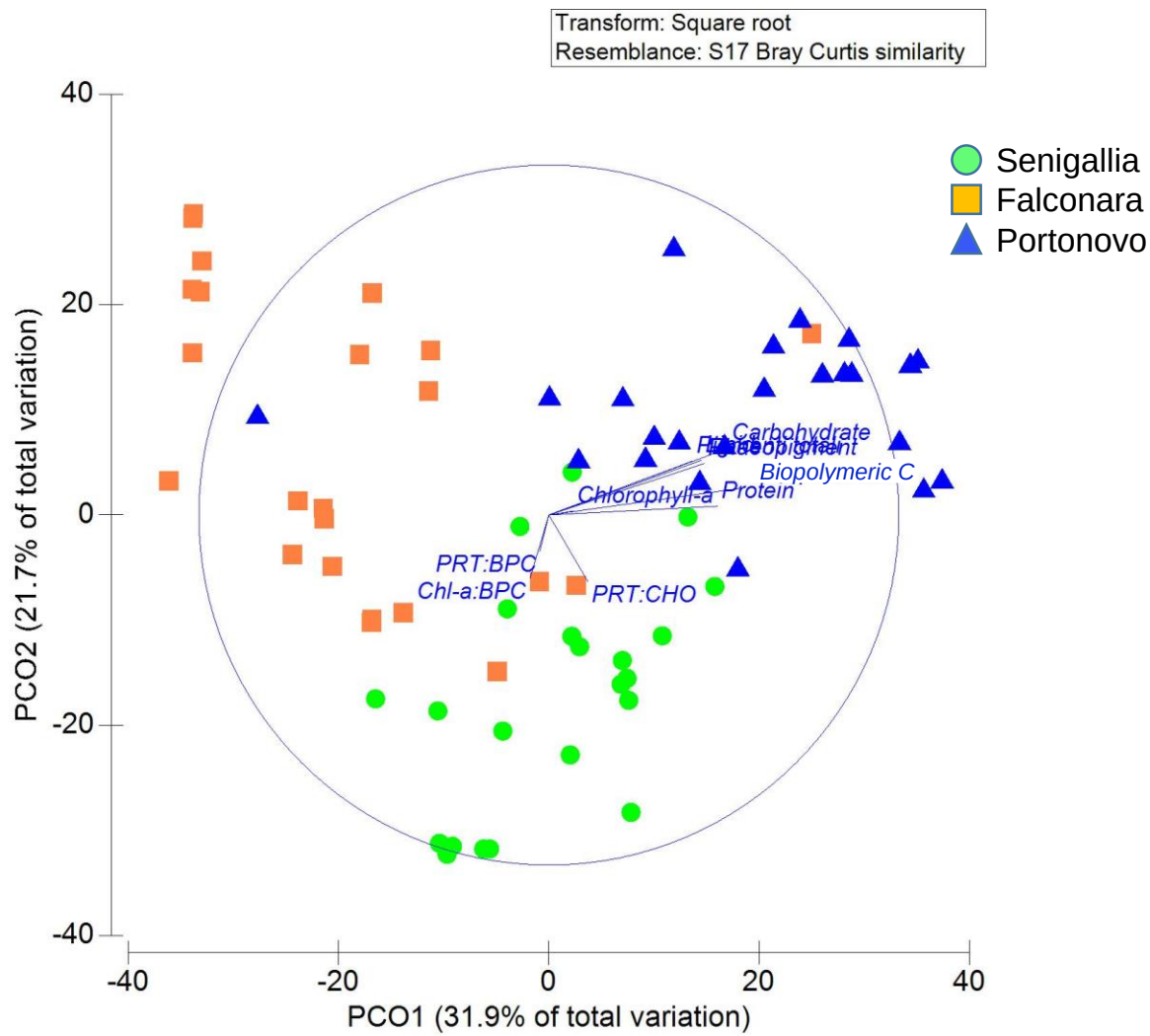
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Figure 3.

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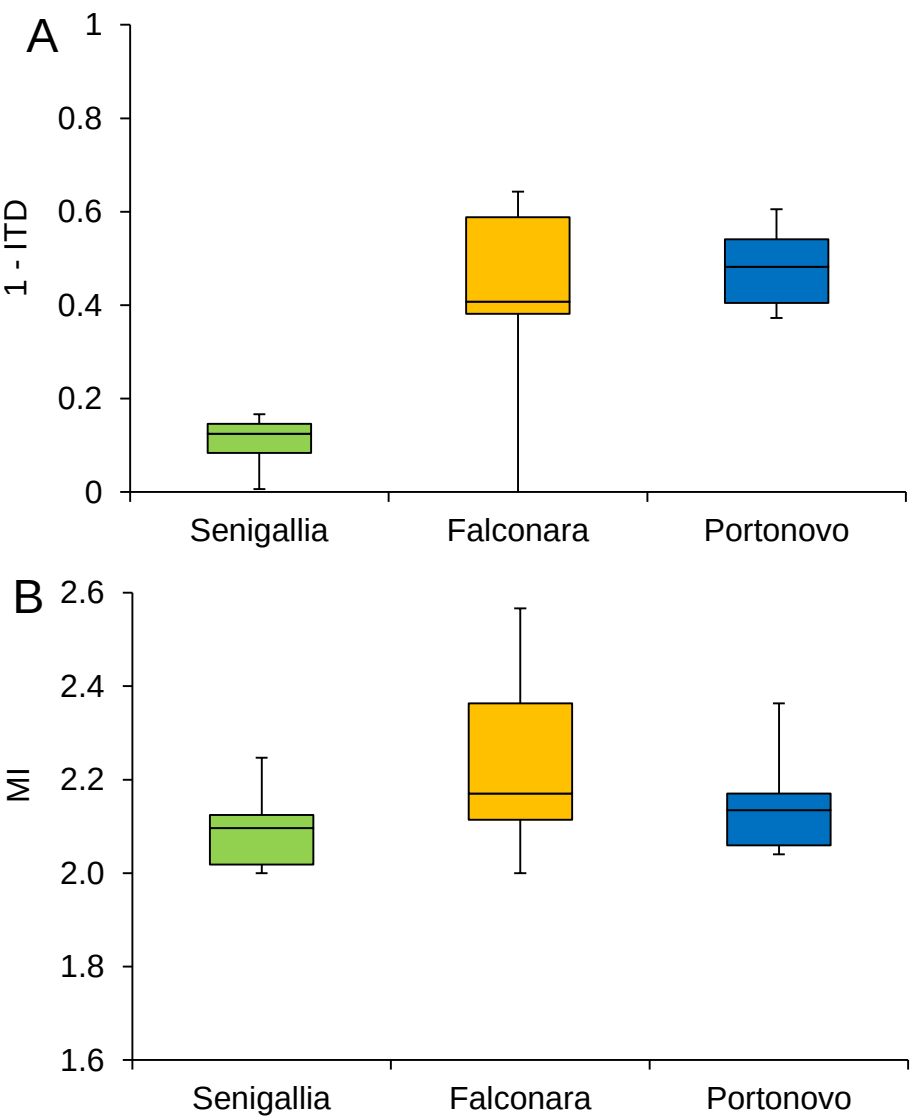
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1015 **Figure 4.**

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1020 **Figure 5.**

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1022 **Supplementary Table S1.** Results of pair wise testing variations in the sedimentary OM biochemical compounds contents, indicators of nutritional
1023 quality and biochemical composition. dF=degree of freedom; MS=mean square; F=F statistic; ***=P < 0.001; **=P < 0.01; *=P < 0.05; ns=not
1024 significant.

	pair wise "Site x Time" testing "Time"			pair wise "Site x Time" testing "Site"							
	Senigallia	Falconara	Portonovo	January 2011	September 2011	November 2011	December 2011	January 2012	May 2012	June 2012	September 2012
Chlorophyll-a	Dec11>Jan11>May12>Sept11,Nov11>Jan12,Jun12,Sept12	Sept12>Nov11>May12>Sept11,Dec11>Jan12,Jan11>Jun12	Dec11,Jan11,Nov11>Sept11,May12,Sept12>Jan12,Jun12	Por>Sen>Fal	ns	Fal,Por>Sen	Sen,Por>Fal	Por>Sen	ns	Sen,Por>Fal	Fal>Por>Sen
Phaeopigment	Jan11,Dec11>Sept11,Nov11,Jan12>May12,Jun12,Sept12	Sept11,May12>Nov11,Sept12>Jan11,Dec11,Jan12,Jun12	Jan11>Dec11,Jan12>Sept11,Nov11>Jun12,Sept12>May12	Por>Sen>Fal	Por>Sen	Por>Sen,Fal	Por,Sen>Fal	Por>Sen>Fal	Fal>Por,Sen	Por>Sen,Fal	Por>Sen
Total phyotpigment	Jan11,Dec11>Sept11,Nov11>Jan12,Sept12>May12,Jun12	Sept11,May12>Sept12>Jan11,Nov11,Dec11,Jan12,Jun12	Jan11>Dec11,Jan12>Sept11,Nov11>Jun12,Sept12>May12	Por>Sen>Fal	Por>Sen	Por>Sen,Fal	Por,Sen>Fal	Por>Sen>Fal	Fal>Por>Sen	Por>Sen,Fal	Por,Fal>Sen
Protein	Jan11,Dec11,May12>Sept12>Nov11,Jan12>Jun12>Sept11	May12,Sept12,Jan12>Jun12,Sept11>Nov11,Dec11	Dec11,Jan12,Sept12,Jan11>Nov11,May12>Jun12>Sept11	Por>Sen>Fal	Por>Sen	Por>Sen>Fal	Por>Fal	Por>Sen,Fal	ns	Por>Sen,Fal	Por>Sen,Fal
Carbohydrate	Jan11,Sept11,May12>Dec11>Nov11,Jan12,Jun12,Sept12	Sept11>May12,Sept12>Jan11,Nov11,Dec11,Jan12>Jun12	Jan11,Sept11,Nov11,Dec11,Jan12>May12,Sept12>Jun12	Por,Sen>Fal	ns	Por>Sen,Fal	Por>Sen>Fal	Por>Sen,Fal	ns	Por>Fal	Por>Sen
Lipid	Jan11,Sept11,Dec11,Sept12>Nov11,Jan12,May12,Jun12	Jan11,Sept11,Nov11,May12,Sept12>Dec11>Jan12,Jun12	Jan11,Sept11>Nov11,Dec11,Jan12,May12,Sept12>Jun12	Por>Sen>Fal	Por>Sen>Fal	Por>Sen,Fal	Por,Sen>Fal	ns	ns	Por>Fal	Por>Sen,Fal
Biopolymeric C	Jan11,Dec11,May12,Sept12>Nov11,Jan12,Sept11	Sept11,Nov11,Jan12,May12,Jun12,Sept12>Jan11,Dec11	Jan11,Sept11,Nov11,Dec11,Jan12,Sept12>May12,Jun12	Por>Sen>Fal	Por>Sen,Fal	Por>Sen,Fal	Por>Fal	Por>Sen,Fal	ns	Por>Fal	Por>Sen,Fal
Biochemical camposition	Jan11#Sept11#Nov11,Dec11#Jan12#May12,Sept12	Jan11#Sept11#Nov11,Dec11#Jan12,May12,Jun12,Sept12	Jan11#Sept11#Nov11#Dec11#Jan12#May12#Jun12#Sept12	Sen#Fal#Por	Sen,Fal#Por	Sen#Fal#Por	Sen,Por#Fal	Sen,Fal#Por	Fal#Por	Sen,Fal#Por	Sen#Fal#Por
Chlorophyll-a to biopolymeric C ratio	Nov11,Dec11,Jan12>Jun12,Sept12>May12>Jan11,Sept11	May12>Nov11,Dec11,Jan12,Jun12,Sept12>Jan11,Sept11	Nov11,Dec11,Jan12,Jun12,Sept12>May12>Jan11>Sept11	Fal>Sen	Fal>Por,Sen	Sen,Fal>Por	ns	ns	Fal>Por>Sen	Por,Sen>Fal	Fal>Por,Sen
Protein to biopolymeric C ratio	Nov11,Dec11,Jan12,May12,Jun12,Sept12>Jan11,Sept11	Jan12,Jun12,Sept12>May12>Nov11,Dec11>Jan11,Sept11	Jun12,Sept12>Dec11,Jan12,May12>Nov11>Jan11>Sept11	Sen>Por>Fal	ns	Sen>Por>Fal	Por>Fal	ns	Por>Fal	Por>Fal	ns
Protein to carbohydrate	Jan11,Nov11,Dec11,Jan12,May12,Jun12,Sept12>Sept11	Nov11,Dec11,Jan12,May12,Jun12,Sept12>Jan11>Sept11	Dec11,Jan12,May12,Jun12,Sept12>Jan11,Nov11>Sept11	Sen,Por>Fal	ns	Sen>Por>Fal	Por>Fal	ns	Por>Fal	Por>Fal	Por>Fal

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Supplementary Table S2. Results of pair wise testing variations in the nematode diversity indices and species composition. dF=degree of freedom; MS=mean square; F=F statistic; ***=P < 0.001; **=P < 0.01; *=P < 0.05; ns=not significant.

	pair wise "Site x Time" testing "Time"			pair wise "Site x Time" testing "Site"									
	Senigallia	Falconara	Portonovo	January 2011	May 2011	June 2011	September 2011	November 2011	December 2011	January 2012	May 2012	June 2012	September 2012
SR	Jan11,May11,Jun11,Sept11,May12,Jun12,Sept12>Nov11,Dec11,Jan12	Jan11,May11,Sept11,Nov11,Dec11,Jan12,May12,Jun12,Sept12>Jun11	Sept11>Sept12	ns	ns	Por>Sen>Fal	Por>Sen,Fal	Por,Fal>Sen	Por,Fal>Sen	Por,Fal>Sen	Por>Sen	Por>Sen,Fal	ns
D	Jan11,May11,Jun11,Sept11,May12,Jun12,Sept12>Nov11,Dec11,Jan12	Jan11,May11,Sept11,Nov11,Dec11,Jan12,May12,Jun12,Sept12>Jun11	Sept11,May12>Sept12	ns	ns	Por>Sen>Fal	Por>Sen,Fal	Por,Fal>Sen	Por,Fal>Sen	Por,Fal>Sen	Por>Sen	Por>Sen,Fal	ns
H	Jan11,May11,Jun11,Sept11,May12,Sept12>Dec11,Jan12,Jun12>Nov11	Jan11,Sept11,Dec11,Jan12,May12,Jun12,Sept12>May11,Nov11>Jun11	Jun11,May11,Jun11,Sept11,Nov11,Sept12>Dec11,Jan12,May12,Jun12	ns	Por>Fal	Por>Sen>Fal	Por,Fal>Sen	Por,Fal>Sen	Fal>Por>Sen	Fal>Por>Sen	ns	Por,Fal>Sen	Por>Sen,Fal
J	Jan11,May11,Jun11,Sept11,Dec11,Jan12,May12,Jun12,Sept12>Nov11	Jan11,May11,Sept11,Dec11,Jan12,May12,Jun12,Sept12>Nov11>Jun11	Jan11,May11,Jun11,Sept11,Nov11,Sept12>Dec11,Jan12,May12,Jun12	ns	Por,Sen>Fal	Por>Sen>Fal	Por,Fal>Sen	Por,Fal>Sen	Fal>Por>Sen	Fal>Por,Sen	ns	ns	Por>Sen,Fal
ES100	Jan11,May11,Jun11,Sept11,May12,Sept12>Jan11,Nov11,Dec11,Jan12	Jan11,May11,Sept11,Nov11,Dec11,Jan12,May12,Jun12,Sept12>Jun11	Jun11,May12>Sept12	ns	ns	Por>Sen>Fal	Por>Sen,Fal	Por>Fal>Sen	Por,Fal>Sen	Por,Fal>Sen	Por>Sen	Por>Sen,Fal	ns
1-ITD	Jan11,May11,Jun11,Sept11,May12,Jun12,Sept12>Nov11,Dec11,Jan12	Jan11,Sept11,Dec11,Jan12,May12,Jun12,Sept12>Nov11,May12,Jun12	Jan11,May11,Jun11,Sept11,Nov11,Dec11,Jan12,Jun12,Sept12>May12	Por>Sen	Por>Fal	Por>Sen>Fal	ns	Por,Sen>Fal	Por,Fal>Sen	Por,Fal>Sen	Por,Fal>Sen	Por,Fal>Sen	Por>Fal>Sen
MI	May11,Jun11>May12,Jun12>Jan11,Sept11,Nov11,Dec11,Jan12,Sept12	Jan11,Sept11,Dec11,Jun12>Nov11>May11,Jun11,May12,Jun12,Sept12	Sept12>Jan11,May11,Jun11,Sept11,Nov11,Dec11,Jan12,May12,Jun12	Por,Fal>Sen	Por>Sen,Fal	ns	Fal>Sen>Por	ns	Fal>Por>Sen	Fal>Por>Sen	Por>Sen	Por>Sen	Por>Fal>Sen
Species composition	Jan11,May11#Jun11#Sept11,May12,Jun12,Sept12>Nov11,Dec11,Jan12	Jun11#Jan11,May11#Sept11#Nov11#Dec11,Jan12#May12,Jun12#Sept12	May11#Jun11#Sept11#Dec11,Jan12#May12#Jun12#Sept12#Dec11,Jan12	ns	Por#Sen#Fal	Por#Sen#Fal	Por#Sen#Fal	Por#Sen#Fal	Por#Sen#Fal	Por#Sen#Fal	Por#Sen,Fal	Por#Sen#Fal	Por#Sen#Fal

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1031 **Supplementary Table S3.** Species retrieved and their relative abundance percentages from each site/period.

	January 2011			May 2011			June 2011			September 2011			November 2011			December 2011			January 2012			May 2012			June 2012			September 2012			
	Sen	Fal	Por	Sen	Fal	Por	Sen	Fal	Por	Sen	Fal	Por	Sen	Fal	Por	Sen	Fal	Por	Sen	Fal	Por	Sen	Fal	Por	Sen	Fal	Por	Sen	Fal	Por	
	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	
<i>Acantholaimus</i> sp.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.3	0.0	0.3	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Ammotheristus</i> sp.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Belbolla</i> sp.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Chaetonema</i> sp.1	1.0	1.0	2.0	0.7	2.0	0.0	0.7	0.0	0.0	0.0	4.3	0.0	0.0	0.0	0.0	0.0	9.0	0.3	0.0	9.0	0.3	0.0	0.3	0.0	0.0	0.3	1.7	0.0	0.3	2.7	0.0
<i>Chaetonema</i> sp.2	0.0	0.0	0.0	0.0	0.0	1.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Desmodora</i> sp.1	0.0	1.3	3.3	0.0	0.0	1.7	0.0	0.0	1.0	0.3	0.0	5.0	0.3	1.3	3.3	0.7	0.0	3.0	0.7	0.0	3.0	0.7	0.0	0.7	0.3	0.0	3.3	0.7	0.0	7.0	
<i>Diodontolaimus</i> sp.1	3.0	0.7	1.7	2.0	1.3	1.7	0.7	0.0	0.7	0.3	1.0	3.7	0.0	0.0	0.0	0.0	23.7	0.7	0.0	24.0	1.3	2.3	1.7	3.7	7.3	19.3	8.7	1.0	3.3	0.0	
<i>Dorylaimopsis</i> sp.1	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Eleutherolaimus</i> sp.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.3	0.0	0.0	4.7	0.0	0.0	0.0	0.0	
<i>Enoploides</i> sp.1	1.7	3.3	7.7	0.7	2.0	4.0	1.7	0.0	15.0	0.7	4.3	2.0	0.0	7.3	6.7	0.7	14.3	2.7	0.7	14.0	2.0	0.3	1.7	11.7	1.0	2.7	12.0	0.3	12.7	3.3	
<i>Gnomoxyala</i> sp.1	1.7	0.0	0.3	0.0	0.0	0.3	0.0	1.3	1.7	0.0	0.0	0.3	0.0	0.0	0.0	0.0	4.3	0.0	0.0	4.7	0.0	0.3	3.7	0.3	0.0	1.3	0.7	1.0	0.0	0.0	
<i>Halalaimus</i> sp.1	0.0	4.3	0.7	3.3	1.3	0.3	9.7	0.0	0.0	6.0	11.3	0.0	0.0	4.0	0.0	0.3	1.0	0.0	0.3	1.0	0.0	3.7	2.0	0.7	1.0	0.3	0.0	1.0	1.7	1.3	
<i>Hopperia</i> sp.1	4.7	8.0	11.0	0.3	0.0	12.3	0.0	0.0	8.7	0.0	0.0	15.3	0.0	0.3	5.3	0.0	0.0	9.0	0.0	0.0	9.0	0.0	0.0	3.3	0.0	0.0	2.0	0.7	0.0	3.3	
<i>Marylynnia</i> sp.1	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	7.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Marylynnia</i> sp.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Marylynnia</i> sp.5	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Mesacanthoides</i> sp.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Metalinhomoeus</i> sp.1	1.7	0.7	0.3	0.0	0.0	0.0	1.0	0.0	0.0	0.7	0.0	0.3	0.3	0.0	2.7	0.0	3.3	0.7	0.0	3.3	0.7	0.7	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0	
<i>Metalinhomoeus</i> sp.3	5.0	7.7	15.3	9.0	0.0	34.0	2.0	0.0	6.7	0.3	2.3	4.7	0.0	0.0	16.3	0.0	0.0	6.3	0.0	0.0	6.3	5.0	0.0	1.3	0.3	0.3	3.0	4.7	0.0	4.3	
<i>Molgolaimus</i> sp.1	0.0	0.0	0.0	0.0	1.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.7	3.3	0.0	0.0	0.0	0.0	0.0	0.7	0.0	
<i>Nemanema</i> sp.1	0.0	0.0	0.0	1.7	0.0	5.3	2.3	0.0	1.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.0	0.0	0.7	0.0	0.0	0.7	0.7	0.3	0.3	1.7	0.0	0.0	
<i>Neotonchus</i> sp.1	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Odontophora</i> sp.1	4.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0	1.0	0.3	0.0	1.0	0.3	0.0	1.3	0.0	0.0	0.0	0.0	0.0	4.3	0.0	
<i>Oncholaimellus</i> sp.1	0.0	4.0	2.7	0.0	1.0	0.0	0.0	0.0	0.0	0.0	3.7	0.0	0.0	0.0	0.0	0.0	8.3	0.0	0.0	8.7	0.0	0.0	0.3	0.0	0.0	7.7	0.0	0.3	1.7	0.0	
<i>Oxystomina</i> sp.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.3	0.0	1.0	0.3	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.7	0.0	
<i>Paralongicyatholaimus</i> sp.1	0.0	0.0	0.0	0.0	0.7	0.3	0.0	0.0	0.0	0.0	3.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.7	
<i>Paralongicyatholaimus</i> sp.5	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.0	6.3	0.3	25.7	3.7	0.0	0.0	0.0	1.0	0.0	1.0	1.0	0.0	1.0	0.0	0.0	0.3	0.0	0.0	4.3	0.0	0.0	22.0	
<i>Paramesonchium</i> sp.1	0.3	1.0	0.3	0.0	2.7	3.0	0.0	0.0	0.3	1.0	5.0	3.0	0.0	2.7	2.3	0.0	0.3	1.0	0.0	0.3	1.0	1.7	16.7	1.0	0.0	6.7	0.0	0.7	1.7	5.3	
<i>Paramonohystera</i> sp.1	39.7	44.7	34.3	34.3	75.3	12.0	50.3	98.7	15.3	43.0	31.0	12.7	84.7	58.0	13.0	57.3	25.3	9.0	58.0	25.3	8.7	26.7	28.7	27.3	53.7	41.3	44.7	56.7	60.0	25.3	
<i>Paramonohystera</i> sp.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Paramonohystera</i> sp.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.7	0.0	1.7	0.0	
<i>Pierrickia</i> sp.1	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Procamacolaimus</i> sp.1	0.0	0.3	0.7	0.3	2.0	0.7	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.3	0.0	0.0	1.3	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.3	1.0	0.0	
<i>Retrotheristus</i> sp.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.7	0.0	0.0	
<i>Sabatieria</i> sp.1	30.7	17.3	9.0	35.7	9.3	19.0	21.3	0.0	34.7	40.3	7.0	38.3	12.7	19.0	39.3	32.0	5.3	54.0	31.7	5.0	54.0	49.7	30.7	40.0	34.3	13.7	16.3	21.3	6.7	15.0	

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<i>Setosabatieria</i> sp.1	5.3	0.7	0.0	11.0	0.0	0.3	9.0	0.0	1.0	5.0	0.7	3.0	2.0	0.0	2.3	7.3	0.0	1.3	7.0	0.0	1.3	6.0	1.0	2.7	1.0	0.0	0.7	7.0	1.3	0.7
<i>Sphaerolaimus</i> sp.1	0.0	0.0	0.0	0.0	0.0	0.7	0.0	0.0	3.7	0.0	0.0	0.0	0.0	0.0	2.7	0.0	0.0	4.0	0.0	0.0	4.3	0.0	0.0	2.0	0.0	0.0	2.3	1.7	0.0	10.0
<i>Sphaerolaimus</i> sp.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Subsphaerolaimus</i> sp.1	0.7	3.7	6.0	0.0	0.0	0.3	0.0	0.0	1.7	0.3	0.0	5.7	0.0	0.3	3.3	0.0	0.0	0.7	0.0	0.0	0.7	0.0	0.0	0.3	0.0	0.0	0.3	0.0	0.0	1.3
<i>Subsphaerolaimus</i> sp.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Synonchiella</i> sp 1	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Synonchiella</i> sp.2	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Thalassomonhystera</i> sp.1	0.0	0.0	0.0	0.7	0.0	2.7	0.7	0.0	1.3	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.0	4.7	0.7	0.0	4.7	0.0	0.3	4.0	0.0	0.0	0.0	0.0	0.0	0.3
<i>Theristus</i> sp.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Wieseria</i> sp.1	0.3	0.0	4.0	0.0	0.0	0.0	0.0	0.0	0.3	1.3	0.0	1.7	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

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1034 **Caption of supplementary figures:**

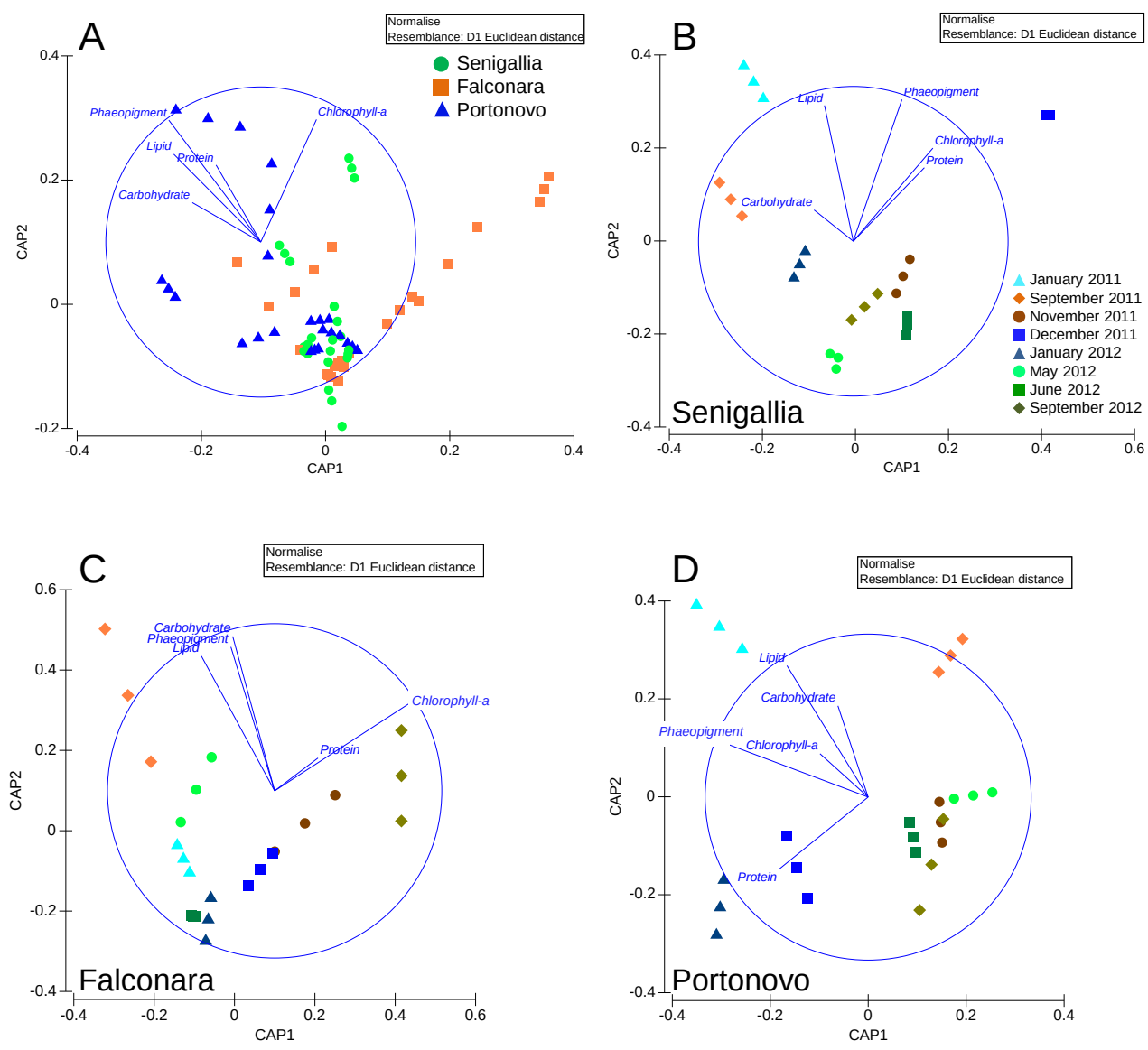
1035 **Supplementary Figure S1.** Output of canonical analysis of principal coordinates (CAP) on
1036 sedimentary organic matter biochemical composition at all sites (A), and separately at Senigallia (B),
1037 Falconara (C) and Portonovo (D).

1038 **Supplementary Figure S2.** Output of canonical analysis of principal coordinates (CAP) on
1039 sedimentary organic matter nutritional quality at all sites (A), and separately at Senigallia (B),
1040 Falconara (C) and Portonovo (D). (Chla-BPC = algal fraction of BPC, PRT:BPC = protein fraction
1041 of BPC, PRT:CHO = protein to carbohydrate ratio).

1042 **Supplementary Figure S3.** Trophic structure of nematode assemblages at Senigallia (A), Falconara
1043 (B) and Portonovo (C). 1A = one-selective (bacterial) feeders, 1B = non-selective deposit feeders,
1044 2A = epistrate or epigrowth (diatoms), 2B = predators/omnivores.

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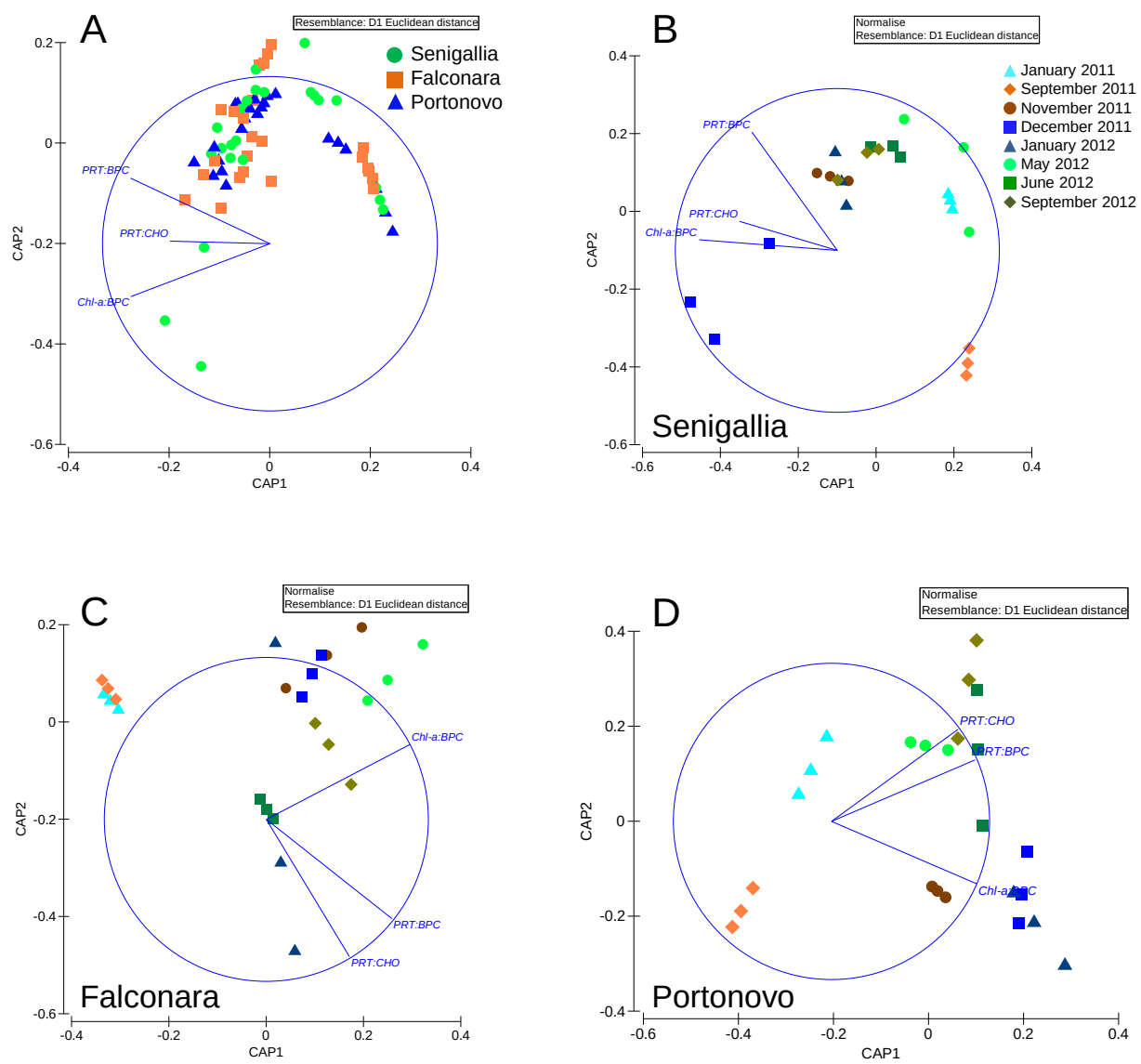
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1049 **Supplementary Figure S1.**

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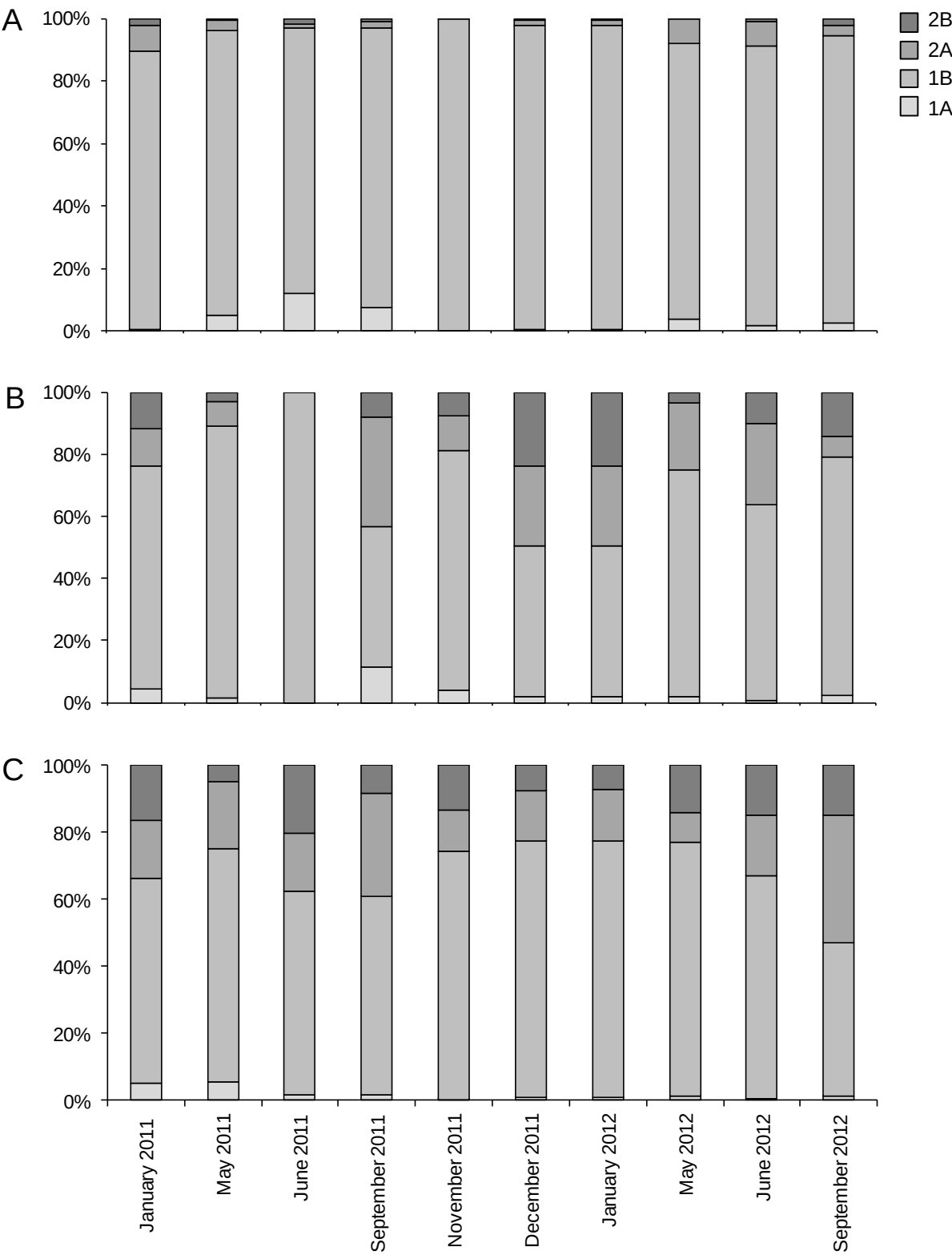


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1054 **Supplementary Figure S2.**

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1059 **Supplementary Figure S3.**