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

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ABSTRACT

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

Keywords: sedimentary organic matter biochemical composition, ecological quality status (EQS), 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.

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

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

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

At the same time, the assessment of the environmental quality status in the Adriatic Sea still largely depends upon the indicators and tools (e.g., biotic component) utilized, so that it requires the simultaneous use of a wide range of ecological indicators (Uusitalo et al., 2016). Macrofaunal biodiversity, for instance, whose ecological traits have been widely associated to environmental alteration, is commonly utilized for the classification of the ecological status of marine benthic ecosystems (Borja et al., 2008). Nevertheless, more recently, meiofauna, due to their high diversity and standing stocks, high turnover rates and lack of larval pelagic dispersal, have attracted increasing attention as a tool for detecting anthropogenic impact and for ranking the environmental quality status of different marine ecosystems (Danovaro et al., 1995; 2000; 2009b; Mazzola et al., 1999; 2000; La Rosa et al., 2001; Mirto et al., 2002; 2010; 2014; Fraschetti et al., 2006; 2016; Pusceddu et al., 2007; 2011; 2014a; 2016; Gambi et al., 2009; Moreno et al., 2011; Alves et al., 2013; 2015; Bianchelli et al., 2010; 2016a; 2016b). Meiofauna, in fact, are very sensitive to environmental disturbances, particularly to organic enrichment and eutrophication (Bianchelli et al., 2016a), at temporal scales much narrower than those generally exhibited by macrofauna. Previous studies, indeed, highlighted the influence of changes in the trophic status of marine sediments on the meiofaunal biodiversity under different environmental conditions and ecological alteration (Pusceddu et al., 2007, 2011; Bianchelli et al., 2010; 2013; 2016a). Such a relationship is not consistently positive, as the pattern of meiofaunal biodiversity responses varies depending upon the levels of the benthic trophic status (Pusceddu et al., 2007; Bianchelli et al., 2016a).

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

2. Materials and methods

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.

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

For the purpose of this study, sediment samples were collected over >20 months (from January 2011 to September 2012) with ca. bi-monthly sampling intervals (i.e., January, May, June, September, November, December 2011, January, May, June and September 2012), by means of a Van Veen grab (sampling surface 0.15 m^2), on board of the R/V Actea. Only deployments in which the sediments resulted undisturbed were utilized for sampling. Sediment samples collection and storage were carried out following the procedures reported in Danovaro (2010) and detailed in Bianchelli et al. (2016). At each site and time, sediment samples were collected from three true and independent replicates, for all the investigated variables.

2.2 Biochemical composition of sediment organic matter

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

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

The algal fraction of the BPC pools was estimated as percentage contribution of total phytopigment (expressed as C equivalents) to BPC (Pusceddu et al., 2009). The percentage contributions of protein (expressed as C equivalents) to BPC and the values of the protein to carbohydrate ratio were used as indicators of sedimentary organic matter nutritional quality (Pusceddu et al., 2009).

2.3 Nematode biodiversity

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

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

2.4 Nematode functional traits

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

The trophic habits of the nematode assemblages were defined according to the individual stoma 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 = $g_1^2+g_2^2+g_3^2…+g_n^2$, 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 $\Sigma\, \nu\,(i)\, f\,(i)$, where ν 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).

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

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

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

2.6 Indicators of ecological quality based on nematode biodiversity

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

2.7 Statistical analyses

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

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

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

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

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

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

3. Results

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

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

3.1 Biochemical composition and nutritional quality of sedimentary organic matter

Organic matter content, algal and protein fractions of biopolymeric C (BPC) and values of the protein to carbohydrate ratio in the sediment were significantly higher at Portonovo and/or Senigallia than at Falconara in all sampling times, with few exceptions. At all sites, all biochemical variables displayed significant temporal variations, though with varying patterns for the different variables (Supplementary Table S1). At most sampling times the biochemical composition of sediments varied significantly among sampling sites (Supplementary Table S1). The differences in the biochemical composition among sites were mostly due to OM contents in the sediments at Portonovo, which were higher than those in all other sites in almost all sampling times (Supplementary Figure S1A). At each site, the biochemical composition of sediments varied significantly also among sampling times, with variables responsible for the observed temporal changes varying among the three sampling sites (Supplementary Figure S1B-D). At most sampling times, the OM nutritional quality varied among sites mostly because of the very high values of the algal contribution to BPC at Falconara and the highest values of the protein fraction of BPC and protein to carbohydrate ratio at Senigallia and Portonovo (Supplementary Table S1). Differences in the nutritional quality of sedimentary OM were also associated with values of the algal fraction of BPC, which peaked up in different sampling times at each site (in November 2011-January 2012 at Senigallia and Portonovo, in May at Falconara). The lowest values of the protein fraction of BPC and protein to carbohydrate ratio occurred in January and September 2011 at all sites, whereas significant peaks occurred in different times at the three sites (Supplementary Table S1). Temporal variations in the OM nutritional quality were driven by different combinations of variables at the three sampling sites (Supplementary Figure S2B-D).

3.2 Indicators of benthic trophic status

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

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

3.3 Nematode biodiversity

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

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

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

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

Overall, a total of 16 exclusive species were encountered in this study: three at Senigallia (*Dorylaimopsis* sp1, *Neotonchus* sp1, *Paramonohystera* sp2), 7 at Falconara (*Ammothenstus* sp1, *Belbolla* sp1, *Eleutherolaimus* sp1, *Mesacanthoides* sp1, *Synonchiella* sp1, *Synonchiella* sp2, *Theristus* sp1) and 6 at Portonovo (*Chaetonema* sp2, *Marylynnia* sp3, *Marylynnia* sp5, *Pierrickia* sp1, *Sphaerolaimus* sp4, *Subsphaerolaimus* sp2).

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

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

3.4 Nematode functional (trophic) diversity and life strategy

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

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

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

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

3.5 Indicators of ecological quality status (EQS)

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

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

The temporal variability of the EQS ranking at each site is reported in Table 8B. At Senigallia, the indicators H’ and ITD change with sampling times, consistently. At Falconara, all indicators change with sampling time. At Portonovo, MI, c-p and ITD change with sampling times. In some sampling times (e.g., September 2011 at Falconara, June 2011 at Portonovo), very different ranking (e.g., from bad to good in January and June at Portonovo) is reported, depending on the indicator used.

4. Discussion

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

The Adriatic Sea represents one of the most complex basins along European seas. A recent integrated assessment of the Adriatic Sea status, based on marine biodiversity indicators, revealed poor conditions for this basin (Uusitalo et al., 2016). According to the sampling strategy applied in the present study, our attempt was to utilize a combined approach to assess the environmental status of sites affected by different anthropogenic activities, from the putatively most impacted (Falconara) to the less impacted one (Portonovo). Pelagic-benthic coupling is a key process in determining the trophic condition of benthic systems (Giordani et al., 2002), and the accumulation of organic matter in surface sediments is important in determining the environmental conditions in which the meiofauna 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 (protein, carbohydrate and BPC contents) aspects of OM enrichment processes (Pusceddu et al., 2009).

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

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

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

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

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

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

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

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

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

4.3 Relationships between nematode biodiversity and benthic trophic status

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

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

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

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

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

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References

Alves A.S., Adão H., Ferrero T.J., Marques J.C., Costa M.J., Patrício J. (2013) Benthic meiofauna as indicator of ecological changes in estuarine ecosystems: the use of nematodes in ecological quality assessment. Ecological Indicators 24, 462–475. DOI: http://dx.doi.org/10.1016/j.ecolind.2012.07.013

Alves A.S., Caetano A., Costa J.L., Costa M.J., Marques J.C. (2015) Estuarine intertidal meiofauna and nematode communities asindicator of ecosystem's recovery following mitigation measures. Ecological Indicators 54, 184–196. DOI: http://dx.doi.org/10.1016/j.ecolind.2015.02.013

Alvisi F., Giani M., Ravaioli M., Giordani P. (2013) Role of sedimentary environment in the development of hypoxia and anoxia in the NW Adriatic shelf (Italy). Estuarine, Coastal and Shelf Science 128, 9–21. DOI: doi.org/10.1016/j.ecss.2013.05.012

Anderson M.J. (2001) A new method for non-parametric multivariate analysis of variance. Austral Ecology 26, 32–46. DOI: 10.1111/j.1442-9993.2001.01070.pp.x

Anderson M.J. (2003) DISTLM Forward: A FORTRAN computer program to calculate a distance-based multivariate analysis for a linear model using forward selection. Department of Statistics, University of Auckland, New Zealand.

Anderson M.J., Gorley R.N., Clarke K.R. (2008) PERMANOVA+ for PRIMER: Guide to Software and Statistical Methods. PRIMER-E: Plymouth, UK.

Apitz S.E., Elliott M., Fountain M., Galloway T.S. (2006) European environmental management: moving to an ecosystem approach. Integrated Environmental Assessment and Management 2, 80–85. DOI: 10.1002/ieam.5630020114

Armenteros M., Pérez-García J.A., Ruiz-Abierno A., Díaz-Asencio L., Helguera Y., Vincx M., Decraemer W. (2010) Effects of organic enrichment on nematode assemblages in a microcosm experiment. Marine Environmental Research 70, 374–382. DOI: http://dx.doi.org/10.1016/j.marenvres.2010.08.001

ARPAM (2014) Relazione sullo stato di qualità dei corpi idrici marino-costieri per l'anno 2013. ARPAM, Ancona, Italy. http://www.arpa.marche.it/images/PUBBLICAZIONI/relazione%20corpi%20idrici%20marino%20costieri%202013.pdf

ARPAM (2015) Relazione sullo stato di qualità dei corpi idrici marino-costieri anno 2014. ARPAM, Ancona, Italy. http://www.arpa.marche.it/images/PUBBLICAZIONI/Relazione%20mare%202014.pdf

Balsamo M., Albertelli G., Ceccherelli V.U., Coccioni R., Colangelo M.A., Curini-Galletti M., Danovaro R., D'Addabbo R., Leonardis C., Fabiano M., Frontalini F., Gallo M., Gambi C., Guidi L., Moreno M., Pusceddu A., Sandulli R., Semprucci F., Todaro M.A., Tongiorgi, P. (2010) Meiofauna of the Adriatic Sea: current state of knowledge and future perspective. Chemistry and Ecology 26, 45–63. DOI: 10.1080/02757541003705492

Bianchelli S., Pusceddu A., Canese S., Greco S., Danovaro R. (2013) High meiofaunal and nematodes diversity around mesophotic coral oases in the Mediterranean Sea. PLoS One 8 (6), e66553. DOI: https://doi.org/10.1371/journal.pone.0066553

Bianchelli S., Pusceddu A., Buschi E., Danovaro R. (2016a) Trophic status and meiofauna biodiversity in the Northern Adriatic Sea: Insights for the assessment of good environmental status. Marine Environmental Research 113, 18–30. DOI: http://dx.doi.org/10.1016/j.marenvres.2015.10.010

Bianchelli S., Buschi E., Danovaro R., Pusceddu A. (2016b) Biodiversity loss and turnover in alternative states in the Mediterranean Sea: a case study on meiofauna. Scientific Reports 6: 34544. DOI: 10.1038/srep34544

Bianchelli S., Gambi C., Zeppilli D., Danovaro R. (2010) Metazoan meiofauna in deep-sea canyons and adjacent open slopes: A large-scale comparison with focus on the rare taxa, Deep Sea Research Part I: Oceanographic Research Papers 57 (3), 420-433

Bongers T., Alkemade R., Yeates G.W. (1991) Interpretation of disturbance-induced maturity decrease in marine nematode assemblages by means of the Maturity Index. Marine Ecology Progress Series 76, 135−142. DOI: 0.3354/meps076135

Bongers T, Ferris H. (1999) Nematode community structure as a bioindicator in environmental monitoring. Trends in Ecology and Evolution 14(6), 224−228. https://doi.org/10.1016/S0169-5347(98)01583-3

Borja A., Bricker S.B., Dauer D.M., Demetriades N.T., Ferreira J.G., Forbes A.T., Hutchings P., Jia X., Kenchington R., Marques J.C., Zhu C. (2008) Overview of integrative tools and methods in assessing ecological integrity in estuarine and coastal systems worldwide. Marine Pollution Bulletin 56, 1519–1537. DOI: http://dx.doi.org/10.1016/j.marpolbul.2008.07.005

Borja A., Elliott M., Andersen J.H., Cardoso A.C., Carstensen J., Ferreira J.G., Heiskanen A., Marques J.C., Neto J.M., Teixeira H., Uusitalo L., Uyarra M.C., Zampoukas N. (2013) Good Environmental Status of marine ecosystems: What is it and how do we know when we have attained it? Marine Pollution Bulletin 76: 16–27. DOI: http://dx.doi.org/10.1016/j.marpolbul.2013.08.042

Chen C.A., Soo C.L., Balsamo M., Semprucci F. (2018) An approach based on nematode descriptors for the classification of ecological quality (EcoQ) of the Malaysian coasts. Marine Biodiversity 48: 117-126. DOI: https://doi.org/10.1007/s12526-017-0813-1

Clarke K.R., Gorley R.N. (2006) PRIMER V6: User Manual/Tutorial. PRIMER-E, Plymouth, UK.

Coll M., Piroddi C., Steenbeek J., Kaschner K., Lasram,F.B.R., Aguzzi J., Ballesteros E., Bianchi C.N., Corbera J., Dailianis T., Danovaro R., Estrada M., Froglia C., Galil B.S., Gasol J.M., Gertwage R., Gil J., Guilhaumon F., Kesner-Reyes K., Kitsos M.-S., Koukouras A., Lampadariou N., Laxamana E., de la Cuadra C.M.L.-F., Lotze H.K., Martin D., Mouillot D., Oro D., Raicevich S., Rius-Barile J., Saiz-Salinas J.I., Vicente C.S., Somot S., Templado J., Turon X., Vafidis D., Villanueva R., Voultsiadou E. (2010) The biodiversity of the Mediterranean Sea: Estimates, patterns, and threats. PLoS ONE 5, 8: e11842. DOI: 10.1371/journal.pone.0011842

Coll M., Piroddi C., Albouy C., Ben Rais Lasram F., Cheung W.W.L., Christensen V., Karpouzi V.S., Guilhaumon F., Mouillot D., Paleczny M., Palomares M.L., Steenbeek J., Trujillo P., Watson R., Pauly D. (2012) The Mediterranean Sea under siege: spatial overlap between marine biodiversity, cumulative threats and marine reserves. Global Ecology and Biogeography 21: 465–480. DOI: 10.1111/j.1466-8238.2011.00697.x

Costanza R., d'Arge R., de Groot R., Farber S., Grasso M., Hannon B., Naeem S., Limburg K., Paruelo J., O'Neill R.V., Raskin R., Sutton P., van den Belt M. (1997) The value of the world's ecosystem services and natural capital. Nature 387, 253-260. DOI:10.1038/387253a0

Costanza R. (1999) The ecological, economic, and social importance of the Oceans. Ecological Economics 31, 199–213. DOI: http://dx.doi.org/10.1016/S0921-8009(99)00079-8

Costanza R., de Groot R., Sutton P., van der Ploeg S., Anderson S., Kubiszewski I., Farber S., Turner R.K. (2014) Changes in the global value of ecosystem services. Global Environmental Change 26, 152-158. DOI: https://doi.org/10.1016/j.gloenvcha.2014.04.002

Cozzi S., Giani M. (2011) River water and nutrient discharges in the northern Adriatic Sea: current importance and long term changes. Continental Shelf Research 31, 1881−1893. DOI: http://dx.doi.org/10.1016/j.csr.2011.08.010

Cozzi S., Falconi C., Comici C., Cermelj B., Kovac N., Turk V., Giani M. (2012) Recent evolution of river discharges in the Gulf of Trieste and their potential response to climate changes and anthropogenic pressure. Estuarine, Coastal and Shelf Science 115, 14−24. DOI: http://dx.doi.org/10.1016/j.ecss.2012.03.005

Danovaro R. (2010) Methods for the study of deep-sea sediments, their functioning and biodiversity. CRC Press Taylor & Francis Group.

Danovaro R., Gambi C. (2002) Biodiversity and trophic structure of nematode assemblages in seagrass systems: evidence for a coupling with changes in food availability. Marine Biology 141, 667–677. DOI 10.1007/s00227-002-0857-y

Danovaro R., Fabiano M., Vincx M. (1995) Meiofauna response to the Agip Abruzzo oil spill in subtidal sediments of the Ligurian Sea. Marine Pollution Bulletin 30, 133–145. DOI: http://dx.doi.org/10.1016/0025-326X(94)00114-O

Danovaro R., Gambi C., Manini E., Fabiano M. (2000) Meiofauna response to a dynamic river plume front. Marine Biology 137, 2, 359–370. DOI: https://doi.org/10.1007/s002270000353

Danovaro R., Gambi C., Luna G.M., Mirto S. (2004) Sustainable impact of mussel farming in the Adriatic Sea (Mediterranean Sea): evidence from biochemical, microbial and meiofaunal indicators. Marine Pollution Bullettin 49, 325–333. DOI: https://doi.org/10.1016/j.marpolbul.2004.02.038

Danovaro, R., Gambi, C., Dell’Anno, A., Corinaldesi, C., Fraschetti, S., Vanreusel, A., Vincx, M., Gooday, A.J. (2008) Exponential decline of deep-sea ecosystem functioning linked to benthic biodiversity loss. Current Biology 18, 1–8. DOI: http://dx.doi.org/10.1016/j.cub.2007.11.056

Danovaro R., Fonda Umani S., Pusceddu A. (2009a) Climate change and the potential spreading of marine mucilage and microbial pathogens in the Mediterranean Sea. PLoS ONE 4(9), e7006. DOI: http://dx.doi.org/10.1371/journal.pone.0007006

Danovaro R., Gambi C., Höss S., Mirto S., Traunspurger W., Zullini A. (2009b) Case studies using nematode assemblage analysis in aquatic habitats. In Wilson M.J., Kakouli-Duarte T. (2009) Nematodes as environmental indicators, pp. 146-171. DOI 10.1079/9781845933852.0000

Danovaro R., Carugati L., Berzano M., Cahill A.E., Carvalho S, Chenuil A., Corinaldesi C., Cristina S., David R., Dell'Anno A, et al. (2016) Implementing and innovating marine monitoring approaches for assessing marine environmental status. Frontiers in Marine Science 3: 213. DOI: https://doi.org/10.3389/fmars.2016.00213

Dell'Anno A., Mei M.L., Pusceddu A., Danovaro R. (2002) Assessing the trophic state and eutrophication of coastal marine systems: a new approach based on the biochemical composition of sediment organic matter. Marine Pollution Bulletin 44, 611–622. DOI: http://dx.doi.org/10.1016/S0025-326X(01)00302-2

Dell’Anno A., Pusceddu A., Langone L., Danovaro R. (2008) Biochemical composition and early diagenesis of organic matter in coastal sediments of the NWAdriatic Sea influenced by riverine inputs. Chemistry and Ecology 24, 75–85. DOI: 10.1080/02757540701814580

Di Camillo C.G., Cerrano C. (2015) Mass mortality events in the NW Adriatic Sea: phase shift from slow- to fast-growing organisms. PLos One 10 (5), e0126689. DOI: http://dx.doi.org/10.1371/journal.pone.0126689

Duplisea D.E., Hargrave B.T. (1996) Response of meiobenthic size-structure, biomass and respiration to sediment organic enrichment. Hydrobiologia 339, 161−170. DOI: 10.1007/BF00008924

Essink K., Keidel H. (1998) Changes in estuarine nematode communities following a decrease of organic pollution. Aquatic Ecology 32, 195–202. DOI: 10.1023/A:1009901207217

European Parliament and Council (2008) Directive 2008/56/EC of the European Parliament and of the Council of 17 June 2008 establishing a framework for community action in the field of marine environmental policy - Marine Strategy Framework Directive. CELEX reference 32008L0056.

Fraschetti S., Gambi C., Giangrande A., Musco L., Terlizzi A., Danovaro R. (2006) Structural and functional response of meiofauna rocky assemblages to sewage pollution. Marine Pollution Bulletin 52, 540−548. DOI: http://dx.doi.org/10.1016/j.marpolbul.2005.10.001

Fraschetti S., Guarnieri G., Gambi C., Bevilacqua S., Terlizzi A., Danovaro R. (2016) Impact of offshore gas platforms on the structural and functional biodiversity of nematodes. Marine Environmental Research 115:56-64. DOI: 10.1016/j.marenvres.2016.02.001

Gambi C., Bianchelli S., Perez M., Invers O, Ruiz J.M. (2009) Biodiversity response to experimental induced hypoxic-anoxic conditions in seagrass sediments. Biodiversity and Conservation 18, 33–54. DOI: :10.1007/s10531-008-9433-1

Gasparovic B. (2012) Decreased production of surface-active organic substances as a consequence of the oligotrophication in the northern Adriatic Sea. Estuarine, Coastal and Shelf Science 115, 33–39. DOI: http://dx.doi.org/10.1016/j.ecss.2012.02.004

Giani M., Djakovac T., Degobbis D., Cozzi S., Solidoro C., Fonda Umani S. (2012) Recent changes in the marine ecosystems of the northern Adriatic Sea. Estuarine, Coastal and Shelf Science 115, 1−13. DOI: http://dx.doi.org/10.1016/j.ecss.2012.08.023

Giere O. (1979) The impact of oil pollution on intertidal meiofauna. Field study after the La Coruna spill, May, 1976. Cahiers de Biologie Marine 20: 231−251.

Giordani P., Helder W., Koning E., Miserocchi S., Danovaro R., Malaguti A. (2002) Gradients of benthic–pelagic coupling and carbon budgets in the Adriatic and Northern Ionian Sea. Journal of Marine Systems 33: 365−387. DOI: 10.1016/S0924-7963(02)00067-2

Halpern B.S., Walbridge S., Selkoe K.A., Kappel C.V., Micheli F., D'Agrosa C., Bruno J.F., Casey K.S., Ebert C., Fox H.E., Fujita R., Heinemann D., Lenihan H.S., Madin E.M.P., Perry M.T., Selig E.R., Spalding M., Steneck R., Watson R. (2008) A global map of human impact on marine ecosystems. Science 319, 948–952. DOI: 10.1126/science.1149345

Halpern B.S., Frazier M., Potapenko J., Casey K.S., Koenig K., Longo C., Stewart Lowndes J., Rockwood R.C., Selig E.R., Selkoe K.A., Walbridge S. (2015) Spatial and temporal changes in cumulative human impacts on the world’s ocean. Nature Communications 6: 7615. DOI: 10.1038/ncomms8615

Hannachi A., Elarbaoui S., Khazri A., D'Agostino F., Sellami B., Beyrem H., Gambi C., Danovaro R., Mahmoudi E. (2016) Effects of antifouling booster biocide Irgarol 1051 on the structure of free living nematodes: a laboratory experiment. Environmental Science: Processes Impacts 18, 832−843. DOI:10.1039/C5EM00631G

Heip C., Vincx M., Vranken G. (1985) The ecology of marine nematodes. Oceanography and Marine Biology, an Annual Review 23, 399–489.

Hooper D.U., Chapin F.S., Ewel J.J., Hector A., Inchausti P., Lawton J.H., Lodge D.M., Loreau M., Naeem S., Schmid B. (2005) Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. Ecological Monograph 75, 3–35. DOI: 10.1890/04-0922

Hummel H., Frost M., Juanes J.A., Kochmann J., Castellanos Perez Bolde C.F., Aneiros F., et al. (2015) A comparison of the degree of implementation of marine biodiversity indicators by European countries in relation to the Marine Strategy Framework Directive (MSFD). Journal of the Marine Biological Association of the United Kingdom 95, 1519–1531. DOI: https://doi.org/10.1017/S0025315415000235

Kamburska L., Fonda-Umani S. (2006) Long-term copepod dynamics in the Gulf of Trieste (Northern Adriatic Sea): recent changes and trends. Climate Research 31, 195–203. DOI:10.3354/cr031195

La Rosa T., Mirto S., Mazzola A., Danovaro R. (2001) Differential responses of benthic microbes and meiofauna to fish-farm disturbance in coastal sediments. Environmental Pollution 112, 3, 427–434. DOI: https://doi.org/10.1016/S0269-7491(00)00141-X

Levin L.A. (2003) Oxygen minimum zone benthos: adaptation and community response to hypoxia. Oceanography and Marine Biology 41, 1–45.

Loreau M., Naeem S., Inchausti P., Bengtsson J., Grime J.P., Hector A., Hooper D.U., Huston M.A., Raffaelli D., Schmid B., Tilman D., Wardle D.A. (2001) Biodiversity and ecosystem functioning: current knowledge and future challenges. Science 294, 804–808. DOI: 10.1126/science.1064088

Lorenzen C.J., Jeffrey S.W. (1980) Determination of chlorophyll and phaeopigments spectrophotometric equations. Limnology and Oceanography 12, 343–346. DOI: 10.4319/lo.1967.12.2.0343

Losi V., Ferrero T.J., Moreno M., Gaozza L., Rovere A., Firpo M., Marques J.C., Albertelli G. (2013) The use of nematodes in assessing ecological conditions in shallow waters surrounding a Mediterranean harbour facility. Estuarine, Coastal and Shelf Science 130, 209–221. DOI: http://dx.doi.org/10.1016/j.ecss.2013.02.017

Mahmoudi E., Essid N., Beyrem H., Hedfi A., Boufahja F., Vitiello P., Aissa P. (2005) Effects of hydrocarbon contamination on a free living marine nematode com- munity: results from microcosm experiments. Marine Pollution Bulletin 50 (11), 1197–1204. DOI:10.1016/j.marpolbul.2005.04.018

Mahmoudi E., Essid N., Beyrem H., Hedfi A., Boufahja F., Aissa P., Vitello P. (2008) Mussel-farming effects on Mediterranean benthic nematode communities. Nematology 10, 323−333.

Margalef D.R. (1958) Information theory in ecology. International Journal of General Systems 3: 36–71.

Mazzola A., Mirto S., Danovaro R. (1999) Initial fish-farm impact on meiofaunal assemblages in coastal sediments of the Western Mediterranean. Marine Pollution Bulletin 38, 12, 1126−1133. DOI: https://doi.org/10.1016/S0025-326X(99)00142-3

Mazzola A., Mirto S., La Rosa T., Danovaro R., Fabiano M. (2000) Fish farming effects on benthic community structure in coastal sediments: analysis of the meiofaunal resilience. ICES Journal of Marine Science 57: 1454−1461. DOI: 10.1006/jmsc.2000.0904

McArdle B.H., Anderson M.J. (2001) Fitting multivariate models to community data: a comment on distance-based redundancy analysis. Ecology 82, 290−297. DOI: 10.2307/2680104

Merckx B., Goethals P., Steyaert M., Vanreusel A., Vincx M., Vanaverbeke J. (2009) Predictability of marine nematode biodiversity. Ecological Modelling 220,1449–1458. DOI: 10.1016/j.ecolmodel.2009.03.016

Micheli F., Halpern B.S., Walbridge S., Ciriaco S., Ferretti F., Fraschetti S., Lewison R., Nykjaer L., Rosenberg A.A. (2013) Cumulative human impacts on Mediterranean and Black Sea marine ecosystems: assessing current pressures and opportunities. PLoS One 8 (12), e79889. DOI: http://dx.doi.org/10.1371/journal.pone.0079889

Mirto S., La Rosa T., Gambi C., Danovaro R., Mazzola A. (2002) Nematode community response to fish-farm impact in the western Mediterranean. Environmental Pollution 116, 203−214. DOI: http://dx.doi.org/10.1016/S0269-7491(01)00140-3

Mirto S., Bianchelli S., Gambi C., Krzelj M., Pusceddu A., Scopa M., Holmer M., Danovaro R. (2010) Fish-farm impact on metazoan meiofauna in the Mediterranean Sea: analysis of regional vs. habitat effects. Marine Environmental Research 69, 38–47. DOI: http://dx.doi.org/10.1016/j.marenvres.2009.07.005

Mirto S., Arigò C., Genovese L., Pusceddu A., Gambi C., Danovaro R. (2014) Nematode assemblage response to fish-farm impact in vegetated (*Posidonia oceanica*) and non-vegetated habitats. Aquaculture Environment Interactions 5, 17–28. DOI: https://doi.org/10.3354/aei00091

Moens T., Vincx M., 1997. Observations on the feeding ecology of estuarine nematodes. Journal of the Marine Biological Association of the United Kingdom 77, 211–227. DOI: https://doi.org/10.1017/S0025315400033889

Moens T., Bouillon S., Gallucci F., 2005. Dual stable isotope abundances unravel trophic position of estuarine nematodes. Journal of the Marine Biological Association of the United Kingdom 85, 1401–1407. DOI: https://doi.org/10.1017/S0025315405012580

Moreno M.., Ferrero T.J., Gallizia I., Vezzulli L., Albertelli G., Fabiano M. (2008) An assessment of the spatial heterogeneity of environmental disturbance within an enclosed harbour through the analysis of meiofauna and nematode assemblages. Estuarine, Coastal and Shelf Science 77, 565-576. DOI: https://doi.org/10.1016/j.ecss.2007.10.016

Moreno M., Semprucci F., Vezzulli L., Balsamo M., Fabiano M., Albertelli G. (2011) The use of nematodes in assessing ecological quality status in the Mediterranean coastal ecosystems. Ecological Indicators 11, 328–336. DOI: http://dx.doi.org/10.1016/j.ecolind.2010.05.011

Mozetič P., Francé J., Kogovšek T., Talaber I., Malej A. (2012) Plankton trends and community changes in a coastal sea (northern Adriatic): bottom-up vs. topdown control in relation to environmental drivers. Estuarine, Coastal and Shelf Science 115, 138–148. DOI: http://dx.doi.org/10.1016/j.ecss.2012.02.009

Neher D.A., Darby B.J. (2009) Chapter 4: general community indices that can be used for analysis of nematodes assemblages. In: Wilson M., Kakouli-Duarte T. (eds) Nematodes as environmental indicators. CABI Publishing, Wallingford, p 107−123.

Neira C., Sellanes J., Levin L.A., Arntz W.E. (2001) Meiofaunal distributions on thePeru margin: relationships to oxygen and organic matter availability. Deep-Sea Research I 48, 2453–2472. DOI: https://doi.org/10.1016/S0967-0637(01)00018-8

Nicholson M.D., Jennings S. (2004) Testing candidate indicators to support ecosystem-based management: the power of monitoring surveys to detect temporal trends in fish community metrics. ICES Journal of Marine Science 61, 35–42. DOI: https://doi.org/10.1016/j.icesjms.2003.09.004

Pielou E.C. (1975) Ecological diversity. John Wiley, New York.

Piroddi C., Coll M., Liquete C., Macias D., Greer K., Buszowski J., Steenbeek J., Danovaro R., Christensen V. (2017) Historical changes of the Mediterranean Sea ecosystem: modelling the role and impact of primary productivity and fisheries changes over time. Scientific Reports 7:44491. DOI: 10.1038/srep44491

Platt H.M., Warwick R.M. (1983) A synopsis of the freeliving marine mematodes. Part I. British enoplids. Cambridge University Press, Cambridge.

Platt H.M., Warwick R.M. (1988) A synopsis of the free-living marine nematodes. Part II: British chromadorids. Cambridge University Press, Cambridge.

Pusceddu A., Dell'Anno A., Fabiano M. (2000) Organic matter composition in coastal sediments at Terra Nova Bay (Ross Sea) during summer 1995. Polar Biology 2, 288–293. DOI: 10.1007/s003000050446

Pusceddu A., Dell'Anno A., Danovaro R., Manini E., Sarà G., Fabiano M. (2003) Enzymatically hydrolyzable protein and carbohydrate sedimentary pools as indicators of the trophic state of 'detritus sink' systems: a case study in a Mediterranean coastal lagoon. Estuaries 26, 641–650. DOI: 10.1007/BF02711976

Pusceddu A., Fraschetti S., Mirto S., Holmer M., Danovaro R. (2007) Effects of intensive mariculture on sediment biochemistry. Ecological Applications 17, 1366–1378. DOI: 10.1890/06-2028.1

Pusceddu A., Dell'Anno A., Fabiano M., Danovaro R. (2009) Quantity and bioavailability of sediment organic matter as signature of benthic trophic state. Marine Ecology Progress Series 375, 41–52. DOI: https://doi.org/10.3354/meps07735

Pusceddu A., Bianchelli S., Gambi C., Danovaro R. (2011) Assessment of benthic trophic status of marine coastal ecosystems: significance of meiofaunal rare taxa. Estuarine, Coastal and Shelf Science 93, 420–430. DOI: http://dx.doi.org/10.1016/j.ecss.2011.05.012

Pusceddu A., Bianchelli S., Martín J., Puig P., Palanques A., Masqué P., Danovaro R. (2014a) Chronic and intensive bottom trawling impairs deep-sea biodiversity and ecosystem functioning. Proceedings of the National Academy of Sciences of the United States of America 111, 24, 8861–8866. DOI: 10.1073/pnas.1405454111

Pusceddu A., Gambi C., Corinaldesi C., Scopa M., Danovaro R. (2014b) Relationships between meiofaunal biodiversity and prokaryotic heterotrophic production in different tropical habitats and oceanic regions. PLoS ONE 9(3): e91056. DOI:10.1371/journal.pone.0091056

Pusceddu A., Fraschetti S., Scopa M., Rizzo L., Danovaro R. (2016) Meiofauna communities, nematode diversity and C degradation rates in seagrass (*Posidonia oceanica* L.) and unvegetated sediments invaded by the algae *Caulerpa cylindracea* (Sonder). Marine Environmental Research 119, 88-99. DOI: 10.1016/j.marenvres.2016.05.015

Rockström J., Steffen W., Noone K., Persson Å., Chapin F.S., III, Lambin E.F., Lenton T.M., Scheffer M., Folke C., Schellnhuber H.J., Nykvist B., de Wit C.A., Hughes T., van der Leeuw S., Rodhe H., Sörlin S., Snyder P.K., Costanza R., Svedin U., Falkenmark M., Karlberg L., Corell R.W., Fabry V.J., Hansen J., Walker B., Liverman D., Richardson K., Crutzen P., Foley J.A. (2009) A safe operating space for humanity. Nature 461, 472-475. DOI:10.1038/461472a

Sandulli R., Semprucci F., Balsamo M. (2014) Taxonomic and functional biodiversity variations of meiobenthic and nematode assemblages across an extreme environment: a study case in a Blue Hole cave. Italian Journal of Zoology 81, 508–516. DOI: http://dx.doi.org/10.1080/11250003.2014.952356

Schratzberger M., Whomersley P., Warr K., Bolam S.G., Rees H.L. (2004) Colonisation of various types of sediment by estuarine nematodes via lateral infaunal migration: a laboratory study. Marine Biology 145, 69–78. DOI 10.1007/s00227-004-1302-1

Schratzberger M., Maxwell T.A.D., Warr K., Ellis J.R., Rogers S.I. (2008) Spatial variability of infaunal nematode and polychaete assemblages in two muddy subtidal habitats. Marine Biology 153, 621–642. DOI 10.1007/s00227-007-0836-4

Seinhorst J.W. (1959) A rapid method for the transfer of nematodes from fixative to anhydrous glycerine. Nematologica 4, 67−69. DOI: 10.1163/187529259X00381

Semprucci F., Balsamo M. (2014) Free-living marine nematodes as bioindicators: past, present and future perspectives. Trends in Environmental Science 6, 17−36.

Semprucci F., Sbrocca C., Rocchi M., Balsamo M. (2014) Temporal changes of the meiofaunal assemblage as a tool for the assessment of the ecological quality status. Journal of the Marine Biological Association UK 95(2), 247−254. DOI: 10.1017/S0025315414001271

Semprucci F., Losi V., Moreno M. (2015a) A review of Italian research on free-living marine nematodes and the future perspectives in their use as ecological indicators (EcoInd). Mediterranean Marine Science 16, 352−365. DOI: http://dx.doi.org/10.12681/mms.1072

Semprucci F., Frontalini F., Sbrocca C., Armynot du Châtelet E., Bout-Roumazeilles V., Coccioni R., Balsamo M. (2015b) Meiobenthos and free-living nematodes as tools for biomonitoring environments affected by riverine impact. Environmental Monitoring Assessment 187(5), 1−19. DOI 10.1007/s10661-015-4493-7

Semprucci F., Balsamo M., Frontalini F. (2015c) The nematode assemblage of a coastal lagoon (Lake Varano, southern Italy): ecology and biodiversity patterns. Scientia Marina 78(4), 579−588. DOI: http://dx.doi.org/10.3989/scimar.04018.02A

Steyaert M., Moodley L., Nadong T., Moens T., Soetaert K., Vincx M. (2007) Responses of intertidal nematodes to short-term anoxic events. Journal of Experimental Marine Biology and Ecology 345, 175−184. DOI: http://dx.doi.org/10.1016/j.jembe.2007.03.001

UNEP - United Nations Environmental Programme (1992) Convention on biological diversity. United Nations Environmental Programme, Nairobi, Kenia.

Uusitalo L., Blanchet H., Andersen J.H., Beauchard O., Berg T., Bianchelli S., et al. (2016) Indicator-Based Assessment of Marine Biological Diversity – Lessons from 10 Case Studies across the European Seas. Frontiers in Marine Sciences 3, 159. DOI: 10.3389/fmars.2016.00159

Vezzulli L., Fabiano M. (2006) Sediment biochemical and microbial variables for the evaluation of trophic status along the Italian and Albanian Continental Shelves. Journal of the Marine Biological Association of the United Kingdom 86, 27−37. DOI: 10.1017/S0025315406012823

Vezzulli L., Moreno M., Marin V., Pezzati E., BartoliM., Fabiano M. (2008) Organic waste impact of capture-based Atlantic bluefin tuna aquaculture at an exposed site in the Mediterranean Sea. Estuarine, Coastal and Shelf Science 78, 369−384. DOI: http://dx.doi.org/10.1016/j.ecss.2008.01.002

Warwick R.M., Platt H.M., Somerfield P.J. (1998) Free-living marine nematodes. Part III. British Monhysterids. Synopses of the British fauna (new series), Vol 53. Field Studies Council, Shrewsbury.

Wieser W. (1953) Die Beziehung zwischen Mundhöhlengestalt, Ernährungsweise und Vorkommen bei freilebenden marinen Nematoden. Arkiv for Zoologi 4, 439−484.

Worm B., Barbier E.B., Beaumont N., Duffy J.E., Folke C., Halpern B.S., Jackson J.B.C., Lotze H.K., Micheli F., Palumbi S.R., Sala E., Selkoe K.A., Stachowicz J.J., Watson R. (2006) Impacts of biodiversity loss on ocean ecosystem services. Science 314, 787−790. DOI: 10.1126/science.1132294

Zeppilli D., Sarrazin J., Leduc D., Arbizu P.M., Fontaneto D. et al. (2015) Is the meiofauna a good indicator for climate change and anthropogenic impacts? Marine Biodiversity 45, 505−535. DOI:10.1007/s12526-015-0359-z

Caption of Figures

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

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

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

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

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

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

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).

Table 2. OM sedimentary contents and nutritional quality (A): concentration of phytopigments (chlorophyll-a, phaeopigments and total phytopigments), protein, carbohydrate, lipid, BPC, chlorophyll-a and protein to BPC and protein to carbohydrate ratios. Nematode diversity indexes (B): species richness (SR), index of Margalef (D), equitability (Pielou's index, J), Shannon-Wiener information function (H'), expected species number 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

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

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**
Chlorophyll-a	Site	2	0.8	5.1	*	2.1
	Time	7	3.9	23.3	***	30.5
	Site × Time	14	2.4	14.4	***	55.1
	Residual	48	0.2			12.3
Phaeopigment	Site	2	7.9	122.2	***	22.8
	Time	7	3.1	48.0	***	23.6
	Site × Time	14	2.2	33.5	***	49.0
	Residual	48	0.1			4.5
Total phyotpigment	Site	2	6.9	96.0	***	19.7
	Time	7	3.1	43.4	***	23.5
	Site × Time	14	2.3	32.2	***	51.8
	Residual	48	0.1			5.0
Protein	Site	2	9.8	25.2	***	31.0
	Time	7	2.1	5.4	***	15.0
	Site × Time	14	1.3	3.3	**	23.4
	Residual	48	0.4			30.7
Carbohydrate	Site	2	7.3	20.9	***	23.5
	Time	7	3.6	10.2	***	29.1
	Site × Time	14	1.1	3.0	**	19.1
	Residual	48	0.3			28.3
Lipid	Site	2	8.7	82.7	***	27.4
	Time	7	4.8	45.2	***	39.6
	Site × Time	14	1.1	10.3	***	25.0
	Residual	48	0.1			8.1
Biopolymeric C	Site	2	12.1	34.4	***	37.7
	Time	7	1.7	4.9	***	11.7
	Site × Time	14	1.3	3.6	***	23.6
	Residual	48	0.4			27.1
Biochemical camposition	Site	2	34.6	32.1	***	21.1
	Time	7	17.4	16.2	***	27.6
	Site × Time	14	8.0	7.4	***	35.0
	Residual	48	1.1			16.3
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

B)		**DF**	**MS**	**F**	**P**	**% explained variance**
SR	Site	2	4232.0	36.0	***	23.0
	Time	9	822.7	7.0	***	13.1
	Site × Time	18	910.2	7.7	***	44.2
	Residual	60	117.6			19.7
D	Site	2	4923.1	34.4	***	29.7
	Time	9	808.2	5.6	***	13.4
	Site × Time	18	679.7	4.7	***	32.2
	Residual	57	143.2			24.7
H	Site	2	3506.7	48.3	***	25.4
	Time	9	634.6	8.7	***	13.5
	Site × Time	18	694.9	9.6	***	44.4
	Residual	57	72.6			14.9
J	Site	2	868.9	16.8	***	15.9
	Time	9	179.5	34.6	**	8.0
	Site × Time	18	308.4	59.5	***	48.1
	Residual	57	51.8			28.0
ES100	Site	2	3979.6	36.0	***	31.8
	Time	9	571.4	5.2	***	12.3
	Site × Time	18	496.1	44.9	***	30.6
	Residual	57	110.5			25.3
1-ITD	Site	2	17198.0	53.3	***	51.0
	Time	9	897.3	2.8	**	5.6
	Site × Time	16	947.4	2.9	**	17.0
	Residual	56	322.6			26.3
MI	Site	2	718.5	77.2	***	43.4
	Time	9	39.8	4.3	***	6.8
	Site × Time	16	54.5	5.9	***	27.7
	Residual	56	9.3			17.1
Species composition	Site	2	20169.0	49.6	***	2.0
	Time	9	2323.3	5.7	***	12.2
	Site × Time	18	1812.4	4.5	***	26.8
	Residual	60	407.0			23.3

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

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

Among Sites	Contrast	Dissimilarity %	Average dissimilarity %	Responsible species
January 2011	Senigallia vs Falconara	46.2	46.6	*Metalinhomoeus* sp3, *Paramonohystera* sp1, *Hopperia* sp1
	Senigallia vs Portonovo	48.4		*Sabatieria* sp1, *Metalinhomoeus* sp3, *Subsphaerolaimus* sp1
	Falconara vs Portonovo	45.1		*Metalinhomoeus* sp3, *Paramonohystera* sp1, *Hopperia* sp1
May 2011	Senigallia vs Falconara	51.6	54.6	*Setosabatieria* sp1, *Metalinhomoeus* sp3, *Paramonohystera* sp1
	Senigallia vs Portonovo	47.4		*Hopperia* sp1, *Setosabatieria* sp1, *Metalinhomoeus* sp3
	Falconara vs Portonovo	64.9		*Metalinhomoeus* sp3, *Paramonohystera* sp1, *Hopperia* sp1
June 2011	Senigallia vs Falconara	59.9	63.2	*Sabatieria* sp1, *Halalaimus* sp1, *Paramonohystera* sp1
	Senigallia vs Portonovo	50.6		*Paramonohystera* sp1, *Halalaimus* sp1, *Hopperia* sp1
	Falconara vs Portonovo	79.2		*Paramonohystera* sp1, *Sabatieria* sp1, *Enoploides* sp1
September 2011	Senigallia vs Falconara	50.4	51.0	*Paralongicyatholaimus* sp5, *Sabatieria* sp1, *Chaetonema* sp1
	Senigallia vs Portonovo	44.7		*Hopperia* sp1, *Paramonohystera* sp1, *Halalaimus* sp1
	Falconara vs Portonovo	57.8		*Hopperia* sp1, *Sabatieria* sp1, *Halalaimus* sp1
November 2011	Senigallia vs Falconara	39.9	49.1	*Marylynnia* sp1, *Enoploides* sp1, *Sabatieria* sp1
	Senigallia vs Portonovo	57.9		*Paramonohystera* sp1, *Metalinhomoeus* sp3, *Sabatieria* sp1
	Falconara vs Portonovo	49.5		*Paramonohystera* sp1, *Metalinhomoeus* sp3, *Sabatieria* sp1
December 2011	Senigallia vs Falconara	66.5	63.5	*Diodontolaimus* sp1, *Enoploides* sp1, *Sabatieria* sp1
	Senigallia vs Portonovo	53.7		*Paramonohystera* sp1, *Hopperia* sp1, *Metalinhomoeus* sp3
	Falconara vs Portonovo	70.2		*Sabatieria* sp1, *Diodontolaimus* sp1, *Hopperia* sp1
January 2012	Senigallia vs Falconara	66.7	64.0	*Diodontolaimus* sp1, *Sabatieria* sp1, *Enoploides* sp1
	Senigallia vs Portonovo	54.8		*Paramonohystera* sp1, *Hopperia* sp1, *Metalinhomoeus* sp3
	Falconara vs Portonovo	70.6		*Sabatieria* sp1, *Diodontolaimus* sp1, *Hopperia* sp1
May 2012	Senigallia vs Falconara	45.3	44.3	*Paramesonchium* sp1, *Metalinhomoeus* sp3, *Paramonohystera* sp1
	Senigallia vs Portonovo	39.9		*Enoploides* sp1, *Thalassomonhystera* sp1, *Hopperia* sp1
	Falconara vs Portonovo	47.6		*Paramesonchium* sp1, *Enoploides* sp1, *Hopperia* sp1
June 2012	Senigallia vs Falconara	42.8	44.7	*Oncholaimellus* sp1, *Sabatieria* sp1 , *Paramesonchium* sp1
	Senigallia vs Portonovo	40.2		*Enoploides* sp1, *Paralongicyatholaimus* sp5, *Sabatieria* sp1
	Falconara vs Portonovo	51.0		*Oncholaimellus* sp1, *Enoploides* sp1, *Paramesonchium* sp1
September 2012	Senigallia vs Falconara	47.4	51.0	*Enoploides* sp1, *Sabatieria* sp1, *Metalinhomoeus* sp3
	Senigallia vs Portonovo	45.7		*Paralongicyatholaimus* sp5, *Paramonohystera* sp1, *Sphaerolaimus* sp1
	Falconara vs Portonovo	60.0		*Paralongicyatholaimus* sp5, *Sphaerolaimus* sp1, *Paramonohystera* sp1

Between Times	Contrast	Dissimilarity %	Avg dissimilarity %	Responsible species
Senigallia	January 2011 vs May 2011	36.9	31.2	*Hopperia* sp1, *Odontophora* sp1, *Metalinhomoeus* sp3
	May 2011 vs June 2011	26.4		*Halalaimus* sp1, *Metalinhomoeus* sp3, *Sabatieria* sp1
	June 2011 vs September 2011	28.7		*Sabatieria* sp1, *Nemanema* sp1, *Setosabatieria* sp1
	September 2011 vs November 2011	37.0		*Sabatieria* sp1, *Paramonohystera* sp1, *Halalaimus* sp1
	November 2011 vs December 2011	24.0		*Sabatieria* sp1, *Paramonohystera* sp1, *Setosabatieria* sp1
	December 2011 vs January 2012	15.4		*Sabatieria* sp1, *Paramonohystera* sp1, *Paralongicyatholaimus* sp5
	January 2012 vs May 2012	40.0		*Paramonohystera* sp1, *Metalinhomoeus* sp3, *Sabatieria* sp1
	May 2012 vs June 2012	39.6		*Paramonohystera* sp1, *Metalinhomoeus* sp3, *Sabatieria* sp1
	June 2012 vs September 2012	33.2		*Metalinhomoeus* sp3, *Setosabatieria* sp1, *Diodontolaimus* sp1
Falconara	January 2011 vs May 2011	42.5	48.1	*Paramonohystera* sp1, *Hopperia* sp1, *Halalaimus* sp1
	May 2011 vs June 2011	45.3		*Sabatieria* sp1, *Paramesonchium* sp1, *Chaetonema* sp1
	June 2011 vs September 2011	71.4		*Paralongicyatholaimus* sp5, *Paramonohystera* sp1, *Halalaimus* sp1
	September 2011 vs November 2011	52.1		*Paralongicyatholaimus* sp5, *Marylynnia* sp1, *Paramonohystera* sp1
	November 2011 vs December 2011	59.5		*Diodontolaimus* sp1, *Chaetonema* sp1, *Oncholaimellus* sp1
	December 2011 vs January 2012	17.6		*Metalinhomoeus* sp1, *Oncholaimellus* sp1, *Diodontolaimus* sp1
	January 2012 vs May 2012	56.1		*Paramesonchium* sp1, *Diodontolaimus* sp1, *Sabatieria* sp1
	May 2012 vs June 2012	44.3		*Diodontolaimus* sp1, *Sabatieria* sp1, *Oncholaimellus* sp1
	June 2012 vs September 2012	43.8		*Enoploides* sp1, *Diodontolaimus* sp1, *Paramonohystera* sp1
Portonovo	January 2011 vs May 2011	47.1	34.3	*Metalinhomoeus* sp3, *Nemanema* sp1, *Paramonohystera* sp1
	May 2011 vs June 2011	37.5		*Metalinhomoeus* sp3, *Paralongicyatholaimus* sp5, *Enoploides* sp1
	June 2011 vs September 2011	31.6		*Enoploides* sp1, *Sphaerolaimus* sp1, *Paramesonchium* sp1
	September 2011 vs November 2011	35.8		*Metalinhomoeus* sp3, *Paralongicyatholaimus* sp5, *Diodontolaimus* sp1
	November 2011 vs December 2011	35.0		*Metalinhomoeus* sp3, *Thalassomonhystera* sp1, *Sabatieria* sp1
	December 2011 vs January 2012	21.5		*Thalassomonhystera* sp1, *Sphaerolaimus* sp1, *Paramonohystera* sp1
	January 2012 vs May 2012	32.9		*Paramonohystera* sp1, *Enoploides* sp1, *Metalinhomoeus* sp3,
	May 2012 vs June 2012	31.7		*Sabatieria* sp1, *Thalassomonhystera* sp1, *Paralongicyatholaimus* sp5
	June 2012 vs September 2012	36.0		*Diodontolaimus* sp1, *Paralongicyatholaimus* sp5, *Paramesonchium* sp1

Table 6. Results of DistLM forward carried out to ascertain the role of different environmental variables on nematode species composition, index of trophic diversity (1-ITD) and maturity index (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

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	*Chromadorella*, *Sabatiera* and *Polysigma* (more tolerant to extreme conditions).	*Desmoscolex* and *Bolbolaimus* (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, *Pontonema vulgare*, *Pierrickia*, *Dorylaimopsis*, *Sabatieria*, *Oncholaimellus*, *Oxystomina*, *Ptycholaimellus*, *Comesomoides*, *Daptonema*, *Setosabatieria*, *Polysigma.*	Enoploids, *Latronema*, *Elzalia.*	Species richness declines, trophic diversity increases.	Review in Danovaro et al., 2009b
	Sabatieria, *Dorylaimopsis* and *Oxystomina* (increase dominance). *Pierrickia* and *Ptycholaimellus* (no differences).	*Setosabatieria*, *Latronema* and *Elzalia.*	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
	Daptonema and *Prochromadorella* (increase dominance). *Microlaimus* (indicator of stress conditions).	*Richtersia*, *Desmoscolex* and *Halalaimus* (highly sensitive to biodeposition). *Desmodora* (indicator of pristine conditions).	Reduced biodiversity.	Mirto et al., 2014
Organic pollution	*Eudiplogaster pararmatus*, *Dichromadora geophila* (diatom feeders, increased abundance and dominance).	*Sabatieria ssp.*(sensitive to extreme decrease of oxygen availability). *Viscosia* and *Halichoanalaimus* (predators). *Leptolaimus papilliger*, *Daptonema sp.* (indicators of change in food conditions). *Halalaimus sp.* (indicator of less stressed environment). *Innocuonema tentabundum*, *Halalaimus gracilis*, *Hypodontolaimus balticus* and *Ptycholaimellus ponticus* (indicator of less stressed, more stable environment). *Enoplus littoralis* (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 dicrease, MI and trophic diversity do not vary.	Review in Danovaro et al., 2009b; Fraschetti et al,. 2006
Harbour area - low contaminants and organic matter content	*Chromadorita, Chaetonema, Marylynnia, Belbolla, Enoplolaimus*		Low diversity and high dominance found, despite the relatively low levels of contamination (probabily 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 *Sabatieria, Daptonema, Comesa and Terschellingia.* Other genera: *Oncholaimellus, Thalassoalaimus, Spirinia, Neotonchus, Microlaimus, Ptycholaimellus, Eleutherolaimus, Molgolaimus.*		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	*Dorylaimopsis, Metacyatholaimus, Pierrickia, Diplopeltoides, Leptolaimus, Halalaimus, Pselionema, Desmoscolex,Sphaerolaimus, Rhips, Gnomoxyalia, and Tricoma.*		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	*Daptonema spp.*, *Marylynnia spp.*, *Sabatieria spp.* and *Terschellingia spp.*	*Tricoma spp.*, *Desmoscolex spp.*, *Quadricoma spp.*, *Halalaimus spp.*		Vezzulli et al., 2008
Hydrocarbon impact	*Daptonema*, *Viscosia* (less sensitive or even tolerant to oil hydrocarbon stress).	*Chromaspirina*, *Hypodontolaimus*, *Oncholaimellus*, *Paracanthonchus*, *Setosabatieria*, *Xyala* (immediately disappeared after oil spill. Recovered rapidly and appeared to be opportunist).	No effect on trophic diversity (non selective impact).	Danovaro et al., 1995
	Enoplolaimus litoralis (Became extremely abundant)			Giere, 1979
		Setosabatieria, *Sabatieria*	Higher trophic diversity, increase of persisters	Fraschetti et al., 2016
			Late response of community structure and trophic diversity	Frithsen et al., 1985
Diesel impact	*Hypodontolaimus colesi*, *Daptonema trabeculosum*, *Daptonema fallx*, *Marylynnia stekhoveni* (opportunistic or diesel-resistant).	*Chaetonema*, *Pomponema*, *Oncholaimus campylocercoides.*		Mahmoudi et al., 2005
Heavy metals	*Axonolaimus*, *Sabatieria*, *Monhystera*, *Theristus* (indicators of stress conditions).			Gyedu-Ababio et al., 1999
Physical disturbance	*Sabatieria pulchra*, *Sabatieria punctata*, *Daptonema tenuispiculum*, *Enoplolaimus spp*, *Theristus spp.*		Diversity declines.	Review in Danovaro et al., 2009b
Anthropogenic pressures (population density, harbors, dredging activities) on estuaries	*Sabatieria*, *Daptonema*, *Terschellingia*, *Paracomesoma. Daptonema, Sabatieria* and *Dichromadora* tolerant to wide salinity range.			Alves et al., 2013

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 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 the three sites is highlighted in light grey.

<table>
<tr><th>A)</th><th></th><th colspan="5">Ecological quality status</th><th></th></tr>
<tr><th></th><th>Indicator</th><th>Bad</th><th>Poor</th><th>Moderate</th><th>Good</th><th>High</th><th>Source</th></tr>
<tr><td rowspan="5">Proposed thresholds</td><td>MI</td><td>≤2.2</td><td>2.2≤MI<2.4</td><td>2.4≤MI<2.6</td><td>2.6≤ MI<2.8</td><td>>2.8</td><td>Moreno et al., 2011</td></tr>
<tr><td>c-p</td><td>c-p 2>80%</td><td>c-p 2>60% and c-p 4<3%</td><td>c-p 2≥50% and 3<c-p 4<10%</td><td>c-p 2≥50% and c-p 4>10%</td><td>c-p 2≤50% and c-p 4>10%</td><td>Moreno et al., 2011</td></tr>
<tr><td>H'</td><td>0<H'≤1</td><td>1<H'≤2.5</td><td>2.5<H'<3.5</td><td>3.5<H'<4.5</td><td>>4.5</td><td>Moreno et al., 2011</td></tr>
<tr><td>ITD</td><td>1</td><td>0.6<ITD≤0.8</td><td>0.4<ITD≤0.6</td><td>0.25<ITD≤0.4</td><td>0.25</td><td>Moreno et al., 2011</td></tr>
<tr><td>Sensitive/ Tollerant genera (>10%)</td><td>*Paracomesoma, Terschellingia, Sabatieria group*</td><td>*Daptonema/Theristus, Paralongicyatholaimus, Parodontophora, Odontophora*</td><td>*Anticoma, Desmodora, Spirinia, Marylynnia , Prochromadorella*</td><td>*Halalaimus, Setosabatieria, Ptycholaimellus*</td><td>*Desmoscolecidae, Microlaimus, Richtersia, Oncholaimus, Pomponema, Epacanthion*</td><td>Moreno et al., 2011</td></tr>
<tr><td rowspan="5">Senigallia</td><td>MI</td><td>2.0-2.2</td><td></td><td></td><td></td><td></td><td>present study</td></tr>
<tr><td>c-p</td><td>86<c-p 2<100%</td><td></td><td></td><td></td><td></td><td>present study</td></tr>
<tr><td>H'</td><td colspan="2">0.5-1.8</td><td></td><td></td><td></td><td>present study</td></tr>
<tr><td>ITD</td><td colspan="2">0.7-1</td><td></td><td></td><td></td><td>present study</td></tr>
<tr><td>Sensitive/ Tollerant genera (>10%)</td><td>*Sabatieria sp1 (13-50%)*</td><td>*Theristus sp1 (0-3%), Paralongicyatholaimus sp5 (0-1%), Odontophora sp1 (0-4%)*</td><td>*Desmodora sp1 (0-1%)*</td><td>*Halalaimus sp1 (0-10%), Setosabatieria sp1(1-11%)*</td><td></td><td>present study</td></tr>
<tr><td rowspan="5">Falconara</td><td>MI</td><td colspan="3">2.0-2.6</td><td></td><td></td><td>present study</td></tr>
<tr><td>c-p</td><td colspan="3">55<c-p 2<100% and 0<c-p 4<11%</td><td></td><td></td><td>present study</td></tr>
<tr><td>H'</td><td colspan="2">0.1-2.1</td><td></td><td></td><td></td><td>present study</td></tr>
<tr><td>ITD</td><td colspan="4">0.3-1</td><td></td><td>present study</td></tr>
<tr><td>Sensitive/ Tollerant genera (>10%)</td><td>*Sabatieria sp1 (0-31%)*</td><td>*Paralongicyatholaimus sp1 (0-1%), Odontophora sp1 (0-4%)*</td><td>*Desmodora sp1 (0-1%), Marylynnia spp (0-7%)*</td><td>*Halalaimus sp.1 (0-11%), Setosabatieria sp1(0-1%)*</td><td></td><td>present study</td></tr>
<tr><td rowspan="5">Portonovo</td><td>MI</td><td colspan="2">2.0-2.4</td><td></td><td></td><td></td><td>present study</td></tr>
<tr><td>c-p</td><td colspan="3">64<c-p 2<92% and 0<c-p 4<6%</td><td></td><td></td><td>present study</td></tr>
<tr><td>H'</td><td></td><td>1.8-2.1</td><td></td><td></td><td></td><td>present study</td></tr>
<tr><td>ITD</td><td></td><td></td><td colspan="2">0.4-0.6</td><td></td><td>present study</td></tr>
<tr><td>Sensitive/Tollerant genera (>10%)</td><td>*Sabatieria sp1 (9-54%)*</td><td>*Odontophora sp1 (0-0.3%), Paralongicyatholaimus sp5 (0-22%)*</td><td>*Desmodora sp1 (1-7%), Marylynnia spp (0-1%)*</td><td>*Halalaimus sp.1 (0-1%), Setosabatieria sp1(0-3%)*</td><td></td><td>present study</td></tr>
</table>

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

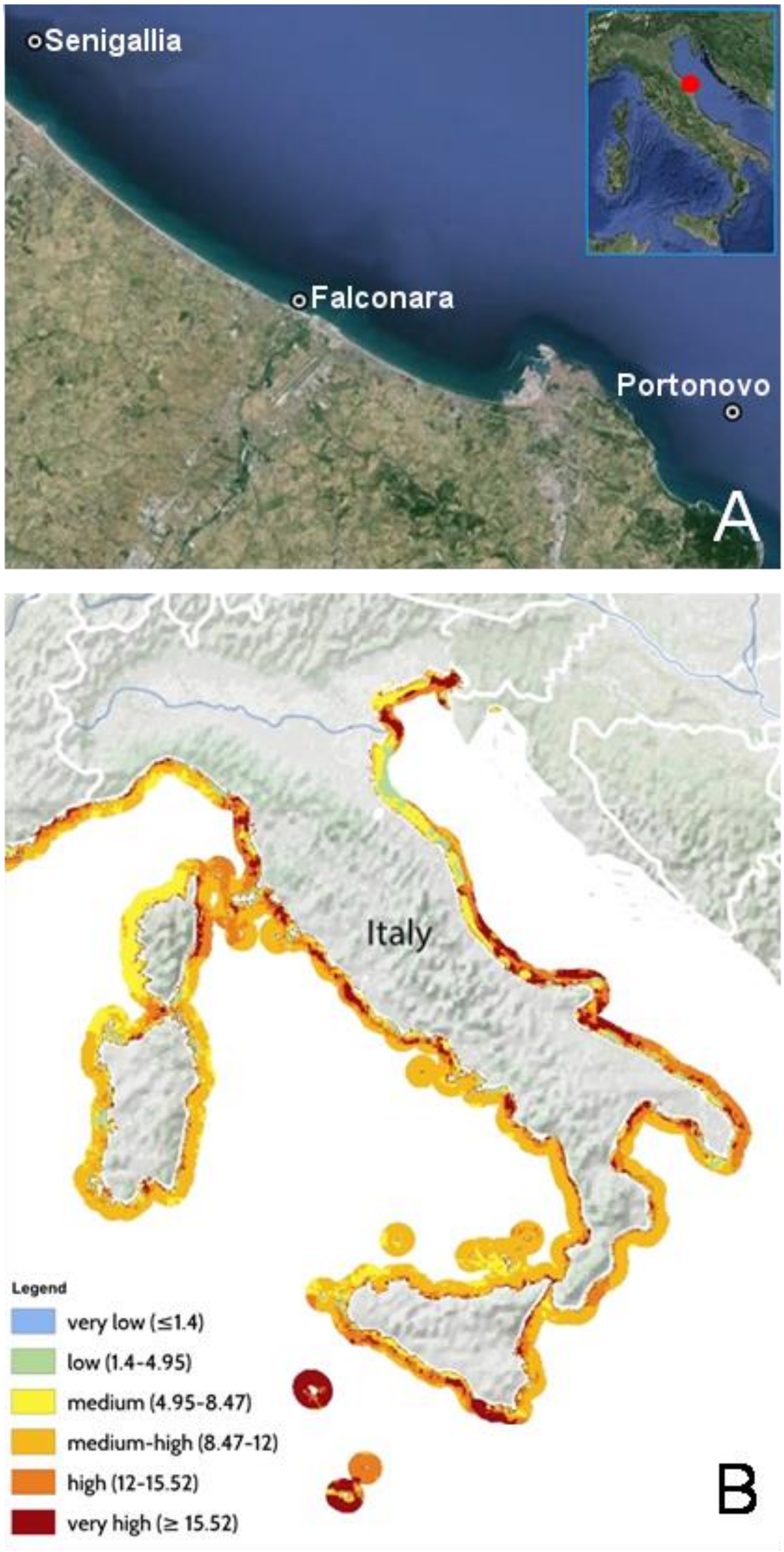

Senigallia
Falconara
Portonovo
A
Italy
Legend
very low (≤1.4)
low (1.4-4.95)
medium (4.95-8.47)
medium-high (8.47-12)
high (12-15.52)
very high (≥ 15.52)
B

Figure 1.

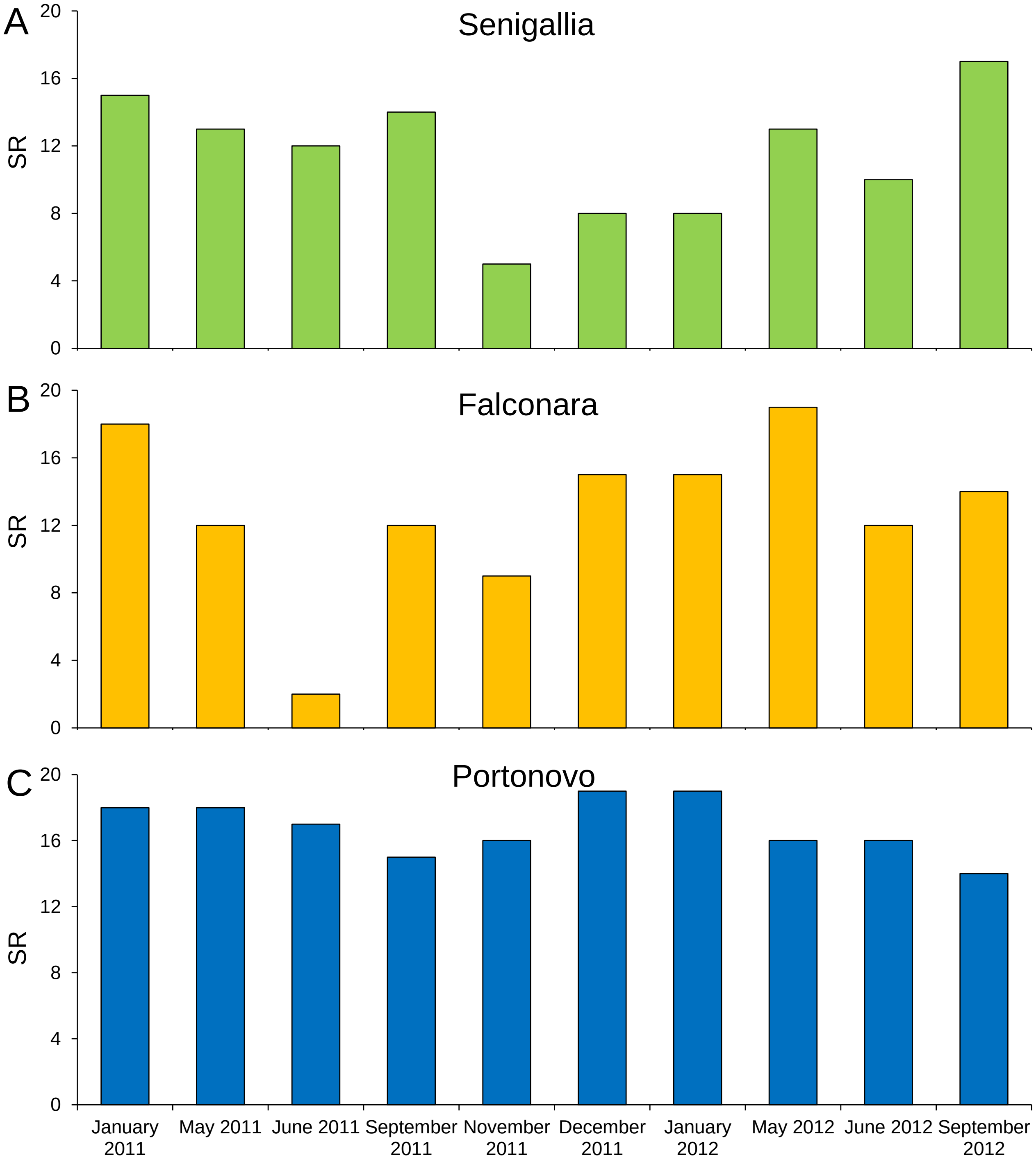

A
Senigallia
B
Falconara
C
Portonovo
SR
0
4
8
12
16
20
January 2011
May 2011
June 2011
September 2011
November 2011
December 2011
January 2012
May 2012
June 2012
September 2012

Figure 2.

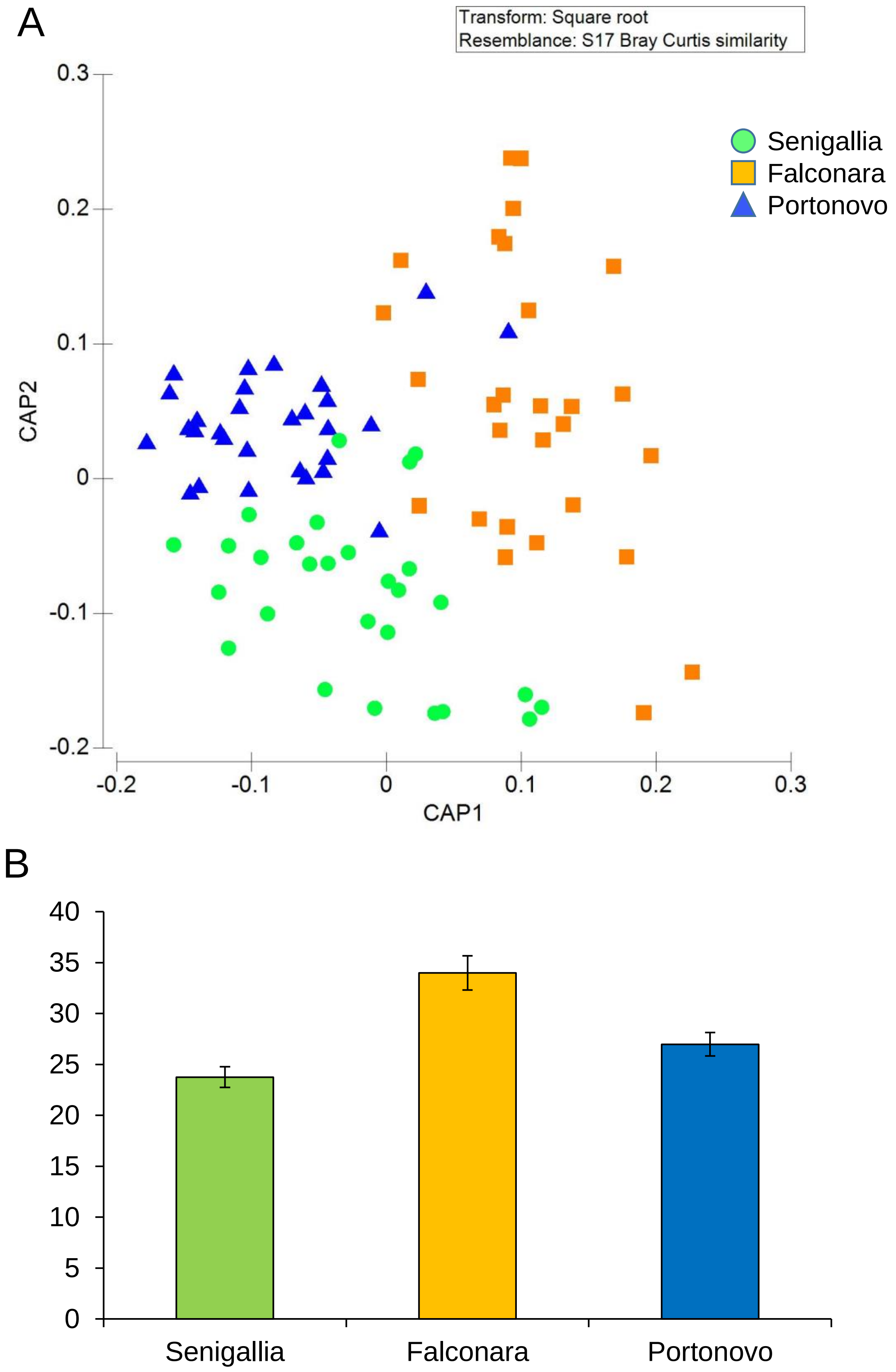

A
Transform: Square root
Resemblance: S17 Bray Curtis similarity
Senigallia
Falconara
Portonovo
CAP2
0.3
0.2
0.1
0
-0.1
-0.2
CAP1
-0.2
-0.1
0
0.1
0.2
0.3
B
40
35
30
25
20
15
10
5
0
Senigallia
Falconara
Portonovo

Figure 3.

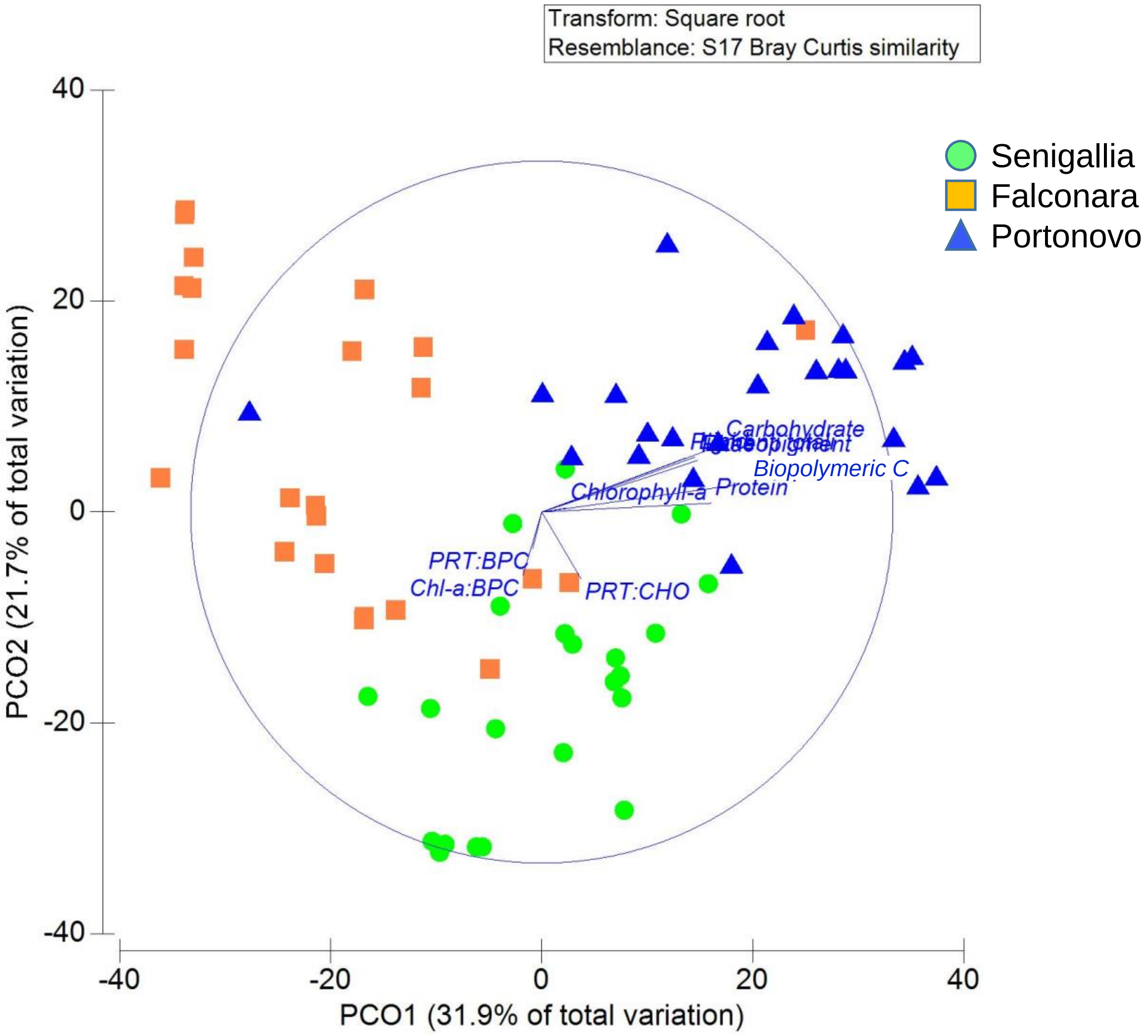

Transform: Square root
Resemblance: S17 Bray Curtis similarity
Senigallia
Falconara
Portonovo
Carbohydrate
Phaeopigment
Biopolymeric C
Chlorophyll-a
Protein
PRT:BPC
Chl-a:BPC
PRT:CHO
40
20
0
-20
-40
PCO2 (21.7% of total variation)
-40
-20
0
20
40
PCO1 (31.9% of total variation)

Figure 4.

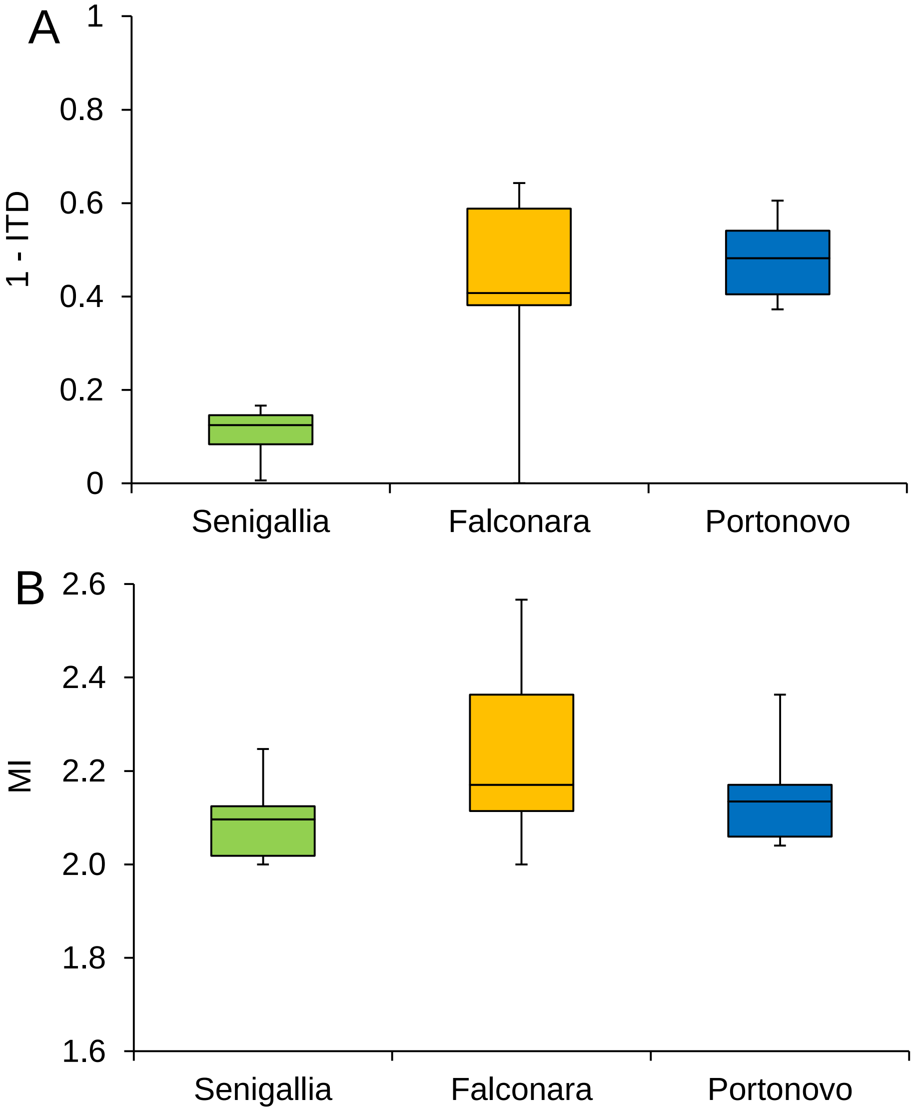

A
1
0.8
0.6
0.4
0.2
0
1 - ITD
Senigallia
Falconara
Portonovo
B
2.6
2.4
2.2
2.0
1.8
1.6
MI
Senigallia
Falconara
Portonovo

Figure 5.

Supplementary Table S1. Results of pair wise testing variations in the sedimentary OM biochemical compounds contents, indicators of nutritional 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 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

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,May,12,Jun12,Sept12>Nov11	Jan11,May11,Sept11,Dec11,Jan12,May12,Jun12,Sept12>Nov11>Jun11	Jan11,May11,Jun11,Sept11,Nov11,Sept12>Dec11,Jan12,May,12,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,Jun12	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

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 %
Acantholaimus 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
Ammotheristus 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
Belbolla 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
Chaetonema 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	0.3	1.7	0.0	0.3	2.7	0.0
Chaetonema 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
Desmodora 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
Diodontolaimus 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
Dorylaimopsis 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
Eleutherolaimus 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
Enoploides 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
Gnomoxyala 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
Halalaimus 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
Hopperia 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
Marylynnia 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
Marylynnia 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
Marylynnia 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
Mesacanthoides 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
Metalinhomoeus 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
Metalinhomoeus 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
Molgolaimus 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
Nemanema 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
Neotonchus 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
Odontophora 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
Oncholaimellus 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
Oxystomina 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
Paralongicyatholaimus 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
Paralongicyatholaimus 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
Paramesonchium 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
Paramonohystera 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
Paramonohystera 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
Paramonohystera 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
Pierrickia 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
Procamacolaimus 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
Retrotheristus 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
Sabatieria 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

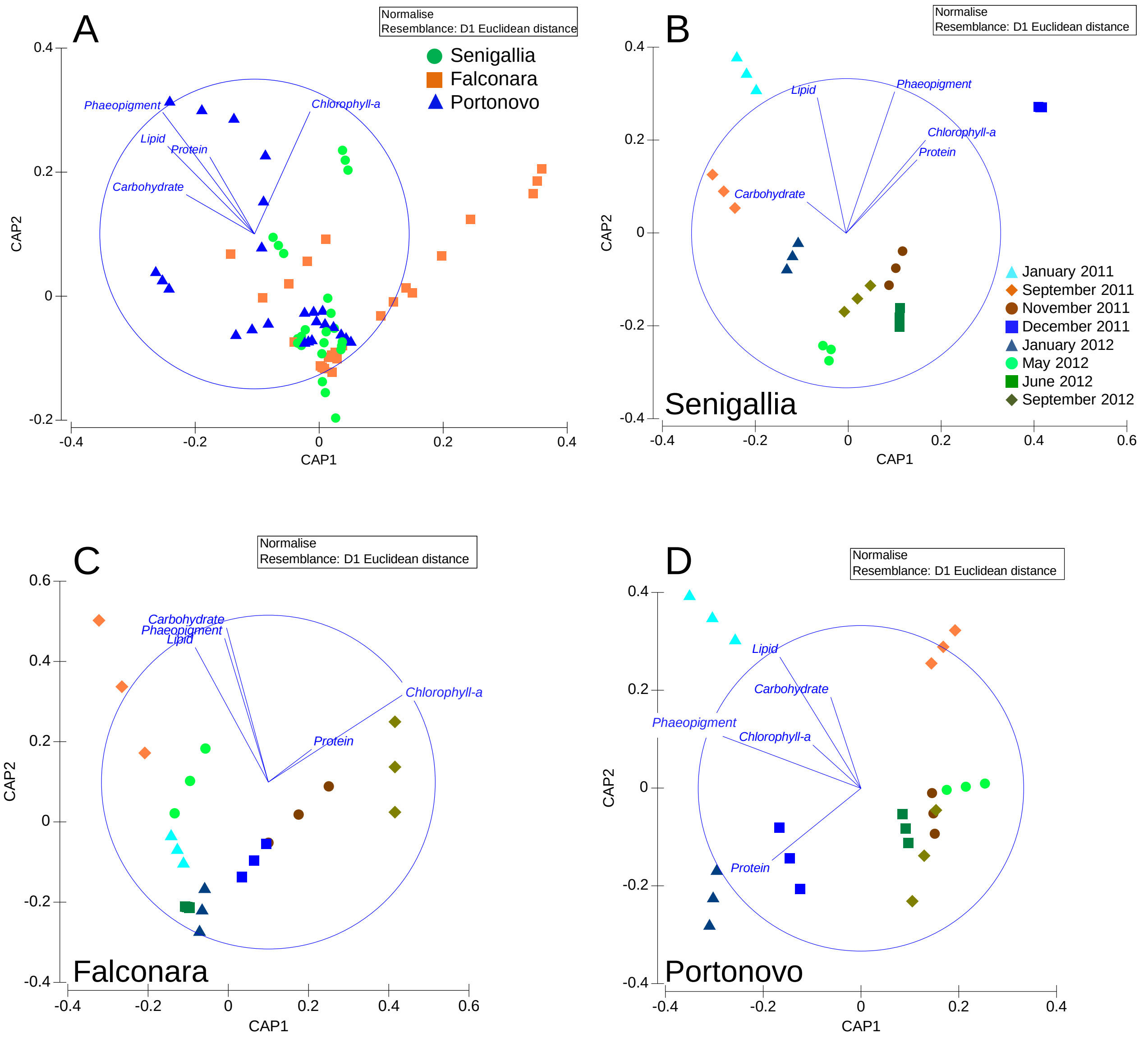
Setosabatieria sp.1	5.3	0.7	0.0	11.0	0.0	0.3	9.0	0.0	1.0	5.0	0.7	3.0	2.0	0.0	2.3	7.3	0.0	1.3	7.0	0.0	1.3	6.0	1.0	2.7	1.0	0.0	0.7	7.0	1.3	0.7
Sphaerolaimus sp.1	0.0	0.0	0.0	0.0	0.0	0.7	0.0	0.0	3.7	0.0	0.0	0.0	0.0	0.0	2.7	0.0	0.0	4.0	0.0	0.0	4.3	0.0	0.0	2.0	0.0	0.0	2.3	1.7	0.0	10.0
Sphaerolaimus sp.4	0.0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

Caption of supplementary figures:

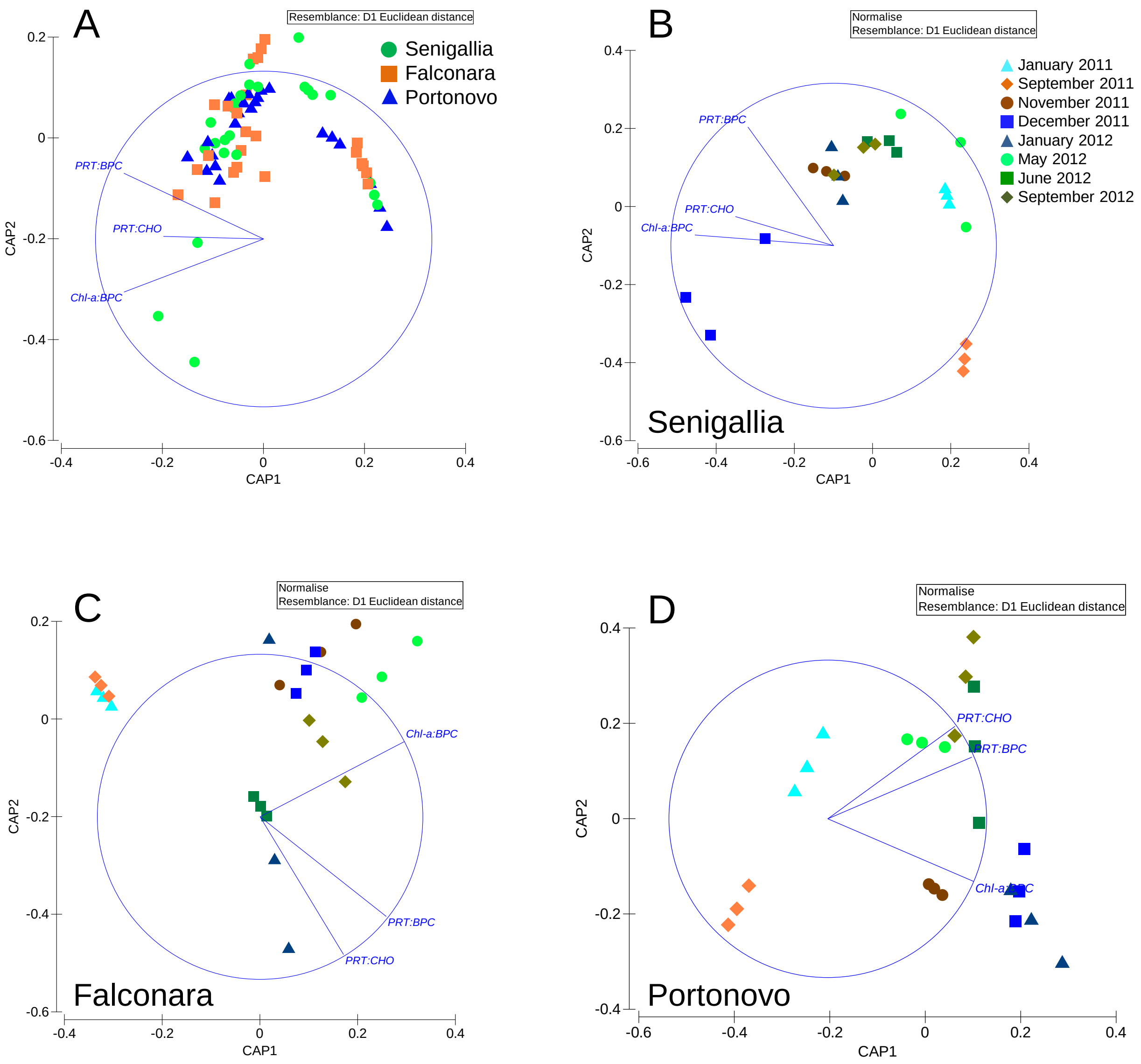
Supplementary Figure S1. Output of canonical analysis of principal coordinates (CAP) on sedimentary organic matter biochemical composition at all sites (A), and separately at Senigallia (B), Falconara (C) and Portonovo (D).

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

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



Supplementary Figure S1.



Supplementary Figure S2.

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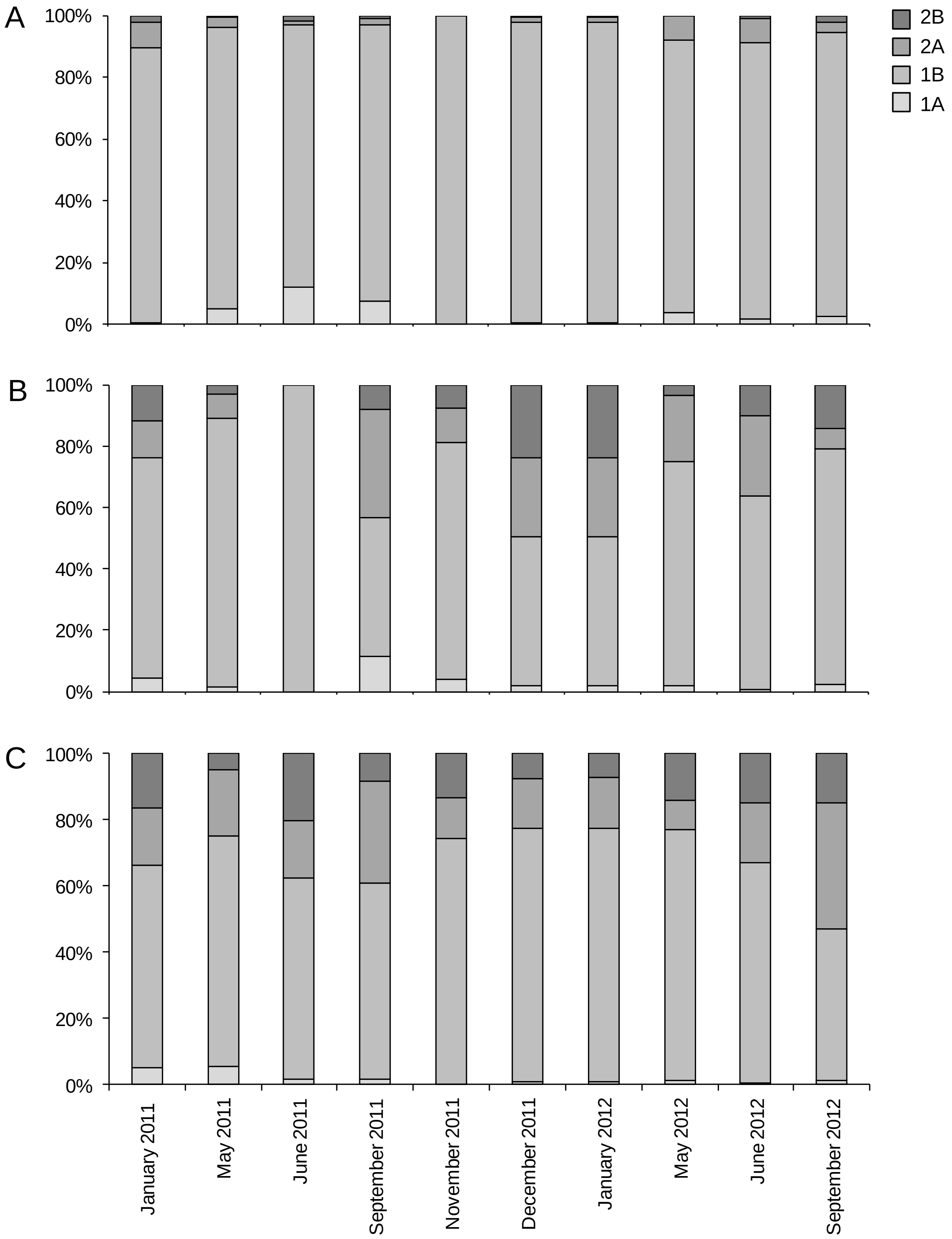

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1059 **Supplementary Figure S3.**