Ecological Indicators 95 (2018) 270–287

Contents lists available at ScienceDirect

Ecological Indicators

journal homepage: www.elsevier.com/locate/ecolind

Original Articles

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

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ARTICLE INFO

Keywords:
Sedimentary organic matter biochemical composition
Ecological quality status (EQS)
Marine Strategy Framework Directive

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.

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

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https://doi.org/10.1016/j.ecolind.2018.07.032
Received 8 March 2018; Received in revised form 13 July 2018; Accepted 16 July 2018

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,b). 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 (Merckx 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 utilized 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,b; 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 (Fig. 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 (Fig. 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. bimonthly 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. (2016a). At each site and time,

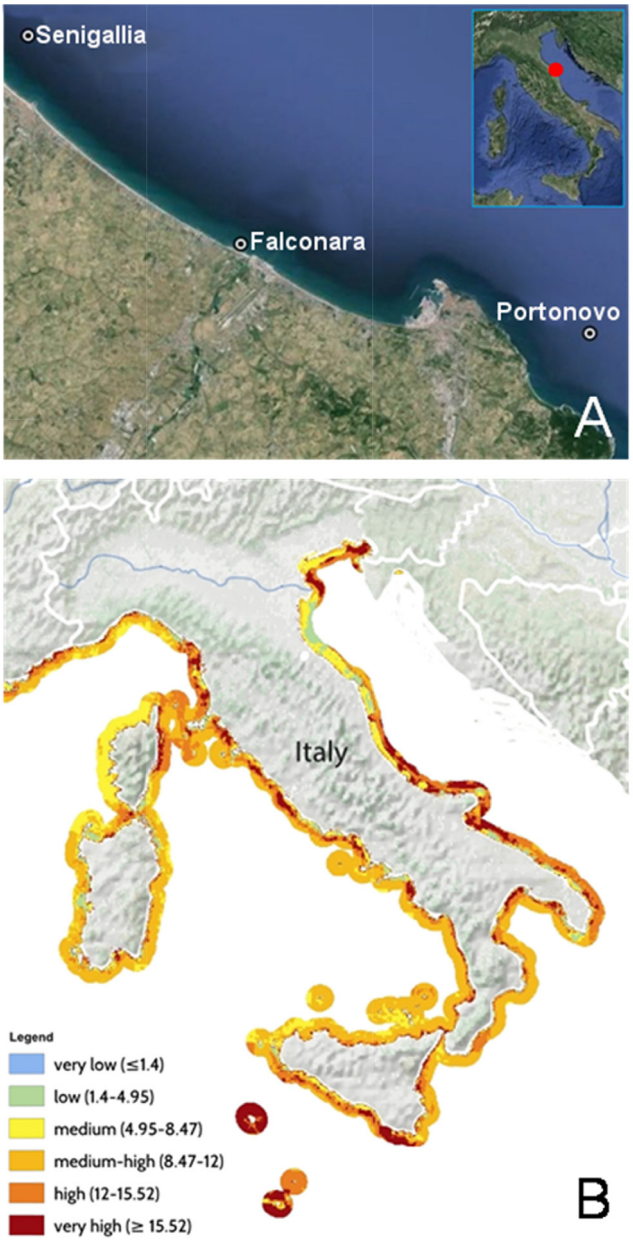

Fig. 1. (A) Location of the sampling sites (Senigallia, Falconara and Portonovo) in the Northern Adriatic Sea (the red dot indicates the study area). (B) Spatial distribution of cumulative impacts to the territorial waters of Italian seas, modified from Micheli et al. (2013) [Impacts considered: artisanal fishing, fishing (demersal, pelagic, destructive, non-destructive, high bycatch, low bycatch), benthic structures (oil rigs), coastal aggradation (coastal renourishment), coastal engineering (coastal defense and harbours), 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]. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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 40 μg C 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 mg C mg^{-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 = $g_1^2 + g_2^2 + g_3^2 \ldots + g_n^2$, 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,

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)

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., 2009). 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, 2001, 2003; Anderson et al., 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).

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) site	Time	Chlorophyll-*a*		Phaeopigment		Total phytopigment		Protein		Carbohydrate		Lipid		Biopolymeric C		Chlorophyll-*a* to BPC		Protein to BPC		Protein to carbohydrate ratio	
		μg g^{-1}		μg g^{-1}		μg g^{-1}		mg g^{-1}		mg g^{-1}		mg g^{-1}		mg g^{-1}		%		%			
		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) Site	Time	SR			D			H (log$_e$)			J			ES (100)			1-ITD			MI		
		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

(*continued on next page*)

Table 2 (*continued*)

B) Site	Time	SR			D			H (log_e)			J			ES (100)			1-ITD			MI		
		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)
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

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 data 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 Fig. 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 data Fig. 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 data 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 data Table S1). Temporal variations in the OM nutritional quality were driven by different combinations of variables at the three sampling sites (Supplementary data Fig. S2B–D).

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.ecolind.2018.07.032.

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.

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

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

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 (Fig. 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; Fig. 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

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.

A)	Indicator	Benthic trophic status: 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

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 (Fig. 3B). The analysis of principal coordinates (PCO; Fig. 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 data Fig. 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 data Table S2, Fig. 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, Fig. 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

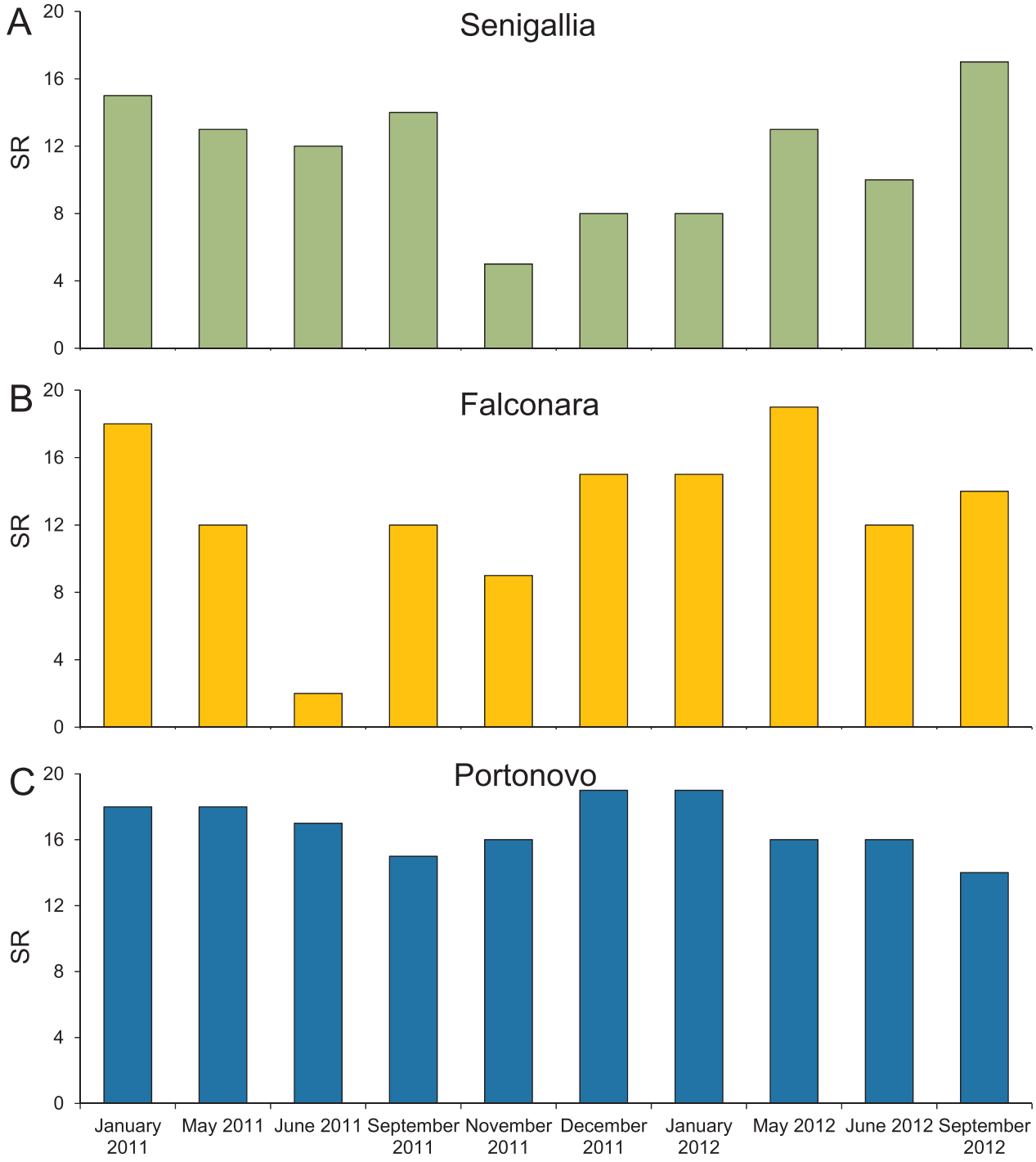

Fig. 2. Temporal variation of nematode species richness (SR) at Senigallia (A), Falconara (B) and Portonovo (C). (SR values calculated cumulatively from the 3 replicates are reported. Average values ± sd are reported in Table 2.)

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).

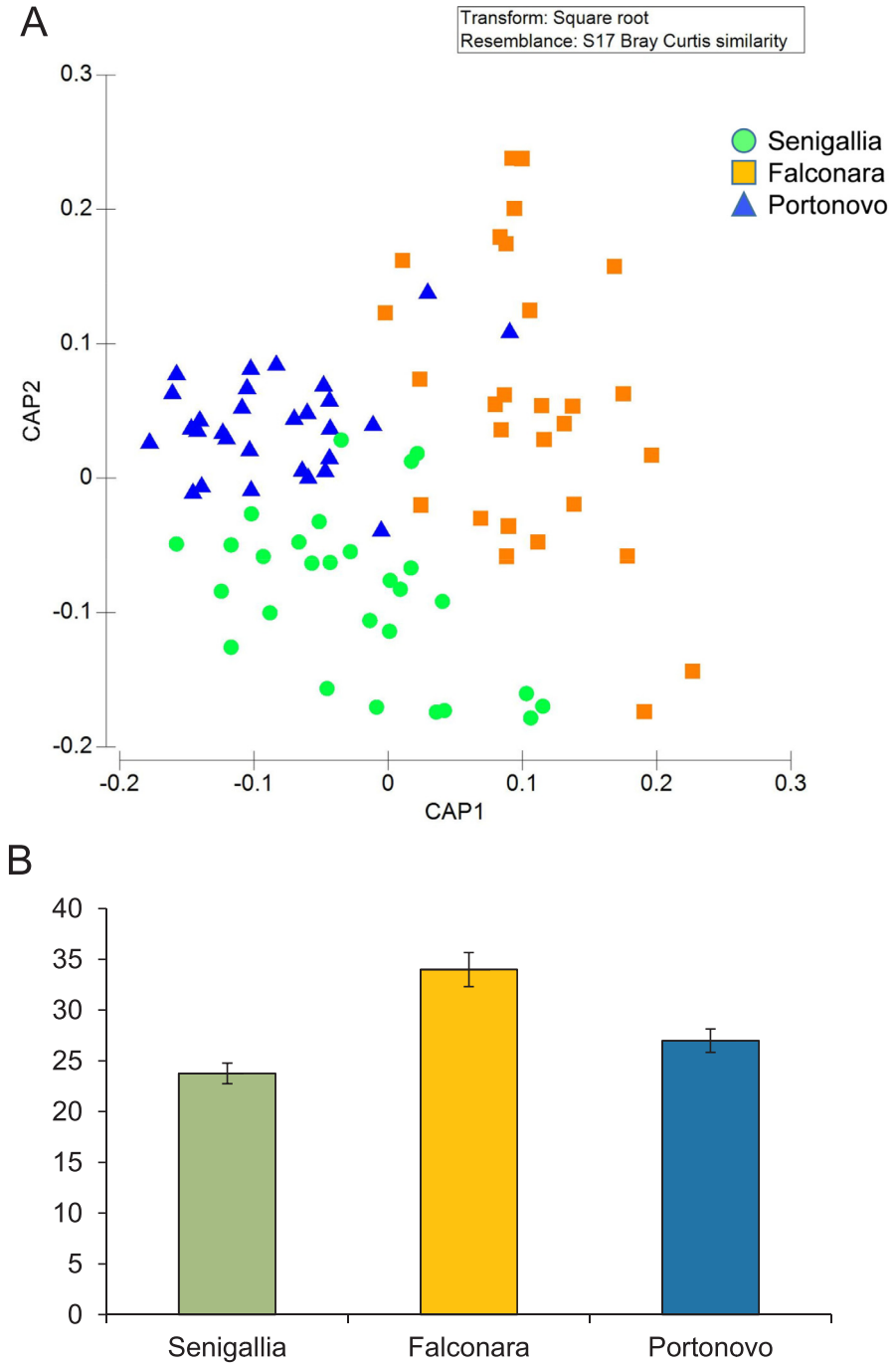

Fig. 3. Output of CAP (A) and PERMDISP on temporal variations (B) in the nematode species composition.

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,b; 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 utilized in the

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

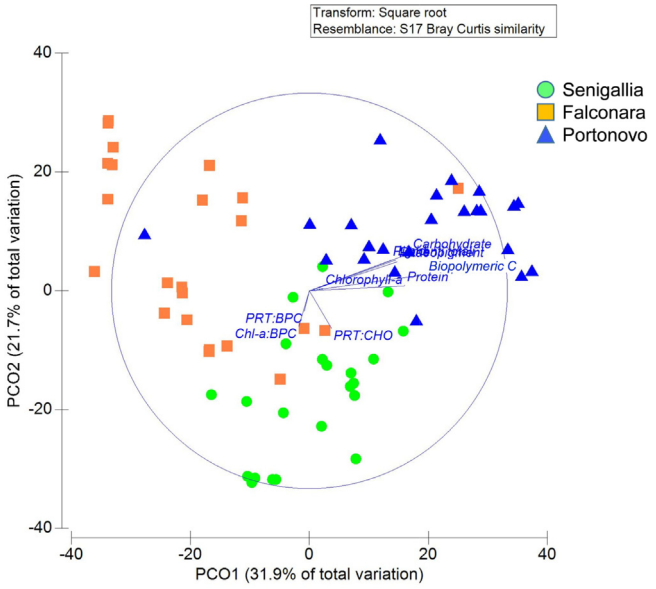

Fig. 4. Output of PCO, to identify sedimentary OM variables mostly responsible for the differences in the composition of nematode assemblages.

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

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).

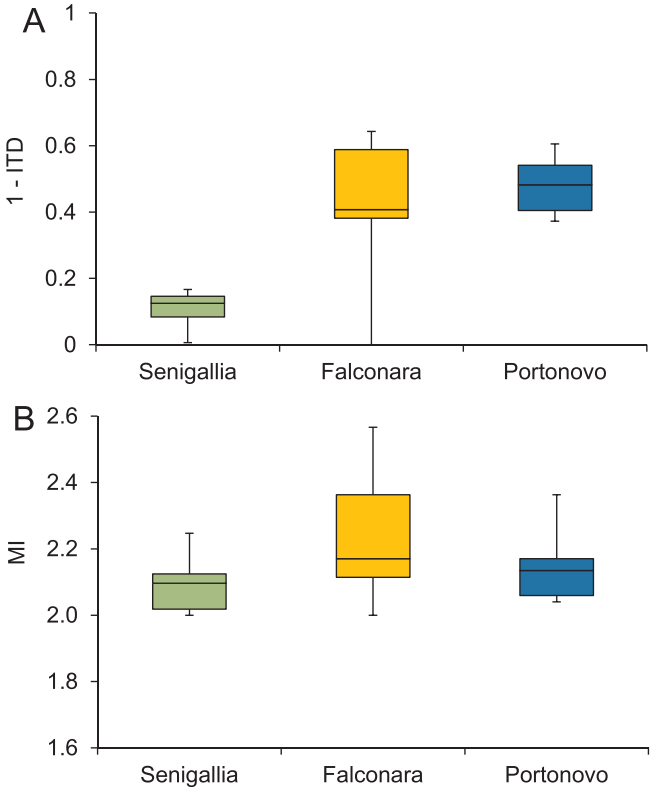

Fig. 5. Distribution of 1-ITD and MI values at Senigallia, Falconara and Portonovo. Reported are minimum, maximum, median values and standard error bars.

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

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)4
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) and 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)

(*continued on next page*)

Table 7 (*continued*)

Impact typology	Tolerant genera/species	Sensitive genera/specie	Effects on the overall assemblages	Reference
Physical disturbance	*Sabatieria pulchra, Sabatieria punctata, Daptonema tenuispiculum, Enoplolaimus spp., Theristus* spp.		Diversity declines	Review in Danovaro et al. (2009b)
Anthropogenic pressures (population density, harbours, dredging activities) on estuaries	*Sabatieria, Daptonema, Terschellingia, Paracomesoma. Daptonema, Sabatieria* and *Dichromadora* tolerant to wide salinity range			Alves et al. (2013)

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

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.

A)

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

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; Boufahjaa 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,b). 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.

Acknowledgements

This study is part of the projects DEVOTES, funded by the European Union under the 7th Framework Programme 'The Ocean of Tomorrow' Theme (grant agreement no. 308392, www.devotesproject.eu) and MERCES, funded by the European Union's Horizon 2020 research and innovation programme (grant agreement no. 689518, http://www.merces-project.eu). Marta Miatta (Newfoundland and Labrador's University, Memorial University) is aknowledged for helping during the laboratory analyses.

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