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Influence of natural and anthropogenic disturbances on foraminifera and free-living nematodes in four lagoons of the Po delta system



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ABSTRACT

The natural high productivity of river lagoons has always attracted human activities of high economic value such as aquaculture. Notwithstanding, these environments are also prone to contamination, excessive organic enrichment and hypoxic/anoxic events due to the synergy between natural (e.g. fluctuations of oxygen, temperature and salinity) and anthropogenic factors (e.g. accumulation of pollutants carried by the river). In order to minimize any economic loss and potential risk for human health, river lagoons are kept under surveillance by means of monitoring campaigns that rarely include foraminifera and nematoda despite their suitability as ecological indicators. In May 2016, these two assemblages were investigated within the framework of the project RITMARE in four lagoons of the Po Delta system (Adriatic Sea) in terms of taxonomic composition, biodiversity and biological traits. Dominated by a few tolerant taxa (Ammonia tepida and Haynesina germanica for foraminifera; Daptonema, Tershellingia and Sabatieria for nematoda), both assemblages seemed adapted to conditions of environmental instability mainly ascribable to natural disturbance factors rather than to anthropogenic contamination, as indicated by the DISTLM outputs. Furthermore, the stochastic occurrence of abnormal tests and the presence of numerous organisms in subsurface sediment levels confirmed a limited effect of synthetic organic compounds (e.g. aromatic hydrocarbons and polybrominated diphenyl ethers) and trace metals on foraminifera. Focusing on nematoda, the assemblage seemed more sensitive in reflecting the peculiar environmental conditions of each lagoon and the degree of confinement, as indicated by the significant intra- and interlagoon variability detected for the majority of the tested metrics. In particular the significantly less biodiverse assemblage dominated by r-strategist (i.e. pioneer) organisms at the inner and middle stations of Scardovari may represent the nematoda response to recent hypoxic/anoxic conditions that often occur in the inner areas of this lagoon because of its low water renewal. Both assemblages showed to be suitable for the implementation of monitoring programs; from the study of foraminifera, a relatively low chemical contamination was inferred while, from that of nematodes, recent events of oxygen deficiency were detectable.

1. Introduction

Foraminifera and free-living nematoda represent, respectively, the most diverse and abundant protozoans (Sen Gupta, 1999) and metazoans (Giere, 2009) of the meiofauna. Together, these benthic assemblages occupy a unique position in the benthic food web since they are at the same time both consumers of a range of carbon sources and a food source for secondary consumers (Schratzberger and Ingels, 2018). Being characterized by unique peculiarities (i.e. high abundance and diversity, small organism size that ensures a limited sampling effort,

rapid turnover cycles, widespread distribution, limited mobility and lack of larval dispersal; Balsamo et al., 2012), these assemblages exhibit measurable responses to a wide plethora of disturbance factors (Alve, 1995; Scott et al., 2001; Frontalini and Coccioni, 2011; Semprucci et al., 2015; Zeppilli et al., 2015). Foraminifera are reported as sensitive to chemical contamination and in particular to trace metals (Coccioni et al., 2009; Frontalini et al., 2018). Stressed assemblages display lowered abundances, biodiversity loss, dwarfism and increased abnormalities of the tests (for review, see Frontalini and Coccioni, 2011; Balsamo et al., 2012). Similarly, free-living nematodes show shifts in

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their taxonomic and trophic composition in relation to organic enrichment, anoxia (Taheri et al., 2015) and elevated concentrations of contaminants as polycyclic aromatic hydrocarbons-PAHs (Moreno et al., 2011; Losi et al., 2013) or polychlorinated biphenyls-PCBs (Franzo et al., 2018).

The suitability of foraminifera and nematoda as target assemblages for monitoring studies favored the development of dedicated indices that are relatively easy to apply and to understand by managing authorities. Furthermore, some of these metrics are based not only on their taxonomic composition but also embrace ecological aspects as the trophic guilds and the reciprocal percentage of r- and k-strategists. Focusing on foraminifera, a widely used metrics is the Foraminiferal Abnormalities Index (FAI), which is based on the percentage of abnormal specimens over the whole population (Coccioni et al., 2009). For what concerns nematodes, popular indices are the Index of Trophic Diversity (ITD; Heip et al., 1985) and the Maturity Index (MI; Bongers, 1990; Bongers et al., 1991). Although criticized for the relatively ambiguous results obtained especially in case of anthropogenic disturbance (for review, see Semprucci et al., 2015), ITD is still widely adopted and focuses on nematodes trophic guilds while MI takes into account the reciprocal proportions of colonizer (r-strategists) and persister (k-strategists) nematodes.

Despite their ecological role and suitability for providing information about potential ongoing environmental changes, foraminifera and nematoda have been never synoptically investigated in highly dynamic environments such as river lagoons. These habitats, or more in general the Transitional Environments (TEs), are substantially influenced by freshwater flows due to their vicinity to river mouths and, at the same time are also partly saline due to the proximity to the sea (Water Framework Directive, 2000/60/EC). Due to these peculiarities, TEs are naturally stressed areas because of the high degree of variability in their physico-chemical characteristics such as oxygen, temperature and salinity (McLusky and Elliott, 2004, 2007). Furthermore, at the same time TEs are subjected to anthropogenic stress because prone to the accumulation of contaminants and organic matter carried by the river. In these particular environments, the study of foraminifera and nematoda, both in terms of taxonomic composition and main dedicated indices, may integrate the monitoring actions in detecting potential negative phenomena (e.g. hypoxia/anoxia, contamination of the sediments) that frequently characterize the TEs and that can negatively affect both the ecosystem and human productive activities such as aquaculture.

The Po River is the most important Italian watercourse and its drainage basin contributes to more than 35% of Italian agricultural, livestock and industrial production (Casatta et al., 2016). These human activities originate important loads of both organic matter and contaminants making the Po River one of the main vectors of nutrients, trace metals and a multitude of other compounds to the northern Adriatic Sea (Giani et al., 2012). At its confluence into the sea, several TEs, including river lagoons, form the Po deltaic system. Here, the compounds carried by the river tend to accumulate in the sediments, entailing a potential risk to both natural communities and human health. The concern about the latter derives from the fact that over the years the high productivity of the Po Delta lagoons has favored the establishment of aquaculture activities addressed at the production of seafood for humans. Among them, the intensive cultivation of the Manila clam (Ruditapes philippinarum) represents the leading shellfish activity in the area that makes Italy the second world-highest producer after China (FAO, 2014). In virtue of its high ecological and economic value, the area is kept under surveillance by means of regular monitoring campaigns performed by the competent authorities as the Regional Environmental Protection Agencies (ARPA). Although several environmental variables and communities are included in these monitoring plans, to date foraminifera and nematoda have never been considered.

In the present study, we investigated these two assemblages in four

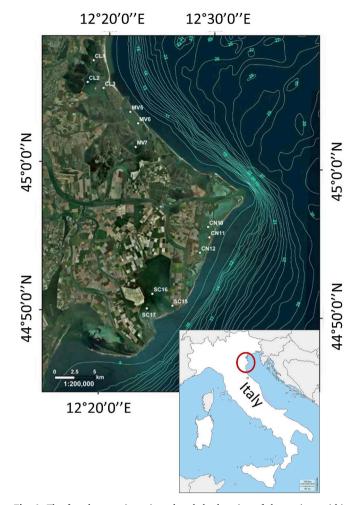


Fig. 1. The four lagoons investigated and the location of the stations within each lagoon along an increasing confinement gradient.

lagoons of the Po River Delta that were chosen to represent potentially different environmental conditions (Fig. 1). While Marinetta-Vallona (MV) and Canarin (CN) receive direct freshwater inflows from the Po River, Caleri (CL) and Scardovari (SC) show more marine features because mainly subjected to the seawater ingression through the mouths that connect these lagoons with the sea. For both assemblages, the abundance, taxonomic composition and biodiversity were investigated. Furthermore, the calculation of exclusively dedicated indices was performed: FAI for foraminifera; ITD and MI for nematoda.

The following questions were addressed:

- Did foraminifera and nematoda show an intra- and/or an inter-lagoon variability?
- 2. Did such differences give any indication about ongoing or recent disturbance factors (natural and/or anthropogenic) for the two assemblages?
- 3. Could the study of foraminifera and nematoda make more effective the monitoring of river lagoons?

2. Materials and methods

2.1. Study site

The Po River is one of the largest rivers of the Mediterranean Sea in terms of freshwater fluxes, with an average discharge of $1500\,\mathrm{m}^3\,\mathrm{s}^{-1}$ (Ludwig et al., 2009). At its delta, situated in the northwestern boundary of the Adriatic Sea, are located seven lagoons. Four of them

were investigated in the present study: Caleri (CL), Marinetta-Vallona (MV), Canarin (CN) and Scardovari (SC) following a North-South direction (Fig. 1). While MV and CN receive direct freshwater inflows from some branches of the Po River, CL and SC are influenced only indirectly by the freshwater since it is mainly transported by the river plume and enters in the lagoons through the connections (mouths) with the northern Adriatic Sea. For a comprehensive description of the hydrodynamic characteristics of the lagoons, see Maicu et al. (2018).

The northernmost lagoon Caleri is $11\,\mathrm{km}^2$ wide with an average depth of 1.5 m. Its hydrodynamics depends largely on the water exchange with the sea that occurs through the only mouth. At its southernmost corner a narrow passage connects Caleri to Marinetta lagoon and a very small amount of freshwater comes from a pumping station in the northern corner. Since directly connected to each other, Marinetta (3.5 km², depth 1-1.5 m) and Vallona (9.1 km², depth 1.5 m) form a single transitional system that is influenced by both the seawater entering through the two mouths and by the freshwater coming from the Po di Levante, a deltaic branch of the Po River. Canarin presents only a small mouth to the sea located in its northern part (max depth 2.5 m). Freshwater enters the lagoon in the southern corner through a narrow channel from the Po di Tolle and through minor tributaries originating from the primary branch of the Po River, the Po di Pila, which accounts for more than 60% of the freshwater discharge and sediment load of this Italian river (Syvitski et al., 2005). Scardovari is the largest embayment of the present study (32 km²) and is located between the Po di Tolle and the Po di Gnocca branches. It receives Po freshwater indirectly, through the wide mouths that connect the lagoon to the Adriatic Sea. The northern area is characterized by an average depth of \sim 2.5–3 m and by low hydrodynamic conditions that lead to dystrophic events especially during late summer (ARPAV, 2016). On the contrary, the southern area (depth $\sim 1.5 \,\mathrm{m}$) is subjected to a more pronounced water exchange due to the vicinity with the mouths. Though to different degrees, all lagoons are exploited for the culture of the Manila clam (R. philippinarum), in particular Vallona from which comes about one-fifth of the total (~200 millions €) clam production of the northern Adriatic Sea (Turolla, 2008; Abbiati et al., 2010).

2.2. Sampling

In each lagoon, three stations located along a gradient of increasing confinement were sampled in May 2016 (Fig. 1). During each sampling, depth, bottom sea temperature and salinity were measured using a multiparametric probe (YSI ECO2 EXP 7 2001) while the sediments were collected by means of a manual box corer with 225 cm² of sampling area. At each station, five cut-off plastic syringes (internal diameter: 2.7 cm, surface area: 5.72 cm²) were used for subsampling the sediment collected by the box corer in order to obtain two and three pseudo-replicates for foraminifera and meiofauna, respectively. The remaining sediment was sampled (top 0-1 cm layer) for environmental variables exhaustively presented in the following studies: Cibic et al. (this issue) for sediment grain-size, Zoppini et al. (this issue) for Biopolymeric Carbon (BPC) and ΣPAHs, Viganò et al. (this issue) for ΣPCBs, polybrominated diphenyl ethers (ΣPBDEs) and the pool of products derived from DDT degradation (ΣDDX). In the present study, these variables (Supplementary Table A1) were included in the statistical analysis in order to explore any possible relationship with the two target assemblages.

2.3. Foraminifera

The top 4 cm of sediments were immediately extruded from each pseudo-replicate and further divided in four sub-samples 1 cm thick (ca. 6 cc). Afterwards, each of them was soaked in > 90% ethanol (Scott et al., 2001; Schönfeld et al., 2012). According to literature (Scott et al., 2001 and references therein), the sub-sampling for the study of foraminifera in subtidal/intertidal environments envisages the collection

of the top 1 cm of the sediment in a $10\,\mathrm{cm}^2$ area (=10 cc). Since the selected sampling tool, i.e. the cut-off syringe, had a smaller area (ca. $6\,\mathrm{cm}^2$), two adjacent pseudo-replicates were collected in order to have additional sediment in case the number of foraminifera specimens in one pseudo-replicate was too scarce (< 300 organisms) and, consequently, not representative.

Once in laboratory, each sediment subsample was washed through a 45 μm sieve and subsequently stored in ethanol to which 1–2 cc of Rose Bengal solution was added (Scott and Medioli, 1980). Constantly retained in a liquid suspension, all samples were inspected in liquid (Scott et al., 2001) after more than 2 weeks according to Schönfeld et al. (2012) and using a stereomicroscope Leica M165C (up to 192X). Literature reports that the largest part of the foraminifera biocenosis in marginal/intertidal/shallow subtidal environments are present at the sediment level 0-1 cm (Scott and Medioli, 1980; Scott et al., 2001; Schönfeld et al., 2012). Since the studies investigating these aspects in shallow subtidal environments are very scarce, all four sub-samples obtained from a pseudo-replicate of each station (cm 0-1, cm 1-2, cm 2-3, cm 3-4) were examined. In case of very abundant biocenosis, a wet splitter (settling column splitter according to Scott and Hermelin, 1993) was used for counting a number of splits sufficient to reach at least 300 specimens. As at each station the total number of foraminifera from one pseudo-replicate was representative (> 300 organisms), the specimens in the second pseudo-replicate were not counted and, therefore, included in the dataset. The results are expressed as number of specimens per volume unit (cc or cm³). The distinction between living (stained) and dead specimens was performed according to the recommendations by Schönfeld et al. (2012) and references therein. At last, deformed specimens were also counted to provide the FAI index, i.e. the percentage of abnormal specimens within the whole assemblage (Coccioni et al., 2009).

2.4. Nematoda

The top 10 cm of the sediment samples were immediately extruded from each pseudo-replicate and preserved in buffered 4% formaldehyde solution using prefiltered seawater stained with Rose Bengal $(0.5\,\mathrm{g\,L^{-1}})$, as described by Danovaro (2010) for meiofauna.

Once in laboratory, the samples dedicated to nematoda were processed according to the method for the extraction of meiofauna from muddy sediments fixed with formalin (Danovaro, 2010). All metazoans passing through a 1 mm sieve but retained by a 30 μ m mesh net were separated from the sediment by centrifugation with Ludox HS-40 (Heip et al., 1985) with a density of 1.15–1.18 g cm $^{-3}$ (Danovaro, 2010).

For each pseudo-replicate, extracted meiofaunal organisms were placed on a Delfuss cuvette and all specimens were counted (abundance was expressed in individuals per $10 \, \mathrm{cm^2}$) and classified into the main groups (Higgins and Thiel, 1988) under a stereomicroscope (Zeiss Discovery V20; final magnification of 40 or 80X). In order to ensure the randomness during collection, the first 100 nematodes encountered in the cuvette were hand-picked out using a fine pin while the remaining ones were counted only. Collected specimens were transferred from formalin to glycerol through a series of ethanol-glycerol solutions and finally mounted on slides in anhydrous glycerin (Seinhorst, 1959). All nematodes on permanent slides were identified at the genus level under a 100x oil immersion objective (Olympus BX51) using the pictorial keys of Platt and Warwick (1983, 1988) and Warwick et al. (1998), as well as the original species descriptions and identification keys available through NeMys (Bezerra et al., 2018).

The trophic structure of nematode assemblage was studied by assigning each genus to one of the following feeding groups (Wieser, 1953): selective (1A) and non-selective (1B) deposit feeders, epigrowth feeders (2A) and predators/omnivores (2B). The Index of Trophic Diversity (ITD) was calculated according to Heip et al. (1985): ITD = $\Sigma\theta^2$, where θ is the percentage contribution of each feeding type. ITD values range from 0.25 (the highest trophic diversity; i.e. each trophic group

Table 1
Abundance of foraminifera, expressed as ind. cm^{-3} in the four sediment levels at all stations. Mean abundance of nematodes, expressed as ind. $10 cm^{-2}$, at all sites. Per each lagoon, the stations are presented from the innermost to the outer site. SD = Standard deviation.

Foraminifera	CL1	CL2	CL3	MV7	MV6	MV5	CN12	CN10	CN11	SC16	SC17	SC15
0–1 cm	93.3	329.2	128.2	45.3	59.4	87.3	56.2	26.0	23.2	117.5	234.7	36.5
1–2 cm	28.9	92.4	5.4	9.4	36.2	15.4	17.5	8.9	4.1	7.5	37.5	8.0
2–3 cm	21.6	50.3	3.9	4.7	17.2	4.4	9.7	5.8	17.0	3.7	16.7	1.9
3–4 cm	24.4	12.2	1.0	0.8	24.2	2.3	5.2	6.5	15.6	3.1	28.1	1.6
Total abundance	168.1	484.1	138.5	60.2	137.0	109.4	88.6	47.2	59.9	131.8	317.0	48.1
Nematoda	CL1	CL2	CL3	MV7	MV6	MV5	CN12	CN10	CN11	SC16	SC17	SC15
Mean abundance	1555.4	554.4	806.8	360.8	1390.5	1609.1	1463.6	1575.4	782.7	1090.3	1686.2	1205.2
SD	872.1	62.6	320.9	106.3	669.1	245.3	404.5	487.7	468.3	490.3	1066.9	964.9

accounts for 25% of the whole nematode assemblage) to 1.0 (the lowest trophic diversity; i.e. one feeding type represents 100% of the assemblage).

The maturity index (MI, Bongers, 1990; Bongers et al., 1991) was calculated as the weighted average of the individual colonizer-persister (c-p) values: MI = $\Sigma v(i)f(i)$, where v is the c-p value of genus i and f(i) is the frequency of that genus. This index is based on the gradual discrimination among r-strategist (colonizers, i.e. c-p1 and c-p2) towards k-strategist nematodes (persisters; i.e. c-p4 and c-p5).

2.5. Statistical analysis

Performed using the PRIMER v7 software package (Clarke and Warwick, 2001) with the PERMANOVA add-on package (Anderson et al., 2008), the same univariate and multivariate analyses were conducted on foraminifera and nematoda datasets. The sediment levels considered for foraminifera were merged prior to any statistical analysis (i.e. top 0–4 cm). Taxonomic diversity indices (d, Margalef, 1986; H' log2, Shannon and Weaver, 1949) were calculated based on the abundances of foraminifera species and nematode genera.

To test for spatial differences in the composition of foraminifera and nematodes, two data matrices based on the abundance of species/ genera at each station were constructed by applying the Bray-Curtis similarity and forth root transformation of data, the latter to scale down densities of highly abundant genera and increase the importance of less abundant ones. For foraminifera, a one-way PERMANOVA test was conducted on the matrix using "lagoon" as a fixed factor and the unrestricted permutation of raw data was performed (9999 permutations). For nematodes, a two-way PERMANOVA routine was performed on the matrix using "lagoon" as a fixed factor and "confinement" as a fixed factor nested in "lagoon". The permutation of residuals under a reduced model was performed (9999 permutations). In both cases, the null hypothesis (i.e. no significant difference among samplings) was rejected when the significance level p was < 0.05. The Monte Carlo permutation p was used when the number of permutations was lower than 150. If significant differences were detected, a posteriori pair-wise comparisons were performed (9999 permutations).

To check for spatial differences of foraminifera abundance, diversity (d and H') and FAI, a one-way PERMANOVA analysis was applied, using the same design described for species composition. For this purpose, another data matrix, based on Euclidean-distance similarity, was built. Similarly, spatial differences of nematodes abundance, diversity (d and H'), MI and ITD were checked by means of a two-way PERMANOVA analysis with the same design described for genera composition and conducted on a data matrix based on Euclidean-distance similarity.

For both assemblages, a non-metric Multidimensional scaling ordination (nMDS) was performed on a Bray-Curtis similarity matrix based on species/genera abundance in order to visualize any difference among stations (Clarke and Green, 1988). Similarity profiles (SIM-PROF) analysis was used to test significant spatial differences (p < 0.05).

In order to determine whether foraminifera and nematoda were influenced by the main environmental variables (Table A.1), a distance-based linear model (DISTLM, McArdle and Anderson, 2001) routine was carried out on foraminifera and nematoda. In both cases, the stepwise selection procedure and the adjusted R^2 was used as a selection criterion to enable the fitting of the best explanatory environmental variables in the model (Anderson et al., 2008). Prior to analysis, the environmental variables were tested for collinearity (Draftsman plot and Spearman correlation matrix). Sand % was omitted from the analysis because tightly correlated with mud % $(r^2 > 0.90)$ and therefore considered redundant. To compensate for skewness, log(X) transformation was performed on contaminants ($\Sigma PBDEs$, $\Sigma PCBs$, ΣDDX and $\Sigma PAHs$); square root transformation was performed on salinity, depth, bottom water temperature and BPC data while silt % and clay % were arcsine-transformed.

3. Results

3.1. Foraminifera

Foraminifera abundance showed a wide variability since the two minima (47.1 ind. cm⁻³ at CN10 and 48.1 ind. cm⁻³ at SC15) were one order of magnitude lower than the two maxima (484.1 and 317.0 ind. cm⁻³ at CL2 and SC17, respectively) (Table 1). Due to such variability, significant differences among lagoons in terms of abundance were not detected as indicated by PERMANOVA outputs (Table 2). With the exception of Canarin, intermediate stations showed higher abundances when compared with the inner (I) and the outer (O) sites. Therefore, a clear pattern along the confinement gradient was not observed. Focusing on the vertical distribution of the organisms, foraminifera were gradually less abundant from the top sediment layer to the subsurface, although the deepest layer was not characterized by the lowest abundance at all stations as at MV6 and at SC17 (Table 1).

Juvenile specimens (i.e. organisms with few chambers and intact protoplasm) were observed only in subsurface sediment levels at all Caleri stations and at SC16 and SC17. Overall, 24 species belonging to 17 genera, were observed (Supplementary Table A2). Considering all sediment levels together, on average ~99% of the whole assemblage was represented by 5 dominant species while the others showed a sporadic occurrence (Fig. 2a). Ammonia tepida was the most abundant species at all stations with the exception of CL1 and SC16, sites characterized by the dominance of Haynesina germanica (Relative Abundance - RA equal to 50.4% and 36.5%, respectively). These two sites showed also RA values of Aubignyna perlucida one order of magnitude higher than at the other stations. Although Cribroelphidium gunteri and Quinqueloculina seminula were observed at almost all stations, the former showed the highest RA at MV6 (21.3%) while the latter characterized mainly the outer stations (16.3% at CL3 and 29.4% at SC15) of the 'marine' lagoons Caleri and Scardovari. In terms of taxonomic composition, a clear pattern was not evidenced along the confinement gradient with the exception of CL1 and SC16, the two inner stations of

Table 2

Outputs of the PERMANOVA main test performed on foraminifera and nematode datasets. Significant differences (* = P < 0.05; ** = P < 0.01; *** = P < 0.001) are indicated in bold. The outputs of the *a posteriori* pair-wise comparisons are reported in Supplementary Table A4 df = degree of freedom; SS = Sum of Squares; MS = Mean Squares; La = factor 'Lagoon'; Co(La) = factor 'Confinement' nested within 'Lagoon'; n.s. = not significant; CL = Caleri; CN = Canarin;

Foraminifera						
Species composition	Source of variation	df	SS	MS	Pseudo-F	P(perm)
opecies composition	lagoon	3	1865.4	621.79	1.3422	0.2255
	Residual	8	3706	463.25	110 122	0.2200
	Total	11	5571.4			
ABU	Source of variation	df	SS	MS	Pseudo-F	P(perm)
	lagoon	3	67828	22609	1.5703	0.2126
	Residual	8	1.15E+05	14398		
	Total	11	1.83E+05			
d	Source of variation	df	SS	MS	Pseudo-F	P(perm)
	lagoon	3	0.61754	0.20585	1.7066	0.2501
	Residual	8	0.96495	0.12062		
	Total	11	1.5825			
H'	Source of variation	df	SS	MS	Pseudo-F	P(perm
	lagoon	3	0.49196	0.16399	0.81318	0.532
	Residual	8	1.6133	0.20166		
	Total	11	2.1052			
FAI	Source of variation	df	SS	MS	Pseudo-F	D(perm
1711	lagoon	3	55 1.9801	0.66003	0.70624	P(perm) 0.6238
	Residual	8	7.4765	0.93457	0.7 002 1	0.0200
	Total	11	9.4566	0.50 107		
Nematoda						
Genera composition	Source of variation	df	SS	MS	Pseudo-F	P(perm)
	Lagoon	3	10479	3493	6.4635	0.0001
	Co(La)	8	13936	1742.1	3.2235	0.0001
	Residual	22	11889	540.43		
A posteriori pair-wise comp	Total parisons	33	36510			
Lagoon	all≠all,**or ***		Co(La)	In CL, O≠M*		
				In MV, I≠M*		
				In CN, I≠M*, O* In SC, O≠I*, M*		
				m 50, 0 / 1 , m		
Abu	Source of variation	df	SS	MS	Pseudo-F	P(perm)
	La	3	6.47E + 05	2.16E+05	0.32499	0.8031
	Co(La)	8	5.71E+06	7.14E+05	1.0752	0.404
	Residual	22	1.46E + 07	6.64E + 05		
	Total	33	2.11E+07			
d	Source of variation	df	SS	MS	Pseudo-F	P(perm)
	La	3	1.2132	0.4044	3.6875	0.0257
	Co(La)	8	2.2828	0.28535	2.602	0.0357
	Residual	22	2.4127	0.10967		
	Total .	33	6.1682			
A posteriori pair-wise comp Lagoon	SC≠CN**, MV*		Co(La)	In SC, O≠I*, M*		
Eugoon	50, 01, 11,		GO(Ea)	In CN, I≠O*		
H′	Source of variation	df	SS	MS	Pseudo-F	P(perm)
	La	3	0.82604	0.27535	3.4859	0.0348
	Co(La)	8	2.6874	0.33592	4.2527	0.0022
	Residual	22	1.7378	0.078989		
	Total	33	5.4871			
A posteriori pair-wise comp						
Lagoon	CL≠ SC *, MV**		Co(La)	In SC, O≠I*, M*		
ITD	Source of variation	df	SS	MS	Pseudo-F	P(perm
	La	3	0.099206	0.033069	8.0328	0.0007
	Co(La)	8	0.22538	0.028172	6.8435	0.0004
	Residual	22	0.090567	0.004117		
A nostariori poir vice co	Total	33	0.46737			
A posteriori pair-wise comp						
Lagoon	$SC \neq CL^{***}, MV^{**}$		Co(La)	In SC, O≠I*, M**		

(continued on next page)

Table 2 (continued)

Foraminifera						
MI	Source of variation	df	SS	MS	Pseudo-F	P(perm)
	La	3	0.43274	0.14425	12.263	0.0002
	Co(La)	8	0.35869	0.044836	3.8115	0.0078
	Residual	22	0.25879	0.011763		
	Total	33	1.1165			
A posteriori pair-wise	comparisons					
Lagoon	MV ≠ CN **, CL**, SC***		Co(La)	In SC, O≠I**, M**		
	$SC \neq CL^{**}$			In CN, I≠M*		
				In MV, I≠M*		

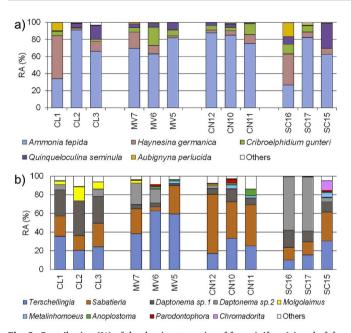


Fig. 2. Contribution (%) of the dominant species of foraminifera (a) and of the dominant nematode genera (b). Per each lagoon, the stations are presented from the innermost (left) to the outer one (right). RA = Relative Abundance.

Caleri and Scardovari, which showed an overall different taxonomic composition from that observed at the other investigated sites.

The nMDS plot clearly separated SC16, SC17 and all Caleri stations from the other investigated sites, confirming the presence of an assemblage that differed in terms of taxonomic composition (i.e. CL1 and SC16) and higher abundance at these stations. On the contrary, a clear separation between inner and outer stations was not evidenced (Fig. 3).

Focusing on species vertical distribution through the four sediment levels, the overall patterns described above were confirmed (Fig. 4).

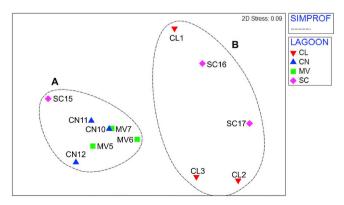


Fig. 3. nMDS ordination based on the abundances of foraminifera species (top $0-5 \, \text{cm}$ of sediment). The groups were identified by the SIMPROF test (p < 0.05).

Furthermore, 4 species were found exclusively in subsurface levels: *Ammonia veneta, Elphidium advenum, Cribroelphidium incertum* and *Quinqueloculina oblonga*. On the contrary, *Reophax nana* was observed only in the top sediment layer of CL3 and SC16.

The overall different taxonomic composition at CL1 and SC16 did not correspond to more diverse assemblages, at least in terms of richness. Focusing on d, in fact, values ranged from 0.77 to 2.03 at SC15 and CL3, respectively. Mean d value was higher in Caleri (1.68) than in the other lagoons, where it varied between 1.10 (Marinetta-Vallona) and 1.33 (Scardovari) (Table 3). Nevertheless, PERMANOVA outputs did not indicate the presence of a more significantly diverse assemblage in Caleri in comparison to the other TEs (Table 2). The lowest value of H' was observed at CL2 (0.63) while the maximum, equal to 2.20, characterized SC16 (Table 3). The lack of significant differences among lagoons also in terms of H' was due to the wide variability among stations within the same waterbody (Table 2). Furthermore, a clear confinement pattern in terms of diversity was not detected.

In order to ascertain the role of the environmental variables (Supplementary Table A1) on foraminifera species composition, the DISTLM routine was performed. The results of the sequential test outputs indicated silt% as the environmental variable that, together with clay%, explained the variability of foraminifera composition (Table 4).

With a maximum value equal to 5.4 ind. cm⁻³ at SC17, abnormal tests showed very low abundances at all stations and belonged only to *A. tepida*, *C. gunteri* and *H. germanica* (Plate I). Their distribution did not follow any kind of pattern along the sediment levels or among/within lagoons (Fig. 4). Ranging from 0.19% (CL1) to 3.44% (CN10), FAI values were quite low at all stations (Table 3) and significant differences among TEs were not detected by the PERMANOVA routine (Table 2).

3.2. Nematoda

Nematode abundance varied between 360.8 ± 106.3 and 1686.2 ± 1066.9 ind. $10\,\mathrm{cm}^{-2}$ at MV7 and SC17, respectively (Table 1). On average, the four lagoons were characterized by comparable mean abundances and a clear pattern along the confinement gradient was not observed. PERMANOVA analysis, in fact, showed that nematode abundance did not significantly differ according to either the factor "lagoon" or the factor "confinement" nested within "lagoon" (Table 2).

Overall, 44 genera of free-living nematodes, belonging to 16 families, were identified (Supplementary Table A3). Although clearly dominant in all lagoons, Daptonema, Sabatieria and Terschellingia showed different RA according to the considered TE. Focusing on the former genus, it was mainly represented by 2 species that clearly displayed a different distribution according to the lagoon. In order to not lose this information, the further separation in Daptonema sp.1 and Daptonema sp. 2 was introduced. In Marinetta-Vallona, the assemblage was dominated by Terschellingia that represented up to 62.7% of all the organisms (MV6). On the contrary, Sabatieria was the most abundant genus in Canarin where it accounted for ~40% of the whole assemblage at all stations. RA values of Daptonema sp. 2 were equal to 56.8% and 57.8% at SC16 and SC17, respectively, while the outer site of

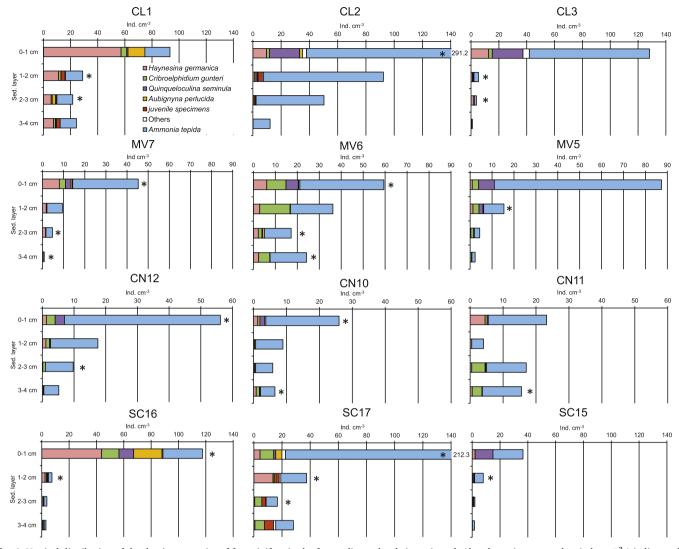


Fig. 4. Vertical distribution of the dominant species of foraminifera in the four sediment levels investigated. Abundance is expressed as ind. cm⁻³.* indicates the presence of abnormal tests.

Scardovari, SC15, was characterized by a more diverse assemblage without a clear dominance of one particular genus. Similarly, in Caleri Daptonema sp.1, Sabatieria and Terschellingia showed comparable RA (Fig. 2b). Beside these dominant genera, several others were observed according to the site: Molgolaimus was more abundant in Caleri only, where it accounted for 15% of the assemblage at CL2; Chromadorita showed the most elevated RA at the outer station SC15. PERMANOVA outputs confirmed that this different genera composition was statistically significant not only among lagoons but also within the same lagoon. Nevertheless, significant differences that mirrored coherently the increasing confinement gradient were observed only in Canarin and Scardovari. As indicated by the a posteriori outputs, in fact, in the former lagoon the inner site (CN12) differed from the other stations while in Scardovari the composition of nematode assemblage at the outer SC15 was statistically different from those observed at SC16 and SC17 (Table 2; Supplementary Table A4).

The nMDS plot graphically displayed the variable interaction highlighted by the nested PERMANOVA between the factors "lagoon" and "confinement" (Fig. 5). Apart from the intrinsic variability among pseudo-replicates, sometimes even for those of the same station, the groups identified by SIMPROF discriminated quite well which lagoon was overall more dissimilar from the others and where the confinement gradient was more pronounced. Group A gathered, in fact, all Caleri pseudo-replicates and all inner sites from the other lagoons, suggesting,

to some extent, similar nematode assemblages. On the contrary, most of the intermediate and outer sites of Canarin and Marinetta-Vallona constituted group B, suggesting a more pronounced confinement gradient within these two lagoons.

Focusing on diversity indices, d ranged from 0.56 ± 0.06 (SC17) to 1.84 ± 0.18 (CN11) while H' varied between 1.54 ± 0.39 (SC17) and 2.65 ± 0.22 (SC15) (Table 3). Biodiversity indices showed similar patterns characterized by higher values at the outer stations and lower ones at the inner sites with the exception of Marinetta-Vallona, where the innermost MV7 was inhabited by a more diverse assemblage in comparison to those observed at MV5 and MV6. The lowest values obtained were referred to SC17 and SC16, i.e. the two sites clearly dominated by Daptonema sp. 2. For what concerns d, PERMANOVA outputs highlighted that Scardovari was statistically different from Canarin and Marinetta-Vallona. Furthermore, a confinement gradient was detected within Scardovari and Canarin where the outer stations showed richness values significantly higher than those measured at the inner sites (Table 2; Supplementary Table A4). Similarly, the assemblage was less biodiverse even in terms of H' in Scardovari and, within this lagoon, the inner stations showed the absolute lowest values (Table 3).

Focusing on nematode trophic diversity, more than 50% of the organisms were non-selective deposit feeders (1B) with the exception of MV6 (Supplementary Table A3). Their dominance was ascribable to the

Richness (d) and	diversity (H'log.	2) indices calcula	Richness (d) and diversity (Hlog2) indices calculated from foraminifera and	nifera and nemat	ode datasets. FA	I values were ca	lculated for fora	minifera while IT	nematode datasets. FAI values were calculated for foraminifera while ITD and MI values for nematoda.	for nematoda.		
Foraminifera CL1	CL1	CL2	CL3	MV7	MV6	MV5	CN12	CN10	CN11	SC16	SC17	SC15
Р	1.38	1.62	2.03	1.22	1.02	1.07	0.89	1.56	0.98	1.64	1.57	0.77
H'(log2)	1.66	0.63	1.56	1.36	1.51	0.97	0.75	0.94	1.14	2.20	1.04	1.28
FAI	0.19	0.23	0.47	1.62	0.83	0.74	1.47	3.44	0.27	0.37	1.69	0.68
Nematoda	CL1	CL2	CL3	MV7	MV6	MV5	CN12	CN10	CN11	SC16	SC17	SC15
р	0.97 ± 0.32	1.06 ± 0.26	1.26 ± 0.15	1.39 ± 0.70	1.35 ± 0.10	1.31 ± 0.44	0.97 ± 0.29	1.15 ± 0.40	1.84 ± 0.18	0.59 ± 0.19	0.56 ± 0.06	1.46 ± 0.19
H'(log2)	2.15 ± 0.38	2.27 ± 0.25	2.44 ± 0.16	2.14 ± 0.26	1.93 ± 0.08	1.68 ± 0.25	1.83 ± 0.48	2.14 ± 0.14	2.40 ± 0.08	1.56 ± 0.27	1.54 ± 0.39	2.65 ± 0.22
Œ	0.50 ± 0.03	0.51 ± 0.06	0.51 ± 0.03	0.50 ± 0.03	0.58 ± 0.06	0.55 ± 0.03	0.62 ± 0.02	0.52 ± 0.04	0.56 ± 0.19	0.79 ± 0.10	0.76 ± 0.08	0.41 ± 0.00
MI	2.43 ± 0.07	2.37 ± 0.13	2.37 ± 0.05	2.41 ± 0.11	2.72 ± 0.11	2.47 ± 0.22	2.22 ± 0.03	2.37 ± 0.09	2.28 ± 0.19	2.12 ± 0.06	2.14 ± 0.06	2.45 ± 0.00

Table 4 Results of the DISTLM sequential test performed on foraminifera and nematode datasets. SS = mean square; F = F statistic; *** = P < 0.001; ** = P < 0.01; * = P < 0.05; n.s. = not significant.

Foraminifera	Adj R^2	SS(trace)	Pseudo-F	P	Prop.	Cumul.
Clay (8-12 phi)	0.35165	1296.9	3.9492	**	23.277	46.954
Silt (4-8 phi)	0.16044	1319.1	3.1021	*	23.676	23.676
BPC	0.38859	478.06	1.5438	n.s.	8.5806	55.534
ΣPCBs	0.43956	490.37	1.7275	n.s.	8.8015	64.336
ΣDDX	0.48317	416.39	1.5907	n.s.	7.4738	71.809
Nematoda	Adj R^2	SS(trace)	Pseudo-F	P	Prop.	Cumul.
ΣPBDEs	0.14383	2785.2	2.8479	**	22.166	22.166
ΣPBDEs Silt (4–8 phi)	0.14383 0.3267	2785.2 1626.3	2.8479 2.1146	**	22.166 12.943	22.166 57.154
Silt (4–8 phi)	0.3267	1626.3	2.1146	*	12.943	57.154
Silt (4–8 phi) ΣPAHs	0.3267 0.4148	1626.3 1372.8	2.1146 2.0537	* n.s.	12.943 10.926	57.154 68.08
Silt (4–8 phi) ΣPAHs ΣDDX	0.3267 0.4148 0.19028	1626.3 1372.8 1455.5	2.1146 2.0537 1.5737	* n.s. n.s.	12.943 10.926 11.584	57.154 68.08 33.751
Silt (4–8 phi) ΣPAHs ΣDDX Depth	0.3267 0.4148 0.19028 0.2329	1626.3 1372.8 1455.5 1314.3	2.1146 2.0537 1.5737 1.5	* n.s. n.s. n.s.	12.943 10.926 11.584 10.46	57.154 68.08 33.751 44.211
Silt (4–8 phi) ΣPAHs ΣDDX Depth BPC	0.3267 0.4148 0.19028 0.2329 0.45639	1626.3 1372.8 1455.5 1314.3 906.03	2.1146 2.0537 1.5737 1.5 1.4591	* n.s. n.s. n.s. n.s.	12.943 10.926 11.584 10.46 7.2109	57.154 68.08 33.751 44.211 75.291

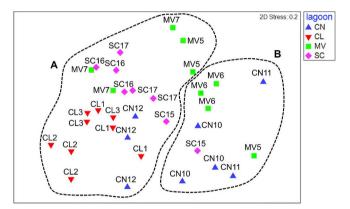


Fig. 5. nMDS ordination based on the abundances of nematode genera. The groups were identified by the SIMPROF test (p < 0.05).

elevated abundances of the main genera, i.e. Sabatieria, Daptonema sp.1 and Daptonema sp. 2, that belong, in fact, to this trophic group. Selective deposit feeders (1A) were the second most abundant organisms at all stations and the dominant ones at MV6 due to the high numbers of Terschellingia. Epigrowth feeders (2A) and predators/omnivores (2B) accounted for RA values 1 or 2 orders of magnitude lower than 1A- and 1B-nematodes and slightly higher abundances were observed at the outer stations (e.g. CN11 and SC15). Due to this trophic structure of the assemblage, the resulting ITD values varied between 0.41 \pm 0.0 (SC15) and 0.79 \pm 0.10 (SC16). Nematodes showed similar trophic diversity in all lagoons with the exception of Scardovari, where the pronounced dominance of Daptonema sp. 2 at SC16 and SC17 lowered consistently the trophic diversity of the assemblage, resulting, therefore in the highest ITD values (Table 3). PERMANOVA outputs supported these findings since Scardovari was statistically different from both Caleri and Marinetta-Vallona and its outer station differed from the inner ones (Table 2; Supplementary Table A4).

Ranging between 2.12 ± 0.06 (SC16) and 2.72 ± 0.11 (MV6) (Table 3), MI varied significantly both among lagoons and along the increasing confinement (Table 2; Supplementary Table A4). In particular, the differences were more pronounced between Marinetta-Vallona and Scardovari due to the higher % of c-p3 and c-p2 nematodes in the former and in the latter waterbody, respectively. Focusing on the cp classes composition, in fact, the assemblage was dominated by colonizers especially at SC16 and SC17 where c-p2 organisms reached RA > 80%. On the contrary, the community was to some extent more

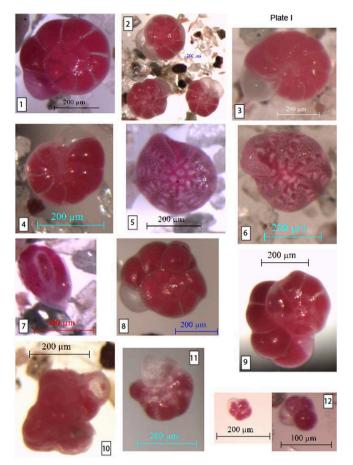


Plate I. Normal (1–7) and abnormal (8–12) foraminifera specimens belonging to the dominant species observed in the four lagoons. It is worthy the protoplasm very dense and well stained, in particular in the deeper sediment levels. 1. *A. tepida* (CN12, cm 0–1); 2. *A. tepida* (MV6, cm 3–4); 3. *H. germanica* (CL1, cm 0–1); 4. *H. germanica* (CL1, cm 3–4); 5. *E. gunteri* (CN12, cm 0–1); 6. *E. gunteri* (CN12, cm 3–4); 7. *Q. seminula* (CL1, cm 0–1); 8. *A. tepida* with deformed test (MV6, cm 3–4); 9. *A. tepida* with deformed test (twins) (SC15, cm 1–2); 10. *A. tepida* with deformed test (twins) (SC17, cm 0–1); 11. *E. gunteri* with deformed test (CL3, cm 1–2); 12. Juvenile specimens (CL1, cm 1–2). A scale bar in micrometers is provided.

mature at MV5 and MV6 due to the higher % of c-p3 nematodes (> 60%).

DISTLM results of the sequential test performed on nematode dataset indicated Σ PBDEs and silt% as the two environmental variables that significantly influenced the data cloud (Table 4).

4. Discussion

For the first time foraminifera and nematoda, the two most abundant and biodiverse meiobenthic assemblages, were studied in 4 lagoons of the Po River, i.e. one of the main deltaic systems of the Mediterranean Sea. Their abundance and taxonomic composition were investigated together with the biodiversity (d and H') and the calculation of indices exclusively developed for these assemblages: FAI for foraminifera, MI and ITD for nematoda. Resulting intra- and inter-lagoon differences were related to the main environmental variables in order to explore the meiobenthic response to natural and/or anthropogenic disturbance factors and, therefore, the suitability of these two assemblages for detecting ongoing and recent environmental changes.

From a taxonomic point of view, foraminifera and nematoda showed similar compositions to those already reported in other TEs (Albani and Serandrei Barbero, 1982; Zampi and D'Onofrio, 1987; Hohenegger et al., 1989; Albani et al., 2007; Carboni et al., 2009; Melis

and Covelli, 2013; Benito et al., 2016 for foraminifera; Villano and Warwick, 1995; Semprucci et al., 2014; Jouili et al., 2017 for nematoda). Focusing on the former organisms, the assemblage did not change remarkably over time since, to some extent, it was composed of the same species observed in previous studies carried out in Po Delta lagoons (D'Onofrio et al., 1976; Asioli et al., 1988; Asioli, 1993; Coccioni, 2000). As in other TEs, both assemblages were dominated by a few taxa (Ammonia tepida and Haynesina germanica for foraminifera; Daptonema, Tershellingia and Sabatieria for nematoda), reported as opportunistic and tolerant to environmental stress as organic enrichment and chemical contamination (Frontalini and Coccioni, 2011 for a review: Semprucci et al., 2015). The organisms, however, were likely not subjected to a massive organic enrichment, at least in comparison with the findings previously reported in other TEs. In the present study, in fact, even the highest BPC value ($\sim 6 \text{ mg C g}^{-1}$ at CN12) was clearly lower than the amounts measured by Pusceddu et al. (2007) in the lagoons of Venice (7.50 \pm 0.87 mg C g⁻¹), Goro (12.44 \pm 1.64 mg C g^{-1}) and Lesina (16.21 \pm 1.40 mg C g^{-1}). An alarming situation was detected neither in terms of chemical contamination. In the studied sediments, the contents of legacy (SDDX, SPCBs) and emerging pollutants (SPBDEs) showed, with some exceptions, low/moderate levels of contamination (Viganò et al., this issue) and a potential ecotoxicological risk due to metal concentrations was observed only for Ni (Zonta et al., this issue). Although DISTLM routine indicated ΣPBDEs among the environmental variables that shaped the nematode assemblage, for both communities the role of the studied contaminants likely was not more important than that exerted by other abiotic (natural) factors, such as sediment grain-size fractions (Table 4), suggesting that, overall, the anthropogenic stress represented potentially by these compounds was relatively limited. This was further corroborated by the percentage of abnormal foraminifera, which tend to be more abundant in relation to contaminants such as trace metals and hydrocarbons (Yanko et al., 1994, 1998; Samir, 2000; Di Leonardo et al., 2007; Bergamin et al., 2009; Coccioni et al., 2009; Frontalini et al., 2010; Bouchet et al., 2018). In the present study, abnormal specimens showed a stochastic occurrence at all stations, leading to FAI values lower than those calculated in other TEs (Goro, up to 37%, Coccioni, 2000; Venice, up to 14.3%, Coccioni et al., 2009; Lesina and Orbetello, up to 12.9%, Frontalini et al., 2010; Grado-Marano, up to 19.1%, Melis and Covelli, 2013), confirming a limited effect of the measured contaminants on these organisms. In the four lagoons, therefore, the presence of scarcely diverse assemblages dominated by a few tolerant taxa could be ascribable mainly to the natural stress represented by pronounced fluctuations of physico-chemical variables (e.g. salinity, temperature and sedimentation rate) rather than to anthropogenic disturbance factors, in accordance with the 'Estuarine Quality Paradox' (Dauvin, 2007; Elliot and Quintino, 2007).

Focusing on the vertical distribution of foraminifera (Fig. 4), the organisms reached relatively high abundances not only at the surface levels but also at the deeper ones (i.e. up to 45-61% at CN10, CN11, MV6 and CL1), with the exception of CL3 and SC16, where the majority of specimens (93% and 89%, respectively) were in the uppermost sediments (0-1 cm). The presence of foraminifera in sub-surface levels was mainly ascribed to the two dominant species, i.e. A. tepida and H. germanica. While the presence of the former below the top 0-1 cm was in accordance with the negative geotaxis described for this species, H. germanica was reported to show an opposite behavior (Seuront and Bouchet, 2015). The observation of numerous H. germanica specimens in subsurface levels may however be explained by the intense activity of food search reported for both species (Seuront and Bouchet, 2015) and by their enhanced proliferation in sediments with abundant labile organic matter (Murray, 2006; Cesbron et al., 2016). Overall, the investigation of foraminifera vertical distribution demonstrated to add further information to that derived from the sole analysis of the 0-1 cm sediment level. This should promote some caution when the foraminifera standing crop of the uppermost sediment is used for modern

distribution studies as well as for environmental quality assessment (Coccioni, 2000; Frontalini and Coccioni, 2008; Melis and Covelli, 2013).

While foraminifera did not show significant intra- and inter-lagoon variability, nematoda differed significantly among lagoons and the degree of confinement (Table 2; Supplementary Table A4). According to the factor 'lagoon', a posteriori pair-wise comparisons pointed out that Scardovari mainly differed from the other waterbodies for the majority of the metrics. The investigation of the confinement effect within each lagoon (explained by the factor 'confinement' nested within 'lagoon'), clarified that this difference was mainly ascribable to SC16 and SC17. i.e. the inner and middle stations, where the assemblage was not only less biodiverse, but also dominated by r-strategists and non-selective deposit feeders. Since such pronounced intra- and inter-lagoon differences did not clearly mirror particular patterns of the main environmental variables (Supplementary Table A1), the dissimilarities among nematode assemblages might represent the response of these organisms to recent but not still ongoing disturbance events, whose occurrence should be sought in the peculiar hydrodynamics of each lagoon. By applying an unstructured 3D numerical model, Maicu et al. (2018) recently described that Scardovari is characterized by a very low water renewal as suggested by the Water Renewal Times (WRT) that the authors have estimated not only between 8 and 16 days, but also up to 30 days in the inner part of the lagoon during August and September. At SC16 and SC17 the dominance of r-strategist (i.e. pioneer) nematodes in assemblages characterized by a low biological and trophic diversity may represent, therefore, the response of these organisms to recent and frequent hypoxic/anoxic events that tend to occur in the inner part of this lagoon (ARPAV, 2015).

In accordance with our main findings, dissimilar responses of nematoda and foraminifera to oxygen deficiency were reported in a longterm in situ experiment during which both assemblages were exposed to anoxia (Langlet et al., 2013, 2014; Taheri et al., 2015). As at SC16 and SC17, significantly lower values of richness (d) and diversity (H') were observed in nematode assemblages exposed to 23- and 307-day anoxia (Taheri et al., 2015). Furthermore, the organisms did not recover quickly but required a considerable time lapse to reach pre-treatment conditions. In the 23-day treatment, the assemblage was not fully recovered even after 30 days while it needed 90 days to recover after a 307-day exposition to anoxia. The authors inferred that the recovery takes place mainly by means of nematode arrival from non-impacted surrounding areas by swimming or settling after passive movement through the water column, rather than by reproduction of survived local individuals (Taheri et al., 2015 and references therein). In Scardovari, the poorly structured assemblage at SC16 and SC17 may be the result of an unaccomplished recovery, likely prolonged by the low water renewal that could limit the arrival of new specimens. On the contrary, foraminifera showed to be more resistant to long-lasting anoxia, both in terms of abundance (Langlet et al., 2013) and taxonomic composition (Langlet et al., 2014). This was confirmed by the lack of significant differences among the assemblage in Scardovari and those observed in the other lagoons for all the tested metrics (Table 2).

Although less accentuated, Marinetta-Vallona exhibits hydrodynamics similar to Scardovari. According to Maicu et al. (2018), Vallona and Marinetta are so hydrodynamically different that the authors treated them separately. Although both waterbodies are characterized by substantial inputs of freshwater, the active hydrodynamics of Marinetta ensures also a substantial mixing with the sea and the formation of a smooth salinity gradient. On the contrary, in Vallona the model indicated that the noticeable volume of freshwater tends to be confined in the inner area of this sub-basin. According to these environmental differences, foraminifera and nematoda at MV7, the station within Vallona, were expected to differ from the assemblages at MV5 and MV6, the sites of Marinetta. Focusing on the former, the assemblage seemed quite homogeneous among the MV stations (Figs. 2a and 3), confirming the tolerance of foraminifera to potential hypoxic

events that may occur at MV7 as already speculated for Scardovari, where the confinement of the water in its inner part is expected to be more pronounced. Also for nematodes, in fact, the differences between MV7 and the two Marinetta sites were not as evident as in Scardovari (Fig. 5). Notwithstanding, the higher RA of *Daptonema* sp. 2 at MV7 (23.1%) than at MV5 (0.2%) and MV6 (14.7%), suggests that, to some extent, within Vallona the environmental conditions were similar to those at SC16 and SC17, where this genus accounted for more than 56% of the whole assemblage (Fig. 2b).

While in Canarin the link between its hydrodynamics and the two benthic communities is unclear, in Caleri the relatively homogeneous environmental conditions (i.e. low WRT values, high flux exchange with the sea and almost zero freshwater inputs that hamper the formation of sharp salinity gradients; Maicu et al., 2018) seem to favor a likewise homogeneous assemblage of nematoda. In the nMDS plot, in fact, all Caleri pseudo-replicates were gathered in Group A (Fig. 5), and PERMANOVA results highlighted significant differences in the genera composition between CL2 and CL3, only (Table 2). Although foraminifera species composition at CL1 to some extent differed from that at CL2 and CL3 (Figs. 2a and 4), this dissimilarity was not very pronounced. In the nMDS plot, in fact, all Caleri stations were grouped within Group B (Fig. 3) and PERMANOVA outputs indicated that, overall, there was no statistical difference among lagoons.

Overall, the study of both foraminifera and nematoda demonstrated to be a promising tool for implementing monitoring actions in Po Delta lagoons. Their taxonomic composition may reflect ongoing or recent disturbance factors as observed for nematodes in relation to hypoxic events. Furthermore, the indices exclusively developed for these two assemblages (FAI, MI and ITD) demonstrated to integrate and support the main findings derived from the study of the assemblage composition. The low FAI values, for instance, confirmed a limited effect of chemical contaminants on foraminifera, in accordance with the lack of significant inter- and intra-lagoon variability observed in terms of taxonomic compositions, diversity and abundance. For what concerns nematodes, the concomitance of low MI and high ITD values at SC16 and SC17 suggested the presence of a poorly structured assemblage dominated by pioneer organisms, indicating that the organisms were likely recovering after a disturbance event.

5. Conclusions

In the four studied lagoons, both foraminifera and nematoda were dominated by a few taxa tolerant to an environmental instability mainly ascribable to natural disturbance factors (i.e. pronounced fluctuations of physico-chemical variables) rather than to anthropogenic contamination and organic enrichment. The stochastic occurrence of abnormal tests, the very low FAI values and the presence of numerous organisms in subsurface sediment levels suggested, in fact, that foraminifera were not particularly affected by the measured synthetic organic compounds and trace metals. Similarly, nematoda seemed not particularly sensitive to these anthropogenic disturbance factors (with the exception of Σ PBDEs), as indicated by the DISTLM outputs. On the other hand, nematoda seemed to reflect more than foraminifera the peculiar environmental conditions in each lagoon and the degree of confinement, as indicated by the significant intra- and inter-lagoon variability detected for the majority of the tested metrics. In particular, the least biodiverse assemblage dominated by r-strategist (i.e. pioneer) genera at the inner stations of Scardovari (SC16 and SC17), suggested a nematodes response to recent hypoxic events that often occur within this waterbody because of its low water renewal. Both assemblages showed to potentially implement future monitoring programs: while from the study of foraminifera we can infer conditions of relatively low chemical contamination, from that of nematoda we can deduce recent conditions of oxygen deficiency.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecss.2019.02.039.

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