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## The nematode assemblage as a tool for the assessment of marine ecological quality status: a case-study in the Central Adriatic Sea

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### Abstract

Free-living nematodes are efficiently used as bio-indicators of anthropogenic disturbance in marine ecosystems. Among various criteria, functional traits may represent useful tools for monitoring environmental impact in coastal areas. A study of nematode assemblages was carried out in three locations of the Central Adriatic Sea (Italy), one at the mouth of the Foglia River (Baia Flaminia) and two enclosed in the Monte San Bartolo Natural Park (Monte Brisighella and Fiorenzuola di Focara). Taxonomic composition and the functional traits of the nematode assemblage revealed a possible influence of the organic load of the Foglia River. Biotic data, as well as environmental parameters, suggested a particularly negative impact of the river on the assemblage at Baia Flaminia. Here, the increasing impact of the river led to a rise in the relative abundance of *r*-strategist genera like *Chromadora*, *Sabatieria* and *Viscosia*. Poor ecological conditions were also present at Brisighella, where the river might exert its influence due to the presence of long shore currents. In contrast, the results revealed that the best ecological quality was at Fiorenzuola di Focara, where the impact of anthropogenic activities was generally irrelevant. This study documents how nematodes can be used as an early warning indicator with which to monitor the health quality of vulnerable littoral areas.

**Keywords:** nematodes, biological indicators, river disturbance, Natural Park, Adriatic Sea.

### Introduction

Urban and industrial activities introduce large amounts of pollutants to the sea, causing permanent and significant disturbances to and having an impact on marine ecosystems. Coastal systems are among the most sensitive and vulnerable marine areas, where economic activities are concentrated and rivers may be important sources of nutrients and pollutants for neighbouring open seas (Moodley *et al.*, 1998; Albertelli *et al.*, 1999; Danovaro *et al.*, 2000; Semprucci *et al.*, 2010).

Sediments are considered to be the most human-impacted domains, and are therefore the target for studies of biodiversity and actions aimed at its preservation. Living organisms are the most appropriate indicators for use in the evaluation of water body quality, because they integrate both the biotic and the abiotic components of an ecosystem through their adaptive responses (Casazza *et al.*, 2002). Benthic communities in particular are an important source of information that can be utilized to investigate and characterize the habitat where they live.

Meiofauna have proved to be an extremely useful

tool for the assessment of the environmental effects of anthropogenic disturbance (e.g. Coull & Chandler, 1992; Austen *et al.*, 1994; Somerfiel *et al.*, 1994; Austen & Somerfield, 1997; Kennedy & Jacoby, 1999). Among meiofaunal taxa, nematodes provide many ways to assess changes in a benthic domain. Indeed, the ecological value of nematodes is related not only to their notable quantitative occurrence in the benthos, but also to their pivotal role within the trophic chains of aquatic ecosystems and their function in stabilizing the effects of shores (Platt & Warwick, 1980). These features, together with the ability to survive in extreme conditions, the short biological cycles and the high stability of populations, produce a more rapid reaction to environmental changes in this community than the response observed in macrobenthos or other meiofaunal groups (Heip *et al.*, 1985; Danovaro *et al.*, 2009; Moreno *et al.*, 2011; Vanaverbeke *et al.*, 2011; Semprucci & Balsamo, 2012). It is for this reason that this group has recently been proposed within the Water Framework Directive (WFD, Directive 2000/60/EC) (Moreno *et al.*, 2011) as an indicator for assessing the ecological quality of marine ecosystems.

The Mediterranean Sea, which has numerous levels of endemism in its coastal zones, is a key area for the study of the influence of natural and anthropogenic changes in biodiversity (Danovaro & Pusceddu, 2007). In the Adriatic Sea, which is a closed basin, these changes may even be enhanced, leading to its greater vulnerability (Balsamo *et al.*, 2010).

In this context, a study of the nematode assemblage has been carried out on the coast of the Monte St. Bartolo Natural Park, which is a valuable but vulnerable area that has not yet been subject to any conservation plan. The aims of this study were to elucidate the nematode assemblage spatial distribution pattern and assess the potential organic enrichment effects caused by the river on this benthic assemblage. To this end, sediment samples were taken at three sites (two stations in each one) located on a gradient away from the river mouth during two different seasons to test the following hypothesis:  $H_0$ , the nematode assemblage, was not significantly influenced by the river at the different sites and stations and showed the same temporal responses.

## Materials and Methods

### Study area and sampling

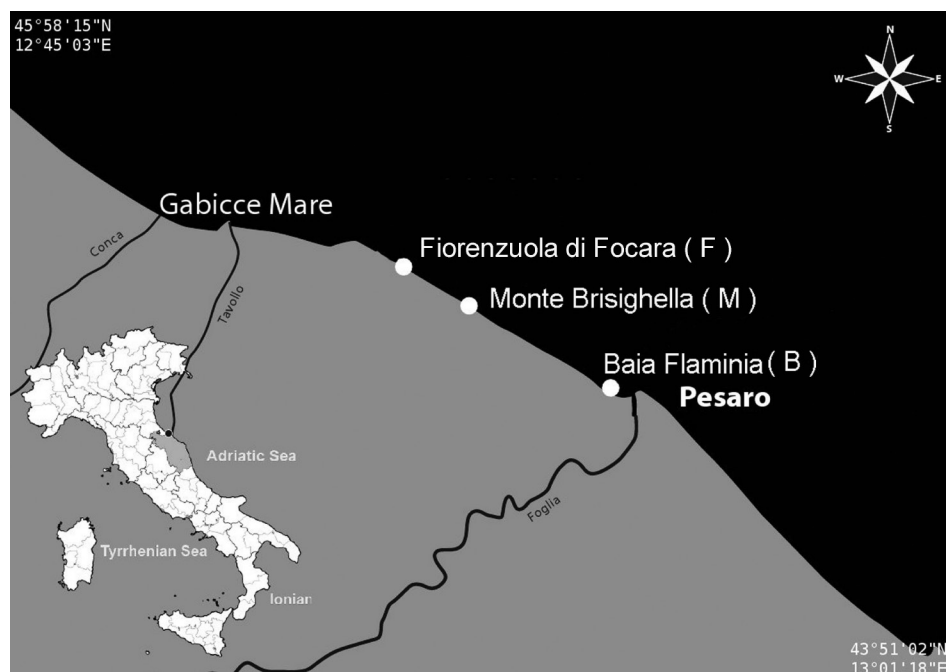
The Adriatic Sea is a basin in the Central Mediterranean, and is characterized by an extremely long and morphologically complex coastline. The hydrology and hydrodynamism, biogeochemical features and ecosystem dynamics of the entire basin have been intensively investigated over recent decades (see Artegiani *et al.*, 1997). The general circulation of the Adriatic Sea is cyclonic,

and mainly determines the distribution of the run-off products introduced by the rivers, even if local currents (i.e. long-shore and rip currents) can assume great importance in the transport of sediment (see Semprucci *et al.*, 2010 for further details).

The Natural Regional Park of Monte San Bartolo (Pesaro, Italy) is located along the coast of the northern Marche and contains the only rocky cliff along the entire northern Adriatic coastline. The park was created in 1997 to protect a terrestrial area of great naturalistic importance that lies along a main bird migratory route, includes the wintering habitats of many bird species, plays host to rare plant species and constitutes an almost complete section of the Messinian stage in Italy. The coastal strip of the park is still poorly known, even if it is characterized by high diversity (Mosci *et al.*, 2002) and the great heterogeneity of its benthic habitats: soft and hard sediments, natural and artificial substrates, and few seagrass (*Zostera*) meadows.

The study area was located between Fiorenzuola di Focara and Pesaro, and the coast of this stretch is partially enclosed in the Natural Park (Fig. 1).

Baia Flaminia (43°55'N–12°53'E) is adjacent to the mouth of the Foglia River and Pesaro harbour. The terminal stretch of the Foglia River (79 km long overall) flows through the city of Pesaro and mainly carries wastewater from urban and industrial areas and livestock farms. The discharge speed is about 7.08 m<sup>3</sup>/s and the inflow has been essentially unchanged over the last few decades (Colantoni *et al.*, 2004) (Fig. 1). The Monte Brisighella (43°56'N – 12°50'E) and Fiorenzuola di Focara (43°57'N–12°49'E) sites are located along the coast of the Monte S. Bartolo Natural Park (Fig. 1).



**Fig. 1:** Geographical position of the study area.

Samples were collected from each site in two different periods, May and November 2003. At Baia Flaminia (Site B), Monte Brisighella (Site M) and Fiorenzuola di Focara (Site F), sediment samples were collected from two stations at depths of 1.5 m (St. 1) and 3 m (St. 2) respectively. Three replicate samples for the quantitative analysis of meiofauna were taken from each station by a SCUBA diver using a hand-held piston corer (6 cm<sup>2</sup>) driven to a depth of 9 cm. An additional sample for grain size analysis was also collected from each site.

#### *Sediment analyses*

A grain size analysis was performed on the collected sediments using a vibro-siever for fractions larger than 63 µm and an X-ray analyzer for the pelite fraction (Sedi-graph 5200, Micrometric). The 'φ' notation (-log<sub>2</sub> size in mm) was used for sediment classification and the various granulometric parameters for statistical analysis (Folk & Wards, 1957).

#### *Nematode treatment*

Samples utilized for meiofaunal analysis were treated with a 7% MgCl<sub>2</sub> aqueous solution for specimen narcotization, fixed in 4% formaldehyde solution in buffered sea-water, stained with Rose Bengal and finally stored for subsequent processing. In the laboratory, the samples were rinsed with a gentle jet of fresh water through a 0.5 mm sieve to separate the macrofauna. They were then decanted, sieved 10 times through a 38 µm mesh and centrifuged three times with Ludox HS30 (specific density 1.18, Heip *et al.*, 1985). About 100 nematode specimens from each replicate were selected at random, transferred in glycerine and mounted as permanent slides (Seinhorst, 1959). Identification at the genus level was performed using a light optical microscope equipped with Nomarski optics (Optiphot-2 Nikon), with the support of pictorial keys (Platt & Warwick, 1983, 1988; Warwick *et al.*, 1998). Nematode trophic groups were defined according to Wieser (1953), while the Index of Trophic Diversity (ITD), based on the proportion of each trophic group, was calculated following Heip *et al.* (1985). Since an environmental disturbance may change the food supply in a given area, an increase of ITD values (due to a marked dominance of a single trophic group) may be indicative of a stress increase. The Maturity Index (MI) was determined for the nematode assemblage as the weighted average of the individual colonizer-persister (c-p) species (Bongers *et al.*, 1991). In particular, Bongers distinguished *r*-strategist species (colonizers or c-p 1), which are more tolerant of environmental variations, and *k*-strategist species (persisters or c-p 5), which are more sensitive. This index has been proposed as a semi-quantitative value that is useful when it comes to revealing ecosystem conditions from the composition analysis of the nematode assemblage.

#### *Statistical analysis*

**Non-metric Multi-Dimensional Scaling (nMDS)** ordinations derived from Bray-Curtis similarity matrices were used to check differences in the structure of the nematode assemblages between the various sites (fourth root transformation). The significance of the differences in the composition of the nematode genera between the sites, stations and periods was tested using an analysis of similarities (2-way crossed ANOSIM site × season and 2-way nested ANOSIM site × station). A SIMPER test (cut-off of 50%) was used to determine the contribution of each nematode genus to total dissimilarity. Shannon's diversity (H') and evenness (J) indices (log<sub>2</sub>) were calculated to describe the nematode (at the genus level) assemblage structures. All of the analyses mentioned above were performed using the Primer v.5 software package (Clarke & Gorley, 2001; Clarke & Warwick, 2001). Possible differences in the univariate measures (abiotic variables, Shannon H' and Pielou J indices, Maturity Index and Index of nematode Trophic Diversity) were evaluated using an analysis of variance (3-way ANOVA). Tukey's multiple-comparison tests were applied when significant differences (p < 0.05) were detected (SPSS v.17 program).

## **Results**

#### *Sediment analyses*

All of the sediments collected were fine sands (Md<sub>φ</sub> 3) (Table 1). In May 2003, the Mz<sub>φ</sub> ranged between 2.02 (F St. 1) and 2.81 (B St. 1): they were generally moderately sorted, had a negative asymmetry at the M and F sites, and a 'tail' of fine sediments at site B. The sand fraction was always dominant (95-98%) followed by mud (2-5%) and gravel (<1%) (Table 1). In November 2003, the Mz<sub>φ</sub> ranged between 2.14 (M St. 2) and 2.81 (B St. 1). The sediments were generally moderately sorted and in this case also displayed a negative asymmetry at the M and F sites and a positive Sk<sub>φ</sub> value at site B. The sand fraction was always dominant (93-97%) followed by mud (2-4%) and gravel (0-3%) (Table 1).

#### *Nematode assemblage*

The nematode assemblage was quite diversified (H': from 2.24±0.04 to 3.02±0.12; J: from 0.74±0.04 to 0.91±0.02) (Fig. 2a, b), and included 55 genera from 21 families. Chromadoridae and Xyalidae were the richest families, with 9 and 7 genera respectively. The most abundant families were Xyalidae (47%), Chromadoridae (14%), Axonolaimidae (9%) and Comesomatidae (8%).

At site B, there were 43 genera belonging to 20 families: the most abundant genera were *Daptonema*, *Paramonhystera*, *Odontophora* and *Chromadora*. At site M, 26 genera from 14 families were recorded, and *Daptonema*, *Paramonhystera* and *Odontophora* were the most abundant. Finally, at site F, 30 genera from 14 families were



**Table 1.** Sedimentological parameters of the studied stations.

Station	Mdφ Mode	Mzφ Mean size	σφ Sorting	Skφ Skewness	Gravel (%)	Sand (%)	Mud (%)
B St. 1 M03	3	2.81	0.63	0.30	0.19	94.84	4.97
B St. 2 M03	3	2.75	0.59	0.30	0.16	95.86	3.98
M St. 1 M03	3	2.15	0.67	-0.11	0.01	97.53	2.47
M St. 2 M03	3	2.09	0.72	-0.08	0.05	97.40	2.55
F St. 1 M03	3	2.02	0.65	-0.02	0.30	97.93	1.77
F St. 2 M03	3	2.19	0.66	-0.24	0.64	97.73	1.64
B St. 1 N03	3	2.81	0.63	0.30	0.58	95.44	3.97
B St. 2 N03	3	2.75	0.59	0.30	0.11	96.35	3.54
M St. 1 N03	3	2.28	0.74	-0.09	0.02	97.48	2.50
M St. 2 N03	3	2.14	0.70	-0.10	0.01	97.58	2.42
F St. 1 N03	3	2.18	0.82	-0.22	1.94	95.84	2.22
F St. 2 N03	3	2.50	0.68	-0.03	3.40	93.48	3.12

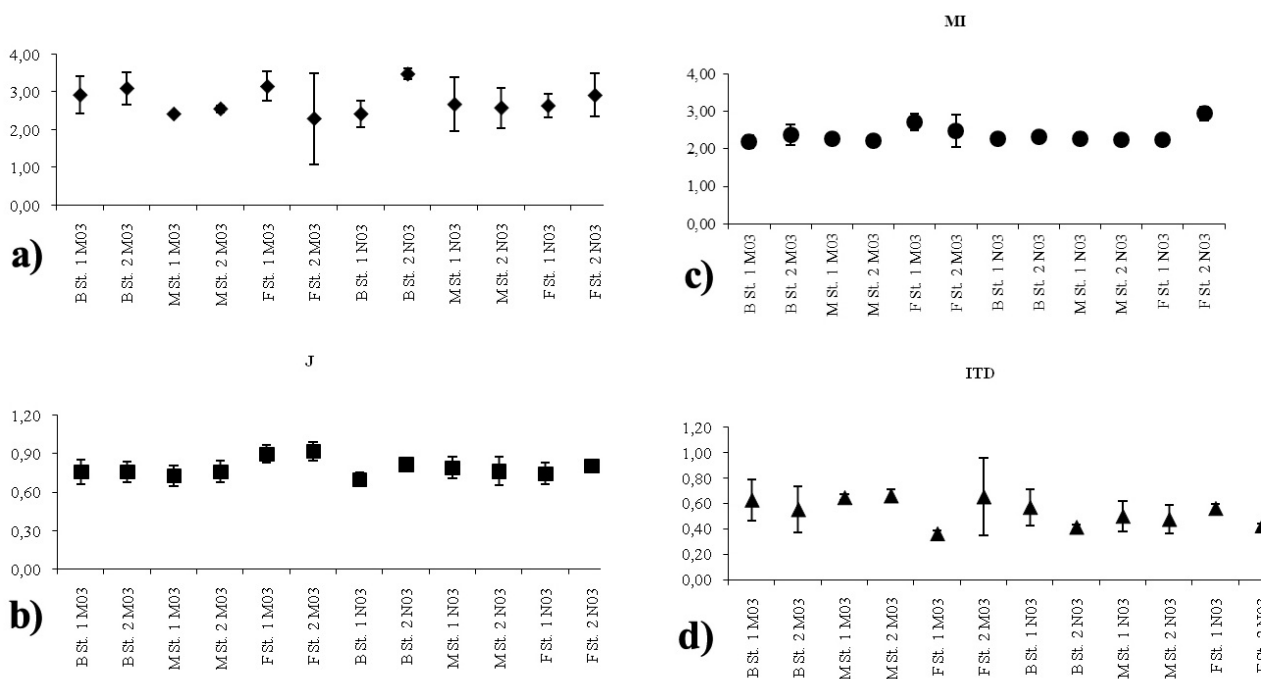
found, and were mainly represented by *Daptonema*, *Odonotophora*, *Oncholaimus*, *Pomponema* and *Epacanthion*.

The Maturity Index values ranged from 2.24±0.04 (site M, May 2003) to 2.94±0.19 (site F, St. 2, November 2003) (Figure 2c).

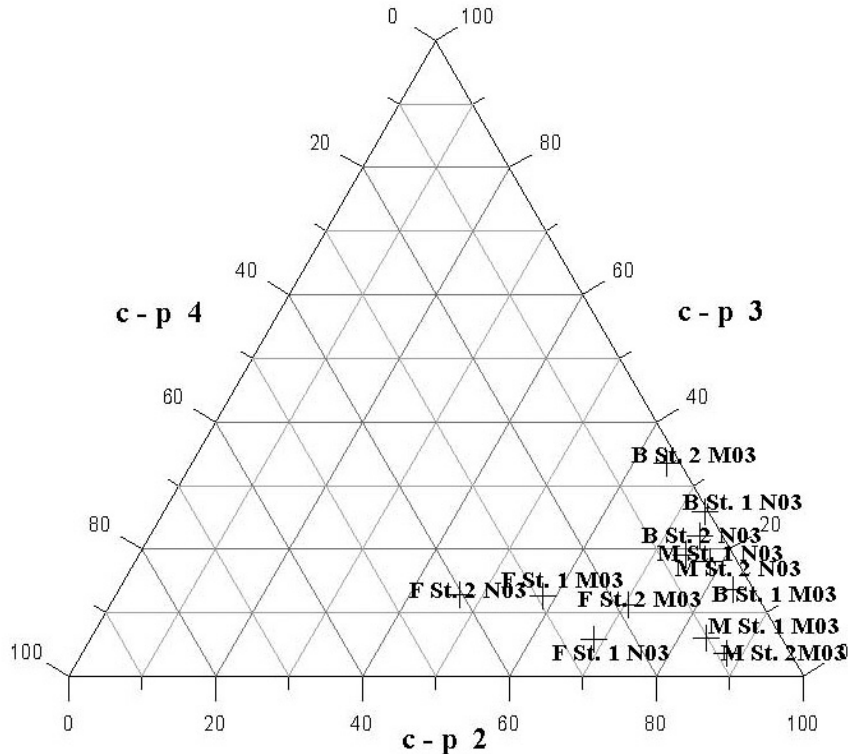
Overall, c-p2 was the most abundant class, with 74% of the specimens, followed by c-p3 (15%) and c-p4 (11%), while the c-p1 and c-p5 classes were totally absent. At site B, c-p2 represented 74% of the individuals, followed by c-p3 (23%) and c-p4 (3%). A similar c-p composition was also recorded at site M, with 80% of c-p2, 13% of c-p3 and 7% of c-p4, while at site F the

c-p2 was 65%, followed by c-p4 (25%) and c-p3 (10%) (Fig. 3).

The nematode assemblage was primarily composed of non-selective deposit feeders (63% of the total assemblage), followed by epistrate feeders and predators/omnivores (18% each) and selective deposit feeders (1%). The non-selective deposit and epistrate feeders were dominant at site B (65 and 26% respectively) and site M (70 and 17% respectively), while non-selective deposit feeders and predators were the groups dominant at site F (54 and 35% respectively). In May, 1B was dominant (66%) followed by 2B (15%) and 2A (18%), while 1B (61%), 2A (21%) and 2B (18%) were more abundant in



**Fig. 2:** a) Shannon Index; b) Pielou Index; c) Maturity Index; d) Index of Trophic Dominance calculated for the nematode assemblage at each station in the study area.



**Fig. 3:** Composition of colonizers-persisters (c-p) at each station and in each period. The c-p1 and c-p5 were excluded because they were not found in the study area.

November. The Index of Trophic Diversity (ITD), which was calculated on the proportion of each feeding type, ranged between  $0.66 \pm 0.05$  (site M St. 2, May 2003) and  $0.36 \pm 0.03$  (site F St. 1, May 2003). Site M (0.57) had the highest values followed by site B (0.54) and site F (0.50) (Figure 2d).

### Statistical analysis

Site B had the greatest mud content (ANOVA,  $p < 0.01$ , Tukey's test: M vs. B  $p < 0.05$  and F vs. B  $p < 0.01$ ). Moreover, no significant differences in grain size were detected either between the stations or between the two sampling periods (ANOVA,  $p > 0.05$ ).

So far as the nematode assemblage is concerned, significant differences between the sites were detected by the ANOSIM ( $R = 0.67$ ;  $p < 0.001$ ). The greatest differences were between site B vs. site F ( $R = 0.82$ ;  $p < 0.001$ ), followed by site B vs. site M ( $R = 0.65$ ;  $p < 0.001$ ) and site F vs. site M ( $R = 0.58$ ;  $p < 0.001$ ). These differences are also visible in the nMDS plot (Fig. 4). Significant differences were also recorded between the two periods (ANOSIM,  $R = 0.49$ ;  $p = 0.001$ ), whereas no difference was found between the stations (ANOSIM,  $p > 0.05$ ).

The genera that mainly contributed to the total dissimilarity between the sites are reported in Table 2. The Maturity Index revealed significant differences, especially between the sites (ANOVA,  $p < 0.01$ ), with higher val-

ues at site F (Tukey's test  $p < 0.05$ ). Similarly, the Pielou index produced results that were significantly different between the sites (ANOVA,  $p < 0.05$ ), with higher values at site F (Tukey's test  $p < 0.05$ ). That index was also significant in the season  $\times$  site interaction, with higher values at site F in May ( $p < 0.05$ ) (Table 3). The ITD seemed to be influenced more by the seasons (ANOVA,  $p < 0.05$ ). No significant differences were detected for the Shannon index (Table 3).

### Discussion

Many human activities commonly take place on or in coastal areas, and they often have an impact on aquatic ecosystems, possibly leading to deterioration in the ecological quality of the sediment. Although it is difficult to separate the influence of human from natural variable effects in field investigations (Tietjen, 1977; Platt *et al.*, 1984; Travizi & Vidakovic 1994), several studies have highlighted the sensitivity of the nematode community to various kinds of anthropogenic disturbances, which seem to alter the nematode assemblages in composition, taxonomic diversity and functional groups (e.g. Austen *et al.*, 1994; Danovaro *et al.*, 1995; Mirto *et al.*, 2002; Schratzberger *et al.*, 2002; Frascchetti *et al.*, 2006; Moreno *et al.*, 2008, 2009; Vezzulli *et al.*, 2008).

The composition of the nematode assemblages described in this study is fully comparable with those re-

**Table 2.** SIMPER comparisons between the stations (cut off 50%).

<b>B vs. M</b>				
<b>Genera</b>	<b>Av.Abund.</b>		<b>Contrib.%</b>	<b>Cum.%</b>
<i>Chromadora</i>	13.33	0.96	6.72	6.72
<i>Pomponema</i>	0.42	5.06	5.72	12.45
<i>Sabatieria</i>	11.24	2.46	5.45	17.9
<i>Viscosia</i>	2.19	0.2	4.54	22.44
<i>Cobbia</i>	1.83	5.96	4.34	26.77
<i>Dichromadora</i>	3.03	5.88	3.94	30.72
<i>Oncholaimellus</i>	1.29	2.20	3.70	34.42
<i>Theristus</i>	2.43	1.13	3.59	38.01
<i>Chaetonema</i>	1.88	0.86	3.37	41.38
<i>Paramesonchium</i>	0.00	1.13	3.26	44.64
<i>Odontophora</i>	8.78	6.98	3.22	47.86
<i>Mesacanthion</i>	1.30	1.69	3.19	51.05
<b>B vs. F</b>				
<b>Genera</b>	<b>Av.Abund.</b>		<b>Contrib.%</b>	<b>Cum.%</b>
<i>Chromadora</i>	13.33	0.63	6.11	6.11
<i>Sabatieria</i>	11.24	0.55	5.58	11.68
<i>Theristus</i>	2.43	18.2	4.84	16.52
<i>Pomponema</i>	0.42	7.02	4.22	20.74
<i>Epacanthion</i>	0.00	5.21	4.18	24.92
<i>Paramonhystera</i>	6.87	5.27	4.18	29.11
<i>Chromaspirinia</i>	0.09	9.14	4.06	33.16
<i>Viscosia</i>	2.19	0.23	3.95	37.11
<i>Oncholaimus</i>	2.00	8.07	3.59	40.7
<i>Cobbia</i>	1.83	1.94	3.07	43.77
<i>Oncholaimellus</i>	1.29	2.70	3.02	46.79
<i>Mesacanthion</i>	1.30	2.59	2.93	49.72
<i>Odontophora</i>	8.78	10.45	2.91	52.63
<b>M vs. F</b>				
<b>Genera</b>	<b>Av.Abund.</b>		<b>Contrib.%</b>	<b>Cum.%</b>
<i>Paramonhystera</i>	15.49	5.27	6.64	6.64
<i>Theristus</i>	1.13	18.2	6.32	12.96
<i>Epacanthion</i>	0.00	5.21	5.41	18.38
<i>Chromaspirinia</i>	0.00	9.14	5.3	23.67
<i>Cobbia</i>	5.96	1.94	4.9	28.57
<i>Oncholaimus</i>	1.28	8.07	4.73	33.3
<i>Dichromadora</i>	5.88	1.95	4.58	37.88
<i>Pomponema</i>	5.06	7.02	4.37	42.25
<i>Daptonema</i>	41.56	13.83	4.28	46.53
<i>Odontophora</i>	6.98	10.45	4.05	50.57

ported for assemblages from the northern Adriatic Sea and silty sand sediments worldwide (Heip *et al.*, 1985; Travizi & Vidakovic 1997; Semprucci *et al.*, 2008, 2010; Travizi, 2010).

The nematode diversity showed values comparable with those reported from other areas of the northern Adriatic, with similar sediment grain size but subject to eutrophication phenomena or waste inputs from rivers (Vidakovic, 1988; Travizi & Vidakovic 1994; Semprucci *et al.*, 2010). The overall values of the Maturity Index recorded in the study area highlighted a notable dominance of general opportunistic nematodes (c-p2), which are known to increase in abundance at times of high stress (Bongers & Bongers, 1998; Gyedu-Ababio & Barid, 2006).

Our results suggest that the ecological quality of the analyzed sites ranges from poor (at sites B and M) to high (site F). In particular, the biological data and the environmental parameters suggest that the Foglia River has a negative influence, especially in the site nearest to its mouth (site B). The significantly higher quantity of mud and the positive sediment asymmetry detected in this site may indicate both generally lower hydro-conditions and the possible effects of the inputs of the Foglia River. Indeed, it is well known that the level of organic content is related to the grain size of sediments and rises in parallel with the increasing mud fraction (Mayer, 1994a, b; Tyson, 1995). The enhancement of these two components may result in the lower permeation of oxygen, increased microbial oxygen uptake/demand and a subsequent build up of toxic by-products (e.g. ammonia, dissolved sulphide) (Florek & Rowe, 1983; Santschi *et al.*, 1990; Fenchel *et al.*, 1998). This can lead to impoverished benthic communities dominated by a few opportunistic species, as supported by the low MI values and the dominance of *r*-strategist genera at site B. This observation also confirms previous investigations (Pianetti *et al.*, 2004) reporting events of faecal contamination on the beaches coming from the river inputs.

The nematode genera that mainly contributed to distinguishing site B from the others were *Chromadora*, *Sabatieria* (*S. pulchra* grp) and *Viscosia*, all of which were particularly abundant. The *Sabatieria* species are well-known for their ability to persist in conditions that are unsuitable for most nematodes (low oxygen content, high sulphide concentration). They are also able to survive and reproduce even at high levels of anthropogenic impact (Hendelberg & Jensen, 1993; Soetaert *et al.*, 1995; Austen & Somerfield, 1997; Steyaert *et al.*, 1999; Somerfield *et al.*, 2003; Schratzberger *et al.*, 2004; Moreno *et al.*, 2008; Vezzulli *et al.*, 2008; Semprucci *et al.*, 2010). The *Chromadora* and *Viscosia* species may be abundant in sediments characterized by high organic content (Danovaro *et al.*, 1995; Schratzberger *et al.*, 2004; Beyrem *et al.*, 2011; Boufahja *et al.*, 2011). The poor ecological quality also found at site M, which is mainly related to the abundance of Xyalidae (*r*-strategists) (Bongers *et*

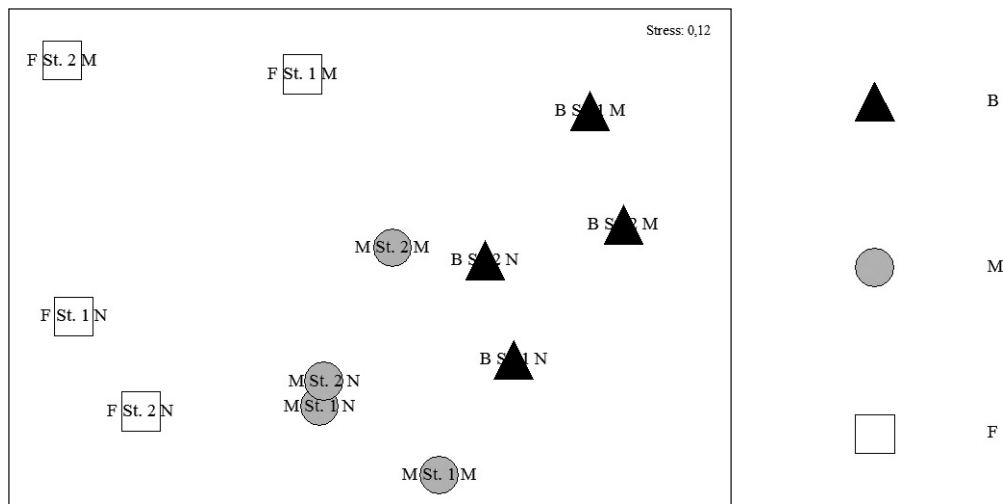


Fig. 4: nMDS plot of the nematode assemblages (fourth root transformed).

al., 1991), could be due to the effects of the long shore currents that transport river runoffs northwards. These local currents can assume great importance in the coastline transport of sediments, pollutants and discharges, as previously documented by Semprucci *et al.* (2010).

Site F, where more *k*-strategist genera like *Pomponema*, *Chromaspirinia* and *Oncholaimus* were detected, had the best environmental conditions (Bongers *et al.*, 1991; Danovaro *et al.*, 1995; Austen & Mc Evoy, 1997; Schratzberger & Warwick, 1998; Mahmoudi *et al.*, 2005) (Table 2; Fig. 3). A similar trend was also revealed by the Index of Trophic Diversity, which showed a good repar-

ation of trophic groups at site F, thus proving that there was good heterogeneity of food resources in the sediments at the site.

When it comes to the seasonal variations of the nematode assemblage, these are probably related to a different food supply. Indeed, there was an increase of epistrate feeder genera in November. This is probably related to the major water column concentrations of Chl-*a*, which were recorded at the Foglia River in that period (Table 4). This is a trophic parameter that might indicate an increased river water supply due to rainfall, which is a hypothesis that is also supported by the lower salinity

Table 3. 3-way ANOVA results conducted on the Shannon ( $H'$ ), Pielou ( $J$ ) and Maturity indices (MI) and the Index of trophic diversity (ITD).

Factor interaction	$H'$		$J$		MI		ITD	
	F	<i>p</i>	F	<i>p</i>	F	<i>p</i>	F	<i>p</i>
site	-	n.s.	4.64	< 0.05	12.94	< 0.01	-	n.s.
station	-	n.s.	-	n.s.	-	n.s.	-	n.s.
season	-	n.s.	-	n.s.	-	n.s.	4.63	< 0.05
season × site	-	n.s.	3.96	< 0.05	-	n.s.	-	n.s.
site × station	-	n.s.	-	n.s.	-	n.s.	-	n.s.
station × season	-	n.s.	-	n.s.	5.36	< 0.05	4.50	< 0.05
season × station × site	-	n.s.	-	n.s.	9.56	< 0.01	-	n.s.

Table 4. Water parameters reported in the study area (available online in: [http://www.uniurb.it/it/portale/index.php?mist\\_id=0&lang=IT&tipo=IST&page=1170](http://www.uniurb.it/it/portale/index.php?mist_id=0&lang=IT&tipo=IST&page=1170)).

Water Parameters	Temperature (°C)	DO (mg/l)	Salinity (‰)	pH	Chl- <i>a</i> (µg/l)
Pesaro M 03	21.2	7.0	34.7	8.2	0.6
Gabicce M 03	22.6	6.2	34.4	8.2	0.7
Pesaro N 03	11.4	7.6	32.0	8.2	2.1
Gabicce N 03	11.5	8.1	31.9	8.3	4.6



values in November. The increase of the trophic group 2A only at site B and site M in that period could further prove the greater influence of river inputs in those sites.

In conclusion, regular checks of biological assemblages are needed to evaluate the state of health of a coastal area. This is essential for the proper management of the littoral zone without compromising the biodiversity and functionality of the coastal ecosystem. Our results stress the importance of an accurate assessment of the ecological quality of vulnerable littorals. They also confirm that taxonomic composition and the functional traits of nematodes may be useful tools for monitoring anthropogenic disturbance in a coastal system.

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