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SEMPRUCCI F. University of Urbino
LOSI V.
MORENO M.

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A review of Italian research on free-living marine nematodes and the future perspectives on their use as Ecological Indicators (EcoInds)

F. SEMPRUCCI[1], V. LOSI[2] and M. MORENO[2]

[1] Dipartimento di Scienze della Terra, della Vita e dell'Ambiente (DiSTeVA), Università di Urbino, loc. Crocicchia, 61029 Urbino, Italy
[2] Dipartimento di Scienze della Terra, dell'Ambiente e della Vita (DISTAV), Università di Genova, C.so Europa 26, 16132 Genova, Italy

Corresponding author: federica.semprucci@uniurb.it

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Abstract

The use of free-living marine nematodes as ecological indicators (EcoInds) of human impacts has increased greatly in Italy since 1990. This paper is a summary of the Italian research experience in the study of nematode assemblages of shallow water habitats, and provides a breakdown of the most important insights that have been obtained so far. Although nematodes are among the best candidates for the Ecological Quality (EcoQ) assessment in the benthic domain, many guidelines need to be developed and limits overcome. Italian research has certainly contributed to the achievement of this purpose with highly focused local investigations on the effects of specific stressors (riverine and sewage discharge, aquaculture, trace elements and hydrocarbons), but also to a large extent with wider analyses aimed at finding new and valuable tools for monitoring programs and useful nematode descriptors, in line with the European Directives. Currently, the Italian and foreign experiences in this field draw light to the fact that the best nematode descriptors for the EcoQ assessment are the taxonomic composition and life strategy traits. However, nematode worldwide research is running the risk of being marginalized because of the relatively narrow scope of most contemporary studies. Nematode researchers should work in a *nematologist community* in order to better promote the use of nematodes such as EcoInds in the era of the Water Framework Directive (WFD) and Marine Strategy Framework Directive (MSFD). In the present paper, possible steps to obtain this goal are brought to the reader's attention and discussed.

Keywords: Nematoda, monitoring, taxonomic and functional analyses, anthropogenic disturbance, Water Framework Directive, Marine Strategy Framework Directive.

Introduction

Coastal habitats are subject to intense environmental pressure (e.g. fishing impacts, pollution, climate change, eutrophication, and the establishment of alien species) with extensive synergistic effects between natural and human systems. As well as natural fluctuations in abiotic parameters, anthropogenic disturbances can further modify coastal environments, sometimes even to a greater extent than that expected, and produce changes in the biota different from those derived from natural variability alone (Losi *et al*., 2013a). On a world-wide scale, the increasing impact of human activities on marine and estuarine environments has attracted attention towards the need for monitoring, assessing and managing ecological integrity to promote the long-term sustainability of these systems (Borja *et al*., 2008). In the Mediterranean Sea, which is a hot spot of biodiversity (Danovaro *et al*., 2010), numerous threats have resulted in extensive impact especially of the coastal systems (Danovaro & Pusceddu, 2007). Because of its central geographical position within the Mediterranean Sea and its extensive coastline (~7,458 km), Italy is a suitable area where a synthesis of the effects of anthropogenic disturbance on coastal marine systems may be carried out.

Among the EcoQ (Ecological Quality) parameters, biological indicators are fundamental to the classification of water bodies, because they integrate both the biotic and abiotic components of an ecosystem through their adaptive responses (Casazza *et al*., 2002). Sediments store many of the waterborne contaminants from industrial discharge, agricultural watersheds and urban run-off and as a result, pollutant concentrations in the sediments may be considerately higher than in the overlying water column (Coull & Chandler, 1992). Benthic assemblages, living in close contact with sediment particles and interstitial water, are intimately exposed to pollution and may respond to adverse ecological conditions, primarily by undergoing the following: local extinctions, taxonomic and functional structure changes and diversity loss. Since macrobenthos and meiobenthos have different ecological roles in marine ecosystems, they may respond to disturbance at different spatial and temporal levels (Semprucci *et al*., 2013a). Despite the fact that macrofauna are more usually employed as EcoInds (Ecological Indicators) than meiofauna, current knowledge about the meiofauna, and in particular nematodes, allows us to suggest their use as EcoInds (Moreno *et al*., 2011). Nematodes are, in fact, the dominant and most diverse group within the meiofauna (e.g. Balsamo *et al*., 2010; Appeltans *et al*., 2012). Due to their intermediate position in the food web, nematodes significantly affect the other benthic components. Moreover, they are in direct contact with pollutants within the sediment as permanent members of the benthos (see Moreno *et al*., 2011 and references therein). This latter fact, along with their short biological cycles and the high stability of the populations (Platt & Warwick, 1980), makes them a potential tool for detecting a more rapid and unequivocal reaction to environmental changes than macrofauna (e.g. Balsamo *et al*., 2010). Given their great abundance and wide distribution, they are advantageous for statistical analysis with only a limited volume of sediment samples necessary for their study (Balsamo *et al*., 2012 for review).

In the recent decades, Italian research has given relevant and positive feedback on the usage of free-living marine nematodes such as EcoInds. Indeed, among the most comprehensive studies aimed at testing and calibrating nematode parameters for monitoring programs have been performed along the Italian coastline (Moreno *et al*., 2011; Bevilacqua *et al*., 2012a,b).

In the present review, the application of nematodes as EcoInds for pollution and disturbance monitoring in Italy is presented and summarized. Furthermore, the necessity for new potential biological parameters and indicators in order to find innovative and more efficient tools for future research and monitoring projects, is discussed.

Review of the free-living nematode monitoring in Italy

Riverine and sewage discharges

Organic enrichment is an important natural and human induced process in coastal ecosystems (Armenteros *et al*., 2010). Organic matter (OM) can be roughly divided into two main groups: labile OM, that is more easily biodegradable and “naturally” produced, and refractory OM, which is more resistant and difficult to be metabolized and with a low nutritional value. Thus, OM may be a significant source of disturbance only when present in high concentrations. A high OM load may, in fact, cause a lower permeation of oxygen, increased microbial oxygen uptake/demand and a subsequent build-up of toxic by-products (e.g. ammonia, dissolved sulphide) (see Armenteros *et al*., 2010 for review).

In Italy, investigations into the effects of this particular stressor on nematode assemblages were carried out in the Adriatic (Villano & Warwick, 1995; Fabbrocini *et al*., 2005; Semprucci *et al*., 2010a, 2013a), Ligurian (Losi *et al*., 2012), Tyrrhenian (Sandulli & De Nicola, 1990, 1991) and Ionian Seas (Fraschetti *et al*., 2006) (Fig. 1).

Studies conducted on the soft sea beds of the Salerno Gulf and Naples Bay revealed that the effect of sewage discharge may generate substantial changes in the structure of meiofaunal assemblages, increasing nematode and decreasing copepod abundance (Sandulli & De Nicola-Giudici, 1990, 1991). Sandulli & De Nicola-Giudici (1991) reported that nematodes increased in abundance along a gradient of increasing organic enrichment, but the disappearance of this taxon was recorded in the immediate vicinity of the sewage outfall, where the physical and chemical parameters also showed a very low EcoQ. The general nematode enhancement with sewage discharge may be explained with the increased food supply available in those sediments. Indeed, nematodes are generally classified into four groups according to the structure of the buccal cavity: 1A, selective deposit feeders; 1B, non-selective deposit feeders; 2A, epigrowth feeders; and 2B, predators and omnivors (Wieser, 1953). They are the most abundant deposit feeders (1A + 1B) in the benthos domain, and may take advantages of this food supply (Armenteros *et al*., 2010) right up to the moment when the most deleterious effects derived from the organic load occurs.

Also Semprucci *et al.* (2010a, 2013a) reported a significant impact of river runoffs on the meiobenthic and nematode assemblages of the Central Adriatic Sea. In particular, Semprucci *et al.* (2010a) documented that river discharge, especially after spring floods, led to a surge in nematode abundance due to an increase of nutrients and some trace elements such as Pb. Among the faunal parameters used by the authors, there was the Maturity Index (MI). This index is calculated as the weighted average of the individual colonizer-persister (c-p) values (based on the reproduction rates, colonisation ability, tolerance to disturbance of the species) and has been proposed as measure of anthropogenic disturbance (Bongers, 1990; Bongers *et al*., 1991). According to Semprucci *et al.* (2010a), MI appeared to be a good index for detection of the stress induced by river discharge and was more efficient than diversity indices, generally more affected by natural variables such as sediment grain size. The low MI values and especially the dominance of generally opportunistic genera (c–p value of 2) detected in the study area suggested a high stress level in the areas adjacent to the river mouth.

A good efficiency of the MI has been reported also in Semprucci *et al.* (2013a). A clear dominance of *r*-strategists (e.g. *Chromadora*, *Sabatieria* and *Viscosia*), often associated with OM enhancement, mirrored perfectly river impact gradient. In contrast, a higher abundance of *k*-strategist genera like *Pomponema*, *Chromaspirinia* and *Oncholaimus* was present further from the river mouth. Oncholaimids, and in particular the genus *Oncholaimus*, are worthy of special attention because, as has been emphasised by Bongers *et al.* (1991), they combine characteristics of *k*- and *r*- strategists. In this case, as well as in others, it may be fundamental to know the species-specific response to the disturbance, because the various species could have a different level of tolerance to the stressors (Schratzberger *et al.*, 2008a; Armenteros *et al*.,

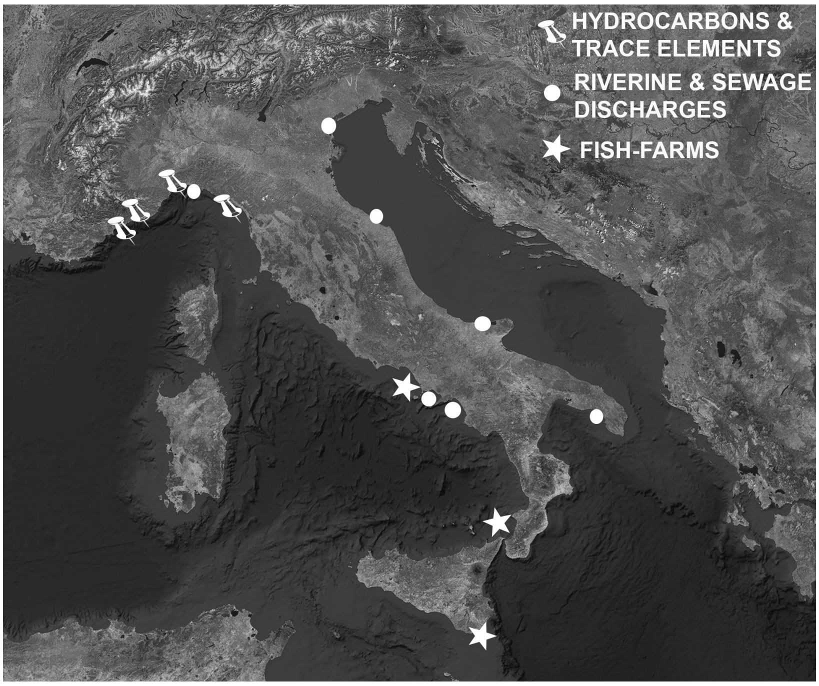

Fig. 1: Italian distribution of the most significant studies involving the responses of nematodes to the different types of disturbances.

2010). The Index of Trophic Diversity (ITD), calculated on the basis of the percentage of nematode feeding types (Heip *et al*., 1985), may give an indication of pollution occurrence when there is an increase of its value (due to a marked dominance of a single trophic guild). In line with that, Semprucci *et al.* (2013a) revealed an increase of ITD with decreasing distance from the river mouth. The authors found also an increase in the abundance of epistrate feeders (2A) which are likely to be positively related to the riverine inputs. Data presented by Danovaro *et al.* (2000) suggested, in fact, that plume inputs and frontal systems, enhancing phytodetritus accumulation and benthic bacterial abundances (two food sources for 2A), might influence density, composition and distribution of meiofaunal assemblages.

Villano & Warwick (1995) investigated the eutrophication effects at the Venice Lagoon (Northern Adriatic Sea). Here, agricultural runoffs caused a proliferation and subsequent decomposition of *Ulva rigida* during the summer period, with a dramatic fall in oxygen levels. Two distinct nematode assemblages were revealed: one associated with higher biomass, and the other one associated with lower biomass or absence of *Ulva*. In particular, *Diplolaimella ocellata*, known to be associated with decaying plant material and implicated in the decomposition process, clearly marked the *Ulva* zone. Here, both diversity (as evidenced by *k*-dominance curves) and species composition varied considerably with time, likely in relation to the *Ulva* growth cycle and decay. In contrast, these same faunal parameters were more stable seasonally in the non-*Ulva* region. Thus, the authors inferred that it is not only likely that the *Ulva* cycle controls seasonal changes in meiofaunal composition, but in turn, given the occurrence of fauna associated with the process of macro-phyte decomposition, the meiofauna has some control over the *Ulva* cycle. This finding is of great importance because it could suggest a crucial role of meiofauna in transitional environment (TE) systems.

An integrated biomonitoring program was carried out for the assessment of the EcoQ of the Lesina lagoon (Southern Adriatic Coast) (Fabbrocini *et al*., 2005). The water parameter levels revealed high primary production, and the sediment and pore water toxicity bioassays recorded from low to moderate and uniformly distributed toxicity levels. *Syringolaimus*, *Terschellingia* and *Sabatieria* were the most abundant genera. The high nematode dominance in sediments characterized by high nitrogen and OM levels suggested the possible exposure of this TE to discharge derived from agricultural and zootechnical activities.

Nematode assemblages showed signs of being affected by sewage discharge in a study on meiofauna of sediments associated with *P. oceanica* meadows (Losi *et al*., 2012). In particular, nematode assemblages associated with three Ligurian *P. oceanica* meadows located in Marine Protected Areas were compared with those associated to three meadows located closely to urbanised coastal areas. Samplings were carried out at the beginning and at the end of the summer season, in order to detect early changes in the meiofaunal assemblages. The number of genera (i.e. genus richness) and H' (Shannon-

Wiener diversity index) of urban meadows decreased in response to OM enrichment. The first differences revealed between urban and MPA meadows were observed as soon as in May, thus suggesting the existence of chronic disturbances affecting urban meadows. Disturbance intensification during the touristic season amplified the differences. In only 4 months, an increase in abundance of *Epsilonema*, *Daptonema* and *Odontophora* was revealed. *Epsilonema* is generally considered a genus sensitive to several types of disturbances (Bongers *et al.,* 1991), but its increase may be explained as an opportunistic response due to its ability to exploit the food released from sewage discharge. Nematodes of MPA meadows showed a more heterogeneous assemblage and was characterized by the presence of sensitive genera, such as *Richtersia*, *Halalaimus* and *Bolbolaimus* indicating good EcoQ. The dominance of 1A appeared closely related to the sewage inputs that favoured development of bacteria in sediments. On the other hand, the dominance of 2A in MPA in May and September was inferred by the authors as a specific feature of *P. oceanica* meadows, consistently with the relevance of vegetal debris, microphytobenthos, epiphytes and phytoplankton inputs of this system type. The higher abundance of predators (2B) in MPA meadows in both periods indicates a more heterogeneous and well-structured trophic assemblage that might imply a higher habitat complexity. Genera composition, H' and ITD were consistent in detecting EcoQ deterioration from May to September in all the urban meadows and Monterosso al Mare meadow, but only the genera composition was able to differentiate EcoQ between MPA and urban meadows already in the month of May. Thus, the authors recommended genera composition as an early warning descriptor of the environmental changes brought about by sewage.

The schematic response of density, trophic and life strategy traits to sewage and riverine discharges in the soft bottoms is reported in Figure 2A.

If the majority of these studies documents the impact of the OM enrichment in the soft sea beds, Fraschetti *et al.* (2006) investigated the response of nematodes to sewage outfall impact in rocky substrata. The authors revealed a nematodes abundance decrease in the impacted site, but no reduction of richness or changes to their taxonomic structure. The decrease of abundance found in this study was in contrast with literature data that reported a higher abundance of nematodes due to the additional food released from sewage discharge and the sedimentation rate (e.g. Armenteros *et al.*, 2010). However, this result was probably related to the notable influence of sewage outfall on the macroalgae assemblage composition that, increasing the structural complexity of the habitat (fractal hypothesis, see Gee & Warwick, 1994 for details), led to an opposite response of the nematode assemblages. Interestingly, the MI did not change significantly between the impacted and the control sites, showing a high percentage of *k*-strategists (c-p value of 4) at both sites. When the trophic style of nematodes was considered, a high abundance of 2A, a group generally associated with a good EcoQ (see for review Semprucci & Balsamo, 2012), was documented in both the localities suggesting a poor influence of sewage discharge also to the trophic guilds (Fig. 2A). However, given the results of all these studies, we can infer that the 2A are more associated to sewage discharges than would be expected.

Aquaculture

The rapid expansion of aquaculture activities may be a potential solution to the problems related to overfishing and fisheries depletion. Nonetheless, it also represents a threat to the integrity of marine ecosystems, releasing nutrients and other wastes into surrounding environments. Aquaculture activities have an impact on local and regional levels (Mirto *et al*., 2010) and recent estimates indicate that, in Mediterranean coastal areas, the release of nutrients from fish farming accounts for between 7 and 10% of the total discharge of nitrogen and phosphorus respectively (see Mirto *et al*., 2012 and references therein). The main impact of aquaculture is the build-up of faeces or pseudofaeces (biodeposition) on sea beds underlying the culture area, with the resulting alteration of sediment grain size, OM content and nitrogen-cycling, which might easily bring about an anoxic environment (e.g. Mirto *et al*., 2002, 2010). Of these environmental changes, particularly oxygen depletion and build-up of toxic products may impact benthic assemblages (Mirto *et al*., 2012).

There have only been four investigation procedures that have focused on nematode assemblages on the Italian coastal systems: three of them have been carried out in the Tyrrhenian Sea (Gulf of Gaeta and Vibo Marina) (La Rosa *et al*., 2001; Mirto *et al*., 2002; Vezzulli *et al*., 2008) and one in the Ionian Sea (Sicily) (Mirto *et al*., 2014) (Fig. 1).

La Rosa *et al.* (2001) documented the effects on bacterial, meiofaunal and nematode assemblages of the fish-farm biodeposition in the Gaeta Gulf (March-October 1997), and they observed a reduction of the meiofaunal abundance after the biodeposition, but only a negligible change in the average meiofauna biomass of the farm sediments. This was particularly evident for nematodes, which dominated the total meiofauna (Mazzola *et al*., 2000). Their biomass was higher below the cage as a result of a selection of tolerant genera of larger size such as *Sabatieria* and *Pierrickia*.

Mirto *et al.* (2002) conducted another study in the same area (from July 1997 to February 1998), in which they documented a significant increase in nematode body weights in OM enriched sediments beneath the cage. However, when the farm was harvested, and biodeposition reduced, the average body weight was similar between samples taken in control and impacted sites. Furthermore, immediately after farm deployment, despite the increased

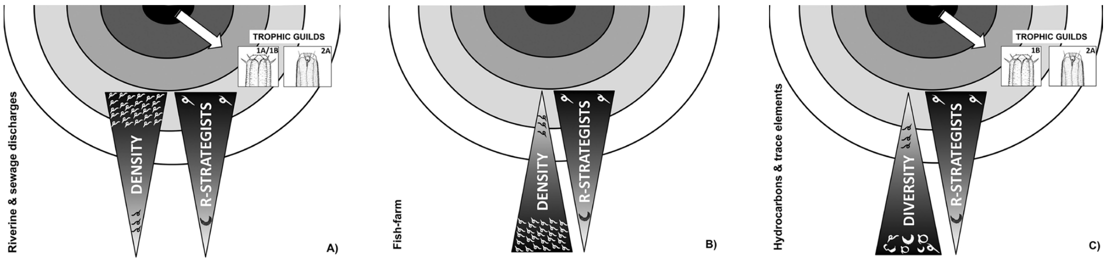

Fig. 2: Schematic response of nematode attributes (abundance, diversity, life strategies and trophic groups) to: A) sewage and riverine discharges; B) fish-farm; C) hydrocarbons and trace elements. In detail, the center of the circles highlights the highest disturbance level, while the triangles directions show increasing or decreasing density, diversity or abundance of r-strategists. The low-high and medium number of nematode cartoons within the density and diversity triangles highlights the low- high and medium density or diversity, respectively. Due the absence of a real impact of fish-farm on the nematode trophic structure, the trophic guilds are not reported in the Figure 2B.

individual size, a strong reduction in abundance beneath the cage resulted in a significant decline of the total biomass. The assemblage structure and composition appeared more useful than other nematode faunal parameters in displaying the impact due to fish-farming in all the seasons, but it did not reveal any type of resilience phenomena. *Setosabatieria* was the genus mainly responsible for the dissimilarity between pristine *versus* fish-farm sediments, and it was dominant at the control site being an EcoInd of good EcoQ. Other sensitive genera were *Latronema* and *Elzalia* disappearing almost completely from farm sediments. In contrast, other genera, i.e. *Dorylaimopsis*, *Sabatieria* and *Oxystomina,* appeared tolerant to farm biodeposition, and took advantage of new adverse conditions. All of them are well-known for their proliferation in stressful conditions or close association with sediments rich in OM (see Mirto *et al.* 2002 for details). The analysis of several functional and diversity indices gave unclear information with the only exception of MI. MI dropped, in fact, as from the first and second month of cage deployment and indicated a good resilience of the assemblages as from the fourth month. In contrast, ITD and various other diversity parameters (namely *k*-dominance curves, genus richness, H’ and J (Pielou Index) did not underscore any clear impact of the farm installations. The authors stressed that the lack of significant differences in trophic structure may be due to a lack of specific trophic group selection by the farm. However, a strong increase in the relative abundance of feeding type 1B as a consequence of OM enrichment was recorded. According to Mirto *et al.* (2002), Wieser’s (1953) classification did not reflect the real trophic structure of assemblages. Trophic classification has been, in fact, largely revised and modified in the last decade (Moens & Vincx, 1997), but the use of this more recent classification is hampered by the low number of species reclassified making its use in ecological surveys impossible.

Mirto *et al.* (2014) investigated the impact of fish farming on the meiobenthic and more specifically the nematode assemblages of *Posidonia oceanica* seagrass meadows and unvegetated soft bottoms (Pachino Bay, Sicily). Among the faunal parameters analysed, MI decreased significantly in the impacted sites in all habitats and regions, while diversity indices did not show a significant impact of the fish-farm. The lack of statistical differences in diversity between impacted and control sites was explained by the authors as a consequence of the contemporary loss of certain sensitive species partially offset by an increase of opportunistic ones in impacted sediments. This hypothesis was supported by the significant decrease of the MI below the farm emphasising an increasing importance of *r*-strategists. Thus, this faunal parameter together with the turnover in species composition, seemed the most sensitive tool to detect the impact of fish farm infrastructures. In particular, the genera *Richtersia*, *Desmoscolex* and *Halalaimus* turned out to be highly sensitive to farm impact, while other genera such as *Daptonema* and *Prochromadorella* were positively related to biodeposition resulting from the installation of the cages.

Different results were documented for an off-shore bluefin tuna fish farm in Vibo Marina, by Vezzulli *et al.* (2008), who recorded no significant variability in H’, J and MI between cages and control sites. This may be related to both the oligotrophic conditions and the exposed nature of the site that was characterized by high water depth and strong hydrodynamic regime. Thus, the study provided good experimental evidence that the development of an offshore industry may be a good solution to minimize the hypertrophic- dystrophic risk associated with aquaculture practice. However, the authors documented the dominance of *Tricoma*, *Desmoscolex*, *Quadricoma* and *Halalaimus* at their control sites, and the dominance of *Daptonema*, *Marylynnia*, *Sabatieria* and *Terschellingia* beneath the farm cages. The first group of species is generally associated with pristine environments, while the second one is well known for its tolerance to stressful conditions (see Vezzulli *et al.*, 2008 and references therein). Given the good results obtained by the taxonomic composition, they recommended sensitive/tolerant genera as good EcoInds for short-term changes observed in the sediments underneath the fish farms.

The schematic response of density and life strategy traits to aquaculture in the soft bottoms is reported in Figure 2B.

Trace elements and hydrocarbons

In marine systems, the major sources of hydrocarbon contamination are oil exploration and production, natural seepage, atmospheric input, tanker accidents, industrial discharge, and urban run-off (Beyrem *et al*., 2010). Although trace elements can have a natural source, the major source is anthropogenic in origin, e.g. mining sites, foundries and smelters, the purification of metals, combustion by-products, traffic and coal, natural gas, paper, and chloro-alkali activities (Balsamo *et al*., 2012).

In addition to their direct effects on nematode assemblages, hydrocarbons also appear to influence nematodes indirectly through effects on the sediment environment such as dissolved oxygen depletion and changes in sediment properties, such as porosity and interstitial sedimentary space (Mahmoudi *et al*., 2005). Also, the accumulation of trace elements in sediments may alter associated microbial assemblages which may in turn influence the meiobenthos through alterations in their food supply (Austen & McEvoy, 1997). Moreover, the combination of these two types of pollutants could have additive or even synergistic effects on the assemblages.

Along Italian coasts, there is only one study focused on hydrocarbon contamination impact on the meiobenthic and nematode assemblages, and it was performed after the Abruzzo oil spill occurred in the Ligurian Sea in April 1991 (Danovaro *et al*., 1995) (Fig. 1). Both the whole meiofaunal assemblage and nematodes showed a significant reaction after the oil spill, but only indices based on nematodes displayed good sensitivity to the oil-induced disturbance. For instance, Hill's diversity index (Hill, 1973) showed a strong reduction, *k*-dominance curves and genus richness as well. However, one month after the oil spill, the nematode diversity level was similar to pre-pollution conditions. The ITD did not change significantly probably due to the scarce influence of the oil pollution on the nematode trophic structure. Nevertheless, a decrease in the abundance of nematode feeding type 1B after the oil contamination took place. This group is likely to be directly affected by oil toxicity as they ingest tar particles and oil emulsion together with the sediment during feeding (Danovaro, 2000). A genus-specific response was also reported: *Chromaspirina*, *Hypodontolaimus*, *Onchalaimellus*, *Paracanthonchus*, *Setosabatieria* and *Xyala* disappeared immediately after the oil spill, but they recovered rapidly appearing to have opportunistic behaviour. In contrast, genera such as *Daptonema* and *Viscosia*, representing ~60% of total nematode abundance in impacted areas, appeared more tolerant to hydrocarbon stress.

Harbours are exposed to multiple contaminants such as toxic compounds, trace elements, hydrocarbons and OM (Losi *et al*., 2013a). Given the increasing occurrence of problems to marine life and human health associated with harbours and all the implications for the EcoQ status, several studies on the effects of the harbours' environmental conditions on nematode assemblages were conducted in the Ligurian Sea (Marin *et al*., 2008; Moreno *et al*., 2008a, b, 2009; Losi *et al*., 2013a,b) (Fig. 1).

Moreno *et al.* (2008a) revealed significant spatial heterogeneity in the abundance and composition of meiofaunal and nematode assemblages inside the Genoa-Voltri harbour in relation to main environmental variables (i.e. Eh, organic matter, polycyclic aromatic hydrocarbons (PAHs), chlorophyll-a and phaeopigments). The individuation of nematode genera indicators of stressful conditions may be important from a management perspective because it could permit the identification and assessment of environmental risk areas that are more exposed or susceptible to problems of hypertrophic-dystrophic or chemical risk in harbours. Therefore, this process might be helpful in the development of good planning and monitoring programs, leading to better management of harbour ecosystems. In particular, the central part of the harbour area, characterized by low Eh values, high OM (also low OM quality), high bacterial densities (TBN) and higher PAHs concentrations, seemed more stressed with a relevant increase of nematodes and consequent decrease in diversity of the general meiofauna. In this same site, the nematode assemblage was dominated by *Terschellingia*, *Sabatieria* and *Paracomesoma*, which have opportunistic behaviour as previously reported. A relation with PAH concentration and OM load was found in the same part of the harbour. Thus, environmental pollution, rather than food supply, seemed to be the main factor responsible for the changes observed in the assemblages of this harbour.

Moreno *et al.* (2008b) compared several meiofaunal indices among sediments of three different harbour-marinas (Genoa-Voltri, Portosole and Marina degli Aregai) to evaluate their usefulness as EcoInds of pollution, and to identify those that best described the environmental quality of such systems characterized by different levels of contamination. In this study, nematode genus-based diversity was correlated with PAHs concentrations, while MI and ITD did not correlate with the concentration of contaminants. The presence of sensitive or tolerant nematode genera appeared particularly informative, making the state of sediment contamination very evident. Generally, in heavily polluted sediments characterized by a low redox potential, the nematode assemblage was dominated by *Terschellingia*, *Sabatieria*, *Paracomesoma* and *Daptonema*. In contrast, in harbour sediments characterized by lower levels of pollution, communities were dominated by *Desmodora* and *Anticoma*. In Marin *et al*. (2008) a multistep indicator-based approach (MIBA) was developed using also the H' based on nematode genera. Worthy of note is the fact that this was the first attempt to use a nematode parameter in defining the EcoQ.

Moreno *et al.* (2009) further investigated the two tourist marinas of Portosole and Marina degli Aregai.

Nematode abundances were significantly higher at Portosole, where higher concentrations of OM were found. At Portosole, the lowest nematode abundances were found to correspond with the highest concentrations of chemicals (namely trace elements, PAHs) and OM suggesting their responsibility for the trend observed. The dominant genera were *Terschellingia*, *Sabatieria*, *Daptonema* and *Paralongicyatholaimus*. The first three genera are well-known as being tolerant, whilst *Paralongicyatholaimus* is poorly known from an ecological point of view. In particular, *Paralongicyatholaimus* showed a significant negative correlation with Cu and it was almost absent at the stations where higher Cu concentrations were found, suggesting Cu-sensitive behaviour for this genus. In contrast, a positive correlation was found between *Ptycholaimellus* and Cu concentration, suggesting this genus is tolerant to Cu. However, contrasting data regarding the pollution sensitivity/tolerance of *Ptycholaimellus* is present in the literature. The significant correlation between assemblage structure and grain size found at Marina degli Aregai and absent at Portosole, was probably due to a stronger influence of the higher concentrations of chemicals present in the latter marina. The ability of nematode assemblages to identify areas subject to a higher level of disturbance suggests that they may greatly help us to focus and to define correct management programs for the harbours.

Vado Ligure (Savona) harbour and its surroundings were studied by Losi *et al.* (2013a). Stations were selected along an anthropogenic disturbance gradient. A high correlation between environmental characteristics (namely water depth, contamination, food availability and sedimentology) and nematode structure response was found. The authors highlighted that, although factors related to sedimentary conditions and food proprieties were important in explaining the spatial distribution and structure of nematode assemblages, the highest portion of nematode variability was explained by contaminants (hydrocarbons and trace elements) and depth. This result was striking, since usually, in non-impacted environments, grain size represents the *'super factor'* (Platt *et al*., 1984) in determining nematode community structure. Univariate measures (richness, diversity, MI and c-p classes) in general reflected the contamination trend, but genus composition gave the best correspondence with the environmental parameters. The multivariate structure of nematode assemblages revealed three distinct sub-areas clearly differentiated by their contamination level and a set of nematode EcoInd genera was selected. *Sabatieria*, *Terschellingia*, *Comesa*, *Oncholaimellus*, *Microlaimus*, *Molgolaimus*, *Daptonema*, *Ptycholaimellus*, *Spirinia*, *Eleutherolaimus*, *Neotonchus* and *Thalassoalaimus* were the genera selected as EcoInds of stressful conditions, while *Chaetonema*, *Marylynnia*, *Chromadorita*, *Belbolla*, and *Enoplolaimus* were chosen as EcoInds of pristine conditions.

Nematode biomass and allometric attributes (i.e. size spectra, body length and width, body shape) were analysed by Losi *et al.* (2013b) at Genoa-Voltri harbour in order to investigate their possible use as EcoInds. The sediment quality was defined by measuring the level of OM enrichment (quantity and biochemical composition of OM matter) and oxygen stress (redox potential). Nematode biomass spectrum (NBS) was highly valuable in determining differences in the EcoQ on a hundred-metre spatial scale. High peaks in the NBS were observed in the more OM-rich and oxygen-stressed sites probably in relation to a lower nematode diversity, with the predominance of tolerant genera such as *Paracomesoma* and *Sabatieria*. Among allometric variables, body length was found to be negatively correlated with oxygen concentrations and positively with OM, whilst the length/width ratio (L/W) was negatively related to oxygen concentrations and OM quality. These results suggest that the allometric attributes analysed represent indicators of nematode functional adaptation to the changing environmental conditions. Such promising results obtained by the authors in this study allow them to suggest the use of biomass and allometric attributes as an alternative method to the taxonomic approach, which is time-consuming and requires taxonomic expertise.

Semprucci *et al.* (2014) classified for the first time the EcoQ of the TE of Varano. The lake did not display high values of trace elements or OM enrichment. However, the nematodes appeared to be mainly represented by general opportunistic species (those having a c-p value of 2), while MI showed unclear results. Since the enrichment of some trace elements may be toxic for living organisms, the Pollution Load Index (PLI) was calculated taking into account As, Cd, Co, Cr, Cu, Ni, Pb, Zn and Mn. PLI compares the total concentration of a given element in the environment with the one expected when excluding anthropogenic contributions (Tomlinson *et al*., 1980). In this regard, a clear relationship between PLI and MI was not found in the area. This could be explained by the environmental complexity of TEs as well as the necessity to update the Bongers life history classification scheme. Indeed, a significant correlation with PLI and *Terschellingia longicaudata* was found. This species is generally considered as c-p3, but a high number of studies showed a strong opportunistic behavior of this species (e.g. Armenteros *et al*., 2009; Moreno *et al*., 2009; Beyrem *et al*., 2010; Semprucci *et al*., 2010a). In order to classify the EcoQ status of Varano in accordance with WFD, the authors used the nematode parameters proposed by Moreno *et al.* (2011) and subsequently by Semprucci *et al*. (2014). On the basis of these thresholds, the EcoQ status of most of the stations could be mainly classified as moderate, followed by good and poor status, in agreement with the level of OM enrichment and trace element levels found. Moderate conditions were generally present in the central area of the lake, while in front of the canal mouths characterized by water discharge, a poor EcoQ was observed. Since TEs are among the most vulnerable coastal habitats worldwide, the definition of TEs EcoQ status could open up new relevant perspectives for their conservation and management.

The schematic response of diversity, trophic and life strategy traits to hydrocarbons and trace elements is reported in the Figure 2C.

Efficiency of nematode attributes in the EcoQ assessment in relation to European Directives

The Water Framework Directive (WFD 2000/60/EC) and Marine Strategy Framework Directive (MSFD 2008/56/EC) aim at defining the baseline of a good EcoQ status of European water bodies. For the WFD, a variety of indices, target values and reference setting approaches for assessing the EcoQ status have been developed, intercalibrated and discussed. Among the several indicators used by the MSFD there are several indices based on macroinvertebrates. Monitoring the status of marine environments is, in fact, traditionally based on macrofauna surveys, for which standardised methods have been established, whilst only few attempts to use meiofauna have been made (Moreno *et al*., 2011; Semprucci *et al*., 2014).

Nematodes, in particular, have already been employed in environmental quality assessment studies and have been proved to be suitable indicators for pollution-induced disturbances of benthic ecosystems (Coull & Chandler, 1992; Bongers & Ferris, 1999; Höss *et al*., 2011). Nevertheless, they are currently not considered as a requirement for the assessment of the ecological status of the benthic environment according to the WFD and MSFD. The use of nematode indices, especially in soft sea beds, can close the gap in current monitoring programs, which are now mainly based on macrobenthic invertebrates (Höss *et al*., 2011), revealing different and complementary aspects of the factors structuring benthic ecosystems. This is fundamental in ecological status assessment (Vanaverbeke *et al*., 2011; Semprucci *et al*., 2013b).

Hereafter, the nematode attributes analysed in relation to specific stressors in the studies previously described will be discussed in order to figure out which descriptors have the greatest potential in the EcoQ assessment.

Taxonomic structure

All aforementioned study cases point to the usefulness of taxonomic structure to assess the response of nematode assemblage to organic and chemical pollution. This is also confirmed by investigations carried out in other geographical regions and on different type of disturbances (i.e. Gyedu-Ababio *et al.,* 1999; Armenteros *et al.,* 2010; Alves *et al.,* 2013). Therefore, the taxonomic structure appears to be a promising tool used to assess the EcoQ in line with the WFD.

Moreno *et al.* (2011) compared the use of different ecological indices, including H’, MI, c-p%, ITD and presence of sensitive/tolerant genera in a study addressing several Italian coastal sites. They found that the latter proved to be the best tool for the evaluation of EcoQ status. Other studies found that the analysis of nematode assemblages at the genus level represents the most informative tool in relation to the EcoQ: in the study of Losi *et al*. (2013a) the analysis of the nematode assemblage at the level of genus revealed the best connection between environmental status and biological response, and in Losi *et al.* (2012) only the genera analysis brought to light early changes in the community due to the touristic pressure. The same results are reported by Vezzulli *et al*. (2008) and Mirto *et al*. (2014) in relation to the fish farm impact and by Schratzberger *et al*. (2009) in relation to physical stress.

In the study of Moreno *et al.* (2011), the choice of sensitive/tolerant genera was carried out on the basis of the Italian and available foreign literature and then validated by the data of the study. *Terschellingia*, *Paracomesoma*, *Daptonema* and *Sabatieria* were, for example, selected as indicators of a poor ecological quality status because of their well-known tolerance to pollution (Danovaro et al., 1995; Austen & Somerfield, 1997; Warwick *et al*., 1997; Schratzberger *et al*., 2006; Steyaert *et al*., 2007; Semprucci *et al*., 2013a, 2014). These genera were found to be made up of more than 10% of the nematode assemblage in the disturbed sites, whereas they were not so abundant, or were even absent, in the undisturbed sites. The adaptation of these nematode genera to stressed conditions was also made evident by the strongly positive correlations with total organic matter or chemicals that were detected in the sites under investigation. In contrast, the genera selected as indicators of good ecological quality status (e.g. Desmoscolecidae, *Richtersia*, *Pomponema*, *Epacanthion*), as documented by other authors, revealed negative correlations with organic enrichment and chemicals, and were also present in higher densities at the more pristine sites (Heip *et al*., 1985; Bongers *et al*., 1991; Mahmoudi *et al*., 2005). However, the limited number of genera suggested by the authors does not always allow for its use in habitats or geographical regions characterized by a different nematode community composition (Semprucci *et al*., 2014). Thus, the set of genera recommended in this study has to be implemented in order to create a more comprehensive list (in a sort of tolerance/sensitivity spectrum) of EcoInds. Furthermore, the new selected EcoInds should be calibrated considering the possibility that nematode species (and greatly the genera) could be differently affected by different stressor types as well as natural variables such as grain size (Semprucci *et al*., 2010b).

Maturity Index

In a good portion of the reported study cases, MI responded to the stressors, proving to have the potential as quality status indicator (Mirto *et al*., 2002, 2014; Semprucci *et al*. 2010a, 2013a). In agreement with those results, Moreno *et al.* (2011), evaluating several nematode indices for EcoQ assessment, found that c-p composition and MI were among the best tools to evaluate the EcoQ.

However, MI and c-p classes are sometimes unable to identify the dominant stressor when multiple stressors are at play and the c-p list has to be updated (see Balsamo *et al*., 2012 for review). Indeed, MI was initially proposed for the study of terrestrial and freshwater habitats (Bongers, 1990), and only then included marine and brackish ecosystems (Bongers *et al*., 1991). However, the lack of empirical evidence regarding life strategies of most marine nematode genera has led to the c-p being used conservatively and implies some limitations especially in its use in marine ecosystems. However, MI and c-p classes proved to be good tools in the detection of environmental disturbance inflicted by chemicals in several studies. Also OM enrichment (derived from sewage discharge or aquaculture) and eutrophication (by riverine outfalls), adding more weight to their usefulness as indicators (see also Essink & Keidel, 1998; Gyedu-Ababio & Barid, 2006; Alves *et al*., 2013). Furthermore, MI, being a single measuring stick able to give an overview of the life styles of the nematode assemblages, and one which is easily communicated to possible environmental managers, this index could be efficiently used for the WFD evaluation.

Diversity indices

Among diversity descriptors, H' (α- diversity, the basic unit of diversity, as the total number of species present in a sample or within a habitat) is certainly one of the best candidates for the EcoQ class definition due to its wide use in nematode ecology.

In the study of Moreno *et al.* (2011), H' gave quite good results although not in all cases. For example, H' of harbour sites were moderate, probably because the unfavourable conditions at these sites persisted for a long time, enabling the nematodes to adapt.

However, H' responded efficiently to the stressors only in some of the reported case studies. Diversity indices may respond to human stress, giving information on its effect on the ecosystems, but they are not always consistent. Links between diversity and disturbance are often very complex. An example of this may be the 'intermediate disturbance hypothesis' for which intermediate levels of disturbance may favour the assemblage biodiversity (Connell & Slatyer, 1977; Salas *et al*., 2006). Also the effect of natural variables such as sedimentological features may greatly influence the biodiversity of nematode assemblages (Semprucci *et al*., 2010a, b). Consequently, there are relevant limitations in the use of diversity indices in ecological and, in particular, WFD assessments. A way to avoid possible problems due to the influence of grain size could be the definition of specific thresholds of the EcoQ classes for each sediment type. This could reduce possible biases in the interpretation of the results. Accordingly, H' should be used with caution and only as a complementary assessment tool of the genus composition and life strategy traits.

Alternatively, human impact on assemblages can be detected through *β*-diversity and taxonomic distinctness. The former expresses the extent of change in community composition at varying positions along a gradient (turnover), or the heterogeneity of assemblages within any spatial, temporal, or environmental extent (variation) (Anderson *et al*., 2011); the latter is a biodiversity measure based on the taxonomical relatedness of species (Warwick & Clarke, 1995) that has been applied successfully for the exploration of benthic biodiversity patterns (see Semprucci & Balsamo, 2012 for review).

Promising results come from the use of *β*-diversity as variation which can unveil patterns of change in assemblages that can remain unnoticed considering other components of diversity. Indeed, the nematode assemblages along the depth gradient analyzed by Bevilacqua *et al.* (2012a) showed no significant variations in *γ*-diversity (diversity measured on a regional scale) and *α*-diversity, while *β*-diversity revealed significant and higher heterogeneity of shallower assemblages suggesting that local factors, such as variability in vegetation, food supply, OM load, natural and human disturbance, may exert a greater influence on nematodes at shallower depths. Although *β*-diversity could provide information fundamental to the understanding of the effects of natural and anthropogenic disturbances on natural assemblages and may be applied to environmental assessments, the quantification of assemblage heterogeneity in assessing patterns of *β*-diversity is still largely unexplored and needs further investigation (Bevilacqua *et al.,* 2012a). Therefore, at the moment, the specific WFD requirements for the EcoQ classification make the implementation of *β*- diversity as a faunal descriptor in accordance with the European Directives still problematic.

Bevilacqua *et al.* (2012b) assessed the potential of taxonomic distinctness to discern perturbed and unperturbed sites by analysing nematode assemblages found along the Italian coastline. They found that the index is strongly affected by natural variability, habitat features, and biogeographic context. Moreover, since specific areas or habitats may have taxonomic distinctness values that are intrinsically lower than others, significantly reduced taxonomic breadth does not necessarily point to perturbed conditions (Costa *et al*., 2010; Bevilacqua *et al.,* 2011). Disentangling intrinsic effects on taxonomic distinctness from those related to human perturbations appears difficult. Thus, the use of taxonomic distinctness as an indicator of human impacts should be treated with caution and only as a complementary assessment tool in monitoring programs. Furthermore, this approach is not helpful in assessing the EcoQ status of systems according to the WFD (Salas *et al*., 2006; Prato *et al*., 2009).

Index of trophic diversity and feeding types

ITD cannot be considered as a good index for the detection of anthropogenic disturbance, as too few investigations have shown its good performance in ecological assessments (e.g. Semprucci *et al*., 2013a). Several authors have reported an ambiguous influence on the ITD of various stressors (e.g., oil spill, biodeposition related to fish farms, physical disturbance), suggesting that these types of stresses do not have a real impact on the trophic structure of the nematodes (Danovaro *et al*., 1995; Mirto *et al*. 2002; Schratzberger *et al.,* 2003, 2009; Alves *et al.,* 2013).

Additionally, the classification of feeding types, as described by Wieser (1953), confining nematode genera to a single trophic status (Heip *et al*., 1985), may not represent the real complexity of nematodes feeding habitats (Moens & Vincx, 1997), with trophic plasticity being described for most feeding types (Moens *et al*., 2005; Schratzberger *et al*., 2008b). Furthermore, the level of tolerance/sensitivity of the trophic groups should be reevaluated: for instance the 2A trophic group (epistrate feeders) showed a greater tolerance than expected in several surveys (e.g. riverine and sewage discharges and hydrocarbon disturbance).

Biomass and allometric attributes

In addition to the above cited functional indices, NBS and allometric attributes should also be considered. Vanaverbeke *et al*. (2003) reported alterations in nematode biomass size spectra (NBS) as responses to oxygen stress and phytoplankton sedimentation events. Another informative parameter is represented by the nematode shape, which was suggested to be related to the available food supply but also with the biogeochemical conditions of the sediment (e.g., oxygen stress) (Tita *et al*., 1999; Soetaert *et al*., 2002; Vanaverbeke *et al*., 2004). Results from the Italian studies under scrutiny here showed that the NBS and allometric attributes could represent a promising tool for ecological assessment, at least for harbour monitoring purposes, showing good results in the discrimination of the EcoQ of different harbour areas (Losi *et al*., 2013b). An advantage in the use of these measures is that they are tools that require a small time-frame and user-expertise. Although nematodes are particularly suitable as EcoInds in freshwater, marine and terrestrial habitats, a general limitation to their application can be seen in their difficult taxonomic identification which requires the support of experienced taxonomists, and analyses do take a considerable length of time. Thus, nematologists worldwide are trying out alternative methods to assess the effects of anthropogenic disturbances that are quicker and easier to operate.

However, the risk is an excessive simplification of the complexity of the assemblages' responses and at the moment, the biological trait approach may be used to give additive information to taxonomic data (Schratzberger *et al*., 2007; Armenteros *et al*., 2009).

Limits and future perspectives of the usage of nematode in ecological assessment

A general limitation of ecological investigations based on nematodes is related to the difficult and time-consuming taxonomic identification of their species. According to some authors a higher taxonomic level of identification (genus level) could efficiently detect assemblage changes due to human stresses (e.g., Somerfield & Clarke, 1995). For these to be successful, pollution assessment might not have to be conducted at the species level (Warwick, 1988; Danovaro *et al*., 1995) and pollution effects are detectable at even higher taxonomic levels (Warwick, 1988). However, other authors stressed the importance of the taxonomic identification at species level, because the functional roles of nematodes may be highly species-specific, and their identification at a species level appears fundamental for understanding their ecological role or to avoid misinterpretation (see Semprucci & Balsamo, 2012 for details). A possible important challenge for the future is the implementation of molecular techniques, which could make nematode taxonomic identification easier and faster (e.g. Bhadury & Austen, 2010; Derycke *et al*., 2010), but classic morphological and molecular approaches have to be used in integrative ways. A notable quantity of information accrued concerning various aspects of nematode biology and ecology is currently linked with morphology (Abebe *et al*., 2011) and an estimation of the richness or diversity, even if it is a rapid estimation, does not give information on the life cycles nor does it define ecological niches of the species, or give indications of their role within an assemblage or ecosystem (Boero, 2010).

Thus, how should nematologists approach the problem of standardizing the response of nematode descriptors to human impacts? The first step is probably to use all of the literature data available (or at least a reliable selection taken from it) to generate an updated list of nematode species and/or genera based on the proportion of sensitivity/tolerance to different levels and types of disturbances. In this respect, a meta-analysis of a large dataset collected in different geographical regions and habitats, could give new and significant insights into the magnitude and direction of human impacts on nematodes (Schratzberger *et al.*, 2009). The various functional and taxonomic descriptors should be evaluated as well, to try to define to a large extent the specific EcoQ thresholds for the best nematode descriptors. Another step should be the development of controlled laboratory experiments to test the nematode descriptor responses in relation to specific anthropogenic disturbances. Such experiments could guarantee an isolation of the effects of natural and anthropogenic variables on the assemblages. Subsequently, this experimental data could be verified and calibrated in large field studies. Once the best nematode descriptors are identified, molecular diagnostic tools could be used, with significant consequences in terms of time reduction, work and taxonomical specialization required, all points that have played a part in making meiofauna and nematodes generally less studied than macrofaunal components.

According to Moore et al. (2009), "*the resolution of the link between ecology, management and society is essential to ensure that ecological research remains relevant* *to real-world issues, that environmental management is informed by the best science and that society has the best chance possible of achieving its preferred outcomes*". If there is some public interest, probably there are more economic interests and financial support for the monitoring programs (Kennedy & Jacoby, 1999). Also Schratzberger (2012), in a paper on the relevance of meiobenthic research for policy-makers, concluded saying: "*I believe that scientists should share the responsibility of ensuring the effective communication of new and exciting scientific knowledge to decision-makers and the public. Spreading knowledge is the key to changing attitudes and behaviour. Hence, exposing interesting research results to a diverse audience is likely to generate scientific and public debate, thereby inspiring and shaping future meiobenthic research."* Thus, what should be done to attract greater interest from the environmental authorities and public arenas for the phylum of the Nematoda as a bioindicator? Future perspectives in marine nematology cannot be reached without synergistic actions. The International Association of Meiobenthologists (IAM) and the MarBEF (Marine Biodiversity and Ecosystem Functioning) represent good examples of those synergistic actions. The International Association of Meiobenthologists (IAM) brings together researchers from around the world to share and discuss the taxonomy and ecology of the general meiofauna. One of the forums created by the IAM is RoMeio (Reducing environments & Meiofauna), which was brought about with the aim of exchanging and enhancing the knowledge on meiofauna from chemosynthetic deep-sea environments. In particular, within this platform, a forum for exchanging information on taxonomy (traditional and molecular) and ecology of meiofauna from shrinking environments has been created. Another important platform is the MarBEF (Marine Biodiversity and Ecosystem Functioning), which integrates and disseminates knowledge and expertise on marine biodiversity, with links to researchers, industry, stakeholders and the general public. Among the projects covered by MarBEF there is MANUELA (Meiobenthic And Nematode biodiversity Unravelling Ecological and Latitudinal Aspects) that has three main aims: to integrate the fragmented information on the dynamics and functional role of meiofauna; to improve understanding of how the activities of meiobenthic organisms, population dynamics and community assemblages are linked to ecosystem processes; to facilitate meiobenthic research within the MarBEF community and stimulate the interest in meiobenthology. However, these projects are mainly focused on the general meiofauna. Therefore, in order to promote nematodes as biological indicators, further actions should be carried out. An event more focused on nematodes and their use as bioindicators has been organized by the Association of Applied Biologists: the International Symposium on nematodes as environmental bio-indicators (5-6 July 2012 at Ghent University, Ghent, Belgium). The primary 'raison d'être' of this Symposium was to enhance the dissemination of ecological importance of nematodes in freshwater, terrestrial and marine ecosystems. It was also designed to understand the underlying mechanisms leading to nematode community changes. However, as Schratzberger (2012) pointed out: "*Meiobenthic research published between 2007 and 2011 has failed to underpin ecosystem management and conservation practices. This is partly because of the belief amongst decision-makers and the public that microscopic organisms beyond our normal range of perception are ecologically unimportant. Methodological limitations related to the taxonomic identification of small-sized organisms and the narrow scope of many contemporary meiofauna studies are also to blame."* Thus, we suggest other more targeted actions to overcome these limits such as the forum created by the benthic foraminifera specialists, who have created the FOBIMO (FOraminiferal BIo-MOnitoring, Schönfeld *et al.*, 2012) initiative not only to create international protocols for monitoring studies, but also to develop a suit of actions that could promote the use of this meiobenthic component in ecological assessments. However, an additional step could probably be taken. Nematode taxonomists and ecologists, with very few exceptions, work independently from one another. Experienced taxonomists (classic morphologists or molecular supporters) and ecologists have to act as a *community* and to reach common aims: the promotion of the nematode use in new environmental policies and the fostering of increased public awareness of the small-sized organisms. In the era of the WFD and MSFD, stronger collaboration among nematologists is the key in changing our future perspectives and intensifying the impact of our research in society.

Conclusions

Over the last two decades, Italian research has contributed significantly to the advancement of our understanding of marine nematode response to human impact. Italian research has certainly contributed to the achievement of this objective with highly focused local investigations on specific stressors, but also to a large extent with wider analyses aimed at finding new and valuable tools for monitoring programs and useful nematode descriptors in line with European Directives. Although many guidelines have still to be developed and enhanced, nematodes are certainly one of the most promising candidates within the benthic domain to assess the EcoQ of marine ecosystems. Both Italian and foreign research highlight the good efficiency of the taxonomic structure, MI and c-p classes as tools in the monitoring assessment. However, their implementation should be carried out in order to obtain standard protocols for the definition of the EcoQ of the marine ecosystems. In order to reach this goal, more synergistic actions should be carried out. For example the *community* of macrofauna specialists has made great efforts to standardise methods, develop biotic indices and provide a quantitative measure of the state of marine ecosystems. Within the meiobenthic *realm* the foraminifera specialists are a good example of what we should follow as nematologists to increase the impact of our research in monitoring programs and in the collective opinion in general. Thus, nematode researchers should probably work more as a *nematologist community* in order to better promote the use of nematodes as EcoInds in the era of the Water Framework Directive and Marine Strategy Framework Directive.

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