



## Original Articles

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

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## ABSTRACT

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

## 1. Introduction

Oceans represent a major source of goods and services for the human wellbeing (Costanza et al., 1997, 2014) and have long been considered a limitless source of food, energy and benefits (Costanza, 1999). Nevertheless, although the role of the oceans in sustaining human life is widely accepted, the human exploitation of the oceans' resources is increasingly rising beyond acceptable limits, causing a loss in biodiversity, altering ecosystems characteristics and functioning (Halpern et al., 2008, 2015; Worm et al., 2006; Rockström et al., 2009).

To limit biodiversity loss and preserve ecosystem goods and services in coastal areas (or to identify priorities for their ecological restoration),

several directives and legislations have recently focused on the analysis of the ecological quality status of estuarine, coastal or off-shore environments. Among these, after the Water Framework Directive (WFD, 2000/60/EC) in 2000, the European Parliament and the European Union Council enacted in 2008 the Marine Strategy Framework Directive (MSFD, 2008/56/EC), as part of the Integrated Maritime Policy (IMP) adopted by the European Commission in 2007. Through implementing environmental Directives, the European Union has moved towards coordinated and integrated catchment-to-coast management, following the most recent legislation calling for the worldwide application of ecosystem-based approaches to the management and conservation of nature and its resources. The MSFD establishes a

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framework for the development of strategies designed to achieve the Good Environmental Status (GES) in the marine environment, by the year 2020, using 11 qualitative descriptors (biodiversity, non-indigenous species, exploited fish and shellfish, food webs, human-induced eutrophication, sea-floor integrity, hydrographical conditions, contaminants, contaminants in fish, marine litter and introduction of energy/noise; (MSFD, 2008/56/EC)). The MSFD directive is based upon an ecosystem-based approach, with a holistic view on the management and protection of marine ecosystems (Nicholson and Jennings, 2004; Apitz et al., 2006; Borja et al., 2008), focusing on ensuring sustainable use of the seas, and providing safe, clean, healthy and productive marine waters.

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

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

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

At the same time, the assessment of the environmental quality status in the Adriatic Sea still largely depends upon the indicators and tools (e.g., biotic component) utilized, so that it requires the simultaneous use of a wide range of ecological indicators (Uusitalo et al., 2016). Macrofaunal biodiversity, for instance, whose ecological traits have been widely associated to environmental alteration, is commonly utilized for the classification of the ecological status of marine benthic ecosystems (Borja et al., 2008). Nevertheless, more recently, meiofauna, due to their high diversity and standing stocks, high turnover rates and lack of larval pelagic dispersal, have attracted increasing attention as a tool for detecting anthropogenic impact and for ranking the environmental quality status of different marine ecosystems (Danovaro et al., 1995, 2000, 2009b; Mazzola et al., 1999, 2000; La Rosa et al., 2001; Mirto et al., 2002, 2010, 2014; Frascchetti et al., 2006, 2016; Pusceddu et al., 2007, 2011, 2014a, 2016; Gambi et al., 2009; Moreno et al., 2011; Alves et al., 2013, 2015; Bianchelli et al., 2010, 2016a,b). Meiofauna, in fact, are very sensitive to environmental disturbances, particularly to organic enrichment and eutrophication (Bianchelli et al., 2016a), at temporal scales much narrower than those generally exhibited by macrofauna. Previous studies, indeed, highlighted the influence of changes in the trophic status of marine sediments on the meiofaunal biodiversity under different environmental conditions and ecological alteration (Pusceddu et al., 2007, 2011; Bianchelli et al.,

2010, 2013, 2016a). Such a relationship is not consistently positive, as the pattern of meiofaunal biodiversity responses varies depending upon the levels of the benthic trophic status (Pusceddu et al., 2007; Bianchelli et al., 2016a).

Among meiofauna, nematodes typically represent from 50 to over 90% of the total meiofaunal abundance; they are cosmopolitan and their distribution, especially in coastal environments, is strongly influenced by the local environmental characteristics (Merckx et al., 2009). Nematodes are characterized by high levels of structural (i.e., species richness) and functional (trophic) diversity (Balsamo et al., 2010; Moreno et al., 2011; Semprucci and Balsamo, 2014). Due to these characteristics, they have been utilized as indicators of a plethora of different environmental disturbances (Danovaro and Gambi, 2002; Steyaert et al., 2007; Moreno et al., 2008, 2011; Neher and Darby, 2009; Mirto et al., 2014; Pusceddu et al., 2014a; Hannachi et al., 2016): they, for example, are sensitive to hydrocarbon contamination (Danovaro et al., 1995; Mahmoudi et al., 2005; Losi et al., 2013) and organic enrichment (Essink and Keidel, 1998; Frascchetti et al., 2006; Moreno et al., 2008; Gambi et al., 2009), including biodeposition from aquaculture activities (Duplisea and Hargrave, 1996; Mazzola et al., 2000; Mirto et al., 2002; Vezzulli et al., 2008). In particular, previous studies have reported that the amount and the nutritional quality of sedimentary organic matter may affect nematodes biodiversity, and more specifically their taxonomic composition (Moreno et al., 2008; Semprucci et al., 2014, 2015a,b; Bianchelli et al., 2016b).

The aim of this study was to investigate the possibly to use nematode biodiversity and benthic trophic status as simple and reliable indicators of the environmental quality of marine coastal ecosystems. In order to achieve this objective, this study was carried out to analyse the spatial-temporal variations in structural and functional biodiversity of free-living nematodes in the coastal North-Western Adriatic Sea in relation with benthic trophic status (in terms of organic matter sedimentary contents and biochemical composition) and several environmental stressors (seasonal tourism, maritime transport associated with the presence of an oil refinery and river discharges). More specifically, we tested the null hypothesis that nematode assemblages (in terms of structural and functional biodiversity) do not vary among sampling times and sites characterized by the presence of different levels of environmental impacts and sedimentary trophic status.

## 2. Materials and methods

### 2.1. Study areas and sampling

The study area is located in the North-Western sector of the Adriatic Sea, where we considered three coastal sites along the Marche Region coastline (Fig. 1A), at ca. 6 m water depth, subjected to different natural and anthropogenic stressors: Senigallia (maritime traffic and riverine inputs), Falconara (riverine inputs and the presence of a petrochemical industry) and Portonovo (tourism and maritime traffic, Site of Community Importance). Detailed descriptions of the 3 investigated sites are given elsewhere (Bianchelli et al., 2016a) and reported in Table 1.

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

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

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