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FREE-LIVING MARINE NEMATODES COMMUNITY STRUCTURAL CHANGES WITHIN A POST-DREDGING SITE AT THE ROMANIAN SHELF

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Abstract. The paper presents the results of assessment of nematodes community diversity and quantitative structure within an area heavily affected by dredging activities performed in the summer 2016 in a perimeter of 2.7 km^2, at depths between 23–27 m within the circalittoral habitats of the Romanian shelf. The nematodes composition changes after almost three years since the dredging cessation were investigated within the direct impacted area where the physical disturbance of sediments is still clearly detectible and in a reference site. Our results show that the recolonisation process of the impacted area is still ongoing, a lower diversity being noted in the centre than at the fringes of the investigated perimeters. In terms of abundance, the community distribution did not exhibit a clear pattern, a trending biased by substrate type and macrofauna influence being assumed. The attempt of evaluating the ecological quality using Bongers Maturity Index (MI) based on changes underwent by nematodes community reflected the physical disturbance of habitats in general, allowing us to assess the meiobenthic population resilience after such events. In addition, the MI showed a good evidence of the organic pollution and contamination.

Keywords: Romanian Black Sea shelf, free-living marine nematodes, structural changes, post-dredging impact, ecological indicators.

AIMS AND BACKGROUND

Several authors pointed out to the meiobenthos and especially to nematodes as potential indicators of physical disturbance in marine ecosystems[1–3]. A comparative study showed that nematode and macrofauna should be used together in monitoring surveys due to their complementary responses regarding environmental status, which may be explained by different response to stress times of each benthic community[4]. Nematodes are generally considered to be more resilient to physical disturbance than the larger macrofauna[5], being able to better reflect the short to medium term changes, as result of their enhanced turnover rates and of their capacity to recolonise adjacent disturbed patches by actively dispersing vertically

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and horizontally through the sediments[6]. However, the evidences on meiobenthic communities' response to physical disturbance are not consistent; data available to date indicate either a minor[7], negative[8] or even a positive effect[9] of trawling on nematodes. Nematode abundance, production, species richness and diversity decreasing were among the negative effects observed, while a possitive effect was seen in abundance of some species. At the Black Sea level, Urkmez[10] found a good relation of functional diversity of nematodes with the quality status at a Turkish coastal site influenced by anthropogenic pollution.

In the period 2015–2016, large scale sand extraction (2.7 km^2 area) has been carried out at the Romanian littoral within the framework of the project 'Protection and rehabilitation of the Southern part of the Romanian littoral in front of Mamaia, Constanta and Eforie Nord', aiming to protect the front beaches against accelerated erosion by artificial nourishment with sand extracted from the sea. After almost three years since dredging cessation, the physical disturbance of substrate is still visible, deep traces left by dredge operation being identified by means of geophysical investigation. The aim of the current study was to identify the magnitude and level of response of nematodes to the impact produced by dredging (strong siltation of sandy sediments, displacement of sediments, organic enrichment) by employing a comprehensive analysis of their community in terms of composition and life strategies.

EXPERIMENTAL

The meiobenthic samples were collected in July 2018 on board of the R/V 'Mare Nigrum' from six stations (Fig. 1) selected from within and around the dredging area, which is located at depths between 23–27 m within the circalittoral habitats of the Romanian shelf, on a transversal profile to Constanta town. The sampling was performed by cutting off the sedimentary material from the surface of Van Veen by helping of a cylinder of 5 cm^2 area. Immediately after collecting, the samples were preserved in 4–5% formaldehyde seawater buffered and stored in plastic recipients until laboratory analysis. About 200 nematodes (or all individuals if less than 200 were present) were picked at random transferred to glycerine and mounted on slides. For identification there was used the pictorial keys[11,12], NeMys online (http://nemys.ugent.be/)[13]. The number of nematodes was given at 5 cm^2. Taking into account the low number of harpacticoids found in the samples, these were not quantitative estimated but qualitatively. The statistical analysis was carried out by helping of available free software PAST v. 3 (Ref. 14). The Maturity Index (MI)[15] was calculated to measure the impact of disturbances and to monitor changes in the structure and functioning of nematodes assemblages. Based on their specific characteristics, all nematode genera were distributed along a coloniser-persister (c-p) scale. The MI was calculated as the weighted mean of the individual taxon scores. 36 bathymetric profiles using the Elak Nautik SeaBeam Multibeam Echo Sounder Systems 1050D at 50 kHz frequency were performed in the dredging area (Fig. 1) in order to reveal the marks left. The bathymetry transverse profile through the dredged area displayed at the bottom of the bathymetry map shows the furrows left (0.5 to 3 m deep).

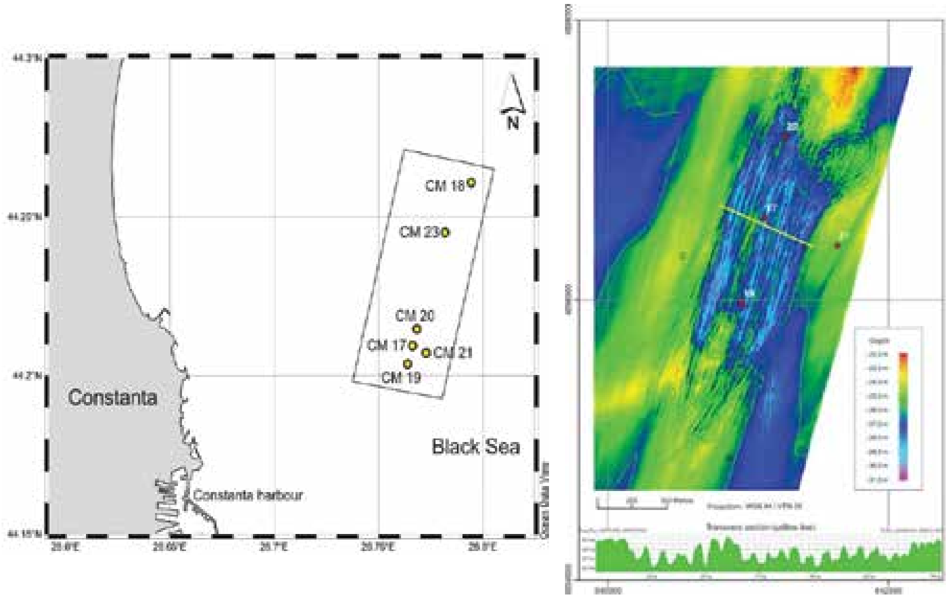

Fig. 1. Sampling sites (right), the bathymetry map and transvers profile through the dredging site (yellow line) (left)

RESULTS AND DISCUSSION

The nematodes within the analysed perimeter was characterised by a relatively low diversity with an average of 15 nematodes taxa (min. 9 – max. 21) belonging to 19 families. The first ranked species after indices of ecological significance (W) and their total abundance (Ab), frequency (*F*%), average density (D_{avg}) and dominance after abundance (*D*%) are displayed in Table 1. The abundances varied between 832 and 128.512 ind.5 cm^{-2} (Table 2).

The nematodes distribution showed an ongoing recolonisation and recovery process of populations within the impacted site, much slower within the inner mixed (mud and sand) habitats (middle of the area) (station CM 17) than in the sandy mud ones situated at its extremities (stations CM 19 and CM 20). Thus, as low as 9 taxa and 3072 ind.5 cm^{-2} were found in the station 17, where *Microlaimus* sp. with 1344 ind.5 cm^{-2} and *Axonolaimus ponticus* with 736 ind.5 cm^{-2} dominated. Comparatively, a higher diversity was recorded within CM 19 and CM 20, with

15 and 21 taxa, respectively. In both stations, *Microlaimus* sp. made up to 25–35% as abundance, being present also in the entire study area, reaching a maximum in the reference station CM 18 (muddy sediments), located in the north eastern part of the perimeter.

Table 1. First eight ranked species after indices of ecological significance (W)

Species	Ab	*F* (%)	Davg	*D* (%)	W
Mesacanthion conicum (Filipjev, 1918) Filipjev, 1927	1792	83.33	358.40	0.98	9.05
Sabatieria abyssalis (Filipjev, 1918)	47360	100.00	7893.33	25.96	50.95
Terschellingia longicaudata de Man, 1907	24560	100.00	4093.33	13.46	36.69
Axonolaimus ponticus Filipjev, 1918	32928	100.00	5488.00	18.05	42.48
Paracanthonchus sp. Micoletzky, 1924	1776	83.33	355.20	0.97	9.01
Sphaerocephalum crassicauda Filipjev, 1918	2528	100.00	421.33	1.39	11.77
Sabatieria pulchra (Schneider, 1906)	3424	100.00	570.67	1.88	13.70
Microlaimus sp.	57008	100.00	9501.33	31.24	55.90

After *S. abyssalis*, the second ranked species after abundance (26%), *A. ponticus* attained almost 20% of total abundance, both species showing a higher preference for the muddy sand sediments. Distribution of the latter seems to be related to the changes underwent within the dredging area, very low densities being accounted in stations CM 19 (96 ind.5 cm^{-2}) and CM 17 (736 ind.5 cm^{-2}) comparative with the one of the station CM 18 (20480 ind.5 cm^{-2}). On contrary, the higher diversity (21 taxa) within the station CM 20 points out to a rapid occupation from the nearby not impacted area of the new habitat niches, consisting of a mix of mud and sand. Should be remarked the presence only in this station of the araeolaimid species *Campylaimus ponticus* and of the enoplid *Leptosomatum sabangense,* though in low number. In the same station, the highest abundance (960 ind.5 cm^{-2}) of the enoplid *M. conicum* has been noted, although pretty numerous populations within the muddy sand habitats were recorded in the station CM 23 as well (480 ind.5 cm^{-2}), situated at some distance away from the impacted area. Similarly, *Dichromadora* was predominantly found in the above mentioned stations. In two of the impacted stations (CM 19 and CM 20), there were found harpacticoids belonging to the genus *Enhydrosoma* and *Amphiascus,* known for their tolerance to disturbance and organic enriched sediments. The poorest community was estimated in the station CM 21, situated eastward of the impacted site, consisting of 832 ind.5 cm^{-2}. Nevertheless, 18 taxa were identified here, among which *M. conicum* reached up to 31% of the total abundance. Instead, there was evinced the weak presence of *Microlaimus* and *A. ponticus*, probably as result of higher fraction of shells.

The non-parametric Kruskal–Wallis test ($p < 0.05$) followed by a Mann–Whitney pairwise test with Bonferroni corrected p-values (after a Shapiro-Wilk normality test was applied to samples distribution) performed revealed spatial significant differences of density ($p < 0.05$) between the samples CM 17, CM 19 and CM 21 and the samples CM 20, CM 23. The differences mainly reflect the spatial gradient of disturbance level, higher in the middle than at the fringes of the dredged area, but also the effect of substrate type.

The estimation of magnitude of impact of dredging activities based on the Bongers Maturity Index (Table 2) revealed a good ecological status within the stations CM 21 and CM 23, a moderate status in the stations CM 19 and CM 20, while the worst situation was found in the station CM 17 and CM 18. In general, a quite clear differentiation of the status between the reference and the dredging impacted sites was shown, excepting the station CM18 due to overwhelming abundance of c-p 2 colonisers (the Comesomatidae, Axonolaimidae and Microlaimidae representatives) as opposite to the station CM 21, where these were encountered in low amount. In general, the c-p 4 and 5 persisters were scarce, only five species belonging to the families Oxystominidae, Leptosomatidae, Enoplidae, Desmoscolecidae and Pandolaimidae being recorded. The thresholds between the ecological statuses were set at 2.00 in between bad and moderate and at 2.20 in between moderate and good, respectively. The thresholds values proposed by Moreno[16] for the Mediterranean Sea and discussed by Urkmez[10] could not be used in the present paper, mostly as result of different habitats type studied. Our study addressed to nematodes within the Romanian circalittoral habitats, populating the mud and sandy mud substrates. It is expected that ecological status classes to have values lower than those proposed for the Mediterranean or the Black Sea coastal area.

Table 2. Species richness, total abundance (ind. 5 cm^{-2}) and calculated MI for each station

Stations	CM19	CM20	CM17	CM23	CM21	CM18
Species richness	15	21	9	20	18	11
Abundance (ind. 5 cm^{-2})	1.840	27.520	3.072	20.688	832	128.512
MI = $\sum$(c-p value*f_i)	2.09	2.12	1.57	2.43	2.45	1.77

MI – the Maturity index (calculated as the weighted mean of the individual taxon scores), vi = coloniser-persister (c-p) value assigned to genus, f_i – frequency of genus i in sample.

RESULTS AND DISCUSSION

Based on six samples collected within an impacted (3 stations) by dredging and within a reference site (3 stations), our study revealed the physical disturbance and the response of nematodes community in terms of changes of qualitative, quantitative and functional composition. After almost three years since dredging cessation, the physical disturbance of habitats was still clearly detected by the geophysical investigation. The initial sandy habitat has underwent almost a complete topographic and granulometric restructuration. Both siltation and the roughness of the landscape increased. This strong perturbation affected profoundly the macrofauna, but also the nematodes, as shown in this paper. Nematodes species richness varied from 9 in the CM 17, located in the middle of the dredged perimeter to 20 and 21 species, respectively at the fringe of it, but still inside the dredging area (CM 20) and within the reference site, in the station situated at 3.6 km northward (CM 23). The abundances were also affected, these being among the lowest in the stations CM 19 and CM 17. However, the spatial distribution of dominant r-strategist taxa (*Microlaimus* sp., *A. ponticus*, *S. abysallis*, *T. longicaudata*), and their abundances witnessess for a slightly reconolisation process or recovery of former populations. For comparisation, k-strategist enoplids (*M. conicum*, *E. littoralis)* were accidentally found in these stations comparing to CM 23. The colonisers (c-p 2) presented in general, a larger proportion of eggs carrying (pregnant) females (e.g. *A. ponticus*, *S. abyssalis*), males and juveniles, while most of the females of *Paracanthoncus* (present in all samples, excepting CM 17) carried offspring. The Maturity Index showed a quite clear differentiation of the ecological status between the reference and the dredging impacted sites. The ecological status in the station CM18 reflected the overwhelming abundance of c-p 2 colonisers (the Comesomatidae, Axonolaimidae and Microlaimidae representatives) as opposite to the station CM 17. Among all, the station CM 18 evinced a higher contamination level with hydrocarbons such as pyren and fluoranten and also with organic matter. Hence, in this case, MI was able also to discriminate with high accuracy the nematodes response to contamination. The diversity and abundance proved also potentially effective in detecting changes in the post-dredging trawling site.

CONCLUSIONS

Lately, the studies dedicated to meiobenthic populations, in general, and the nematodes, in special, have paid more attention to their role as effective indicators of pollution and physical disturbance of habitats[17], boosting the research and application in environmental status assessment according to the The Marine Strategy Framework Directive, especially for the pressure descriptors (6 and 8).

Our study is consistent with the results provided by other similar publications[18,19] in terms of effects observed, in spite of inferred different natural frame and operational conditions. Nevertheless, it is recognised that further knowledge of the functional roles of nematode will be the key to improve the sensitivity and interpretation of biological traits analyses of benthic communities[20]. Many studies revealed that the Maturity Index is not always able to discriminate the different stress intensities or categories. It has been observed[21] that MI best detects the sites affected by different level of disturbance (low from high), especially by organic pollution, but less efficient when coming to discriminate among similar impacted sites. In terms of nematodes response to physical stress it is considered that they are well adapted and many studies[22] confirm this, the reason why the c-p scaling and MI are seen as useless tools in ecological assessment of physical disturbance. However, the MI could be enough confident to reveal the follow-up effects of dredging on the meiobenthos and nematodes, in special. The most important effects are siltation, changing of granulometry of sediments and not ultimately, increased bottom water shear stress due to changes of sediments arrangement (furrows). Since the nematodes are able to penetrate the sediments down to greater depths (e.g. 10 cm[23] or deeper), their habitat is drastically affected in case of physical disturbance. In our case study, the difference in ecological status evinced by the MI between sites with comparative contamination level may support the hypothesis of cummulative dredging and pollution effect on nematodes communities, especially on some k-strategist species.

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