

THE FREE-LIVING NEMATODE COMMUNITY STRUCTURE WITHIN THE ROMANIAN CIRCALITTORAL HABITATS

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Abstract. The paper presents an assessment of nematode community structure and diversity performed in May 2018 within two habitats of the Romanian circalittoral zone. The differences in nematodes distribution as a result of Danube's influence are also highlighted. Tolerant species to organic enrichment dominated the population structure within the terrigenous mud habitat with *Melinna palmata*, while a gradual decreasing of their abundances with habitat changing to mixed sediments with *Dipolydora quadrilobata* and with depth increasing was recorded. In total, 28 taxa belonging to 16 families were identified, of which 16 on mud and 22 on mixed sediments, respectively. The nematode trophic structure dominated by tolerant deposit feeders (84%), similar to the Maturity index (ranged values 0.18 – 0.73), evinced an overall poor ecological quality status of both habitats, which represent over 70% of the two Natura 2000 protected areas located within the perimeter, the Delta Dunării – Marine zone and The South Lobe of *Phyllophora* Field of Zernov.

Key words: Circalittoral habitats, free-living marine nematodes, ecological indices, Danube plume area.

1. INTRODUCTION

Meiofauna plays significant roles in ecosystem being effective in cryptobioturbation, which has far-reaching effects on sediment characteristics and stability, microbial grazing and faecal pellet production, nutrient cycling and meiofauna - macrofauna interactions (Moens and Beninger, 2018). On the other hand, several authors highlighted the changing of abundance, richness and structure of the meiobenthic assemblages with environmental parameters, especially with organic matter content (Mahmoudi *et al.*, 2008; Losi *et al.*, 2013; Kandravicius *et al.*, 2018, Moreno *et al.*, 2011; Danovaro *et al.*, 2000), food availability (Rudnick and Oviatt, 1986), sediments structure and the presence of specific pollutants (Austen and Somerfield, 1997; Dalto *et al.*, 2006; Beyrem *et al.*, 2007; Hermi *et al.*, 2009; Bastami *et al.*, 2017). Due to ability of deposit feeder nematodes to thrive in organic- matter rich sediments, these are regarded as good indicators of eutrophication induced by riverine inputs (Semprucci *et al.*, 2010a). Ürkmez *et al.*, 2014 found also a good relationship of composition of functional groups of nematodes (k and r –strategists) with the percent of organic

matter in sediments. For these reasons, nematodes have been proposed within the Water Framework Directive (Moreno *et al.*, 2011) as an indicator for assessing the ecological quality of marine ecosystems. In the context of existence of two Natura 2000 protected areas, the ROSCI 0066 – Delta Dunării – Marine Zone and the ROSCI 0413 - The South Lobe of *Phyllophora* Field of Zernov, which overlap the study area, an attempt for using nematodes as indicators of ecological status of the area was done.

1.1. STUDY AREA

The study area (L35 – 120B) was located at depths ranging between 27.8 and 43.9 m in the W-SE direction in front of Sfântu-Gheorghe mouth, within the circalittoral zone of the Black Sea, under direct impact of the Danube's plume. When entering into the Black Sea, the Danube brings about 20–30% organic matter, thin particles being carried out with the freshwater surface layer, while larger and heavy particles sink down to the bottom (Berlinsky *et al.*, 2005), thus influencing surface seawater, sediments as well as all compartments of benthic and pelagic food webs

(Bănanu *et al.*, 2007). Moreover, significant sedimentation of biogenic material derived from lysed freshwater diatoms and increased bacterial activity stimulated by the supply of labile organic matter (Ragueneau *et al.*, 2002) strongly affect the biogeochemical processes of the circolittoral sediments within the plume. The average concentration of total organic carbon in the surface sediments in the study area amounted to 1.28%, to 1.64%±0.42 in the muddy circolittoral habitat and to 1.052%±0.36 in the mixed one, respectively.

The two major habitats in the area are shaped by two dominant polychaete species. The tube-dwelling, surface deposit-feeder, ampharetid polychaete, *Mellina palmata*, which thrives in sediments with silt-clay contents that exceed 20% (Dauvin *et al.*, 2007), reached in the studied area densities up to 1,202 indiv. m⁻², forming the *Muddy habitat with M. palmata* (Teacă *et al.*, 2019 *in press*). The species is about 3 to 4 cm in length, making a mucus-lined tube from which it can protrude in order to spread the tentaculate palate over the sediment surface (Băcescu, 1984 in Olafsson *et al.*, 1990). When not feeding, the location of the worm is indicated by a narrow hole (approximately 5 mm in diameter) which is separated from the faecal mound (Olafsson *et al.*, 1990). *Dipolydora quadrilobata* is also a surface deposit-feeder tube-dwelling species occurring at depth range between 35-65 m, where it forms the *Mixed sediments habitat with D. quadrilobata and Mytilus galloprovincialis*. *Dipolydora's* tubes are straight, up to 40 mm long and 1.5 mm wide, built of fine sand grains bound by silt and detrital particles (Surugiu, 2012). Its average density population in the habitat reached 7,550 indiv.m⁻² (Teacă *et al.*, 2019 *in press*).

2. MATERIAL AND METHOD

In the 21-30 May 2018 interval, on board R/V Mare Nigrum, a multicore Mark II equipped with collecting tubes of 10 cm opening diameter was employed for sampling the meiobenthos. Following the observations on sedimentary composition, ten meiobenthic samples have been selected as to reflect the biotope/habitat specific structural nematode community. Thus, 4 samples out of 10 within the muddy sediments and 6 within the mixed ones were collected, within the perimeter L35-120B (named according to international nomenclature) stretching over 295 km² (Fig. 1). At each sampling point, one core was selected to extract the top 5 cm of sediments for further nematodes analysis. The samples were primarily sieved through 125 µm to eliminate the excess of sediments, and the remaining material was conveyed in plastic jars with formalin 10% for preservation. In laboratory, the samples were washed again through 90 µm sieve and 100 – 120 individuals from each sample were randomly selected, transferred using the formalin-ethanol-glycerol technique of Seinhorst (1959) and mounted on slides in a drop of anhydrous glycerol. For taxonomic identification Platt & Warwick (1983, 1988), NeMys online (<http://nemys.ugent.be/>) (Steyaert *et al.*, 2005) were used. The abundance of species was given as total number of individuals of

species in each subsample. The structure of the nematode community was analysed in terms of species richness (S), abundance, the Shannon-Wiener diversity index (H') (Krebs, 1989), dominance and trophic structure. The statistical analysis was carried out by help of of available free software PAST v. 3 (Hammer *et al.*, 2001). The Maturity Index (MI) (Bongers *et al.*, 1991) was calculated to measure the changes in the structure and functioning of nematode assemblages in the two habitats under the Danube's influence. Based on their specific characteristics, all nematode genera were distributed along a colonizer-persister (c-p) scale. The MI calculation is based on the frequency and abundance of families/genus belonging to colonizers (c) and persisters (p) categories (c-p value for each family/ genus has been set by Bongers (1990) after life strategy of nematodes: short life cycle, fast turn-over, characteristic to colonizers, and those with opposite life traits). The c-p takes values from 1 to 5 (from colonizers to persisters). The MI represents the weighted mean of the individual taxon scores. The lower the MI value the higher the level of environmental perturbation. All identified individuals were grouped into four feeding type groups (selective deposit feeders (1A), non-selective deposit feeders (1B), epigrowth feeders (2A), and predators/scavengers (2B)) following Wieser (1953). The nomenclature of species was checked according to the World Register of Marine Species (www.marinespecies.org).



Fig. 1. Meiobenthic samples location within the L-35-120B perimeter

3. RESULTS AND DISCUSSIONS

Totally, 28 taxa belonging to 16 families have been identified, of which 16 taxa on mud and 22 on mixed sediments. The species of Comesomatidae and Linhomoeidae families were the most diverse and abundant, being represented by 3 and 6 taxa, respectively and more than 70% dominance after

abundance. The results of the similarity dendrogram based on the Bray Curtis analyses of not-transformed abundances of the nematode species showed formation of two distinct characteristic communities for the habitats within the area (Fig. 2). The greatest contribution (65.81% after abundance) to the community had the cosmopolitan species *Sabatieria abyssalis* and *Terschellingia longicaudata*, which accounted for a similarity of about 50% between the two habitats. Usually, these inhabit a wide range of sediment types. *S. pulchra* was present only on muddy sediments. Besides *T. longicaudata*, among Linhomoeidae there were identified *Linhomoeus* sp., *Sphaerocephalum crassicauda* and *Megadesmolaimus*, the latter a new genus recorded at the Romanian littoral. Out of them, *Linhomoeus* and *Megadesmolaimus*, were found only on mixed sediments, while the other species showed a similar distribution of their abundances in both habitats. *Axonolaimus setosus* (41.3%) was the second dominant species within the community structure of the muddy habitat, while *Linhomoeus* closely followed by *Bathylaimus cobbi* within the mixed one. Nevertheless, a series of less abundant species contributed at increasing diversity in both habitats. Among these, it is worth mentioning: *Paramonhystera elliptica*, *Eleutherolaimus longus*, *Daptonema* sp., *Odontophora angustilaima*, *Desmolaimus* sp.. Excepting *O. angustilaima*, these recorded low frequencies in the area. In general, the abundances and diversity of strategist species (e.g., *Halalaimus* sp., *Oxystomina clavicauda*, *Sphaerolaimus ostreae*, and *Halanonchus bullatus*) were pretty scarce. This could be related to strategists' lower capacity to withstand the enrichment conditions induced by the riverine input within these habitats.

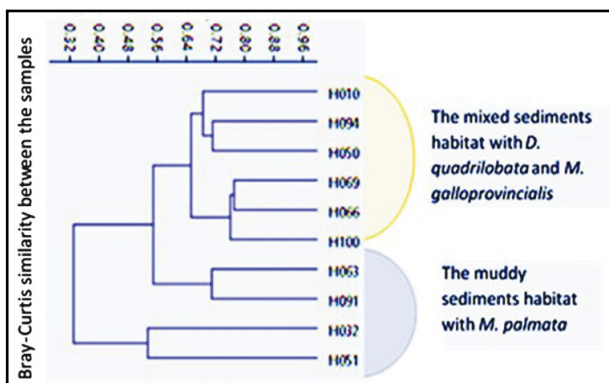


Fig. 2. Bray-Curtis similarity between stations based on the not-transformed nematodes abundance data (right: the two circalittoral habitats identified within the perimeter according to Teacă et al., 2019 in press)

Overall, the non-selective deposit feeders dominated as diversity and abundance (15 species; 84%) (Fig.3). Nevertheless, differentiated distribution of species with similar trophic preferences was noted. For example, the species of non-selective deposit feeders' Axonolaimidae and Comesomatidae seem to avoid overlapping their habitat niche. *A. setosus* was the most abundant in the shallower stations 32 and 51, while in the deeper station 63 and in the one

at the limit with mixed sediments (station 91), its abundances were several times lower. In turn, *O. angustilaima* recorded higher abundances in the stations situated in deeper part of the *Dipolydora quadrilobata* habitat. The Comesomatidae species, *S. pulchra* and *S. longicaudata* reached higher populations on the muddy substrate, while *S. abyssalis* as going deeper towards the mixed substrate. Differences in habitat preferences were also noticed in number and abundance of strategist selective deposit feeders. Thus, out of 7 species, only three of them were common to both habitats. *T. longicaudata* accounted for 65% of the populations within this category. Among other species, *Halalaimus* sp. as opposite to *O. clavicauda*, was mostly found within the mixed habitat in the stations situated in deeper part. Five predator species have been identified within the study area, of which *V. elongata*, *Sphaerolaimus ostreae* and *Mesacanthion conicum* displayed a slight increase in population within the deeper stations of the mixed habitat. Similar to other authors' results (Steyaert et al., 1999, Semprucci et al., 2010b), our study evidenced also an increase in the biodiversity of nematodes in parallel with the grains size of the sediment.

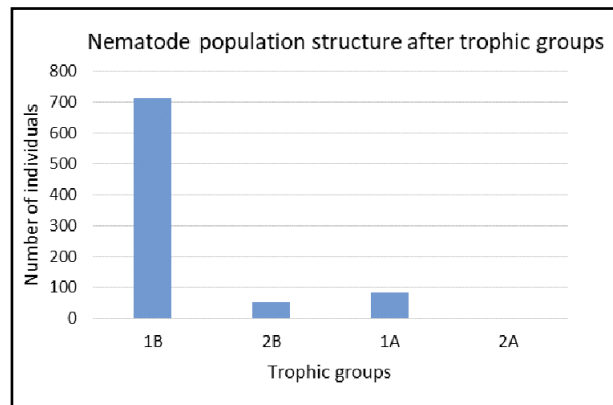


Fig. 3. The trophic structure of nematode populations within the circalittoral habitats of the L-35-120B (selective deposit feeders (1A), non-selective deposit feeders (1B), epigrowth feeders (2A), and predators/scavengers (2B))

3.1. ECOLOGICAL QUALITY ASSESSMENT BASED ON THE NEMATODES' COMMUNITY STRUCTURE

Initially developed to assess the condition of terrestrial and freshwater habitats based on the ecological characteristics of nematode taxa, the Maturity Index (MI) was extended to assessing the condition of marine and brackish sediment (Bongers, 1990). It is usually used to assess the effects of organic enrichment and chemical pollution on the environment. According to MI, the ecological status of both circalittoral habitats was *Bad*, a value of 0.32 for the mud habitat and 0.35 for the mixed one, being calculated. The nematode Shannon Index (H') as well as the percentages of c-p 3 - 5 had also low values, falling within the Moderate, Poor and *Bad* ecological classes, respectively (Table 1).

Table 1. Assessment of ecological status of the two circalittoral habitats based on the nematode population indices (red: Bad Ecological Status (EQ); orange: Poor EQ; green: Good EQ; Blue: High EQ)

Habitat	EcoQ classes	Ecological status	Circalittoral mud with <i>M. palmata</i>	Circalittoral mixed sediments with <i>D. quadrilobata</i> and <i>M. galloprovincialis</i>
Maturity Index	>2.8	High	0.32	0.35
	2.8≤MI<2.6	Good		
	2.6≤MI<2.4	Moderate		
	2.4≤MI<2.2	Poor		
	≤2.2	Bad		
Shannon index (H')	>4.5	High	1.86	1.74
	3.5<H<4.5	Good		
	2.5<H<3.5	Moderate		
	1<H≤2.5	Poor		
	0<H≤1	Bad		
Richness (S)	≥16	High	16	22
	16<S<12	Good		
	8<S<11	Moderate		
	4<S<7	Poor		
	≤4	Bad		
c-p 2 %	0–20 %	High	78	83
	20–40 %	Good		
	40–60 %	Moderate		
	60–80 %	Poor		
	80–100 %	Bad		
c-p 3-5 %	80–100 %	High	21	17
	60–80 %	Good		
	60–40 %	Moderate		
	20–40 %	Poor		
	0–20 %	Bad		
Overall ecological status			Poor	Poor

The indices and the thresholds considered to evaluate the EcoQ (ecological quality) classes according to Danovaro *et al.* (2004), Moreno *et al.* (2011) and Semprucci *et al.* (2014, 2015) are currently widely used to assess the changes in nematode populations in disturbed environmental conditions. Therefore, within the scope of the present work, these were deemed fit for purpose, being applied to the specific case of the Romanian circalittoral under the Danube's discharge influence.

3.2. DISCUSSION

In soft bottom marine sediments where the input of organic matter is high, the role of macro – and meiobenthos in enhancing the remineralisation process increases (Bonaglia *et al.*, 2014; Nascimento *et al.*, 2012). In front of the Danube

Delta, the circalittoral benthic communities are formed of thriving deposit feeders such as *Melinna*, *Dipolydora* and *Prionospio multibranchiata*, making up more than 70% after abundance (Teacă *et al.*, 2019 *in press*). Thus, a competition with meiofauna on resources could be inferred since, in principle, these highly efficient macrobenthic deposit feeders use the same food resource. Nevertheless, according to Gerlach, 1978 this competition is far from being real, because meiofaunal communities by their activities and by excreting metabolic end products induce a bacterial productivity which would not be there without them, and feed on it. Watzin (1983), in turn, found a competition relationship between juvenile forms of macrofauna (Spionidae, Nereididae) and meiofauna (turbellarians, nematodes) for shared space and food. Olafsson *et al.*, 1990, studying the

interaction of nematodes with *Melinna*, found that the faecal casts produced by the latter at the sediments surface had no significant effect neither on their density nor on the diversity.

Similar to other studies (Schratzberger *et al.*, 2007), our results showed a slightly increase in the number of species, genera and trait groups generally with increasing of water depth and sediment heterogeneity of coarse mixed habitat. In turn, no significant ($U = 280.5$; $p = 0.06$) increase of abundances in the same direction has been noticed. Nevertheless, the low abundances of most of the strategist species and trophic groups other than deposit feeders could be explained by the presence of patched microhabitats dominated by tube dwelling suspension and detritus feeders' polychaetes that may advantage the deposit feeders' nematodes. The higher natural unstable environmental conditions usually occurring in the shallower part of the circalittoral give place to colonizers able to "escape" by moving downward into the sediments (Comesomatidae). Hence, the nematode assemblages of silty sediments of *Melinna's* habitat as compared to *Dipolydora's* mixed sediments are relatively homogeneous from functional point of view (poorer trophic diversity).

The results of nematode indices evinced the influence of Danube on shaping the nematode community structure. The threshold values for the Mediterranean Sea suggested by Danovaro *et al.*, 2004, Moreno *et al.*, 2011, and discussed by Ürkmez *et al.*, 2014 were used in the present paper. Our study addressed the nematodes within the Romanian circalittoral habitats, heavily affected especially in the shallower part (19 – 35 m) by the Danube runoffs. The efficiency of MI and of proportion of sensitive and generally opportunistic genera for detection of stress induced by the river discharge have been previously tested by many authors (Semprucci *et al.*, 2013, Frascchetti *et al.*, 2006). Therefore, the EcoQ classes used for the Mediterranean Sea were confidently used for the similar conditions provided by the Danube. As the case of our results, in the central Adriatic Sea (Semprucci *et al.*, 2010b), the r-strategists such as *Sabatieria*, *Terschellingia* and *Viscosia*, dominated the organic matter enhanced sediments. If a comparison is done with the results of ecological status assessment based on the macrozoobenthos communities

(Teacă *et al.*, 2019 *in press*), the nematodes specific indices exhibit similar results. The ecological status is in great proportion influenced by the opportunistic tolerant species, hence the poor condition of both habitats.

4. CONCLUSIONS

Our study revealed the taxonomic structure and abundance of nematodes within two circalittoral habitats from the Romanian shelf characterized by the Danube's influence. The overall diversity was dominated by r-strategists species. Out of a total of 28 taxa belonging to 16 families, 16 have been identified in muddy sediments and 22 in mixed ones, respectively. The Comesomatidae and Linhomoeidae families dominated the species composition and abundance, being represented by 3 and 6 species, respectively and more than 70% dominance. The results of the ecological assessment based on several indices (the Maturity Index, Shannon index – H', species richness (S) and percentage of r-strategists (c-p 1 and c-p 2) and k-strategists (c-p 3 - 5 species) revealed a poor ecological status, which reflects the stress induced by river discharge. The thresholds used to classify the indices values into the ecological classes proposed by different authors for the Mediterranean Sea and applied by Ürkmez *et al.*, 2014 at the coast of Sinop Bay, Black Sea, were used in the present paper, since these have been validated in similar conditions elsewhere. Therefore, we consider that applying them for the circalittoral habitats under the Danube's plume influence is appropriate to assess their ecological status. Nevertheless, further studies should be carried out for the Romanian coastal and shelf habitats to advance the specific threshold values of indices based on nematodes.

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