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CASE STUDY ON NEMATODA TAXOCENOSIS UNDER VARIETY OF WASTE STRESS FOR FOUR STATIONS OF RIA FORMOSA LAGOON, PORTUGAL

A pilot study on meiofauna assemblages was conducted at four stations at Ria Formosa coastal mesotidal lagoon in Southern Portugal. Natural and anthropogenic factors (additional organic input, grain size composition etc.) and their impacts on sedimentary environment (oxygen depletion, hydrogen sulfide contamination) have been discussed, as well as the meiofauna response on organic matter loadings. Bottom deposits from the studied sites of lagoon indicate different levels of organic enrichment – from undisturbed to grossly polluted under periodically anoxia at the deeper sediments layers. Meiobenthos assemblages and Nematoda, particularly, respond on hypoxia and pollution stress by decreasing abundance and diversity. Harpacticoids spatial distribution was associated with their sediment particle size preference of silt-clay and avoidance the sand. Nematoda assemblages have been demonstrated very specific species composition related to environmental factors. Nematoda genera of *Anticoma*, *Leptolaimoides* and *Molgolaimus* registered only under extreme hypoxic/sulfidic sediment conditions at polluted point being tolerant to oxygen deficient and H_2S contamination. *Terschellingia longicaudata* and two species of *Paracomesoma* and *Spirinia* are reported across all studied location, widely distributed in a range of organic enriched lagoon sediments.
Key words: Nematoda, meiobenthos, organic enrichment, hypoxic/sulfidic regimes, coastal lagoon, Southern Portugal

An effect of organic inputs due to pollution and following ecosystem response to nutrient enrichment controlled by the physical and biogeochemical processes in coastal waters [10]. Estuaries and coastal lagoons are especially vulnerable to eutrophication, being the regions of restricted exchange (RRE) with the adjacent ocean and, thus, may accumulate nutrients supplied by the surrounding watershed [4, 12, 16]. It has generally been accepted that organic enrichment at the coastal sediments is a main consequence of pollution (or it caused by natural factors such as currents, waves, suspension, etc.) often induced the hypoxia at the benthic environment [6].

Ria Formosa is a mesotidal coastal lagoon in southern Portugal having the sources of treated and untreated domestic waste contribute to episodic eutrophication affecting on benthic fauna. A number of pressers, such as intensive aquaculture, urbanization etc., brings to undesirable changes occurring in the lagoon system [12]. Recently meiofauna of Ria Formosa lagoon studied quite poor. There have been only two studies [2, 10] concentrated on lagoon meiobenthos communities associated to different levels of pollution and nutrient enrichment.

The classical view of meiofauna response on organic enrichment had demonstrated [14] describing the ecological succession under heavy organic loading when macrofauna reduced or almost absent and nematodes dominated by the metazoans.

A number of studies [1, 15] provide indications that spatial heterogeneity on the scale of a few meters and that the dominant nematode species were tolerant to organic enrichment, such as *Sabatieria pulchra* Schneider, 1906 and *Terschellingia longicaudata* de Man, 1907 [1].

Hypoxia and hydrogen sulphide contamination are commonly assumed as the main stressors due to organic loadings. The most abundant meiofauna taxa are nematodes, also Foraminifera, Ciliate, Polychaeta, Oligochaeta, Crustacea, Platyhelmithes, Gnathostomulida, Kinorhyncha were reported in numerous studies [11, 8, 6]. Species-specific response of nematode assemblages reported in [1] that *Sabatieria pulchra* Schneider, 1906, *Spirinia parasitifera* Bastian, 1865,

Terschellingia communis de Man, 1888, *T. gourbaultae* Austen, 1989 and *T. longicaudata*, 1907 to be distinctive species of eutrophic sediments [5] .

Organic enrichment of the sediments can affect on benthic fauna by opposite ways. From one side, additional nutrient supply can be attractive for benthos as food source and can stimulate more abundant benthic populations [3]. From other side, microbial processes associated with degradation of accumulated organic matter at the sediments drastically changes the bottom environment. As a result, oxygen concentration at the pore water may be reduced, while hydrogen sulfide, being toxic for fauna, may be increased [9]. At grossly polluted sediments even anoxia may be registered [6] that currently important for deeper sediments layers, which are not contacted with surface oxygenated waters. Mentioned that oxygen is a primary respiration requirement for bottom animals, this may cause a fatal effect for infauna.

Our paper will focus on interactions between natural and anthropogenic factors (additional organic input, grain size composition etc.) and their consequences for sedimentary environment of coastal lagoon Ria Formosa, also the meiofauna response on organic loadings.

Materials and Methods. Field experiment as a pilot study on meiofauna communities' structure was conducted at Ria Formosa coastal mesotidal lagoon in Southern Portugal in July 2008. In total four stations – three of them from the sewage discharge sites were chosen to determine the response of meiobenthos assemblages to oxygen regime changes due to hydrogen sulfide contamination at the sediment environment. Location Ancão Basin (st. A) was chosen as baseline site. Ramalhete Channel (st. R) situated near the city waste water plant, Faro Pier (St. F) area has an urban waste outfall and Port area (st. P) has an untreated sewage outfall (Fig. 1).

Sediments from st. P had a strong smell of hydrogen sulfide, also the biofilms at the sediment surface were observed. Ramalhete Channel and Ancão Basin characterized by slow water exchange and there are the sites of shellfish farming and these sediments periodically mixed by shellfisher's diggers.

Sampling was done at one day during the low tide at lower intertidal zone. Three replicates were collected at each station. Plastic syringes with diameter 2 cm were dissected on 0 – 2 and 2 – 4 cm layers (three replicates on each layer) to investigate a meiofauna.

Fig. 1 Study area and sampling stations in July 2008 (Ria Formosa lagoon, Southern Portugal)
Рис. 1 Район исследования и места отбора проб в июле 2008 (лагуна Риа Формоза, юг Португалии)

Sedimentary environment characteristics were analyzed as follow: granulometry composition and particulate organic matter content (POM) at the sediment, redox potential (Eh) and hydrogen sulfide (H_2S) concentration at bottom water. Meiofauna samples were fixed with formalin 10%, stained by Rosa Bengal and then washed through the 63 μm sieves. After meiobenthic organisms were extracted and counted under dissection microscope, Nematodes were prepared to microscopy according to [16]. If present, 100 nematodes from each replicate were mounted for identification for the possible lowest taxonomic level using pictorial key [16], also NeMys database of Ghent University (Belgium).

Simultaneously with meiofauna investigations, the sediment samples were taken from each study site for granulometry composition by sieving according to [18] and particulate organic matter (POM) contents measurements. Graduation of sediment particles less than 0.063 mm were analyzed by Malvern Mastersizer laser diffraction particle size analyzer. POM content was accounted as loss by ignition [10]. Redox potential

was measured by Pt-electrode. H_2S concentration was determined by spectrophotometry [7].

Relationship between environmental variables and meiofauna assemblages collected from each site were described using the correlation-based principal component analysis (PCA). Correlation matrix based on standardized data, consist of H_2S concentration (μM), redox potential (Eh, mV), particulate organic matter content (POM, %), grain size composition (sand, silt and clay proportions, %) as environmental variables and mean densities (ind./0.01m^2) of total meiofauna and major taxa as biological variables.

Results. Sediment physicochemical properties. Water temperature at the different parts of lagoon was varied from 22 to 24 Cº. Salinity measured at the range from 36.2 to 36.6 ‰.

The sediment samples covered a range sandy sediments from muddy sandy gravel (12% of sediment particles <63 μm diameter) to slightly gravelly muddy sand (42% of sediment particles <63 μm diameter). According to [18], the dominant sediment texture at the Ancão Basin site is silt – silt-fine-coarse sand, at the Ramalhete Channel – coarse-medium sand-silt, at the Faro Pier site is medium sand-silt-clay and at the Port Area – coarse-very coarse sand (Fig. 2).

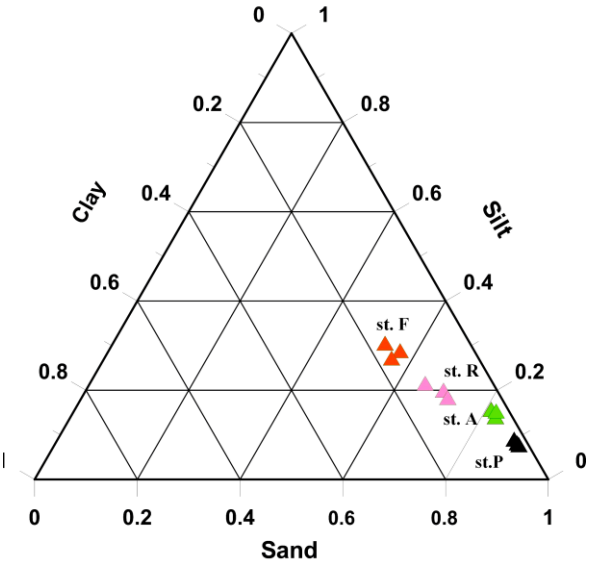

Fig. 2 Sediment texture along the lagoon stations
Рис. 2 Текстура осадков в осадках лагуны

It appears that large variation in the sand proportion is observed across the sites, while the ratio of silt to clay proportion remained relatively constant, with exception st. P.

POM in the sediments samples were determined from 2.54 % the Ancão Basin site at up to 18.50% at the Port station (Ramalhete Channel and Faro Pier has 2.73 and 5.32%), respectively.

The lowest H_2S concentration at the bottom water (0.012 μM) was detected at Ancão Basin and Faro Pier, at Ramlhete station as 0.108 μM and the highest values (0.624 μM) at the Port area. Redox potential measured at Ancão, Faro Pier and Ramlhete was about -135 mV, but at the Port sediments dropped up to -323 mV, that it could indicate the anoxic conditions occurring at the sediments during the low tide.

Meiofauna. Average total meiofauna densities varied in a wide range between 393 ind./0.01 m^2 at the polluted Port station and 30216 ind./0.01 m^2 at Ancão (baseline site). The data were found are normal distributed. Meiofauna total abundances differed significantly across all studied sited (ANOVA, $F(3,8) = 5.83$, $p < .05$). Meiofauna taxonomic composition presented by 11 major taxa (Ciliata, Foraminifera, Mollusca (Bivalvia), Arthropoda (Harpacticoida, Ostracoda, Halacarida, Insecta), Annelida (Polychaeta, Oligochaeta), Turbellaria, Nematoda) with importance contribution of Nematoda in all sites (85 – 93 %). At the Port area meiobenthos assemblages consists of only 3 major taxa (Nematoda, Acarina, Harpacticoida), other of major taxa inhabited Ancão, Ramalhete and Faro Pier sites (9, 7 and 9 number of taxa, respectively).

Vertical distribution of meiofauna has demonstrated the maximum peaks of densities in upper 0 – 2 cm layer, decreasing with sediment depth (Fig. 3). This trend corresponds with classical views on sediments' fauna distribution [6].

From the data in Fig. 2, it is apparent that most abundant meiofauna communities were registered at st. A (Ancão Basin) contrasting to polluted st. P (Port area). Ramalhete Channel (st. R) and Faro Pier site (st. F) show the similar ranges of meiobenthos densities.

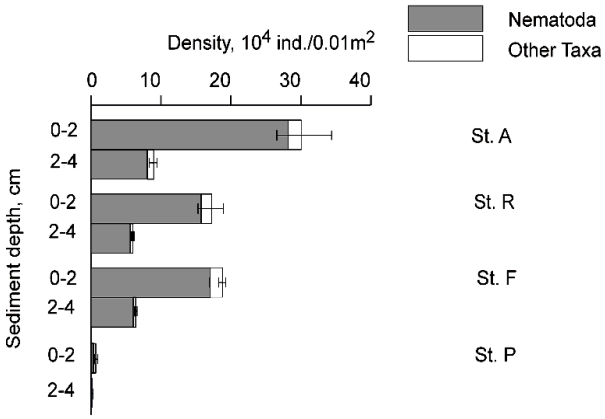

Fig. 3 Vertical profiling of meiofauna distribution along the lagoon stations (average total meiofauna densities with confidential intervals – bars)
Рис. 3 Вертикальное распределение мейофауны в осадках лагуны (средняя численность с доверительными интервалами – планки)

Nematodes. In this study, the clear dominance of free-living marine Nematoda was observed across all station (86 – 92 %). While Nematoda dominated in terms of abundance, we consider the Nematoda community structure by species level on each studied site.

A total of 32 species belonging to 24 genera were encountered at four studied locations of Ria Formosa lagoon. Among them, 3 genera (*Paracomesoma*, *Terschellingia*, *Spirinia*) were found in all stations and 18 genera were registered only in one replicate sample from one location. Genera of *Anticoma*, *Leptolaimoides* and *Molgolaimus* inhabited only the most polluted environment of Port area (Table 1).

T. longicaudata and 2 species of genera *Paracomesoma* and *Spirinia* are common for all locations species.

Discussion. Organic input through the treated and untreated waste waters significantly changes respiration conditions for biota into the sediments. Chosen studied sites located at different parts of lagoon under various anthropogenic loadings that define the local environmental properties.

Table 1 Relative abundance (%) of Nematoda species inhabiting at Ancão site (St. A), Ramalhete Channel (St. R), Faro pier site (St. F) and port area (St. P) in the upper 0–2 cm layer
Табл. 1 Относительное видовое обилие (%) нематод, обитающих в верхних 0 – 2 см донных осадках в исследованных районах

Species inhabiting only one site	St. A	St. R	St. F	St. P
Anticoma sp.1				2
Chromadora sp. 1			1	
Chromadorella sp.1	1			
Cyathoaimus sp1			1	
Leptolaimoides sp.1				1
Leptolaimus sp.1			<1	
Metachromadora sp.2			1	
Metalinhomoeus sp.1			1	
Metoncholaimus albidus Bastian, 1865	10			
Molgolaimus sp.1				13
Neochromadora sp.1			1	
Odontophora sp.1			2	
Paracomesoma sp.2		1		
Paradesmodora sp.1			1	
Prochromadorella sp.1	2			
Paroxystomina sp.1			1	
Rhabdodemania sp.1			1	
Rhips sp.1			1	
Synodontium sp.1			1	
Spirinia sp.1-A		5		
Spirinia sp. 2		<1		
Terschellingia sp.3			2	
Viscosia sp.2			2	
Species inhabiting 2 sites	St. A	St. R	St. F	St. P
Halalaimus sp.1		1	2	
Metacomesoma sp.1	1		2	
Species inhabiting 3 sites				
Metachromadora remanei Gerlach, 1951	1		6	1
Ptycholaimellus sp.1	4	1	2	
Ptycholaimellus ponticus (Filipjev, 1922)	10	1	2	
Terschellingia sp. 2	6	5	5	
Viscosia sp.1	17		2	2
Species inhabiting all sites	St.A	St. R	St. F	St. P
Paracomesoma sp.1	11	2	20	2
Spirinia sp.1	14	77	1	53
Terschellingia longi-caudata de Man, 1907	23	7	48	26

The most exposed by untreated sewage site is the Port area (st. P), where accumulation of degraded material at the sediments leads to H_2S producing by sulfide reducing bacteria (SRB). One of the fatal consequences of it for infauna habiting there may be reducing oxygen in pore water coupled with H_2S contamination. Sedimentary properties at another locations, probably, governed by slow velocity due to far distance from the main channels, where more intensive water exchanges registered. In order to determine the main factors affecting the meiofauna community, PCA analysis was performed.

Meiobenthic communities' response on hypoxic/sulfidic conditions driven by natural and waste stresses was analyzed using the PCA method. Biological variables (total meiofauna and major taxa mean densities (ind./0.1m^2), also the H_2S concentration (μM), redox potential (Eh, mV), particulate organic matter (POM, %) content, grain size composition (sand, silt and clay proportions, %) as environmental variables were used to constrain the correlation matrix. Resulting by the PCA approach, all variables can be summarized in two factor axes that explain 97% of cumulative variances (factor 1 – 81% and factor 2 – 16%). Variables that contribute highly to factor 1 of environmental-meiofaunal relationship are H_2S, Eh, POM, sand-silt-clay percentages and Harpacticoids density. Factor 2 contributed by all variables with weak correlations.

All significant correlations between environmental and biological variables resulted through the PCA procedures are presented in Table 2. Expected exponential relationship between particulate organic matter content (POM) and hydrogen sulfide concentration in bottom waters was found. Observed correlation indicates that accumulation of organic matter at the sediments give rise to H_2S appears. Result from this, available oxygen at the pore water decreased. In this regard, redox potential (Eh) predictably showed negative values and inversely correlated with POM and following H_2S generation at the sediments.

Table 2 Significant correlations between environmental and biological variables (df = 8, p = 0.05, critical value t = 0.63)

Табл. 2 Достоверные корреляции между биотическими и абиотическими переменными (df = 8, p = 0.05, критическое значение t = 0.63)

Variables	H_2S, μM	Eh, mV	POM,%	Sand,%	Silt, %	Clay, %
Meiofauna total, ind./0.1m^2	-0.77	0.76	-0.68	-0.10	0.45	0.42
Nematoda, ind./0.1m^2	-0.74	0.73	-0.64	-0.05	0.40	0.37
Harpacticoida, ind./0.1m^2	-0.63	0.68	-0.76	-0.76	0.80	0.80

However, contrary to expectation, fine sediment fractions (<63 μm of particle size) has not been related with H_2S and POM variables, as well as unexpectedly they are revealed direct correlation with redox potential. In common view, fine sediments would normally be characterized by higher POM content coupled with increasing H_2S concentrations. As a consequence of this, it appears that oxygen depleted, what indicating by Eh values > -100. In our case, the coarse sands were unpredictable attributed to high POM content and, in effect, associated with increasing the H_2S concentrations.

These unsuspected relations between grain size and organic loading at lagoon sediments might be explained by the fact that st. P (Port area) situated in immediate vicinity with pollution source and exposed to organic matter derived from untreated pollution outfall. Sewage input of organic matter was not dispersed away by the tidal waters due to low speed velocity. Observed higher POM percentage registered up to 18.5% at the st. P. This site characterized by higher H_2S concentration in bottom water owing to degradation processes and consequently lowest redox-potential values. All other parts of lagoon sediments revealed more/less typical sedimentary properties for such

environments. It was supposed that Port area more than other locations could be distorted the grain size/POM proportions.

As shown on Fig. 4, the first PCA axes, which explained most of variations in Harpacticoids density, related to the vectors of granulometry composition.

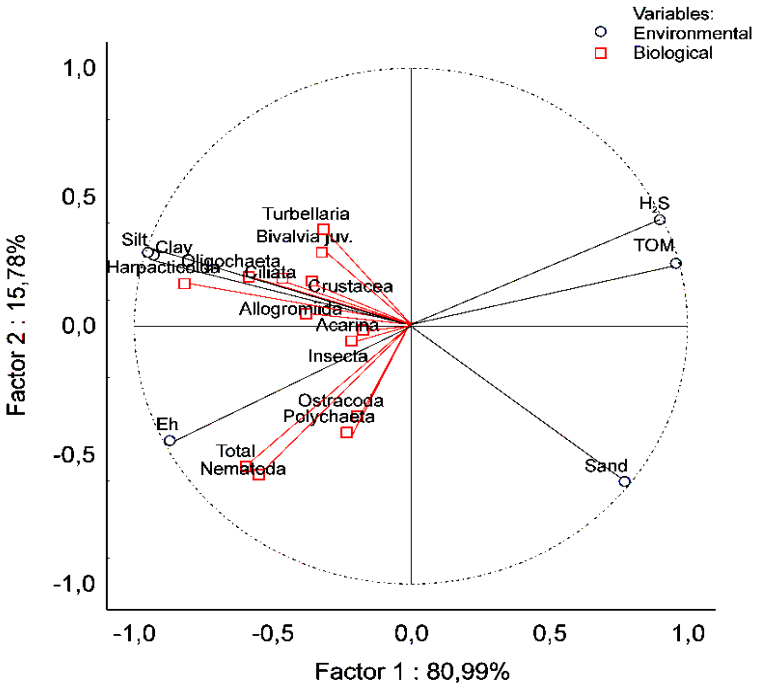

Fig. 4 Ordination of variables the factors axes resulted on PCA
Рис. 4 Проекция переменных на факторных осях по результатам анализа главных компонент

Particularly, Harpacticoids density positively correlated with silt and clay percentages and inversely correlated with sand vector. Such trends can indicate the harpacticoids' preferences on sediment fractions less than 63 μm and avoiding the sands. Total meiofauna and Nematoda densities respond together to environmental pressers – which could be expected given that nematodes composed the meiofauna communities up to 92 % in terms of abundance. Meiobenthos in general and Nematoda abundances, notably, revealed significant correlation to Eh vector, in contrast to H_2S and POM ones (Table 2). This is characteristic benthic respond to environmental hypoxia appears when high level of organic inputs into the sediments increases the H_2S concentration due to degradation processes. Consequently, oxygen demands at sediments drops, that can be easily indicated by Eh measures moving to negative values (< -100 mV).

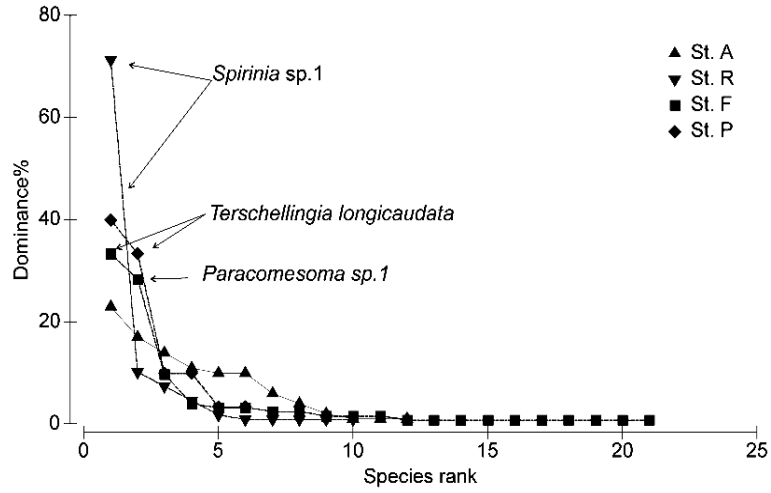

Fig. 5 Dominance structure of Nematoda assemblages from studied lagoon stations
Рис. 5 Структура доминирования таксоцена нематод из исследованных районов лагуны

Nematoda dominance structure was examined by *k*-dominance plot on species rank (Fig. 5). Two sites, both situated near the waste outfalls, show the dominant and subdominant species – *T. longicaudata* and *Spirinia* spp. for st. P and *T. longicaudata* and *Paracomesoma* sp. 1 at st. F.

Despite on sediments at grossly polluted st. P characterized by critical for meiofauna conditions [6] (Eh -323 mV and H_2S presents even at bottom waters), their species ranks' curves has not been indicate the strong communities environmental stress.

Three species of *Anticoma*, *Leptolaimoides* and *Molgolaimus* genera only reported at Port area

under strong hypoxic conditions coupled with H_2S contamination caused by pollution. Their absence at other lagoon locations could probably be explained not only resistance to extreme environments but also by their preferences on coarse sand sediments. *T. longicaudata* and *Spirinia* spp. were counted at st. P as dominant and subdominant, also being common at all studied sites. As illustrates Fig. 5, a dominance curve from Rhamalhete Channel is *j*-shaped, indicating the high dominance of *Spirinia* sp. 1. Such type of curves reported to attribute to disturbed communities under environmental stress. Since st. R characterized by very common sedimentary properties, disturbance of meiofauna could probably be caused by shell-fish farmers' activity. Nematoda assemblages of background site Ancão (st. A) show flatter line signaling low dominance.

The most diverse in terms of species compositions and more sustain-able communities by dominance structure was found the Faro Pier site, despite on its situation near the waste outfall. POM content attributed this location is slightly higher than other sites of lagoon, but not, probably, exceed the critical level when degraded processes leads to deterioration of respiration condition, firstly, decrease the oxygen.

The results of the current study are consistent with those [3, 9] who found that increase of particulate organic matter is positively affected on fauna unless oxygen demand critically decreased [6].

Conclusions. **1.** Relations between sedimentary parameters at four lagoon sites in Southern Portugal, indicate the different levels of organic enrichment at the sediments. Port area is most of all locations exposed by untreated pollution outfall. Intensive input of organic matter through the waste waters has consequence of strong hypoxia, and even periodically anoxia at the deeper layers of sediments. Obviously, that oxygen is available here only at the sediment surface owing to tidal ocean waters comes. Sediments of lagoon impacted by organic input, that can be concluded from distorted grain size/POM proportions, especially at the Port station. Bottom deposits at Ancao, Ramalhete and Faro pier locations could be defined as moderate organic enriched areas. **2.** Total meiobenthos and its' main component – Nematoda assemblages respond on hypoxia and pollution stress by decreasing abundance and diversity. Also Harpacticoids spatial distribution was shown associated with their sediment particle size preference of silt-clay and avoidance the sand. **3.** Meiofauna at grossly polluted Port site appears to be sharply depleted in terms of abundance comparing with other studied lagoon locations. The results on species composition analyzed suggest that Nematoda communities of this site are differentiated from other lagoon locations. It is interesting to mentioned that Nematoda assemblages that existed under oxygen decline in toxic H_2S environments, does not shows strong environmental stress, probably, due to very specific species composition. Genera of *Anticoma, Leptolaimoides* and *Molgolaimus* registered only at this polluted station and, probably, confined to coarse sediments, also being quite tolerant to oxygen deficient and H_2S contamination. *T. longicaudata* and *Spirinia* spp. were dominated in this community. We assume that penetration of nematodes at the deeper anoxic layers had been observed as migrations from the surface into the sediments following the tide cycle. *T. longicaudata, Paracomesoma* sp. 1 and *Spirinia* sp. 1 were reported across all studied location, demonstrating a wide range of tolerance to pollution [16]. **4.** The most diverse in terms of species composition was found the Faro Pier site (st. F), showing the low dominance of community and indicating the sustainable Nematoda population, despite the city wastes outfall is nearby.

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Вивчення таксоцену нематод під впливом забруднення на чотирьох станціях лагуни Ріа Формоза, Португалія. К. О. Іванова. Пілотне дослідження структури співтугрупувань мейофауни проводилося на чотирьох станціях напівзакритої лагуни Ріа Формоза на півдні Португалії. Обговорюється взаємодія природних і антропогенних факторів (накопичення органічної речовини, гранулометричний склад осадів і т.д.), їх наслідки (гіпоксія, зараження сірководнем), а також реакція мейофауни на зміну донної середовища. Осади лагуни розрізняються за рівнем накопичення органічної речовини в точках скиду міських стоків, де реєструється аноксия в глибоких шарах осадів, і у віддалених ділянках лагуни з обмеженим водообміном. Мейобентос, зокрема, нематоди, реагують на гіпоксію і антропогенний стрес значним зниженням чисельності та таксономічного різноманіття. Виявлено, що розподіл гарпактікоід в лагуні пов'язано з їх уподобаннями дрібнодисперсних грунтів і уникнення піщаних. Видовий склад таксоцену нематод

розглядається в контексті впливу факторів донної середовища. Роди нематод *Anticoma, Leptolaimoides* і *Molgolaimus* зареєстровані тільки під впливом гіпоксії і сірководневого зараження осадів і є стійкими до дефіциту кисню і присутності сірководню. *Terschellingia longicaudata* і два види з родів *Paracomesoma* і *Spirinia* відзначені на всіх досліджуваних станціях і повсюдно розподілені в осадах лагуни з різними рівнями органічного забруднення.

Ключові слова: Nematoda, мейофауни, гіпоксія, реакція на накопичення органічної речовини в опадах, лагуна, Південна Португалія

Изучение таксоцена нематод под воздействием загрязнения на четырех станциях лагуны Риа Формоза, Португалия. Е. А. Иванова. Пилотное исследование структуры сообществ мейофауны проводилось на четырех станциях полузакрытой лагуны Риа Формоза на юге Португалии. Обсуждается взаимодействие естественных и антропогенных факторов (накопление органического вещества, гранулометрический состав осадков и т.д.), их последствия (гипоксия, заражение сероводородом), а также реакция мейофауны на изменение донной среды. Осадки лагуны различаются по уровню накопления органического вещества в точках сброса городских стоков, где регистрируется аноксия в глубоких слоях осадков, и в отдаленных участках лагуны с ограниченным водообменом. Мейобентос, в частности, нематоды, реагируют на гипоксию и антропогенный стресс значительным снижением численности и таксономического разнообразия. Обнаружено, что распределение гарпактикоид в лагуне связано с их предпочтениями мелкодисперсных грунтов и избегания песчаных. Видовой состав таксоцена нематод рассматривается в контексте влияния факторов донной среды. Роды нематод *Anticoma, Leptolaimoides* и *Molgolaimus* зарегистрированы только под воздействием гипоксии и сероводородного заражения осадков и являются устойчивыми к дефициту кислорода и присутствию сероводорода. *Terschellingia longicaudata* и два вида родов *Paracomesoma* и *Spirinia* отмечены на всех исследуемых станциях и повсеместно распределены в осадках лагуны с разными уровнями органического загрязнения.

Ключевые слова: Nematoda, мейофауна, гипоксия, реакция на накопление органического вещества в осадках, лагуна, Южная Португалия