Hypoxic Meiobenthic Communities of the Istanbul Strait's (Bosporus) Outlet Area of the Black Sea

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Abstract

Meiobenthos abundance and distribution on the Black Sea shelf and the upper slope area off the Bosporus Strait outlet was studied along a depth transect from 75 to 300 m water depth. This study represents the first detailed quantitative analysis of the structure and distribution of meiobenthos communities in the transition zone from oxic to anoxic conditions in the Bosporus outlet area of the Black Sea. Meiobenthos was present at all investigated depths and includes 21 taxa. Among these the Nematoda was the dominant taxon. The maximum of total meiobenthos was located at 75 m, other peaks occured at 88 m, 103 m, 160 m, 250 m. At 300 water depth of 300 m the amount of the meiobenthos significantly decreased, while the abundance of such groups as the Ciliophora and Nematoda showed a relative increase in abundance extension. Essential taxonomic richness and abundance of meiofauna is observed in the sediment from the surface layer to 5 cm depth. These results suggest that some benthic eukaryotes can tolerate anoxic and sulfidic conditions. Further studies of this region's fauna are needed to yield more information about the taxonomic composition of benthos in the transitional oxic/anoxic water masses of the Black Sea.

Keywords: Meiobenthos, abundance, taxonomic composition, distribution along depths.

Introduction

This work is the first in a series of studies of deep-water meiobenthos in the Istanbul Strait's (Bosporus) outlet area of the Black Sea, conducted within the framework of the EU 7th FP project HYPOX (In situ monitoring of oxygen depletion in hypoxic ecosystems of coastal and open seas, and land-locked water bodies, EC Grant 226213).

The main objectives of studies were to relate the abundances of benthic organisms to changes in the oxygen content of the bottom sediments, to investigate the distribution patterns of different taxonomic groups of meiobenthos along different water depths between 75 m and 300 m, to determine the maximal sediment depth of meiobenthos establish a quantitative occurrence, and to relationships between different taxa and their settlement levels within the sediment. This study represents the first detailed quantitative analysis of the structure and distribution of meiobenthos in the transition zone from oxic to anoxic conditions in the Bosporus outlet area of the Black Sea.

As a silled basin, the Black Sea exhibits a number of remarkable oceanographic and

hydrographic characteristics. The combined effect of great depth (>2000 m), shallow sill depth (35 m) of the Istanbul Strait (Bosporus) outlet, large amount of river water input, and the inflow of warm and saline Mediterranean water creates a distinct basin-wide water-column stratification with a chemocline/ pycnocline at 100 to 150 m, separating an oxic and an anoxic zone. Between the oxic and anoxic waters a suboxic zone is located, which is important for biogeochemical and redox reactions (Başturk et al., 1994; Codispoti et al., 1991; Murray, 1989; 1993). A two-way water-exchange through the Strait of Istanbul reflects the positive freshwater balance of the Black Sea (Özsoy and Ünlüata, 1997). The less saline surface water of the Black Sea flows through the Strait into the Marmara Sea as an uppercurrent (605 km³ yr⁻¹) and the more saline and warm Mediterranean water (305 km³ yr⁻¹) from the Marmara Sea enters the Black Sea as an undercurrent. The Mediterranean water spreads from the Istanbul Strait over the Strait's outlet shelf area of the Black Sea as a few m-thick sheet and then sinks along the continental slope in a series of lateral intermediate depth intrusions (Özsoy and Ünlüata, 1997). In addition to being characterized by the inflow of the warm

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 $(14.5^{\circ}C)$ and saline Mediterranean water (28-34%), the Istanbul Strait's outlet area is influenced by the cyclonic rim current, which is important for the surface circulation of the Black Sea (Oğuz *et al.*, 1993).

The upper water layer in the Bosporus outlet area of the Black Sea has a temperature of 24–25°C in summer and less than 6°C in winter, a salinity of about 15–17‰ and a dissolved oxygen content of 95– 115%. The lower water layer of the Black Sea is depleted in oxygen has a temperature of 8.5–9.1°C, a salinity of 22-23‰ and a hydrogen sulfide concentration of >350 µmol L⁻¹ in the deepest part of the basin (Neretin *et al.*, 2001). An intermediate water mass, commonly referred to as cold intermediated layer (CIL), occurs at 50 to 75 m water depth, with temperatures ranging from 6.5 to 8.8°C and a salinity of 18–22‰.

The morphology of the Istanbul (Bosporus) Strait is characterized by a fan delta, which has been studied in detail by (Flood *et al.*, 2009) using high resolution (HR) bathymetric mapping, HR seismic reflection studies and core stratigraphy. These studies conclude that the fan delta, with its anastomosed distributary channels and sedimentary structure, was constructed under subaqueous conditions by the dense inflow of Mediterranean waters. The channel network construction started 7.5 to 8.0 ka BP (uncalib. C-14 age) and has been active since.

With increasing water depth the "normal" concentration (around 300 µmol L⁻¹) of dissolved oxygen in the water decreases and oxygen deficiency and depletion occurs, which is of environmental importance for the fauna. These types of conditions are referred to as hypoxia and anoxia, respectively. Under hypoxic conditions, many aerobic organisms are affected in their vital activity and studies by (Rosenberg, 1980) showed that the lower level of tolerance for benthic organisms is less than 2 ml L⁻¹ (corresponding to approximately 90 μ mol L⁻¹). This value is often taken as the boundary between normoxia and hypoxia. However, other studies reported that deep-sea benthos can exist at a concentration as low as 1 ml L⁻¹ (corresponding to approximately 45 µmol L⁻¹) (Rosenberg *et al.*, 1991), which makes the supposed boundary of tolerance for low oxygen conditions for benthic fauna vague.

Accordingly, the borders of tolerance were referred to as 2.8 mg L^{-1} (corresponding to 2 ml L^{-1} or 90 µmol L^{-1}) (Modig and Olafsson, 1998), 2–3 mg L^{-1} or 2 mg L^{-1} (corresponding to 1.4 ml L^{-1} or 63 µmol L^{-1}) (Middleburg and Levin, 2009).

Previous Studies

Pioneer studies of benthic fauna in the Bosporus outlet area were done in 1890, where new mollusks species were discovered in this area, which were previously not described from the Black Sea (Ostroumov, 1893; 1894). During benthos studies off Cape Karaburun west of the Istanbul Strait outlet area it was noted for the first time that organisms, specific to the Bosporus area, occured also in the Black Sea west of the Bosporus (Zernov, 1913). Also (Yakubova, 1948) identified 20 Mediterranean benthic species north-west of the Bosporus outlet, which were though to be specific to the Bosporus outlet area. At six stations in the western part of the Bosporus area Băcesco and Mărginneanu (1959) and Băcesco (1960) identified and described 15 species of macrobenthos and 20 species of meiobenthos that were not only new to the Black Sea, but also new to biological literature. As well Kaneva–Abadjiéva (1959) described 12 new mollusk species in the study area.

Several ostracods species specific only to the Bosporus outlet area of the Black Sea were described in the late fifties and early sixties (Caraion, 1959; Marinov, 1962; Shornikov, 1966), including the first notes about living starfish *Marthasterias glacialis* and the description of some features of the echinoderms fauna (Marinov, 1959). The echinoderm fauna and the abundance of basket stars and holothurians species were investigated by K. Vinogradov and V. Zakutsky (1964). Fauna of polychaetes was studied by Dumitrescu (1960; 1962) and Rullier (1963) with the discovery of species new to the Black Sea.

In the sixties research focused on benthic foraminifera in the Bosporus area (depth 80-100m) and three main foraminifera complex were defined based on the dominant foraminifera species. The first complex, dominated by species *Streblus beccarii* was represented by six species, in the second set (represented by 10 species) species of genera *Ammobaculites* are abundant and the third complex consisted of a mixed 32 species group (Didkowsky, 1969).

Subsequently (1991), studies have discovered 79 species of benthic foraminifers, comprising 39 genera and six orders, of which 27 of the new species were unique to the Bosporus region of the Black Sea (Yanko and Vorobyova, 1991).

Additional studies dealing with benthic fauna in the Bosporus outlet area were performed by the Department of Benthos of IBSS, during the July 1958 and June 1980 RV Akademik Kovalevsky cruises in the Bosporus area (Zaika, 1991). By sampling a total of 10 benthic stations at water depths of 70-113 m three ecological communities were distinguished, namely Modiolula phaseolina, located to the northeast of the Bosporus outlet, and the ecological communities Sternaspis scutata and Amphiura stepanovi - Terebellides stroemi located to the northwest of the Strait. Altogether, highest biomass density of *M. phaseolina* and total benthos was observed in the near-Bosporus Black Sea region (Kiseleva and Mikhailova, 1992). These earlier studies indicated that the distribution of Mediterranean species in the Bosporus outlet area was controlled by the Mediterranean outflow through the Istanbul Strait,

which transports the larvae of benthic animals from the Sea of Marmara. These larvae would then settle on the substrate of the shelf and upper slope of the outlet area (Kiseleva, 1959; Kiseleva and Mikhailova, 1992).

Specific communities of benthic organisms adapted to reduced oxygen concentrations occur within this zone of the NW Black Sea (Kiseleva, 1998; Sergeeva, 2011; Sergeeva and Zaika, 2000; Zaika and Sergeeva, 2008). This benthic region is "periazoic zone" termed the (Băcesco and Mărginneanu, 1959) and has been studied in parts of the Black Sea off the coast of Romania (Gomoiu, 2008; Surugiu, 2005), Bulgaria (Marinov, 1978) and Ukraine (Sergeeva and Zaika, 2000; Zaika and Sergeeva, 2008). In recent years, attention has focused on the meio- and micro-benthos living in the deeper, sulphidic part of the Black Sea and there have been several reports of live and active eukaryotes in this hostile environment (Zaika, 2008; Zaika and Sergeeva, 2009).

Materials and Methods

A twelve-day cruise was carried out to the Istanbul Strait's outlet area (Bosporus) of the Black Sea with the R/V 'Arar' from the Istanbul University during $9^{th}-21^{st}$ November 2009. During this cruise we sampled and studied the complete taxa of

meiobenthos communities, covering the size range of 63 μ m to 1.0 mm in the Istanbul Strait outlet area of the Black Sea and water depths of 75 m to 300 m. Fauna was sampled in the area where less saline surface waters of the Black Sea interact with the saline Mediterranean waters, creating a special ecological system and rapid transitions from oxic, hypoxic and anoxic water conditions

Geophysical subbottom profiling and sediment coring were conducted along depth transects from 70 m to 300 m. Nine benthic stations were chosen along this transect, ranging from the oxic to the anoxic zone (Figure 1, Table 1).

Samples for biological studies were collected using a multi-corer and a gravity corer, with which virtually undisturbed sediment samples were obtained. To study vertical and horizontal distribution of meiobenthos at each station, the sediments were sectioned into the following horizontal intervals: 0-1, 1-2, 2-3, 3-5, 5-7 and 7-9 cm. All sediment sections were preserved in 75% alcohol, which is known to preserve morphological structures of fauna without distortion. We avoided prior fixation in formalin in order not to damage calcareous taxa. The sediments were washed through sieves with a mesh size of 1 mm and 63 µm, and stained with Rose Bengal solution before being sorted in water under a microscope for "live" (i.e., stained) organisms. We extracted only those specimens that stained intensely with Rose

41" 42' 41" 39" SEA BLACK 300 41* 33' 41" 30" 411 27 41" 24" 3 . 4 41" 21" • 1 -50-KILOMETRES 10 41* 12* 100

Figure 1. Meiobenthos sampling stations in the Istanbul Strait's (Bosporus) outlet area of the Black Sea (R/V 'Arar' cruise, November 2009).

Station	Water depth, m	Latitude, N	Longitude, E
1	75	41°20,33′	29°06,03′
3	82	41°24,02′	29°03,21′
4	88	41°23,29′	29°12.24′
5	103	41°26,86′	29°12,95′
6	122	41°28,68′	29°14,81′
7	160	41°28,99′	29°15,14′
8	190	41°29,36′	29°15,53′
9	250	41°29,93′	29°16,12′
10	300	41°30,14′	29°16,34′

Table 1. Water depths and coordinates of meiobenthos stations in the Istanbul Strait's (Bosporus) outlet area of the Black Sea (R/V 'Arar' cruise, November 2009)

Bengal and showed no signs of morphological damage. All of the isolated organisms were counted and identified to higher taxa.

The meiobenthos comprises the permanent meiofauna (eumeiobenthos) and the temporary meiofauna (pseudomeiobenthos), the latter representing juvenile stages of macrobenthos (Bougis, 1950; Chislenko, 1961).

High resolution oxygen profiles (increments of 250 µm) were measured with Clark-type microsensors (Revsbech and Ward, 1983) on retrieved cores in the sediment and the overlying water. The microsensors were mounted on a motor-driven micromanipulator and data acquisition was performed with a DAQ-PAD 6015 and a computer. Measurements were performed immediately after core retrieval, however, diffusion of the atmospheric oxygen cannot be completely excluded and thus the measured oxygen concentrations might be slightly higher than the in situ values. Oxygen measurements were only conducted at three stations (88, 103, and 122 m water depth) as at the remaining stations pebbles and shells, densely covering the sediment surface, impeded the penetration of the fragile microsensors (Figure 2).

Results and Discussion

Oxygen concentrations in the sediment and the overlying water and oxygen penetration depth measured at 88 m (St. 4), 103 m (St. 5) and 122 m (St. 6) are presented at Figure 2.

Measurements exhibited water column oxygen concentrations between 0.12 and 0.16 mmol L⁻¹. Although the steepness of the oxygen gradients differed between the profiles, oxygen was rapidly depleted in all cores with a maximal oxygen penetration depth of 1-3 mm. The small differences in penetration depth between cores (Figure 2) can be due to various factors, such as natural heterogeneity of the sediment or different oxygen consumption rates. Presence of oxygen at this depth is in agreement with CTD profiles (data not shown) that were measured during the same cruise in the Bosporus outflow area, however not at the same stations where sediment samples were retrieved. The CTD casts showed that stations in between 150–300 m water depth might have been influence by Mediterranean waters during the sampling period, while stations above 122 m water depth might even permanently have oxygen in the water column. CTD results furthermore indicated that, although the evidence of the Mediterranean water along the main sampling transect north and northeast of the Istanbul Strait's channel was clear, such influence might be absent west of the study area (e.g., Station 3) (Figure 1).

Meiobenthos present between 75 and 300 m included the following 21 taxa: Ciliophora, Gromida, Foraminifera (soft-shell and hard-shell forms), Coelenterata, Nematoda, Kinorhyncha, Oligochaeta, Polychaeta, Turbellaria, Nemertini, Harpacticoida, Cumacea, Amphipoda, Tanaidacea, Ostracoda, Acari, Tardigrada, Bivalvia, Gastropoda, Ophiuroidea, Tunicata (doi:10.1594/PANGAEA.762131 Sergeeva, Mazlumyan). Peaks of meiobenthos abundances were located at 75, 88, 103, 160, 250 m water depth, with abundances decreasing with increasing water depth from 75 to 300 m (Figure 3). The total abundance of meiobenthos ranged from $9 - 1.900 \times 10^3$ ind. $\times m^{-2}$ and the meiobenthos structure was dominated by eumeiobenthos. Pseudomeiobenthos (temporary meiofauna) abundance was elevated at 103 m water depth with the highest abundances of Turbellaria and Bivalvia taxa and at 250 m water depth, where Oligochaeta, Polychaeta and Turbellaria taxa dominated. At 300 m depth the amount of meiobenthos was significantly reduced, while the abundance of such groups as the Ciliophora and Nematoda showed a relative increase (Figure 3).

The considerable decrease in meiobenthos abundance in Station 3 at a shallow depth of 82 m suggests absence of the Mediterranean water at this location as indicated by CTD measurements in this area (data not shown).

Eumeiobenthos made up 83-98% and pseudomeiobenthos made up 2-17% of the bottom community. Studies of the vertical distribution of the meiobenthos community showed that meiobenthos concentrations corresponded 40-80% in the upper sediment layer (0-1cm) at all stations (Figures 4 and 5).

If we compare the density and peaks of meiobenthos in the different sediment layers, uneven



Figure 2. Oxygen concentrations in the sediment and the overlying water at Stations 4-6



Figure 3. Abundance of meiobenthos (103 ind.* m^2) along the depth profile on the Istanbul Strait's (Bosporus) margin outlet area of the Black Sea (RV 'Arar', November 2009).



Figure 4. Share of the eumeiobenthos in the sediment columns.



Figure 5. Share of the pseudomeiobenthos in the sediment columns.

trend can be detected (Figure 6).

Meiobenthos density was decreased in the uppermost sediment layer (0–1cm) at 88m water depth, correlating with low oxygen concentrations and limited oxygen penetration depth. In comparison, at a water depth of 103 m and 122 m water depth oxygen concentrations were slightly higher and oxygen penetrated deeper, correlating with slightly higher abundances. However, considering that the oxygen penetration depth is limited to the upper mm of the sediment (see Figure 2) and the meiobenthos inhabit also deeper sediment layers, we suggest that the meiobenthos might have the ability to adapt to hypoxic/anoxic conditions within the upper sediment column.

If we now compare the content of eumeiobenthos and pseudomeiobenthos in different depths of the sediment, we observe that, as expected, the maximum concentrations of meiofauna usually occur in the surface layer of the sediments, with the 0-2 cm layer containing up to 99% of the total meiobenthos in the study area (see Figures 4 and 5).

Vertical distribution of the pseudomeiobenthos in the sediment exhibit that 10-90 % of fauna lives in the upper layer of the bottom sediment. This can be explained by the life-cycle of pseudomeiobenthos representatives such as Hydrozoa, Oligochaeta, Turbellaria, Polychaeta, Nemertini, Bivalvia, Gastropoda, which dwell in that bottom layer only a part of their life cycle. More even distribution of meiobenthos abundance with sediment depth characterizes the water depths of 75-100 and 250 m (Figure 5), where four main taxa (Oligochaeta, Polychaeta, Turbellaria and Bivalvia) dominated.

The obtained data shows that in the Black Sea benthic biodiversity and changes in faunal patterns mainly depend on the bathymetric gradients (Figure 7). In comparison to the Black Sea Dnieper Canyon area (RV *Meteor* cruise 72/2, February-March 2007), the deep-water meiobenthos in the Bosporus outlet area is taxonomically more diverse (Sergeeva *et al.*, 2011).

At water depths between 75 and 100 m the eumeiobenthos is distributed homogeneously in the different sediment depth layers in the Bosporus outlet area and the maximal abundance of meiobenthos is found at 75 m. Compared with the abundance of fauna at 120 m in the Dnieper Canyon area, the abundance in the Bosporus outlet area at the same depth is four times lower. The break point on the curve of the taxa richness is marked at the 122m depth (Sergeeva *et al.*, 2011).

Our data suggest that the oxic/anoxic transition zone supports a diversity of meiofauna (protozoan and metazoan biota). Although organisms are found in samples from deeper, anoxic/sulfidic areas, in our samples high faunal densities were typically observed in water depths where oxygen disappears, with metazoan taxa (Nematodes and Harpacticoids) and protozoan taxa (Ciliophora, Gromida and Foraminifera), being the most abundant representatives of the eumeiobenthos.

Specific communities of benthic organisms adapted to reduced oxygen concentrations occur within this zone in the NW Black Sea (Kiseleva, 1998; Sergeeva *et al.*, 2011; Sergeeva and Zaika, 2000; Zaika and Sergeeva, 2008). This benthic region, termed the "periazoic zone" (Bacescu, 1963), has been studied in parts of the Black Sea off the coast of Romania (Gomoiu *et al.*, 2008; Surugiu, 2005), Bulgaria (Marinov, 1978) and Ukraine (Sergeeva and, Zaika, 2000, Zaika and Sergeeva, 2008). In recent years, attention has focused on the meio- and microbenthos living in the deeper, sulphidic part of the Black Sea and there have been several reports of live and active eukaryotes from this hostile environment



Figure 6. Meiobenthos in the layers of the bottom sediment in Bosporus area.



Figure 7. Taxa richness of meiobenthos along depth gradient at Bosporus Strait (November 2009)

(Zaika, 2008; Zaika and Sergeeva, 2009).

As during this study oxygen concentration data could nor be obtained from all stations, unfortunately the study does not permit to correlate unevenness of fauna distribution and taxonomic richness of meiobenthos with oxygen concentration. Although it is known (Kiseleva, 1959), that the inflow of the Mediterranean water masses plays a significant role in macrobenthos distribution in the Bosporus region of the Black Sea, our data on the oxygen concentration (0.12 to 0.17 mmol L⁻¹ at depths between 88 and 122 m) and the maximal oxygen penetration depth (1–3 mm) do not testify the considerable effect of the oxygen on the meiobenthos communities in this area.

Essential taxonomic richness and abundance of meiofauna is observed in the sediment from the surface layer to 5 cm depth. The high abundance of different meiobenthos taxa at the depths with low concentration of bottom-water oxygen is undoubtedly not accidental. These concentrations are reminiscent of the 'edge effects' observed at the upper and/or lower boundaries of many continental margin oxygen minimum zones (Levin, 2003; Gooday *et al.*, 2009). Oxygen minimum zone edge effects generally coincide with rising oxygen levels across the upper or lower boundaries, whereas in the Black Sea, and perhaps other anoxic basins, abundance maxima appear where oxygen has almost disappeared (Sergeeva *et al.*, 2011). In both cases the abundance of benthic organisms might be a response to an enhanced food supply.

Conclusions

The meiobenthos present between 75 and 300 m water depth comprised 21 taxa, with Nematoda as the dominant taxon. Meiobenthos distribution differed in the same sediment layers at water depths between 75 and 300m. Maximum meiobenthos abundance was found at 75 m, other peaks occurred at 88 m, 103 m,

160 m, 250 m. The sharp decrease in abundance at 82 m west of the Istanbul Straits channel might be caused by the absence of Mediterranean oxygen-rich water at this location. At a depth of 300 m the amount of meiobenthos was significantly reduced compared to the shallower stations, while the abundance of such groups as Ciliophora and Nematoda showed a relative extension. The vertical distribution of the meiobenthos in the upper 9 cm of the sediment in different water depths of the study area shows that the maximum concentrations of meiofauna are usually found in the surface layers of the bottom sediments with the 0-2 cm layer commonly containing 99% of the total meiobenthos abundance.

On the other hand, our data suggest that some benthic eukaryotes can tolerate anoxic and sulfidic conditions. Further studies of species composition of deep-water meiobenthos fauna in the Bosporus Strait outlet area of the Black Sea are necessary in order to determine, which species are characteristic and indicative of hypoxia /anoxia in the conditions of intruding Mediterranean water masses.

Studies of this region's fauna will yield more information about the taxonomic composition of benthos in the transitional oxic/anoxic water masses of the Black Sea. The specific physiological and biochemical processes that facilitate the survival of eukaryotes in such "extreme" environments are as well important questions for future studies.

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