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## DEEP-WATER BENTHIC POLYCHAETES (*VIGTORNIELLA ZAIKAI* AND *PROTODRILUS* SP.) IN THE BLACK SEA AS INDICATORS OF THE HYDROGEN SULFIDE ZONE BOUNDARY

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**Deep-Water Benthic Polychaetes (*Vigtornietta zaikai* and *Protodrilus* sp.) in the Black Sea as Indicators of the Hydrogen Sulfide Zone Boundary.** Zaika V. E., Sergeeva N. G. — The Black Sea hypoxic layer, situated near the boundary of the hydrogen sulfide zone, is inhabited by the polychaetes *Vigtornietta zaikai*, Kisseleva, 1992 and *Protodrilus* sp., which can serve as indicators of the interface between oxic and anoxic water. The maximum number of polychaetes in the northern part of the Black Sea occur at a depth of 150 m, while near Bosphorus, where the flow of the Marmara Sea water enters the Black Sea, the peak depth varies from 150 to 250 m.

**Key words:** Black Sea, hypoxia, Polychaeta, biological indicators of hydrogen sulfide, abundance, distribution.

**Глубоководные донные полихеты (*Vigtornietta zaikai* и *Protodrilus* sp.) в Чёрном море как индикаторы сероводородной зоны.** Заика В. Е., Сергеева Н. Г. — В Чёрном море, в гипоксическом слое, расположенном у границы сероводородной зоны, обитают полихеты *Vigtornietta zaikai*, Kisseleva, 1992 и *Protodrilus* sp., которые могут служить индикаторами интерфейса оксических/аноксических вод. Пик поселений полихет в северной половине моря постоянно регистрируется на глубинах около 150 м, тогда как у Босфора, где в Чёрное море впадают воды Мраморного моря, глубина пика неустойчива и меняется от 150 до 250 м.

**Ключевые слова:** Чёрное море, гипоксия, Polychaeta, биологические индикаторы сероводорода, численность, распределение.

### Introduction

In the Black Sea the deep waters are permanently anoxic and contaminated with hydrogen sulfide. The hypoxic layer, where the oxygen deficit increases with depth, is situated above the hydrogen sulfide zone. Changes in the composition of benthic communities can be observed where the hypoxic waters impinge on the seafloor and create a gradient of increasing hypoxia. Approaching the boundary of the hydrogen sulfide zone the species composition of macrofauna becomes poorer; large forms occur only occasionally and finally vanish. Gradually, metazoan meiofauna begin to dominate, and together with protozoans, create a distinctive benthic community. For example, in the northern part of the Black Sea at 150 m depth Nematoda dominate the meiobenthos community along with the deep-water polychaetes *Vigtornietta zaikai* Kisseleva, 1992 and *Protodrilus* sp., the largest of which reaching a length of up to 1–1.5 mm (Kisseleva, 1998). The representatives of Hydrozoa and protozoans (Gromida, Foraminifera and Ciliophora) also constitute part of the meiobenthic community. Because the meiobenthic community is characterized by particular species of polychaetes living under conditions of permanent oxygen deficit, we term it the *V. zaikai* community (Zaika et al., 2009).

The consistent presence of *V. zaikai* in every sample of the benthic community bordering the sulfidic zone, and its abundance peak (2–8 thousand specimens/m<sup>2</sup>) at depths of about 150 m (Sergeeva, Zaika, 2000; Zaika, 1999; Zaika et al., 2008, 2009), indicate a good match between this species and the environmental conditions. The second polychaete species, *Protodrilus* sp., possibly has somewhat different environmental requirements, but nevertheless generally is distributed across the same transitional zone. The question therefore arises: can the polychaetes *V. zaikai* and *Protodrilus* sp. be used as biological indicators of the region adjacent to the hydro-

gen sulfide zone? To test this idea, samples were obtained during 2009–2010 across a wide range of depths in three areas of the Black Sea, including the near-Bosporus and north-western regions. Here we present the results of our analyses of these samples.

The present paper was written within the framework of the EC 7th Framework Project “In situ monitoring of oxygen depletion in hypoxic ecosystems of coastal and open seas, and land-locked water bodies” (HYPOX, 0226213) and national theme of the NAS of Ukraine.

### Material and methods

The material was collected in the northern part of the Black Sea during R/V “Maria S. Merian” cruise 15/1 (April–May 2010). Meiobenthos sampling along the Ukrainian north-western shelf was conducted in two areas: I (north-western part of the sea, NWP) and II (Crimean area) (fig. 1).

In the region of the Bosporus Strait (Black Sea), samples were obtained on two occasions: in November 2009 during a cruise of the R/V “Arar” of the Istanbul Technical University and in April 2010 during cruise 15/1 of the R/V “Maria S. Merian” (fig. 2).

Along the Ukrainian shelf sediments samples were taken using a TV multi-corer (TV MUC) in Area I and with a TV MUC and a push-corer (PshC) deployed from the remote operated vehicle “Jago” in Area II. In the Turkish area sediment samples for meiobenthos were taken with a gravity corer and a TV MUC. During sampling, the characteristics of the seafloor (color, presence of detritus and epibionts, etc), as well as sedimentological features in the core samples (color, presence and thickness of oxidized and reduced layers, smell of hydrogen sulfide, presence of macrobenthos, dead shells, etc), were documented.

The sediment cores were sliced into 1-cm-thick layers down to a depth of 5–10 cm depth in order to study the vertical distribution of the fauna. Each layer was washed through two sieves, the upper one with a mesh size of 1 mm, the lower one with a mesh size of 63  $\mu\text{m}$ . All the organisms found in the samples were picked out and recorded. Specimens of the polychaetes *Protodrilus* sp. and *V. zaikai* were counted and separated for further study. Adult and non-mature specimens were distinguished in the population of the former species; in the case of the latter species, the number of segments in every specimen was counted.

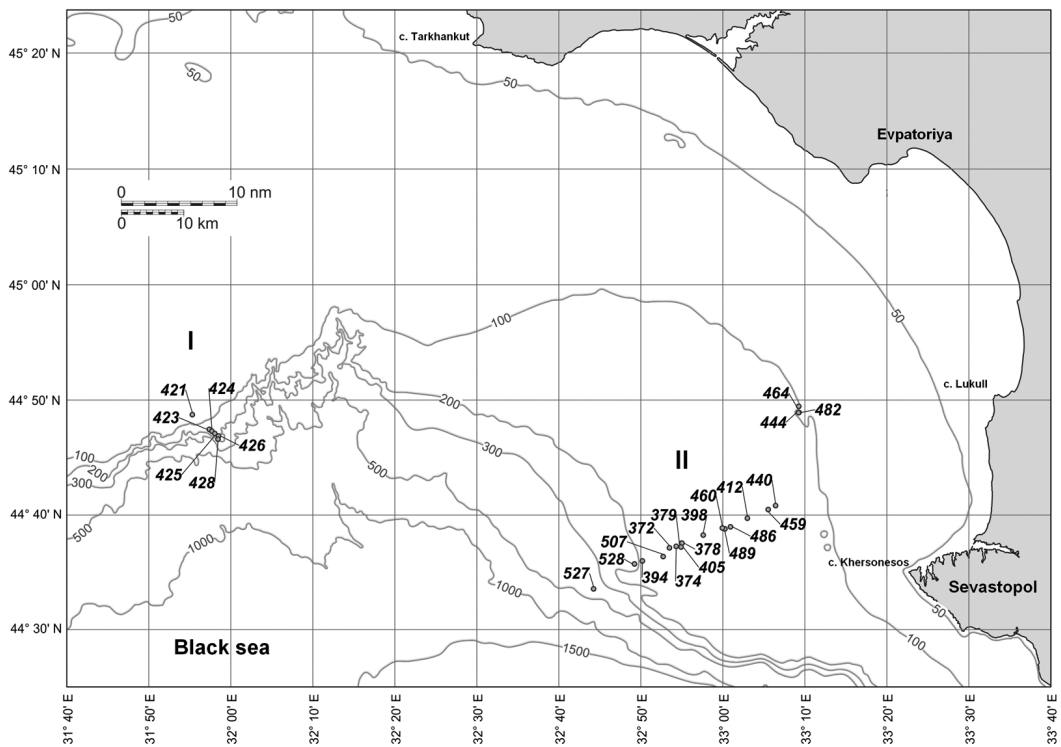


Fig 1. Meiobenthos sampling stations along NW shelf of Ukraine (R/V “«Maria S. Merian»”, April–May 2010): I — NW area, II — Crimean shelf.

Рис. 1. Станции отбора мейобентоса на СЗ шельфе Украины (НИС «Maria S. Merian», апрель–май, 2010): I — СЗ акватория, II — крымский шельф.

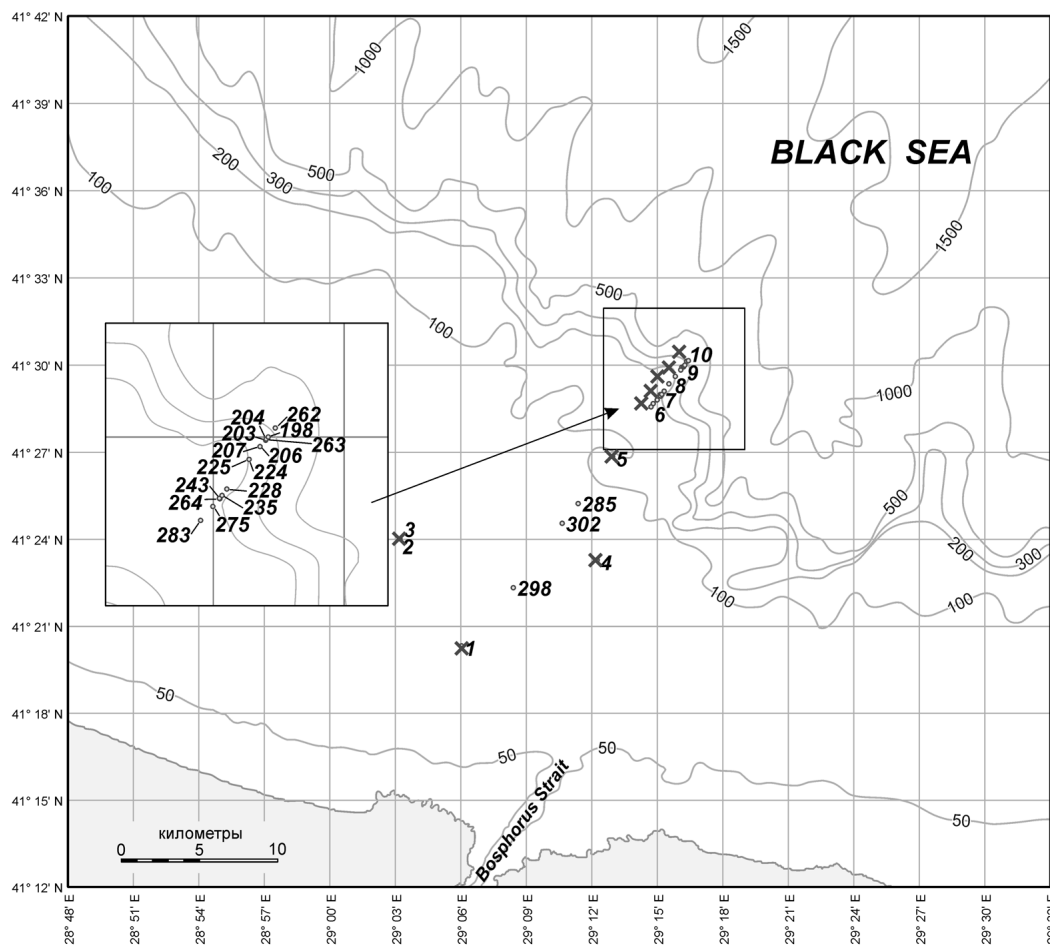


Fig. 2. Meiobenthos stations in the region of the Bosphorus: × — stations 1–10 (R/V “Arar”, November, 2009), o — stations 198–302 (R/V “Maria S. Merian”, April, 2010).

Рис. 2. Мейобентосные станции в районе Босфора: × — станции 1–10 (НИС «Агар», ноябрь, 2009), o — станции 198–302 (НИС «Maria S. Merian», апрель, 2010).

### Results

Along NW shelf of Ukraine, *V. zaikai* was present in sediment samples from 14 stations while *Protodrilus* sp. occurred at 11 stations within the 120–175 m depth range (table 1). The density distribution of these species was not homogeneous along the depth gradient. The maximum abundance of *V. zaikai* was 4.07 and 4.55 thousand spec-

Table 1. Abundance of *V. zaikai* and *Protodrilus* sp. (thousands specimens/m<sup>2</sup>) along the depth profile on the Crimean margin (RV “Maria S. Merian”, April–May 2010)

Таблица 1. Распределение численности (тыс. экз./м<sup>2</sup>) полихет *V. zaikai* и *Protodrilus* sp. по глубине на Крымском полигоне (НИС «Maria S. Merian», апрель–май 2010 г.)

№ station	459	412	460	489	398	424	512	378	379	405	374	372	425	426
Depth, m	120	124	130	138	144	148	150	155	155	157	162	163	163	175
<i>V. zaikai</i>	0.07	1.56	4.07	1.06	4.55	2.47	0.72	1.14	0.28	3.24	1.80	3.59	1.77	1.27
<i>Protodrilus</i> sp.	0.14	0.96	0.36	1.20	0	5.86	0.12	0.42	1.27	0.24	0	0	0.07	0.14

imens/m<sup>2</sup> at 130 and 144 m depth, respectively, compared with 3, 24 and 3, 59 thousand specimens/m<sup>2</sup> at 157 and 163 m depth, respectively, and 1.27 thousand specimens/m<sup>2</sup> at 175 m depth. Densities of *V. zaikai* were 2.98 and 1.98 thousand specimens/m<sup>2</sup> in two sub-samples from 148 m depth (st. 424). This variation could be explained by the differences of sediment structure, especially the surface layers.

At the 148 m depth the abundance of *Protodrilus* sp. (5.65–6.07 thousand specimens/m<sup>2</sup>; mean 5.86 thousand specimens/m<sup>2</sup>) was significantly higher than that of *V. zaikai*. At two stations located at 163 m depth, *Protodrilus* sp. was either absent (Station 372) or present in low abundance (0.07 thousand specimens/m<sup>2</sup>; Station 425), rising slightly to 0.14 thousand specimens/m<sup>2</sup> at the deepest (175 m) site. Hence, peaks of abundance of these polychaetes species were located at particular water depths.

Specimens of *V. zaikai* were collected along the NW shelf of Ukraine on three previous occasions. In February–March 2007 the abundance of this species was 2.6 thousand specimens/m<sup>2</sup> (Zaika et al., 2009), which was similar to its abundance in 2010. Probably, these values were local seasonal abundance minima for *V. zaikai*. Such low densities of this species were not observed in other regions. However, according to the literature data, the maximum abundance during the summer increased to 7.9–8.0 thousand specimens/m<sup>2</sup> in July 1994 and May–June 2007 (Sergeeva, 2004; Zaika et al., 2009).

In the Crimean water area in April–May 2010 *V. zaikai* and *Protodrilus* sp. were observed for the first time within this depths range. The abundances of both species are shown in table 1 and fig. 3.

These data revealed an unusual distribution of the two boundary polychaete species with water depth across the Crimea shelf. Each of the species exhibited its own abundance peak. The abundance of *V. zaikai* increased from a minimum at 120 m depth up to maximum values at 130 and 144 m depth (4.06 and 4.55 thousand specimens/m<sup>2</sup>, respectively). This species was also abundant at somewhat deeper sites (148–163 m). Generally, therefore, it can be concluded that the maximum abundance of *V. zaikai* across the Ukrainian shelf in April–May was between 130 and 163 m water depth, although there were sharp decreases at some stations within this depth range, perhaps due to small-scale differences in the nature of the substrate. *Protodrilus* sp. was also most common within the same depth range, although this species was generally much less abundant than *V. zaikai*. The density peak was observed at 148 m depth, where *Protodrilus* was more abundant than *V. zaikai*.

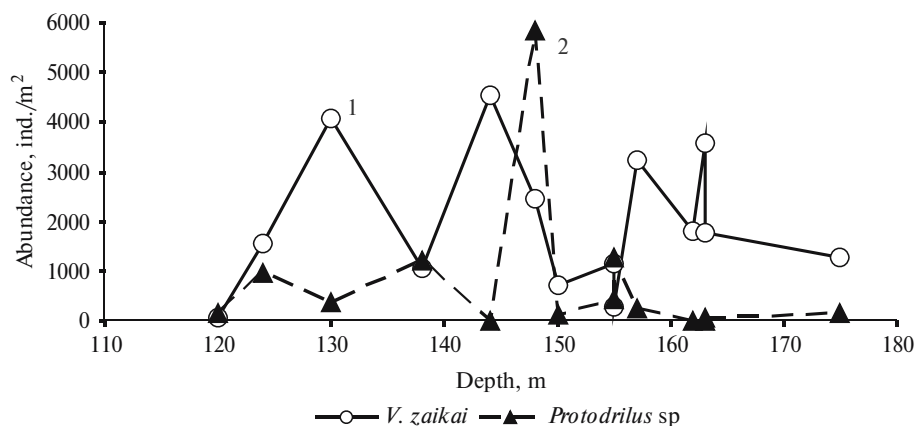


Fig. 3. Distribution of boundary polychaete species, along depth transects of the Ukrainian shelf in the Areas I and II (April–May 2010): 1 – *V. zaikai*; 2 – *Protodrilus* sp.

Рис. 3. Распределение пограничных видов полихет *V. zaikai*, и *Protodrilus* sp. по глубинам украинского шельфа на полигонах I и II (апрель–май 2010 г.): 1 – *V. zaikai*; 2 – *Protodrilus* sp.

A comparison between peaks and troughs of abundance suggests that the two species have somewhat different environmental requirements. It could also be indicative of the local or temporary nature of the boundary zone structure. However, the distribution of *Protodrilus* sp. and *V. zaikai* within the range of depths where oxygen-deficient bottom water impinges on the seafloor emphasizes the role of these species as indicators of hypoxia and the proximity of the hydrogen sulfide zone boundary.

Analysis of all *V. zaikai* data collected from northern part of the Black Sea shows that the abundance of this species was 2.5–8 thousand specimens/m<sup>2</sup> in the north-western area, 4.5 thousand specimens/m<sup>2</sup> across the Crimean shelf, and 11.4–17.1 thousand specimens/m<sup>2</sup> across the Kerch shelf (Zaika et al., 2010). Thus, the highest abundance of *V. zaikai* was recorded in May–June 2007 in the region of Kerch (Zaika et al., 2010).

We compared the age structure of *V. zaikai* populations from the two areas (NW part and Kerch shelf) sampled in May–June 2007. The Kerch area yielded an unusually high proportion (28 %) of juvenile worms with only three body segments as well as specimens with a maximum number of 10 body segments. In the NW area, the polychaetes had up to 14 body segments and the proportion of specimens with three body segments was only 4 % (fig. 4).

The distribution of *V. zaikai* was studied on two occasions in the southern part of the Black Sea near the Bosphorus. During the cruise of R/V “Arar” (November 2009), this species was found for the first time at 250 m depth, although it was absent at shallower sites (75–122, 160 and 190 m). A live gastropod mollusk was also found at 250 m, which suggests that the boundary between hypoxic and anoxic waters and the lower depth limit of the macrobenthos correspond to this depth (Sergeeva et al., 2011). The population density of *V. zaikai* at 250 m depth was 7280 specimens/m<sup>2</sup>. The polychaete penetrated into the sediment to a greater depth than in the northern half of the Black Sea; 25 % of specimens inhabited the 1–2 cm layer and a few were even found in the 5–7 cm layer.

During the cruise of the R/V “Maria S. Merian” in May 2010 both polychaete species were found at a wide range of depths (*V. zaikai* — at 134–250 m, *Protodrilus* sp. — 116–159 m depths) (table 2, fig. 5) in the near-Bosphorus region.

There are three notable features of the results from the 2010 samples: I — the wide range of water depths where *V. zaikai* occurred; II — confirmation of the existence of this species at 250 m depth; III — the “core habitat”, with the maximum of abundance of each species, was located at 150–159 m depth for *V. zaikai* and 134 m depth for

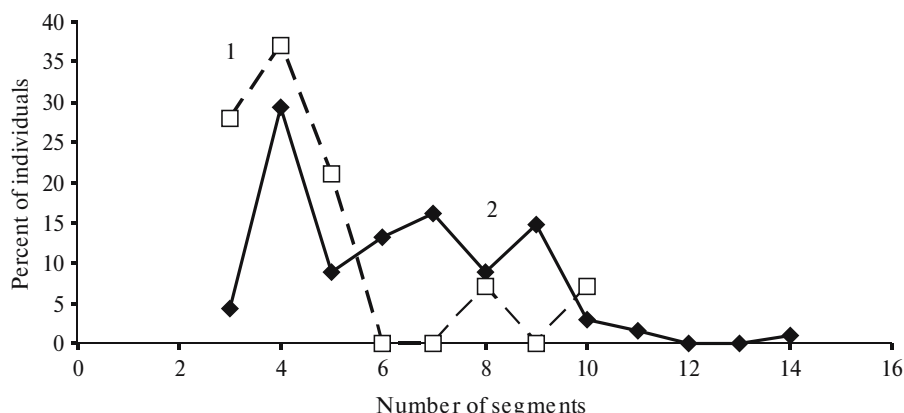


Fig. 4. Distribution of *V. zaikai* population according to the number of body segment during May — June 2007 in two areas: I — NW area; II — Kerch shelf.

Рис. 4. Распределение популяции *V. zaikai* по количеству сегментов в мае — июне 2007 г. на двух полигонах: I — СЗЧ; II — Керченский полигон.

Table 2. Abundance (thousand specimens/m<sup>2</sup>) of the two polychaete species inhabiting the hypoxic/anoxic boundary zone in the near-Bosporus region (RV “Maria S. Merian”, May 2010)

Таблица 2. Численность пограничных полихет (тыс. экз./м<sup>2</sup>) в прибосфорском районе (НИС «Maria S. Merian», май 2010 г.)

№ station / Depth, m	285/116	275/134	264/150	243/153	235/159	228/174	263/250
<i>V. zaikai</i>	0	0.14	7.20	5.22	7.78	0.14	0.14
<i>Protodrilus</i> sp.	0.33	2.61	0	0	0.14	0	0

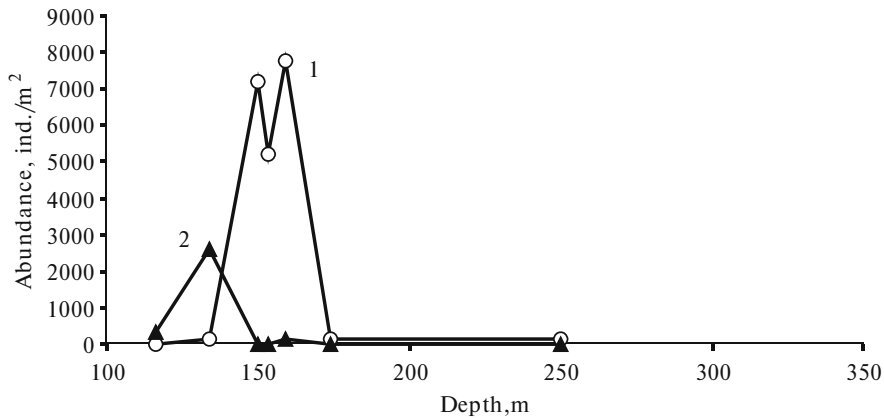


Fig. 5. Abundance of the boundary zone species with depth in the near-Bosporus region: 1 — *V. zaikai*; 2 — *Protodrilus* sp.

Рис. 5. Распределение численности пограничных видов по глубинам в прибосфорском районе: 1 — *V. zaikai*; 2 — *Protodrilus* sp.

*Protodrilus* sp. The term “core of habitat” was first used with reference to the boundary community by Zaika et al. (2008) and is similar to the term “community core” of Kisseleva (1981).

In 2010 *V. zaikai* was found in the 134–250 m depth range while in 2009 it was only recorded at 250 m depth. This depth variation could reflect unstable conditions in the near-Bosporus region. The habitat core of *V. zaikai* was located at a similar depth near the northern shores in 2010 and in other years (Zaika, 2009).

In 2009 the density of *V. zaikai* at 250 m depth was 7.3 thousand specimens/m<sup>2</sup>, which was similar to the maximum value (7.2–7.8 thousand specimens/m<sup>2</sup>) recorded in 2010 in this region. It was suggested that the population core moved down during the year. In the NW area the maximum densities (mean values) were also 7.9–8.0 thousand specimens/m<sup>2</sup> in 1994 and 2007.

The 2009 population consisted mostly of individuals with 3–4 body segments; the largest had only 5–6 segments. In 2010, individuals with 3–5 body segments dominated in the community, while those with 7–8 body segments were rare. These observations suggest that the population was mainly represented by newly settled juveniles, which periodically migrated into the water column. Probably, even juveniles that penetrated into the upper centimeters of the soft sediments of the Bosporus region could easily migrate in case of emergency. It seems likely that the shifting direction of oxygenated bottom currents from the Marmara Sea can change the structure of hypoxic benthic biotopes. In this case it is possible that *V. zaikai* can migrate within the depth range of 134–250 m, forming a habitation core where the oxygen regime is optimal. However, this hypothesis can be proved only by further interdisciplinary studies.

## Discussion

All data concerning the bathymetric distributions of *Vigtorniella zaikai* and *Protodrilus* sp. are summarized in table 3.

The data obtained in different research cruises were not averaged. In table 3 we only include the depths that define the upper and lower limits of occurrence of the two polychaete species. For example, with one exception, the upper occurrence boundary of both species was located in the depth range 116–134 m (usually at 120–130 m). The only exception was an occurrence of *Protodrilus* sp. at 78 m depth, but this could not be considered typical. Unlike the upper boundary, the lower boundary of both species was variable. In general, it was located at a depth of 151–163 m or somewhat deeper at 170–190 m; in the Bosphorus region, however, *V. zaikai* penetrated down to 250 m depth.

Although the maximum densities of the two species coincide rarely, their core habitats have the same overall depth limits (table 3). In addition, in the near-Bosphorus region one survey has shown that their core habitats are located at approximately the same depths as on the north-western shelf, while another survey revealed a shift in the lower boundary of *V. zaikai* to 250 m depth, and *Protodrilus* sp. entirely absent. Of these two polychaete species, *V. zaikai* is usually more numerous than *Protodrilus* sp. and can be found closer to water containing hydrogen sulfide.

A large number of specimens of an unknown polychaete species, and a *Protodrilus* species identified as *P. flavocapitatus*, were reported earlier at three stations located at 150 m depth along the Romanian coast (Bacescu, 1963). We speculate that under oxygen-depleted conditions on the Romanian shelf, the species referred to were *V. zaikai* and *Protodrilus* sp. The ecological and biological characteristics of *P. flavocapitatus* suggest that it was not the representative of the genus *Protodrilus* found on the Romanian shelf (Kisseleva 1998).

We can suggest some preliminary conclusions regarding the influence of physical and chemical parameters. The upper occurrence boundary of the bordering polychaetes species is situated somewhere near the inflection point observed in oxygen profile in the lower part of the oxycline (Stunzhas, 2000). The benthic region under discussion lies beneath the coastal convergence zone, where a deepening of the profiles of all physical parameters takes place during severe winters (Titov, 2000). Thus variations in climatic conditions can shift biological boundaries. The special conditions in the near-Bosphorus region, associated with the influence of water flowing in from the Marmara Sea, has already been mentioned.

We cannot explain the size-age structure of *V. zaikai* populations. As already discussed, juveniles dominated at the study sites in the northern and southern parts of the Black Sea. Based on the data in fig. 4, it appears that the samples from the Kerch area were taken at a period of mass settlement of the polychaete larvae from the plankton. However, the prevalence of younger stages is also characteristic of populations in the near-

**Table 3.** Bathymetric occurrence of boundary polychaete species in NW and near-Bosphorus regions (according to Sergeeva, 2004; Zaika et al., 1999; Zaika, Sergeeva, 2008; Zaika et al., 2009)

**Таблица 3.** Границы встречаемости и глубины ядер поселений для пограничных видов полихет в СЗ и прибосфорском районах (Sergeeva 2004; Zaika et al., 1999; Zaika, Sergeeva, 2008; Zaika et al., 2009)

Boundary / Depth, m		North-Western region		Near-Bosphorus region	
		<i>V. zaikai</i>	<i>Protodrilus</i> sp.	<i>V. zaikai</i>	<i>Protodrilus</i> sp.
Upper occurrence boundary		117–120	78, 124–130	134	116
Lower occurrence boundary	from	151–163	151–157	250	159
	to	175–190	170–175		
Depth of habitat core	from	139–148	138–148	150–160	134
	to			250	

Bosporus region during November 2009 as well as in May 2010. A high percentage of mature forms has never been observed in populations of *V. zaikai*.

There are two possible explanations for this size-age structure. 1) Maturation with further active reproduction followed by the death of mature specimens occurs quickly over a very short time period. 2) The habitats of mature specimens and settled juveniles are spatially separated. Additional data are needed in order to test these hypotheses. We must emphasise that the habitats of *V. zaikai* were not studied during the periods December—January and August—October. Three autumn months are quite long enough for reproduction to occur and the mature specimens to die. However, it implies that the mature stage has a very short mean life span, which does not seem consistent with the long life of *V. zaikai* plankton larvae, which live at the same temperature of 8°C.

At first sight, the second hypothesis seems to be unlikely. Zaika et al. (2009) suggested that the decaying remains of dolphins could serve as a habitat for mature *V. zaikai*. This idea should be tested by analyzing the fauna associated with the cadavers of dolphins decaying on the bottom, especially at the hypoxic depths. The remain of dolphins were not examined in the Black Sea at any depth. Time will tell which of these hypothesis is correct.

Despite these uncertainties about the life histories of *V. zaikai* and *Protodrilus* sp., it is now possible to answer the main question posed in this paper; the two polychaete species considered here can indeed be used in the Black Sea as biological indicators of the boundary of the hydrogen sulfide zone. These species occur only in close proximity to this boundary. Some hydrophysical data suggest that the boundary may fluctuate over time. However, these data are fragmentary, and biological indicators often provide the only method for determining the actual location of the hydrogen sulfide boundary.

Isolated occurrences of the polychaete species can be difficult to interpret, and therefore it is better to base conclusions on mass occurrences of *V. zaikai*, which was recorded closer to sulfidic waters than *Protodrilus* sp. While the lower occurrence boundary of these bordering polychaete species clearly varies with time, fluctuations in the depth of their habitat core provides a more reliable basis for interpreting the long-term dynamics of water-column stratification, mainly in relation to oxygen availability.



Fig. 6. Position of benthic boundary belt occupied by the meiobenthic community dominated by *V. zaikai*.

Рис. 6. Положение пограничного пояса бентали, занятого сообществом мейобентоса с доминированием *V. zaikai*.



Thus, the data obtained on the distribution of the polychaetes species in our study areas allows us to map the approximate location of the boundary belt occupied by a meiobenthic community characterized by a high abundance of *V. zaikai* (fig. 6). At the scale of this map, the hydrogen sulfide zone begins immediately below the belt occupied by the meiobenthic boundary community. The location of this belt should be studied near the western and eastern coasts of the Black Sea, especially in the region of the Georgian — Turkish border.

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