**ABSTRACT**

Present paper reports on the possible existence of recent freshwater fauna in the Black Sea. Based on information available from malacology, ecology, paleontology, stratigraphy, hydrogeology and observations in situ, the presence of freshwater biota on the shelf and continental slope is discussed, including the existence of aerobic life forms in the Black sea deep-water cavity.

**KEY WORDS**

Aerobic; Biodiversity; Ecosystem; freshwater; submarine springs.

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**INTRODUCTION**

The Black Sea is a very specific marine basin with anaerobic water mass spreading from the maximal bottom depth (more than -2200 m) to about -200 m. This water thickness is valuated as about 85% of the whole Black Sea water volume (Zaitsev, 2006) and suggested as absolutely unfit for eukaryotic life (Vinogradov, 1997).

The Black Sea water salinity varies from about 17-18‰ on the inner shelf till 23‰ in the deepest basins' parts (Sorokin, 1982) which excludes any possibilities for freshwater biota inhabitance. Anyway, the possibility of existence of aerobic life "oases" related to fresh groundwater springs in sulfured hydrogen zone was hypothesized, taking into account a few unusual faunistic findings, signs "of island" speciation (Zaika, 2008).

Additionally, on the Black Sea continental shelf bottom surface were repeatedly found out the shells of several gastropod mollusk species typical of freshwaters or brackish complexes (Il’ina, 1966; Golikov & Starobogatov, 1972; Chukhchin, 1984). Most of these species - met nowhere else and described as extinct - supposedly re-deposited from ancient (Neoeuxinian) sediment layers formed in much more freshwater environmental conditions (Golikov & Starobogatov, 1972). However, among the above-mentioned gastropods, only one (Theodoxus pallasi Lindholm, 1924) is known for certain from Neoeuxinian sediment deposits.

During the R/V «Maria S. Merian» (Leibniz Institute for Baltic Sea Research, Germany) expedition, which was conducted in the Black Sea within the framework of the European project HYPOX in 2010, a live gastropod mollusk was sampled at the depth of 250 m (Sergeeva et al., 2011). If one believes that its presence in anoxic zone may be not casual, then it is necessary to admit the existence of oxygen input into the sulfured hydrogen water layer by sources which serve as freshwater springs.

The finding of meiobenthic crustaceans in the deep Black Sea (around -2000 m) and at a depth of 174 m (Korovchinsky & Sergeeva, 2008) may be considered as one of the biological and ecological proofs of the existence of aerobic fauna in anaerobic zone. In 1986, during a test diving on the submarine inhabited vehicle of the USSR Navy at a depth of 600-640 m in the Yalta canyon at bottom, one "oasis" of aerobic life was found
out (Prof. Gevorkyan V.Kh. personal report); this information was under secret for a long time and therefore never published. The most credible reason for the existence of such an “oasis” is the occurrence of a zone of stable and powerful submarine unloading of oxygen-rich groundwaters.

Oceanological and geological researches confirmed the presence of submarine discharge of fresh water zones on the Black Sea continental shelf and slope (Trotsyuk et al., 1988; Shnyukov & Ziborov, 2004). Actually, these zones can create proper conditions for the existence of freshwater or brackish biota, both in aerobic and in anaerobic water masses of the Black Sea.

The aim of the present research, based on geological, biological and ecological data, is to investigate the existence of specific biocenoses in the Black Sea, related to the zones of the submarine unloading of freshwaters. A discovery and description of such a biocenosis would extend knowledge about the Black Sea biodiversity and give new ideas about possible ways of the “oasis” fauna evolution.

**DISCUSSION**

**Geological and oceanological evidence**

Submarine freshwater springs are known from many regions. Very recently, a Max Plank Institute science troop found rich bacterial life connected with freshwater springs even in the Dead Sea (Ioinescu et al., 2012). Black Sea is also suitable for searching submarine sources of freshwater. On geological terms it is possible to distinguish three basic types of submarine sources of groundwater unloading: artesian, karstic and waters of subriverbed flow (Shnyukov & Ziborov, 2004).

Data on the dynamics of the artesian pools’ groundwater opened toward the Black Sea, testify movements of different hydrogeological floors. Actually these pools embrace all coastline of the Black Sea (Shnyukov & Ziborov, 2004). Submarine springs issued from the limestone massifs or other kinds of karsting rocks are widely spread in the Black Sea.

Researches, managing with submarine inhabited vehicles, showed the presence of numerous rocky outcrops from the bottom silty-mud sediments cover on a narrow and steep continental shelf and slope of south Crimea (Bondarev, 2008; 2009). Oceanological researches in 37th voyage R/V “Academician Vernadsky” showed submarine unloading of karsting-crack waters in head parts of many Crimean submarine canyons. The hydrochemical tests of near-bottom water allowed to set that in canyons salinity is lowered notably up to 12, 14, 15, 17‰ (with reference to base-line values of 21-22‰). Particularly, the desaltation of salt waters on the canyons bottom was found out on the continental slope of Turkey, north of Kefken Island and on the extreme north-west of the Turkish shelf (at 300-500 m below the sea level;13-17‰). Signs of desaltation were found also in the canyons of north-west Black Sea (Shnyukov & Ziborov, 2004).

Use of impermeable water samplers, vacuum degassing and chromatographic analysis allowed to set the presence of solved oxygen in the benthic layer of deep-water part of the Black Sea. In the area adherent to the estuarine part of Danube, oxygen in benthic water decreases from 4.2 ml/l in an off-shore zone practically to zero on the shelf edge. As recorded in eight near-bottom samples taken at different depths on the bottom and on the sides of a deep canyon up to 1340 m of depth, below the slope, up to the distal part of canyon fan, oxygen amount ranges from 0.3 to 1.6 ml/l. On the Anatolian side (the cone of dejection of Kyzil-Irmak river) water-solved oxygen was found out in two deep-water stations with bathymetric marks -2064 m and -2003 m, respectively. Measured oxygen concentrations were 0.5 and 0.7 ml•l. At depths of over 2000 m, near-bottom waters contained oxygen, as revealed by tests from the districts of western halistaza zone, interhalistaza zone and the east hollow of the Black Sea (Trotsyuk et al., 1988).

Major part of water samples containing oxygen was collected in submarine canyons or in the surroundings where the underground source of aerobic freshwaters binds to the submarine unloading. All these features do suggest the presence of specific freshwater aerobic biocenoses in the Black Sea is to be considered highly probable.

**Biological and ecological evidence**

Shells of gastropod molluscs of freshwater or brackish habitats were discovered on continental shelf and slope of the Black Sea (Golikov & Staro-
bogatov, 1972), where water salinity is 19-21‰. Their list (Table 1) was recently filled up by one specimen of living gastropod from the near-Bosporus region, where background water salinity exceeds 22‰. Neritidae and Hydrobiidae mostly consist of marine species.

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Locality / inhabitance (after Kantor &amp; Sysoev, 2006, others indicated)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fam. Neritidae: Theodoxus milachevitchi Golikov et Starobogatov, 1966</td>
<td>Crimea offshore/ recent mud, depth 20-60 m Black Sea Neoeuxinian, Vityazeian &amp; Kalamitian layers, recent muds on the depth 18-158 m (Author’s data)/ Aral &amp; Caspian Seas, very freshened littoral spots in the Azov Sea (Golikov &amp; Starobogatov, 1972); rivers of Ural Mountains and Armenia</td>
</tr>
<tr>
<td>Theodoxus pallasi Lindholm, 1924</td>
<td>Crimea offshore, silt on the 20 m depth</td>
</tr>
<tr>
<td>Fam. Hydrobiidae, Subfam. Pyrgulinae: Caspia valkanovi (Golikov et Starobogatov, 1966)</td>
<td>Crimea offshore, mud on the depth of 250m (Author’s data)</td>
</tr>
<tr>
<td>Caspiohydrobia sp.</td>
<td>Crimea, 15 m</td>
</tr>
<tr>
<td>Turricaspia crimeana (Golikov et Starobogatov, 1966)</td>
<td>Crimea offshore, mud on the 80-180 m depth</td>
</tr>
<tr>
<td>Turricaspia iljinae (Golikov et Starobogatov, 1966)</td>
<td>Crimea, Modiolula phaseolina (Philippi, 1844) contained mud.</td>
</tr>
<tr>
<td>Turricaspia nevesskae (Golikov et Starobogatov, 1966)</td>
<td>Crimea offshore, mud on the 80-180 m depth</td>
</tr>
</tbody>
</table>

Table 1. List of freshwater and brackish water gastropods found on the shelf and continental slope of the Black Sea.

However the genus Theodoxus Monfort, 1810 comprises species dwelling exceptionally in fresh or strongly refreshing (up to 5‰) waters of Eurasia. This characteristic allows to use them as bioindicators of freshwater environments (Goodwin, 2006). The Subfamilly Pyrgulinae Brusina, 1882 also includes genera and species from freshwaters (springs, rivers and lakes) of Europe and front Asia (Anistratenko, 1998).

A considerable part of these species inhabits brackish seas – Aral and Caspian. In the Azov-Black Sea basin the subfamily is represented by three genera whose species are usually encountered in the brackish zones of estuaries and rivers (Golikov & Starobogatov, 1972; Kantor & Sysoev, 2006). Hence, substantial differences in water salinity of sampling locations and in typical habitats of mollusk species led to hypothesize a possible introduced origin of shells from a freshwater environment. But, in that case, in the southern part of Crimea littoral, where there are special habitat conditions, these shells should be absent. In addition, these shells are found in the middle and external part of shelf and are absent in littoral zone.

These circumstances and the sub-fossil state of seashells allowed to suppose they to origin from more ancient layers, maybe formed in a freshwater-like environment (Golikov & Starobogatov, 1972). Such environmental conditions existed in a Neoeuxinian period of the Black Sea evolution (Il’ina, 1966); Neoeuxinian layers on the shelf of Crimea are covered by more young ground sedimentations, from 1 to 4 m thick (Shcherbakov et al., 1978). Moving of shells through such a thick layer by means of natural processes is hardly plausible. But even if we assume such a possibility, then in Neoeuxinian deposits these species should be much more abundant than in recent sediments. However, species reported in Table 1 were found only in recent bottom deposits, with the exception of Theodoxus pallasi.

This species is the only one that is really characteristic for Neoeuxinian layers whereas in later deposits is quite rare. It is has been reported that shells of T. pallasi from recent bottom deposits have better saved surface and color pattern compared to those from Neoeuxinian sediments (Il’ina, 1966; Golikov & Starobogatov, 1972).
In our samples there is one *T. pallasi* subfossil specimen from a depth of 158 m from the recent bottom sediment of near-Bosporus region (Fig. 1). Maintenance of colouring of the shell testifies its relatively recent fossilization. Available data allow to suppose that the shells of the freshwater species complex discovered on Black Sea shelf belong to recent living species inhabiting within the limits of the freshwaters biotopes.

In 2010, during the Black Sea international expedition of R/V "Maria S. Merian" (Leibniz Institute for Baltic Sea Research, Germany) in a near-Bosporus region on a depth of 250 m, a live specimen, belonging to the genus *Caspiohydrobia* Starobogatov, 1970, was collected (Fig. 2). As at this depth in the Black Sea there are anoxic conditions, it was hypothesized that the animal had been rescued only by chance (Sergeeva et al., 2011). However if we admit the existence of specific aerobic freshwater fauna in the depths of the Black Sea, then the location of this mollusk corresponds to the biotope formed by a submarine unloading of freshwaters.

In 1986, during test dive of submarine inhabited vehicle of the USSR Navy in the Yalta canyon at a depth of 600-640 m, Dr. Gevorkyan (personal comm.) looked at one "oasis" of eukaryotic supposedly aerobic life. The biotope was characterized by unusually clear (for the Black Sea) water because of the absence, in the water column, of the characteristic particles of organic suspended debris (known as "marine snow"). On the outcropped rocks macrobenthos forms did remind hydroids. The most notable detail of the biota was the occurrence of fishes, exceeding 20 cm in size. The most likely hypothesis for the presence of such a biotope and a biocenosis is the possible existence of a powerful submarine spring of aerobic waters, stably existing since a long time.

Notably, generally speaking, strategy of reproduction [laying eggs attached to the substratum and non pelagic development, (see Chukhchin, 1984; Anistratenko, 1998,] and early ontogenesis peculiarities of these mollusks allow them to exist within the limits of localized biotopes showing parameters contrasting with those of surrounding environments. Another important ecological characteristic for allowing species to inhabit the deep Black Sea is the resistance to hypoxia. Hydrobiidae comprise mollusks adapted to the lack of oxygen (Chukhchin, 1984), and the specimen of *Caspiohydrobia* sp. discovered at a -250 m of depth belongs to this family. In 2002, during the international expedition on R/V Meteor (Germany) to two stations in the north-western part of the Black Sea at the depths of 1900 m and 2190 m, respectively, it was discovered a meiobenthic crustacean species unknown to science. The same species was found out in 2003 in an expedition on R/V "Yantar" (Russia) in north-eastern part of the Black Sea at a 171 m of depth. The specimen (Cladocera: Ctenopoda) allowed to describe a new species, *Pseudopenilia bathyalis* Sergeeva, 2004 and a new respective genus taxon. Consequently, also the *Pseudopenilidae* family Korovchinsky & Sergeeva, 2008 was described.

Hydrochemical analyses of near-bottom water in a deep-water place of sampling of *Pseudopenilia bathyalis* showed the presence of sulfured hydrogen in an amount of 4-12 ml/l at a salinity of 22-23‰.
However, finding out this crustacean species at a depth of 171 m in the hypoxia zone allows to suppose that we deal with an aerobic organism adapted to oxygen-deficit conditions.

Places of submarine water unloading are local phenomena in the Black Sea. Their spatial characteristics and stability in time could be substantially differentiated depending on sources regime. For example, water supplement varies seasonally and may lead to a temporary stop of unloading.

Characters of water springs related to the artesian layers are more stable. In addition, salt and solved gases composition may be different. Spatial structure of freshwater biotope can change as a result of dynamic influences of the surrounding water masses. The innate structure of such biotopes can be non-homogeneous, thus including several biota that differently react to presence/absence of oxygen and of refreshing/salting waters. Hence biotopes and biocenoses of submarine unloading zones may be extremely heterogeneous, various and very vulnerable ecosystems.

CONCLUSIONS

The existence of aerobic life in the deepest part of the Black Sea is traditionally contested and even denied (see Vinogradov, 1997). On the contrary, the present paper strongly supports the hypothesis reporting several evidence on the issue, including the occurrence of the endemic Pseudopenilidae family (Korovchinsky & Sergeeva, 2008) which suggests the evolutionally-long existence of specific fauna in the deep Black Sea. Probably, a freshwater relict fauna could have existed not only from Neoeuxinian time (27-10 thousand years ago) but also earlier.

In fact, the existence of submarine freshwaters springs is independent from the change of sea salinity levels and quantitative and quality composition of this fauna may have undergone transformation during time, depending also on the changes of sea salinity. Oceanological and hydrogeological researches (Shnyukov & Ziborov, 2004; Trotsyuk et al., 1988) reported on desalted waters in near-bottom layers from tide-mark to deep-water cavity bottom of the Black Sea. The general volume of the submarine discharge in the Black Sea is only approximately estimated. However, discharge volumes calculated for single areas show that this volume is ecologically significant. For example, only for the Crimean coast from Balaklava to Simheiz (less than 50 km), karst submarine springs were appraised as about 700 thousand m³/day (Shnyukov & Ziborov, 2004). The same authors assessed the volume of subriver-bed flow as 1/3 of the volume of river flow. At the moment, water unloading in submarine canyons, although being demonstrated as a fact (Shnyukov & Ziborov, 2004), has not been calculated yet (not even preliminarily). The process of the submarine unloading can and must have ecological consequences.

This paper aims at not only discussing and contributing to a deeper knowledge of the refinement of the Black Sea biodiversity and of the recent state of the ecosystem, but also encourages the revision (by several colleagues) of some other aspects of the natural history of this ecosystem.

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