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CONTENTS

Scientific communications

Notes

1871
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SHRIMPS OF THE GENUS *PALAEMON* (CRUSTACEA, DECAPODA, PALAEMONIDAE) OF THE EUROPEAN SEAS

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Shrimps of the genus *Palaemon* (the family Palaemonidae) are among the most active invasive shrimps. To date, six species from this genus inhabit the shelf of seas washing Europe from the Gulf of Bothnia in the north to the Don River mouth in the southeast. Due to global warming and enhanced development of shipping which facilitated the transfer of larvae of these shrimps with ballast water, five out of six species significantly expanded their ranges in a historically short period of time. One of them, *Palaemon macrodactylus*, that was a south-boreal Western Pacific species, became a cosmopolitan. Only *Palaemon xiphias*, a symbiont of a seagrass *Posidonia oceanica*, has preserved its classic Mediterranean–Lusitanian range. The article provides data on the morphology of each of six species and the identification key. Also, the paper describes the history of the formation of new ranges, size composition, features of reproductive biology, and food composition of each species.

Keywords: shrimps, *Palaemon*, invasion, range, European seas, Black Sea

One of the most pressing problems of both theoretical and applied hydrobiology, as well as zoogeography, is the invasion of new species into already existing hydrocenoses and the formation of new ranges for species that seemed to be quite endemic. This process is continuous and unstoppable. For example, 76 species of aquatic and semiaquatic animals and plants have penetrated into water bodies of countries of the Baltic Sea basin since the early XIX century [Olenin, 2005]. The largest water bodies of the Kaliningrad region can serve as evidence of this. Thus, in recent decades, species from the Caspian, Black, and Mediterranean seas and from waters of East Asia and America have penetrated into main water bodies of the region – the Curonian and Vistula lagoons. Out of seven Decapoda species that inhabit water bodies of the Kaliningrad region today (those include two species of shrimps, two species of crabs, and three species of crayfish), the native ones are only a shrimp *Crangon crangon* and one species of crayfish.

The introduction of other two species of crayfish results from accidental or intentional acclimatization. The signal crayfish *Pacifastacus leniusculus*, native to western areas of North America (the Canadian province of British Columbia and the US states of Washington, Oregon, and Idaho), occurs in the Kaliningrad region only in Yantarnoe Lake (Sinyavinsky quarry, formerly Walter amber quarry) located between Yantarny and Sinyavino villages. The spiny-cheek crayfish *Orconectes limosus* is of North American origin as well. It was introduced to Europe in 1890 and colonized all types of water bodies in 21 countries, including Poland, Lithuania, and Belarus. Precisely this species turned out to be a carrier of an oomycete *Aphanomyces astaci*, the causative agent of crayfish plague, and this became a classic case of barbaric acclimatization with catastrophic consequences.

Procambarus clarkii (a so-called red swamp crayfish from Florida) is widely distributed and beloved by aquarists for its bright coloring. This species can carry crayfish plague, is very aggressive towards other crayfish, and actively digs the bottom. It is already clear that the distribution of this American crayfish was a mistake with very unpleasant consequences [Aklehnovich, Razlutskij, 2013; Laurent, 1997; Westman, 2003].

A crab *Rhithropanopeus harrisii* is also of American origin. Its native range is believed to cover fresh and estuarine waters off the eastern coast of North America from the southwest of the Gulf of St. Lawrence (Canada) to Veracruz (Mexico) [Williams, 1984]. Assumably, it migrated from there to waters of the western US. In 1874, the species was registered in Europe [Turoboyski, 1973].

The mitten crab *Eriocheir chinensis* was introduced to Europe from the Yellow Sea in 1912 at the larval stage with ballast water and was found in the Aller River (Germany). From there, it distributed widely in rivers and canals of all Europe from Germany and France to the Mediterranean Sea. In waters of the Kaliningrad region, it was first recorded in 1935 [Bacevièius, Gasiûnaitë, 2008]. In the Black Sea, it was found in 1998, and in the White Sea, in 2010 [Kitaiskii mokhnatorukii krab, 2023].

A shrimp *Palaemon elegans* appeared in waters of the Kaliningrad region in 2002. The first single specimens were caught in the sea almost at the water's edge near Kulikovo village. A year later, it was already caught in relatively large quantities in the Vistula Lagoon, at the Baltic Spit, in a so-called hydro harbor, and in ditches of the Baltiysk forts. The range of this species and other taxonomic, ecological, and faunistic data about it are provided below. There, we would like to note that two more *Palaemon* representatives were found in Polish waters: *P. adspersus* and *P. macrodactylus*. They have not yet been recorded in the Kaliningrad region, but at least the second one may penetrate there in the near future. This governed the necessity to make a "tool" for their rapid and reliable detection in our waters.

The author hopes that this work will be useful as a guide for a wide range of hydrobiologists interested in active invasive species of hydrobionts which include not only representatives of the genus *Palaemon*. Therefore, we considered it necessary to precede the information directly on these species with data on the body morphology of caridean shrimps.

Body structure of caridean shrimps. Shrimps belong to higher crustaceans which are characterized by a constant number of body segments (21); within higher crustaceans, shrimps belong to the order Decapoda, suborder Pleocyemata, and infraorder Caridea [De Grave, Fransen, 2011]. This is a large group of marine, brackish-water, and freshwater arthropods accounting for 3.5 thousand species. The main morphological characters distinguishing them from representatives of other orders of higher crustaceans are structural features of the cephalothoracic shell (carapace). From above and from the sides, it covers the five-segmented head (cephalon) and the eight-segmented thorax. Together, they form the cephalothorax. The lateral surfaces of the carapace (branchiostegites) form the outer walls of the branchial chamber enclosed between the inner surfaces of the branchiostegites and the wall of the cephalothorax. The cephalothorax bears appendages (limbs or their derivatives). Those are divided into three groups. The front five pairs are located within the cephalon. These are two pairs of flagella (antennules and antennae) performing the functions of sensory organs (touch and chemical sense organs) and three pairs of jaws (mandibles and two pairs of maxillae). The thorax bears eight pairs of appendages; the first three ones perform the function of maxillipeds, and five pairs of the walking legs, or pereiopods, perform the locomotor function. The first three pairs (in all representatives of the suborder Dendrobranchiata and in Stenopodidea from Pleocyemata) or two pairs (in Caridea) are armed with claws.

In the gill chamber of decapods, each somite of the thorax and (or) its appendages initially bear four gills named according to their position. The gill sitting on the wall of the segment above the articulated coxa (the first segment of the limb) of this segment is called a pleurobranch. A pair of gills attached to the articular membrane between the body wall and the coxa are arthrobranchs. A gill sitting on the coxa outgrowth serving to ventilate the gill chamber (the epipodit) is called a podobranch.

There are three main types of gills: phyllobranchia, trichobranchia, and dendrobranchia. Phyllobranchia have the simplest structure. This is a stack of leaf-shaped gills sitting in pairs along the axial blood vessel. Trichobranchia are bundles of filiform gills around the axial vessel of the gill. Dendrobranchia are formed by primarily paired gills subdivided into tree-like dissected bundles [McLaughlin, 1980]. Shrimp of the family Palaemonidae to which the genus *Palaemon* belongs have phyllobranchia.

The terminology used to describe shrimps is shown in Fig. 1.

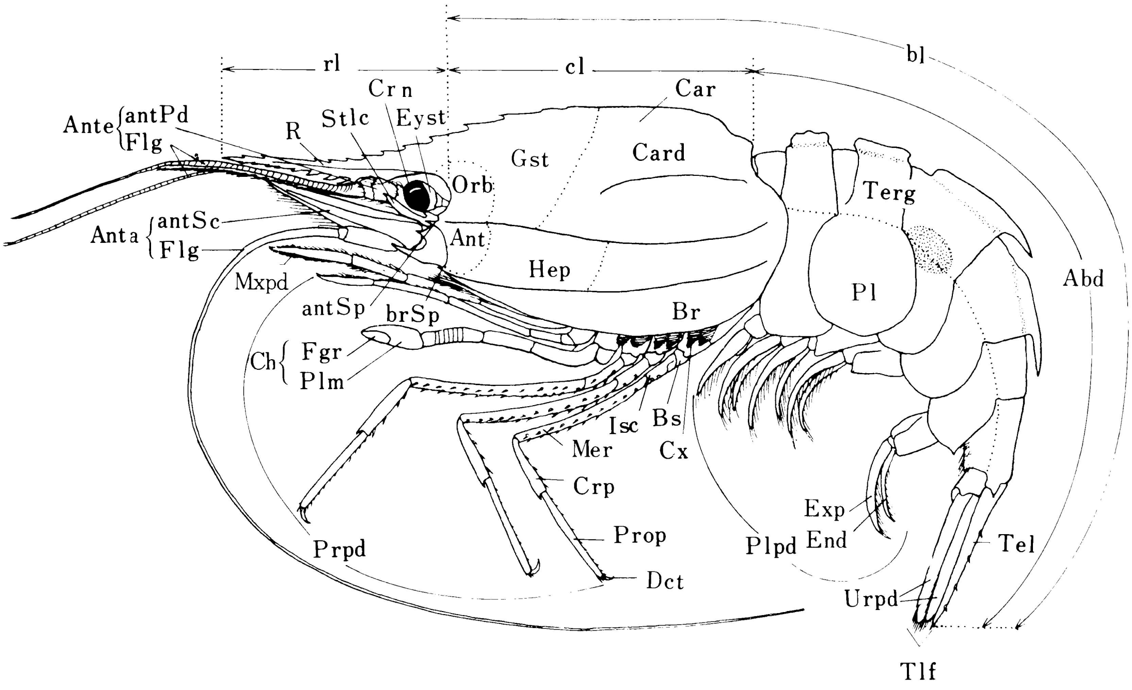

Fig. 1. Terminology used in the identification of shrimps (after [Baba et al., 1986], with additions). AntPd, antennular peduncle; antSC, antennal scale; antSp, antennal spine; bl, body length; Bra, brachial region; brSp, branchiostegal spine; Bs, basis; Car, carapace; Card, cardial region; Ch, chela; cl, carapace length; Crn, cornea; Crp, carpus; Cx, coxa; Dct, dactylus; End, endopod; Exd, exopod; Fgr, finger; Flg, flagellum; Gst, gastric region; Hep, hepatic region; Isc, ischium; Mer, merus; Mxpd, maxilliped; Orb. region, orbital region; Pl, pleuron; Plpd, pleopod; Plm, palm; Prop, propodus; Prpd, pereiopod; Rost, rostrum; rl, rostrum length; Stlc, stylocerite; Tel, telson; Terg, tergit; Tif, tail fan; Urpd, uropod

Shrimps cover a large group of decapods characterized by a unique appearance (so-called shrimp-like) and previously attributed to the former suborder Natantia of the order Decapoda. To date, they are included in the suborders Dendrobranchiata Bate, 1888 (entirely) and Pleocyemata Burkenroad, 1963 (the infraorders Procarididea Felgenhauer & Abele, 1983, Stenopodidea Spence Bate, 1888, and Caridea Dana, 1852) [De Grave, Fransen, 2011; Martin, Davis, 2001]. In September 2011, those accounted for 4,048 species [De Grave, Fransen, 2011].

Representatives of this infraorder play an extremely important role in ocean ecosystems. They inhabit the entire World Ocean from the supralittoral to the abyssal zone; they colonized freshwater basins on the Earth's surface and beneath it (in caves). Among them, there are burrowing and shelter-dwelling shrimps, epibenthic, bottom-dwelling, and pelagic ones. They can be commensals and have very complex relationships with other representatives of the fauna (for example, cleaner shrimps from different taxonomic groups, snapping shrimps from the family Alpheidae cohabiting with fish, or shrimps from the subfamily Pontoniinae which are coral symbionts). The role of shrimps in food webs, as well as their role as objects of fishing and mariculture, is well known.

And, as mentioned above, some of them play a significant role as invasive species. Out of them, representatives of the family Palaemonidae absolutely dominate – the largest family of shrimps (981 species, of which 41 are *Palaemon* ones [De Grave, Fransen, 2011]).

The family Palaemonidae covers shrimps with no longitudinal sutures along the entire length of the carapace. The telson bears two or three pairs of spines. Antennules have two completely separated flagella and an additional branch (the third one). Mandibles usually with a cutting process. Maxilla 1 with an unusually large median lobe of the coxa; maxilla 2 without or with one-two endites (internal branches of the limb). The exopodite of maxilliped 1 with a flagellum. Maxilliped 2 bears no large and tightly seating setae on the marginal segment; the maxilliped's third segment from the end is fused with the second one and with the wider following segment. Pleopods 2 of males have a copulatory organ (appendix masculina, the male process) [Chace, Bruce, 1993].

The family is divided into two subfamilies: Palaemoninae and Pontoniinae. Representatives of the first one usually have the posterior margin of the telson armed with two pairs of spines and bearing two or more submedial setae; maxillipeds 3 usually have two arthrobranchs.

Diagnosis of the genus *Palaemon* (after [Chace, Bruce, 1993; Kobyakova, Dolgopol'skaya, 1969]). The rostrum without a protruding basal ridge; on the anterior margin of the carapace, there are two spines: the upper antennal and the lower branchiostegal. In some species, the branchiostegal spine may be slightly offset from the anterior margin of the carapace. A groove ("longitudinal suture") extends backwards from the branchiostegal spine slightly curving downwards. There is no spine in the liver area of the carapace ("liver spine"). The fourth thoracic sternite has a thin median process; mandibles normally have a two- or three-segmented palp. Three posterior pairs of pereiopods have a simple finger which is shorter than the propodus. The endopodite of pleopods 1 of a male has no marginal protrusion.

As already mentioned, in the world fauna, 41 species of *Palaemon* shrimps are currently known. This genus is very widespread in the World Ocean. Its representatives usually inhabit the littoral zone. In water basins with very poorly developed tidal phenomena, its species are found from the water's edge. *Palaemon* representatives inhabit saline water, brackish water, and freshwater of tropical and temperate climate zones. Some species have limited commercial value, primarily as a bait for hook fishing.

To date, there are 6 species of this genus in European seas.

Key to identify shrimp species of the genus *Palaemon* from European waters

(after [D'Udekem d'Acoz et al., 2005; Kulish, 2021], with additions)

1. Branchiostegal spine located on the anterior margin of the carapace 2
– Branchiostegal spine slightly offset from the anterior margin of the carapace . . . . *Palaemon xiphias* Risso, 1816
2. Rostrum not curved or slightly curved upwards, not very thinned distally, and armed with teeth along 85–100% of its dorsal margin; merus of pereiopods 2 shorter than the carpus or equal to it 3
– Rostrum noticeably curved upwards and thinned distally; armed with teeth along 50–65% of its dorsal margin (*i. e.*, one third of the distal part of the dorsal margin is unarmed); merus of pereiopods 2 is 1.25 times the length of the carpus; the rostrum usually with 6–8 dorsal teeth (excluding the subapical one), 2 of which are located on the cephalothorax behind the orbital margin; the distance between the first and second teeth 1.5 times greater than between the second and third ones; mandibular palp three-segmented; dactylus of pereiopods 2 is 0.7 times as long as a palm or more . . . . . . *Palaemon serratus* (Pennant, 1777)
3. Not less than 7 (usually more) dorsal teeth (excluding the subapical one); 2 or 3 of them are located on the cephalothorax behind the orbital margin; the posterior margin of the dorsal teeth is directed obliquely upward; in just-caught specimens, the lower half of the rostrum has no red pigment spots 4
– There are fewer dorsal teeth (usually 5–6, excluding the subapical one); one of them is located on the cephalothorax behind the orbital margin, and the second is often located right on it; the posterior margin of the dorsal teeth is directed parallel to the dorsal side of the rostrum; in just-caught specimens, the lower half of the rostrum with red pigment spots; mandibular palp three-segmented; dactylus of pereiopods 2 is 0.8 times the length of a palm . . . . . . *Palaemon adspersus* Rathke, 1837
4. Rostrum usually with 7–9 dorsal teeth (excluding the subapical one) 5
– Rostrum usually with 10–12 dorsal teeth (excluding the subapical one); 2 or 3 of them are located on the cephalothorax behind the orbital margin; the distance between the first and second teeth is 1.5–2 times greater than between the second and third ones; the proximal part of the rostrum is not very convex ventrally; the short branch of the external flagellum of antennules is equal to their peduncle; dactylus of pereiopods 2 is 0.7 times the length of a palm; mandibular palp three-segmented; dactylus of pereiopods 3 very slender, approximately 0.9 times as long as the carpus . . . . *Palaemon macrodactylus* Rathbun, 1902
5. Two posterior dorsal teeth located on the cephalothorax behind the orbital margin; the distance between the first and second teeth 1.5 times greater than between the second and third ones; the proximal part of the rostrum protrudes noticeably ventrally (but not always); the short branch of the external flagellum of antennules is 0.7 times as long as their peduncle; mandibular palp three-segmented; dactylus of pereiopods 2, possibly, longer and slenderer on soft substrates than on hard ones . . . . *Palaemon longirostris* H. Milne Edwards, 1837
– Three posterior dorsal teeth located on the cephalothorax behind the orbital margin; the distance between the first and second teeth equal to the distance between the second and third ones; the proximal part of the rostrum protrudes strongly ventrally; the short branch of the external flagellum of antennules fused for approximately 50% of its length; the dactylus of pereiopods 2 is 0.4 times the length of the palm; mandibular palp two-segmented . . . . *Palaemon elegans* Rathke, 1837

Ecological and faunistic characteristics of shrimp of the genus *Palaemon* in European waters

***Palaemon adspersus* (Rathke, 1837)** (Fig. 2A). **Diagnosis** (after [Köhn, Gosselck, 1989], with additions). The length of the rostrum is approximately equal to the length of the carapace; the distal part of the rostrum extends beyond the level of the distal margin of the scaphocerites by approximately the length of the section in front of the distal tooth of the rostrum ventral side. The rostrum with a simple or double apical end, armed from above with five-six, rarely seven teeth, one of which, the most posterior, is on the carapace, behind the orbital margin. The rostrum lower (ventral) side is armed with three, rarely four teeth, with dark brown, to black, chromatophores (according to [D'Udekem d'Acoz et al., 2005], with red spots). Its width is not greater than the width of the upper half of the rostrum. Antennae with two flagella, the outer one with a flagella-like outgrowth fused with the base of the flagella. Mandibles with a three-segmented palp. The length of the claw fingers is ¾ the length of its palm, and the carpus of the pereiopod 2 is shorter than the merus.

Range. The range covers Eastern Atlantic, where the species reaches N60°, the Irish Sea, and the Atlantic waters of Morocco. In the Mediterranean Basin, it is found in the Western Mediterranean, the Alboran, Adriatic, Ionian, and Aegean seas, in the Sea of Marmara, and in the Eastern Mediterranean (Levant) [D'Udekem d'Acoz, 1999]. It inhabits the Black Sea and Sea of Azov [Kulish et al., 2018]. In 1930, *P. adspersus* was accidentally introduced in the Caspian Sea and stayed there. In the Baltic Sea, the species is distributed up to the Southern Finland [Köhn, Gosselck, 1989] and Gdansk [Inyang, 1977/78a, b], and is absent only in the Gulf of Bothnia [D'Udekem d'Acoz, 1999]. It is quite common (but not abundant) in the Bay of Gdansk: there, in summer 1975–1976, the density of the shrimp varied 1 to 17 specimens *per* 100 m^2 at a depth of 1–3 m [Wiktor et al., 1980]. Its low abundance in the Bay of Gdansk is confirmed by other researchers as well [Lapińska, Shaniawska, 2006]. This species inhabits depths of 0.1–10 m together with shrimps *Crangon crangon* and *P. elegans* making up only 2% of the total abundance of caught shrimps of the three species. It has not yet been registered in waters of the Kaliningrad region (our unpublished observations since 2002). The Bay of Gdansk seems to be the eastern border of *P. adspersus* distribution in the Southern Baltic.

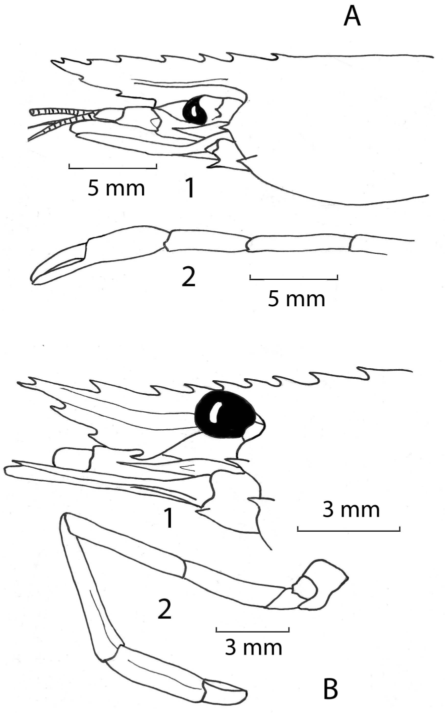

Fig. 2. A, *Palaemon adspersus*; B, *Palaemon elegans*: 1, cephalothorax, side view; 2, the 2nd pereiopods

Ecological characteristics. A shallow-water shrimp. In the northern Black Sea and in the Sea of Azov, it is associated with eelgrass beds [Evchenko et al., 2015] which is likely to result from the fact that it is most abundant in the shallowest areas of lagoons and estuaries inhabiting thickets of *Zostera marina*, *Ruppia* sp., and *Potamogeton* sp. [Kobyakova, Dolgopol'skaya, 1969]. Accordingly, this shrimp prefers relatively soft sediments. In this part of its range, *P. adspersus* is a commercial species; the latest known estimate reports that its stock is 100 t [Evchenko et al., 2015]. Off the southern coast of the Black Sea (Sinop Peninsula, Turkey), it is found in almost the same conditions. Since the material was sampled in January–February, the shrimp occurred at depths of about 30 m, where its abundance was very low; it migrated to the shore only at relatively high water temperature [Bilgin, Samsun, 2006]. The connection of *P. adspersus* with thickets, primarily *Zostera* ones, is also observed in other parts of its range. M. Gutu [1980] reports that the species was found in its thickets off the coast of Romania, and A. Berglund [1983] reports that it was encountered in Gulmar Fjord (western coast of Sweden), in Northern Denmark, and in waters of France from its northern coast to Arcachon (the southern area of the Bay of Biscay). In such cases, this shrimp was registered on sandy sediments, but it was also recorded in rocky depressions of the intertidal zone filled with water, among brown algae (*Fucus* sp.). In the Gulf of Gdansk, it is noted at depths of 0.5–1.0 m and also among thickets [Lapińska, Shaniawska, 2006; Wiktor et al., 1980]. Euryhaline species. It occurs in water with salinity from 5.5‰ (according to [Köhn, Gosselck, 1989]) or from 7–8‰ to oceanic values [Berglund, 1983; Kobyakova, Dolgopol'skaya, 1969], but prefers brackish-water habitats.

It is difficult to describe in detail *P. adspersus* size composition both because its size varies from region to region and because various researchers measure it differently and do not always report how they determined it. We found no conversion factors from the length of the carapace to the total body length.

The timing of the reproductive period is also very variable. Specifically, in a shallow (the depth averages 0.8 m) lagoon of Missolonghi (the Mediterranean Sea, N38°15′, E21°05′), abundant in aquatic vegetation and organic matter accumulated in the substrate, egg-bearing females are found from January to mid-June. Their highest abundance is registered in April. The duration of egg incubation depends on water temperature: at the beginning of the reproductive period, at +13 °C, it takes 42–47 days, and in the middle, when the value is about +20 °C, it takes 22–25 days. The reproductive period is limited to August, for water temperature reaches its maximum at this time. Year-round observations of fluctuations in the size composition have shown as follows: during the first six months of life, both males and females reach a total body length of 21 mm, and at the age of 1 year, both are of 24 mm. Then, the growth of males slows down; by 3 years (the maximum age), males have a body length of 48 mm, and females, 54 mm (assumably, the total body length was measured from the posterior margin of the orbits to the posterior margin of the telson). In winter, the growth almost stops [Klaoudatos, Tsevis, 1987]. These researchers indicate referring to literature sources that in waters of Denmark and Eastern Germany, the reproductive period lasts from May to late August. The mean realized fecundity of *P. adspersus* off the southern coast of the Black Sea is (1,963 ± 144) eggs (the range is 758–3,710). The mean egg sizes (short–long axis) at initial and late stages of embryonic development are 0.58–0.74 and 0.62–0.85 mm, respectively [Bilgin, Samsun, 2006].

In different parts of the range, biological parameters of this species vary, and this also affects the lifespan: it can exceed 3 years. The end of the reproductive period in mid-June, when water temperature is higher than +21 °C, suggests why *P. adspersus* range is limited to temperate and subtropical climatic zones: reproductive activity is possible at water temperature within +13…+20 °C [Klaoudatos, Tsevis, 1987].

Its diet includes detritus, algae, polychaetes, crustaceans, small molluscs, and juvenile fish [Köhn, Gosselck, 1989]. N. Inyang [1977/78b] expands the list of plant remains (in addition to algae, it contains diatoms and *Zostera*). When listing crustaceans, the researcher indicates the occurrence of decapods, as well as copepods, amphipods, and mysids, which together make up about 40% of all the food consumed. The second place is occupied by detritus (35.7%), and the third one, by polychaetes (18.3%).

To study food composition of *P. adspersus* in the Black Sea, the shrimp was sampled in the area of the Lebyazhy Islands of the Karkinitsky Bay, at a depth of less than 1.5 m, in September 2016, from commercial trap nets [Burukovsky, 2019]. The specimens had a body length (from the posterior margin of the orbits to the end of the telson) of 31.5–58.1 mm (males were of 33.9–44.1 mm, with a mode of 37 mm). The sex ratio was approximately 1 : 8 (11.7% of males and 82.3% of females). Females were represented by two groups: shrimps with gonads at maturity stage II had a modal size of 37 mm, and shrimps with gonads at maturity stage III, 47 mm. Therefore, in September, some of females already mated and molted, and vitellogenesis began in their gonads.

It turned out that *P. adspersus* is a benthic feeder and an euryphagous species. Its food spectrum covers a wide range of food objects: from detritus and plant remains to gastropods, higher crustaceans, including shrimps, and fish. In terms of the way it obtains food, it is primarily a gatherer – a detritivore and necrophage, with 70% of the volume of the virtual food lump occupied by detritus and corpses of higher crustaceans. At the same time, towards polychaetes, it behaves as an attacking predator, and towards gastropods, as a grazing one [Burukovsky, 2022a]. Comparison of food composition of this shrimp in the Karkinitsky Bay [Burukovsky, 2019] with that in the Baltic Sea [Inyang, 1977/78b], in Atlantic waters [Figueras, 1986], and Mediterranean waters [Guerao, 1993–1994] of the coast of Spain reveals its spatial and temporal quasi-stability. Based on all of the above, *P. adspersus* should be classified as an opportunistic predator of the subtidal zone [Burukovsky, 2019, 2022a].

It has not yet been encountered off the coast of the Kaliningrad region. The reason is probably as follows. In terms of temperature and salinity, waters washing the shores of this region are similar to those in the neighboring Gulf of Gdansk. However, sediments differ: along the coast of the Kaliningrad region, those are of accumulative origin and are composed of sands brought by a longshore current from the southwest. The bottom of coastal shallows is also composed by medium-sized sands with an admixture of gravel. There is almost no silt in sediments [Blashchizhin, 1976]. Apparently, due to this, there are no permanent *Zostera* and *Fucus* thickets in waters. In some areas, storms regularly wash ashore thickets of both *Zostera* and *Fucus*. It is believed to indicate the temporary nature of their existence [Gubareva et al., 2006]. Probably, this is why *P. adspersus* larvae (undoubtedly carried by the current into our waters) cannot find conditions there for the formation of permanent settlements.

Palaemon elegans **Rathke, 1837** (Fig. 2B). **Diagnosis** (after [Köhn, Gosselck, 1989], with additions). The rostrum is straight or slightly curved upwards. The maximum width of its lower plate is greater than that of the upper one. The rostrum is approximately equal in length to the carapace; its distal end usually does not extend beyond the distal margin of the scaphocerites and is most often bifurcated. The dorsal side of the rostrum bears seven to ten teeth, usually eight-nine. Out of them, three (rarely two) are located on the carapace behind the eye orbit. The lower side of the rostrum is armed with three-four teeth. Mandibles have a two-segmented palp (not a three-segmented one, as in other European species of the genus *Palaemon*). The carpus of the pereiopod 2 is usually longer than the merus and shorter than the claw. The length of the claw fingers is noticeably shorter than the length of its palm.

Range (after [D'Udekem d'Acoz, 1999], with additions). It covers Eastern Atlantic. In the north, the shrimp inhabits areas from the south and east of Norway, where it reaches N60°. In the east, the distribution limit is the Baltic Sea (up to the Gulf of Finland [Katajisto et al., 2013]), all coasts of Britain, waters along the southern coast of the North Sea, the Bay of Biscay, waters of Galicia (Spain), Portugal, Southwestern Spain, and Atlantic waters of Morocco. *P. elegans* was found on the Argen Bank (Mauritania) [Schaffmeister et al., 2006]. At one time, the shrimp was also believed to inhabit the Cape Verde islands and areas along the entire tropical coast of Western Africa [Fransen, 2023]; later, it was proven that this is *P. vicinus* – a species closely related to *P. elegans* [Ashelby, 2009]. It is registered off Madeira Island, the Azores, and Canary Islands. It inhabits all the seas in the Mediterranean Basin, and also it occurs in the Black Sea and Sea of Azov [Borcea, 1929; Caspers, 1951; Kobyakova, Dolgopol'skaya, 1969; Gutu, 1980]. In 1954–1956, during the acclimatization of the mullet in the Aral Sea, *P. elegans* accidentally penetrated there from the Caspian Sea and formed in the Aral Sea dense commercial aggregations [Kobyakova, Dolgopol'skaya, 1969; Vinogradov, 1968]. It survived the death of the Aral Sea and two crisis situations in the remaining "stub." It survived until the early XXI century. Unfortunately, the researcher does not report whether the shrimp occurs there now [Plotnikov, 2021].

It is found in continental waters of Israel and Iraq, where, the same as in the Caspian Sea, it penetrated because of accidental introduction [Plotnikov, 2021].

Outside the Atlantic Ocean basin, *P. elegans* was registered in the Suez Canal. There, it was among so-called Lessepsian migrants named after an engineer Ferdinand de Lesseps, who built the Suez Canal between the Mediterranean and Red seas (opened in 1869). Therefore, it is not accidentally recorded in the Red and Arabian seas and in Persian Gulf.

Ecological characteristics. It inhabits depths from the water's edge down to 5 m and prefers seagrass thickets and depressions of the rocky substrate where there is water during low tide [D'Udekem d'Acoz, 1999; Gutu, 1980]. A marine species, but also occurring in brackish water and freshwater. Mostly confirming this, H. Caspers [1951] adds as follows: off the coast of Bulgaria, single small individuals are found at depths down to 18 m. Off the coast of Romania, it is found at depths of 6–12 m on silty–sandy bottom together with *Crangon crangon* [Borcea, 1929].

P. elegans from the Sea of Azov is called a rockpool shrimp [Evchenko et al., 2015; Kulish et al., 2018], as it prefers relatively hard bottoms, stone accumulations, and baths on rocky littorals [Berglund, 1983; Kobyakova, Dolgopol'skaya, 1969]. However, the same as *P. adspersus*, this species can inhabit seagrass thickets, often together with *P. adspersus*. In the Sea of Azov, it is noted everywhere: from the Perekop Bay to the Kerch Strait. In this water body, its usual length in catches is 7–8 cm (the technique of measurement is not reported), and weight is up to 2.5 g. There, *P. elegans* inhabits the coastal zone among stones and algae, especially *Cystoseira* and *Zostera*.

In waters of Morocco, egg bearing lasts February to July [Lagardére, 1971]. Off the coast of Bulgaria and in the Sea of Azov, the shrimp reproduces May to August, and the peak of spawning occurs in the second half of May – in June [Caspers, 1951; Evchenko et al., 2015]. A female lays 160–3,600 eggs on pleopods carrying them for 1–1.5 months [Evchenko et al., 2015]. During summer, each female is capable of carrying eggs 3–4 times. Off the southern coast of the Black Sea, the mean fecundity of *P. elegans* is (1,057 ± 88) eggs (the range is 308–2,628). The mean egg sizes (short–long axis) at initial and late stages of embryonic development are 0.45–0.57 and 0.48–0.71 mm, respectively [Bilgin, Samsun, 2006].

The exact timing of the reproductive season of the shrimp in waters of the Kaliningrad region has not yet been established. However, it is clear that females can spawn there at least three times during summer.

In the Gulf of Gdansk located to the west of the Kaliningrad region, *P. elegans* is especially abundant on hard and rocky substrates or near concrete piers. The species is often found among filamentous green and brown algae growing on hard substrates [Janas, 2005].

In waters of the Kaliningrad region, it is distributed along the entire coast; this species has inhabited the entire Russian area of the Vistula Lagoon, as well as ditches and canals of the Baltiysk old forts. Its biotope is similar to that described above. In summer, both in the sea and in the lagoon, the shrimp is recorded at shallow depths from the water's edge; in the lagoon, it definitely prefers harder substrates. For example, it is abundant in the so-called hydro harbor of the Baltic Spit, where it inhabits almost the water's edge: cavities under dilapidated concrete lining of edges of the hydro harbor and aggregations of filamentous algae (own observations). The strip along the sea coast, where *P. elegans* is not so abundant, but also occurs, is characterized by medium-sized sands with an admixture of gravel [Blashchizhin, 1976]. The species tolerates significant desalination and withstands salinity fluctuations up to 35‰ and even up to 45‰ (at a temperature of +10 °C). In the Baltic Sea, it occurs at salinity from 15–20‰ (at its western border, in the Kattegat area) to 6–8‰ (in the Baltic Sea) and even to 2‰ (in the Gulf of Bothnia) [Janas, Mańkucka, 2010]. Larvae prefer stable temperatures of about +18 °C for their normal development [Inyang, 1977/78a, b]. In the Black Sea, the shrimp performs seasonal migrations moving from shallows to depths down to 30 m [Kobyakova, Dolgopol'skaya, 1969; Evchenko et al., 2015].

In the Kaliningrad Gulf and Primorskaya Bay of the Vistula Lagoon, the length of the shrimp carapace varied 2.1 to 11.8 mm. Considering the shape of the size composition curves, the total lifespan of *P. elegans* reaches three years. Sex differentiation occurs in the first year of life. Males were not recorded among three-year-old individuals. Assumably, they do not survive (at least, most of them) to this age. Females grow faster than males, and in size groups of 7–8 mm, there are already no males. Juveniles begin to appear in July, but mass replenishment of shrimp settlements occurs in September [Tsigvintsev, 2008].

In the Vistula Lagoon, detritus and plant remains prevailed in full stomachs of *P. elegans*. Detritus looked as a grayish-brown suspension or flakes, and in full stomachs, as a flocculent mass.

Plant remains were represented by higher plants with leaf scraps and their periphyton and by algae. Living plants were not noted among plant remains. They always bore traces of more or less prolonged maceration. In periphyton, diatoms, green algae, and blue-green algae were found belonging to five classes: Pennatophyceae, Centrophyceae, Chlorophyceae, Chlorococcophyceae, and Hormogoniophycea. A total of 23 species were identified.

Most often, shrimps of all size groups contain detritus (88.9%) and chironomid larvae (52.1%). In the virtual food lump, detritus prevailed (making up ⅔ of its volume, 70.4%), as well as plant remains (17.6%). These two food objects, along with periphyton algae, occupy 99.8% of the reconstructed mean volume of the food lump.

Consequently, *P. elegans* is a typical epibenthic shrimp behaving in the Vistula Lagoon as a benthic feeder which prefers a pretty narrow range of bottom objects. Its food also includes planktonic organisms (single cyclops and rotifers), but they are accidental. Detritus and plant remains, accompanied by periphyton algae, completely dominate the shrimp stomachs. In the Vistula Lagoon, *P. elegans* seems to serve currently as the main consumer of detritus of plant origin at different stages of its formation – together with associated periphyton. Apparently, *P. elegans* has no serious competitors among autochthonous species of the lagoon [Burukovsky, 2012, 2022b]. It does not compete with another, "older" invader of the Vistula Lagoon: the crab *Rhithropanopeus harrisii* which prefers there living higher plants with no signs of periphyton [Burukovsky, 2022a].

Palaemon longirostris **H. Milne Edwards, 1837** (Fig. 3). **Diagnosis** (after [Smaldon, 1993; Zooplankton and Micronekton, 2023]). The rostrum straight or slightly curved upwards and protruding beyond the scaphocerite; end of the rostrum sometimes bifurcated and armed with seven-eight dorsal and three-four (rarely five) ventral teeth. Two dorsal teeth are located behind the posterior margin of the orbits; the distance between the first and second teeth is approximately 1.5 times greater than between the second and remaining distal teeth.

The carapace with antennal and branchiostegal spines. Antennae have three flagella; the short branch of the external flagellum is approximately 0.66 (⅔) the length of the antennular peduncle and merges with the long flagellum for approximately ⅓ of its length. The outer margin of the stylocerite straight or slightly convex; its anterior margin convex. The scaphocerites reach the distal half of the carpus of pereiopods 2, sometimes extend somewhat further; the apical spine does not extend beyond the distal margin of the lamellar part of the scaphocerite. Maxillepedes 3 with the exopodite. Mandibles with a three-segmented palp. The finger of pereiopods 2 is approximately 0.4–0.5 times the length of the palm of the claw, but is variable; the carpus is equal in length to the merus or slightly longer. Telson with two pairs of lateral spines.

Note. In 1968, the species *Palaemon garciacidi* was described [Zariquiey Álvarez, 1968]. Its range was limited to waters of Southern Portugal and Southeastern Spain and to the Atlantic waters of Morocco [D'Udekem d'Acoz, 1999]. Interestingly, J.-P. Lagardére [1971] noted that the variability of the rostrum structure in this species overlaps with that of *P. longirostris*. Therefore, he considered *P. garciacidi* Zariquiey Álvarez, 1968 to be not an independent species, but a subspecies *P. longirostris* sp. *garciacidi* Zariquiey Álvarez, 1968. A comparative morphological and genetic study of shrimps from different populations attributed to these two taxa showed that *P. longirostris* and *P. garciacidi* should be considered one species, and the differences between shrimps from various habitats do not exceed the limits of interpopulation variability [Cartaxana, 2015]. Based on the priority criterion, this is *P. longirostris*.

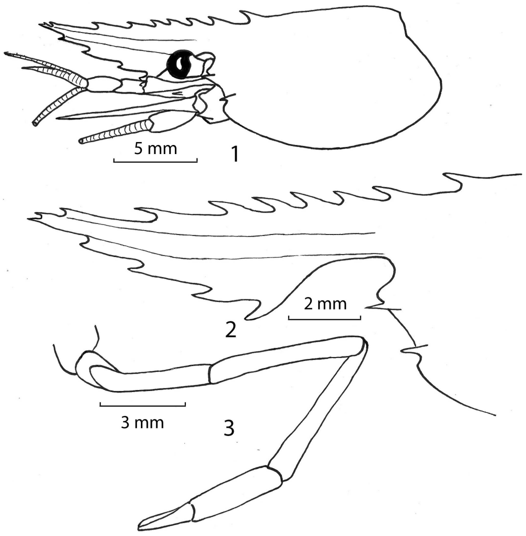

Fig. 3. *Palaemon longirostris*: 1, cephalothorax, side view; 2, rostrum, side view; 3, the 2nd pereiopods

Range. The species inhabits the Eastern Atlantic, where it is recorded from Northeastern Germany to Morocco [D'Udekem d'Acoz, 1999; Lagardére, 1971]. Within this area, it is occasionally found south, southeast, and southwest of England [Smaldon, 1993]. The shrimp is registered in the Bay of Biscay and in Portuguese waters. There are poorly confirmed signals that it occurs in various parts of the Mediterranean, *inter alia* off the Aegean coast of Turkey and in waters of Israel. It was recently noted off the southern coast of the Black Sea (Sinop Peninsula) [Sezgin et al., 2007] and off the southwestern coast of the Crimea, in the estuary of the Chernaya River, at a depth of 3–4 m, on silted shell rock [Statkevich, 2019]. Assumably, *P. longirostris* has already settled in the Black Sea, and its new findings should be expected.

The size of egg-bearing females (the carapace length) is 7.1 to 14.6 mm. In waters of England, they spawn April–May to August [Smaldon, 1993]. Further south, in France, in the Gironde River, egg-bearing females begin to occur earlier, March to July, and spawn at least once a season. They carry 78–1,391 eggs on pleopods; the mean value is (547 ± 234) eggs [Béguer et al., 2010]. Off the coast of France, *P. longirostris* is considered a commercial species with an annual catch of 36–82 t [Béguer, 2010]. Further south, in waters of Morocco, the reproductive period begins earlier, in January, and lasts until July, with a peak in April–May [Lagardére, 1971].

This species tends to act as a predator preferring the most accessible food objects. Juveniles in the Gironde estuary feed mainly on copepods (*Eurytemora affinis* and *Acartia bifilosa*) and adult mysids (*Mesopodopsis slabberi* and *Neomysis integer*). At the same time, they can feed on amphipods (*Gammarus zaddachi*), polychaetes, juvenile decapods, and detritus. There is a tendency to necrophagy. Larvae feed on diatoms [Béguer, 2010].

***Palaemon macrodactylus* Rathbun, 1902** (Fig. 4). **Diagnosis** (after [D'Udekem d'Acoz et al., 2005]). The rostrum straight and extending beyond the distal margin of the antennular peduncle; rather narrow in its proximal third. Its dorsal side armed with 9–15 teeth, most often 10–12 (excluding the sub-apical one). Two-three teeth of the dorsal series located on the carapace behind the level of the orbits. The first tooth separated from the second one by a distance 1.5–2 times (sometimes more) exceeding the distance between the second and third teeth. The size of the gap between the teeth gradually increases from front to back. The distance between two distal teeth approximately equal to that between two proximal ones (or less). The dorsal teeth of the rostrum directed obliquely upwards. The ventral side of the rostrum armed with three-five teeth. There is a branchiostegal spine on the anterior margin of the carapace. The short branch of the outer flagellum of the antennule is equal in length to the antennular peduncle. Mandibular palps three-segmented. The length of the claw fingers is 0.7 of the palm length. The length of the propodus of pereiopods 2 is equal to the length of the carpus. On pereiopods 3–4, the length of the fingers is almost equal to the length of the carpus.

History of the formation of the modern range of the species. The homeland of *P. macrodactylus* is the Far East. There, the northern border of its range is Peter the Great Bay. Further south, the shrimp is found off the coast of South Korea and China, up to the Yangtze River mouth, and inhabits waters of Japan [Ashelby et al., 2013; Marin, 2013]. It is described from waters of Taiwan as well [Chan, Yu, 1985].

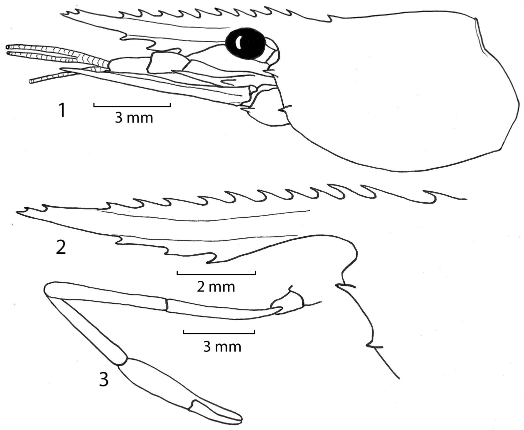

Fig. 4. *Palaemon macrodactylus*: 1, cephalothorax, side view; 2, rostrum, side view; 3, the 2nd pereiopods

The first data on *P. macrodactylus* outside its native range were published by W. Newman in 1963 (cited after: [Ashelby et al., 2013]), *prior* to its finding in waters of Taiwan. It was recorded in San Francisco Bay, and it was already a settled species at that time there. W. Newman believed that *P. macrodactylus* had been inhabiting this area since at least 1954 and was introduced to San Francisco Bay during the Korean War. Anyway, it was recorded there in commercial quantities in 1957 and was used as a fishing bait [Ashelby et al., 2013]. To date, this species is distributed in the Eastern Pacific as far north as Boundary Bay (it is located on the border between the Canadian province of British Columbia and the US state of Washington) and as far south as the Los Peñasquitos Lagoon (San Diego County, 120 miles south of Los Angeles) [Ashelby et al., 2013].

In the 1960–1970s, *P. macrodactylus* was registered in Australia. The history of its occurrence there remains unclear. First, it was noted in Mannering Lake in the state of New South Wales (Southeastern Australia), and later, it was encountered in the vicinity of Port Adelaide on the southern coast of the continent. Moreover, it is assumed that the settlement in Southeastern Australia has not survived to this day [Ashelby et al., 2013].

In the 1990s, the shrimp appeared in European waters. It was first found in the River Thames. Currently, *P. macrodactylus* has settled in a vast area of the Northeastern Atlantic: from the southern area of the North Sea in the north to Southern Spain [Ashelby et al., 2013; D'Udekem d'Acoz et al., 2005; González-Ortegón et al., 2006]. It is still unclear how the species penetrated into the Northeastern Atlantic: directly from Asia or from San Francisco.

As for the Baltic Sea, in the paper [Ashelby et al., 2013], the researchers note that this sea can be considered a risk region for *P. macrodactylus* introduction.

In 2002, the shrimp was found in waters of Romania (Black Sea), in the Port of Constanța, and then in Bulgaria, in the brackish-water Lake Varna which is connected to the sea [Micu, Niţă, 2009; Raykov et al., 2010]. As assumed, the species was carried there from Rotterdam with ballast water.

Around the same time (at least, since 2001), *P. macrodactylus* was registered in the New York estuary network. In the first samples taken that year, there were egg-bearing females, *i. e.*, it was already an established settlement. The region is characterized by such a high density of shipping that it was impossible to determine where this species came from: San Francisco or new settlements in European waters [Ashelby et al., 2013; Warkentine, Rachlin, 2010].

In the same 2001, there was a report on *P. macrodactylus* record in the Southeastern Atlantic, in the port of Mar del Plata (Argentina). There, the shrimp inhabits waters with a mean salinity of 32–33.7‰; it has already distributed over 120 km to the south and to the north, to the borders of Uruguay [Spivak et al., 2006].

In 2005, the species was registered in the Mediterranean Sea: first, in its western part, in waters of the Balearic Islands [Ashelby et al., 2013]; in May 2012, in the Venice lagoon (the northern Adriatic Sea, Italy) [Cavraro et al., 2014]. After this, the "conquest" of the Black Sea was only a matter of time. Indeed, in 2009, the shrimp was noted in Bulgaria, in the above-mentioned Lake Varna formed as a result of global geological shifts and connected to the sea; then, it was recorded in waters of Romania [Micu, Niţă, 2009]. Finally, in July 2018, *P. macrodactylus* was first caught in waters of the European Russia: in the Kerch Strait area (Sea of Azov, coast of the Chushka Spit, Krasnodar Krai) [Timofeev et al., 2019]. A few years later, its settlements were found in the Don River estuary [Matishov et al., 2022]. Consequently, the species definitely settled in the Black Sea and Sea of Azov.

To date, *P. macrodactylus* can be considered a cosmopolitan of temperate and subtropical waters, although in the south of the Asian part of its range, it reaches tropics in waters of China and Taiwan. It can be assumed that the "conquest" of the World Ocean by this species is not yet complete.

Ecological characteristics. Undoubtedly, successful distribution of this species may result not only from significantly developed shipping, but also from its tolerance to a wide range of temperatures and salinities and to hypoxic conditions. Moreover, *P. macrodactylus* is a great osmoregulator and, accordingly, can inhabit freshwater or almost freshwater areas, as in California, and areas with completely marine conditions, as in Mar del Plata [Spivak et al., 2006].

In waters of the Northern Europe, *P. macrodactylus* prefers brackish estuaries, where it inhabits areas of port walls and yacht moorings, as well as spots where floating garbage and a reed (*Phragmites australis*) are accumulated. This is probably why this species, the same as *P. adspersus*, has not yet penetrated into waters of the Kaliningrad region and Lithuania (due to features of currents and sedimentogenesis of these areas, see above).

The peculiarities of life cycle vary greatly from area to area. In the native part of the species range, the breeding season lasts mid-April to early October; in California, egg-bearing females occur May to August; and in Argentina, the breeding season is confined to the southern summer, October to March. Two-year-old *P. macrodactylus* females lay their eggs on pleopods earlier than one-year-old females. One-year-old shrimps lay less than 1,000 eggs, and older ones lay 500 to 2,800. Representatives of each age group can produce at least two generations *per* year, while in laboratory-controlled conditions, they can spawn five to nine times in a row.

The growth rate of females is the highest in the first year of life with a spurt (acceleration) before spawning. The next year, growth slows down. Sexual differences emerge when the shrimps reach the length of 20 mm; females grow faster than males and are larger. Lifespan in Japanese waters is two-three years. In Mar del Plata, the species lives less than in the native part of its range, and this is due to stressful conditions of their habitat in highly saline water [Ashelby et al., 2013].

Stomachs of *P. macrodactylus* contain remains of mysids, copepods, amphipods, barnacles (Balanidae), polychaetes, small bivalves, fish larvae, and insects. They account for 75–93% of stomach contents [Ashelby et al., 2013]. Therefore, the species is considered omnivorous, although it seems clear that it is, first of all, a predator and, possibly, a predator-gatherer (according to [Burukovsky, 2022b]).

***Palaemon serratus* (Pennant, 1777)** (Fig. 5). **Diagnosis** (after [Smaldon, 1993]). The rostrum noticeably curved upwards; its end usually bidentate. In adults, dorsal teeth do not reach the distal third of the rostrum. Six or seven dorsal teeth, and four-five ventral ones; two dorsal teeth located behind the posterior margin of the orbits. The carapace with antennal and branchiostegal spines. Antennae have three flagella; the short branch of the outer flagellum of antennules is approximately 0.85 times the length of the antennular peduncle, and the fused part of the flagellum is 0.20–0.25 times the length of the long flagellum. The outer margin of the stylocerite is convex, but may be slightly concave; in very large individuals, the anterior margin of the stylocerite is convex, and the apical spine is long and strong. The scaphocerite reaches half the length of fingers of pereiopods 2 or the distal end of the finger in juveniles; the apical spine of the scaphocerite does not extend beyond the anterior margin of its plate. Maxillipeds 3 reach half the length of the scaphocerite or are slightly shorter; there is the exopodite. Mandibles with three-segmented palps. Dactylus of pereiopods 2 equals 0.5 times the length of the propodus; merus is 1.25 times the length of the carpus. Telson with two pairs of lateral spines.

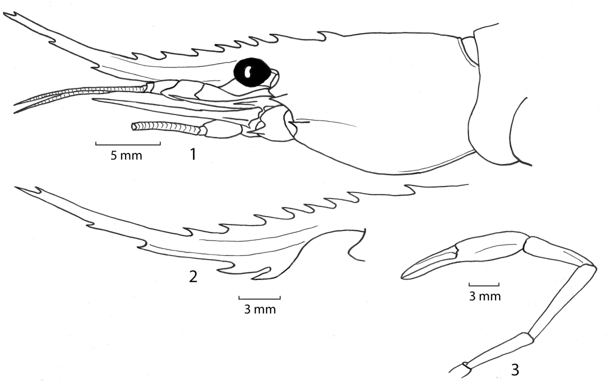

Fig. 5. *Palaemon serratus*: 1, cephalothorax, side view; 2, rostrum, side view; 3, distal part of the 2nd pereiopod

Range. Off the coast of Britain, it is common in the south, southwest, and west and rare in the northeast, although single specimens have been recorded off Northumberland. The shrimp was encountered off the western, southwestern, and southeastern shores of Ireland [Smaldon, 1993]. It occurs in the North Sea, in waters of France: off both coasts of the Cotentin Peninsula and in the Bay of Biscay. *P. serratus* was registered off the coast of Northern Spain (Galicia) and Portugal, off the southwestern coast of Spain, Canary Islands, and the Madeira. It was noted off the coast of Morocco up to Western Sahara. The species was found almost everywhere in the Mediterranean Basin, Sea of Marmara, and Black Sea. Interestingly, *P. serratus* is quite rare in the Black Sea. It was reported from the coast of the Crimea, Lake Varna, and Sukhumi Bay [Kobyakova, Dolgopol'skaya, 1969]; also, it was recorded in underwater karst caves of the Western Crimea (Cape Tarkhankut) [Kovtun, Makarov, 2011].

Ecological characteristics. The shrimp is common along rocky shores, in tidal baths, but often also near shores unprotected and moderately protected from waves, as well as in the subtidal zone among algae (in South Wales, England, among *Laminaria digitata* and *Fucus serratus* [Grenfell, 2013]), and in *Zostera* thickets. *P. serratus* can occur in the same tidal baths as *P. elegans*, but it does not go as far into the littoral zone as the second species [Smaldon, 1993].

It inhabits depths from the water's edge down to 40 m, where it spends winter months. At these depths, females prefer rocky substrates, and males choose silty soils. In summer, the shrimp migrates to estuaries and reaches there the peak of abundance by September; then, is goes back. The reason is this species actively avoids open sea areas (water is too cold in winter and warm in summer). Males are the first to migrate both to estuaries and back [Grenfell, 2013].

The maximum total length is 110 mm; usually, it is slightly less than 100 mm (for some reason, the total body length was measured by this author from the posterior margin of the orbits to the posterior margin of the sixth abdominal segment, and not the telson, as is accepted by most researchers. – *R. B.*). The species is characterized by sexual dimorphism of body size. The mean size of males is 7.5 cm; females, 9 cm. One of the reasons is that males reach sexual maturity at the age of 6–7 months, while females reach sexual maturity at 9–10 months [Grenfell, 2013].

In British waters, the breeding season lasts usually November to June, with some large females laying their eggs in July, August, and September [Smaldon, 1993]. In the Ebro River delta (the Western Mediterranean), the breeding season begins in August, when females with mature gonads appear in shallows overgrown with *Zostera*. However, the proportion of egg-bearing females is low there. They may migrate to other habitats [Guerao, Ribera, 2000].

Palaemon xiphias **Risso, 1816** (Fig. 6). **Diagnosis.** The anterior end of the rostrum always extends beyond the anterior margin of the scaphocerite, and its distal part is curved upward. The rostrum has seven dorsal teeth (rarely eight or six), one subterminal, and five ventral (rarely four). Two posterior dorsal teeth are located on the carapace behind the posterior margin of the orbits. The branch of the short flagellum of the antennule is fused for approximately ⅕ of its length. The palp of the mandible is three-segmented. The claw finger of the pereiopods 2 is significantly longer than the palm of the claw and slightly longer than the carpus. The branchiostegal tooth is located at a distance from the anterior margin of the carapace [Lagardére, 1971; Pesta, 1918].

Range. An inhabitant of the Eastern Atlantic, including water areas of Canary Islands and Madeira. It is registered almost everywhere in the Mediterranean Sea: in the Alboran, Adriatic, Aegean, and Ionian seas and off the coast of Northern Africa from Egypt to Morocco. Wherever it is found, it is closely related to thickets of a seagrass, primarily *Posidonia oceanica* and to a lesser extent *Zostera marina* and *Cymodocea nodosa*. In fact, the range of this species is limited to the range of *Posidonia*: the Mediterranean Sea and nearby areas of the Eastern Atlantic [Posidoniya, 2023].

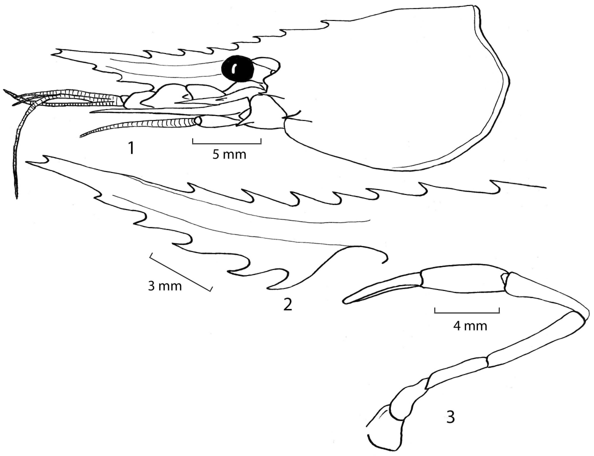

Fig. 6. *Palaemon xiphias*: 1, cephalothorax, side view; 2, rostrum, side view; 3, the 2nd pereiopods

Ecological characteristics. *Posidonia* usually grows in large colonies forming, together with other seagrasses, unique underwater meadows in sea bays and bights at depths from the littoral zone down to 30 m, sometimes even down to 50 m. Wherever these sea meadows are mentioned, *P. xiphias* is mentioned as well. This species is often called *Posidonia* prawn. In the Adriatic Sea, the shrimp is found at depths of 2 to 6 m. In this area, it begins laying eggs on pleopods in May–June [Karlovac, 1969; Pesta, 1918]. The size composition and reproductive biology of this species were best studied in 1989–1990 in the Western Mediterranean: in the Alfacs Bay near the Ebro River delta (Spain) [Guerao et al., 1994]. There, the total body length measured from the end of the rostrum to the end of the telson, was 21–70 mm in females and 19–50 mm in males.

The shrimp growth was observed during the whole year and was faster in summer than in winter. Sexual dimorphism in size composition was noted throughout life, starting with the smallest sizes. In overwintered individuals, especially in females, the growth rate decreases in May which coincides with the beginning of the reproductive season. Already in June, more than 90% of females with a body length of 48–66 mm lay their eggs on pleopods. In October, very few large females remain in the population. A chiasm is formed between them and the next generation of females with the greatest length of 46 mm. Then, the reproductive season ends. The lifespan of *P. xiphias* is 14–17 months. The main part of each generation is born in July. Females can lay eggs on pleopods two to five times depending on water temperature. At +21 °C, embryonic development lasts for 20 days, and at +28 °C, for 12 days. The number of eggs on pleopods varied from 318 in a female with a body length of 37.9 mm to 2,750 in a shrimp with a body length of 69 mm [Guerao et al., 1994].

In terms of food composition, *P. xiphias* is a benthic feeder. Its stomachs most often contain bottom-dwelling higher crustaceans (amphipods, mysids, and isopods); along with them, although much less frequently, bivalves, gastropods, polychaetes, and echinoderms occur. Detritus is found in every second or third stomach. Copepods are recorded quite often (in every third or fourth stomach) (these might be bottom-dwelling harpacticoids. – *R. B.*). The frequency of their occurrence is the highest in juveniles and decreases by 4 times with a gain in the body size of the shrimp. The same is true for the frequency of occurrence of mysids. Other higher crustaceans are more common in larger individuals. Not very often, plant remains are also noted in stomachs [Guerao, 1995]; their role is sharply reduced during winter, when they are replaced chiefly by bottom animals [Sitts, Knight, 1979].

DISCUSSION

Currently, six shrimp species of the genus *Palaemon* inhabit European waters from the Gulf of Bothnia to the Sea of Azov. The set of six objects is not easy to analyze: there are relatively few items for this. On the other hand, it seems that the stimulus (or, precisely, the set of such stimuli) for the emergence of processes in the environment driving the formation of modern habitats of these species was common: global warming which opened the way for European species of the genus *Palaemon* to the north and the presence of permanent routes for "carriers" of reservoirs for their larvae – ships with their ballast water.

As a result, these six species are divided, conventionally, into three groups: a cosmopolitan *P. macrodactylus*, a persistent native of the Mediterranean–Lusitanian zoogeographic area *P. xiphias*, and four other species that cannot be attributed to either the first or second group.

The aboriginality of *P. xiphias* is determined by a clear external factor: symbiosis with a higher plant *Posidonia oceanica*, one of the most interesting species of marine herbaceous plants (so-called seagrasses) common along the coast of the Mediterranean Sea and nearby areas of the Atlantic Ocean. This underwater grass has all the characters of a land plant: it has roots (very long ones, 1.5 m, to hold on more tightly to the seabed), leaves (ribbons up to 0.5 m), stems (those are also roots, only above-the-sand ones, and also up to 1.5 m long), flowers, seeds, and fruits. The area of *Posidonia* underwater meadows in the Mediterranean Sea alone is 3% of its surface area (75,000 km^2) [Posidoniya, 2023]. *P. xiphias* distribution and features of its biology are inseparably connected with these underwater meadows, and *vice versa*: this *Palaemon* species is not known outside *Posidonia* colonies. Accordingly, until *Posidonia oceanica* penetrates into the Black Sea, *P. xiphias* will not occur there. Assumably, in the foreseeable future, there will be no replenishment of *Palaemon* fauna of the Black Sea.

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I would like to thank A. Kulish: he helped me a lot by sending his articles and his colleagues' publications focused on shrimps from the genus *Palaemon* sampled by him in the Sea of Azov, as well as some other necessary papers on the same issue. Also, I would like to thank I. Dovgal who read the manuscript and made a number of important comments on its design.

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КРЕВЕТКИ РОДА *PALAEMON* (CRUSTACEA, DECAPODA, PALAEMONIDAE) ЕВРОПЕЙСКИХ МОРЕЙ

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Креветки рода *Palaemon* (семейство Palaemonidae) — одни из самых активных инвазивных видов креветок. На шельфе морей, омывающих Европу от Ботнического залива на севере и до устья Дона на юго-востоке, сегодня обитают шесть видов креветок из данного рода. В результате глобального потепления и усиленного развития судоходства, облегчивших перемещение личинок этих креветок с балластными водами, в исторически краткий промежуток времени пять из шести видов существенно увеличили размеры своих ареалов, а один из них, *Palaemon macrodactylus*, из южнобореального западнопацифического вида превратился в космополита. Лишь *Palaemon xiphias*, симбионт морской травы *Posidonia oceanica*, сохранил свой классический средиземноморско-лузитанский ареал. В статье представлены сведения о морфологии, истории формирования новых ареалов, размерах, особенностях репродуктивной биологии и составе пищи каждого вида, а также приведены ключи для определения.

Ключевые слова: креветки, *Palaemon*, инвазия, ареал, европейские воды, Чёрное море

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PHENOMENON OF THE “LARGE” HORSE MACKEREL APPEARANCE IN THE BLACK SEA: VERSIONS AND PROOFS

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The paper provides an overview of literature data and results of the authors’ own investigations on the phenomenon of a sudden mass appearance of a “large” (“giant”) form of the horse mackerel (*Trachurus*, Carangidae) (LM) in the Black Sea in the late 1940s and its unexpected disappearance in the mid-1960s. This phenomenon was of great interest and attracted attention of many researchers hypothesizing that it was a new source of information for understanding processes of the organisms’ adaptation and important issues of microevolution and speciation. Different approaches to the study of this fact are analyzed, including determination of the LM origin and clarification of its taxonomic status. On their basis, three mutually exclusive versions are proposed. According to the first one, the LM is a new species of the Mediterranean origin; according to the second one, it is an ecomorph of *Trachurus mediterraneus ponticus*; and according to the third one, it is a hybrid of *T. mediterraneus mediterraneus* and *T. mediterraneus ponticus*. Based on the investigation on thermal resistance of isolated muscles and serological analysis of tissue proteins, Yu. Altukhov and co-authors identified the LM as an independent species of the Mediterranean (and, possibly, Atlantic) origin which appeared in the Black Sea because of a sudden expansion of its range after a sharp increase (outburst) in abundance. The main evidence for the Black Sea origin of the LM is the fact as follows: all stages of its life cycle, *i. e.*, reproduction, growth of juveniles, feeding, and wintering of adults, occur in the Black Sea; moreover, its large specimens were known earlier. Belonging of the Black Sea “small” horse mackerel and LM to the same species was confirmed by the electrophoretic studies on protein composition of their blood serum. The idea of the hybrid origin of the LM was first proposed by I. Dobrovolov based on an investigation of electrophoretic spectra of nonspecific muscle esterases in *T. mediterraneus ponticus* and *T. mediterraneus mediterraneus*. According to our data, the third version of the LM origin seems more probable than other ones. Occurrence of *T. mediterraneus mediterraneus* in the Black Sea and its hybridization with *T. mediterraneus ponticus* are manifestations of the mediterranization; this process has intensified due to increasing anthropogenic load on the ecosystem of Azov–Black Sea Basin and ongoing disruption of freshwater balance resulting from overregulation of river flows and sea salinization. None of three versions can be refuted or confirmed by molecular genetic methods, since there is currently no LM in the Black Sea. Thus, the phenomenon of the “large” horse mackerel appearance in the Black Sea is still unexplained.

Keywords: Black Sea “large” horse mackerel, versions, Mediterranean origin, Black Sea origin, hybrid origin

The “large” (“giant”) horse mackerel unexpectedly appeared in enormous abundance in the Black Sea in the late 1940s and suddenly disappeared in the early 1960s, and this phenomenon aroused great interest of many researches. In relatively short period, about 10–15 years, dozens of articles were published discussing its origin, systematics, morphology, biology, physiology, and biochemistry, as well as characteristics of behavior and migrations, stock assessment, and prospects for its fishery. Such attention was due to at least two factors. The first one seemed to be related to high economic importance of the “large” horse mackerel as a valuable food object. In this regard, accurate estimates of total stock were supposed to serve as a necessary condition for organization of rational exploitation and forecasting. Commercial fishing of the “large” horse mackerel began in 1953, and about 15 thousand tons were caught already in 1956. To compare, maximum catches of the main commercial fish of the Black Sea, the European anchovy *Engraulis encrasicolus* (Linnaeus, 1758), did not exceed 16 thousand tons in the 1950s. Thus, the “large” form prevailed in the Black Sea fishery with the stock that was estimated to be 2–3 million tons [Tikhonov, 1959]. The second factor governing the interest of researchers was as follows: studies of the “large” horse mackerel were supposed to provide new information for better understanding of the organism adaptation, microevolution, and speciation. To date, there is no common concept on the origin of the “large” form, reasons for its occurrence in the Black Sea, and relationships with the Black Sea horse mackerel.

The aim of this paper is to systematize and generalize versions of the “large” horse mackerel origin and its appearance in the Black Sea.

History of research and versions of origin. A short period of the “large” horse mackerel occurrence in the Black Sea has the following chronology. In spring 1948, aggregations of large fish occupying an area of about 800 sq mi were noted from the aircraft searching for marine biological resources (fish and dolphins) off the coast of Georgia [Biologiya i promysel, 1955]. It turned out to be the “large” horse mackerel, 50 cm in length. Next year, it appeared in catches off the Caucasian coast. In 1950, the “large” form spread along the Caucasian coast and southern coast of the Crimea up to the Cape Chersonesus and occurred in the Sea of Azov [Biologiya i promysel, 1955; Tikhonov, 1958, 1959]. Meanwhile, a part of a population wintered off the southern coast of the Crimea. In 1952, the “large” horse mackerel was found off the Anatolian coast and also in the northwestern Black Sea: off the coasts of Romania and Bulgaria. In 1954–1955, only single specimens were recorded in the Black Sea: in the Bosphorus, Sea of Marmara, and Dardanelles [Nümann, 1956]. At the same time, in 1953–1954, the “large” form spread throughout the Black Sea and covered open deepwater areas.

However, since 1955, the range of the “large” horse mackerel was shrinking. This process was accompanied by a decrease in its abundance as a result of natural mortality and fishing and also due to absence of abundant generations. In 1963, total stock dropped by at least 20–25 times compared to that of 1956. Since 1965, the statistics of fishery has no differentiation of the Black Sea horse mackerel into “small” and “large” ones because of extremely low abundance of the second form.

The occurrence of the “large” form in the Black Sea caused debates on its taxonomic status and origin. To date, there are three main versions of the Black Sea “large” horse mackerel origin: Mediterranean, Black Sea, and hybrid ones.

Version of the Mediterranean origin of the “large” horse mackerel. The first to propose this version was Yu. Aleev. Based on similarity in body shape and growth rate of the “large” horse mackerel and horse mackerel from the Mediterranean Sea, the researcher considered that the “large” form is a migrant from the Mediterranean Basin and suggested to name it the Mediterranean horse mackerel [Aleev, 1952]. Later, after detailed investigations of the genus *Trachurus*, he concluded that the “large” horse mackerel was a local herd of *Trachurus mediterraneus ponticus*, along with other three herds (northern, eastern, and southwestern ones), and identified it as a southern herd [Aleev, 1957]. He tended to associate rapid growth of the “large” horse mackerel with more favorable water temperature off the Anatolian coast and better nutritional conditions.

Yu. Altukhov supported the version of the Mediterranean origin. With his co-authors, he applied cytophysiological (thermal resistance of isolated muscles) and biochemical (serological analysis of tissue proteins) techniques for studying the relationship between the "large" horse mackerel and the Black Sea "small" one [Altukhov, 1962a, b, 1967; Altukhov, Apekin, 1963; Altukhov, Mikhalev, 1964]. Based on results, these researchers drew conclusions about the level of differences between the species, and B. Ushakov's concept served as the ground. According to it, thermal resistance of tissues is the species criterion of poikilotherms [Ushakov, 1959]. However, in subsequent studies, differences in thermal resistance were registered not only on the level of species and populations, but also on the level of smaller groups representing different taxonomic ranks, with fish included [Yablokov, 1987]. Yu. Altukhov searched for evidence for the Mediterranean origin of the "large" horse mackerel and even for the Atlantic one. He attempted to explain its mass appearance in the Black Sea by a sudden range expansion resulting from a sharp increase in the species abundance.

N. Revina and T. Saf'yanova supported the version of the Mediterranean origin as well. Based on Yu. Altukhov's data on significant immunological and cytophysiological differences between the "small" and "large" forms and also considering the lack of abundant recruitment of the "large" horse mackerel herd over several years, these researchers suggested that its mass occurrence could result from an outburst outside the Black Sea [Revina, Saf'yanova, 1966a, b]. A possible reason for this phenomenon could be a response to climate change, in particular, intensification of oceanographic processes in the North Atlantic in the 1950s covering the Mediterranean Sea [Izhevsky, 1964]. In the opinion of these authors, the "large" horse mackerel might be a Mediterranean and even Atlantic migrant.

Version of the Black Sea origin of the "large" horse mackerel. V. Tikhonov adhered to the version of the Black Sea origin [Biologiya i promysel, 1955; Tikhonov, 1958, 1959]. His position was based on data of W. Nümann [1956]: according to the latter one, the "large" horse mackerel was noted by Turkish fishermen engaged in fishing off the coasts of Bulgaria, the Crimea, and Georgia since ancient times. Moreover, he relied on facts that all stages of this fish life cycle, *i. e.*, reproduction, growth of juveniles, feeding, and wintering of adults, occur in the Black Sea. Another evidence was the absence of the "large" form in the Mediterranean Sea. According to V. Tikhonov, the "large" horse mackerel was not widespread earlier because of its low abundance. To confirm, he referred to a work of S. Zernov [1913] who recorded the capture of a 40 cm long horse mackerel off the Caucasian coast.

A study of protein composition of the blood serum for six horse mackerel species of two genera, *Trachurus* and *Decapterus*, from the Black, Mediterranean, and Red seas by electrophoresis method revealed no differences between the Black Sea "small" and "large" forms [Golovko, 1964; Kulikova, 1968; Shulman, Kulikova, 1966].

Investigating morphological, ecological, and behavioral characteristics of the Black Sea horse mackerel, R. Shaverdov concluded as follows: the Black Sea "small" and "large" forms were the ecotypes of *T. mediterraneus ponticus* that differed in terms of nutritional conditions alone [Shaverdashvili, 1976; Shaverdov, 1964]. In his opinion, the horse mackerel as the facultative predator was an active migrant, grew faster, and, consequently, became larger during the years of elevated abundance of its main food object, the European anchovy.

Based on results on the variability of plastic traits, Yu. Slynko and co-authors [2018] defined the "large" and "small" horse mackerel as distinct in-population morphological forms that differed in feeding type.

Version of the hybrid origin of the "large" horse mackerel. A Bulgarian researcher I. Dobrovolov analyzed electrophoretic spectra of nonspecific muscle esterase of the Black Sea *T. mediterraneus ponticus*, Mediterranean *T. mediterraneus mediterraneus*, and "large" horse mackerel and proposed the version of the hybrid origin of the latter one [Dobrovolov, 1988, 2000; Dobrovolov, Manolov, 1983]. In accordance with it, the “large” horse mackerel is the result of hybridization of the Black Sea and Mediterranean subspecies that could occur in both the Marmara and Black seas. Its truly large size, high growth rate, noticeable abundance, and other ecological advantages over the Black Sea horse mackerel, in opinion of the author, are due to heterosis effect which is most prominent among the first-generation (F_1) hybrids and quickly disappears in subsequent generations.

Earlier, we applied an ecological approach involving studies of the relationship between populations and changes in their structure, external (physical and geographical conditions and biotic factors), spatial, and temporal ones, with shifts in external conditions [Yablokov, 1987] and also put forward the version of the hybrid origin of the “large” horse mackerel [Zuyev, Melnikova, 2003]. Considering possible reasons for the occurrence of the “large” form in the Black Sea, we reviewed the hydrological situation in the region in the 1940s. Specifically, data on long-term changes (1923–1985) in the Black Sea hydrological regime were analyzed, namely total river flows [Gidrometeorologiya i gidrokhimiya morei SSSR, 1991]. As known [Samodurov, Ivanov, 1998], river flow is the main component of freshwater balance in the Black Sea. It determines the intensity of water exchange with the Sea of Marmara *via* the Bosphorus due to two opposite directional flows: the surface one transferring water with lower salinity from the Black Sea and the bottom one transferring saline Mediterranean water to the Black Sea. An increase in river flow to the Black Sea during high-water years leads to a rise in thickness of the upper desalinated layer and, accordingly, to weakening of the lower Bosphorus flow, whilst a decrease in river flow during low-water years is accompanied by weakening of the upper Black Sea current and a gain in the lower Bosphorus flow. In low-water years, 2–2.5 times more Mediterranean Sea water usually penetrates into the Black Sea with the lower Bosphorus flow as compared to the long-term mean value [Bogdanova, 1972]. Such years are the most favorable for the invasion of flora and fauna representatives from the Mediterranean Sea into the Black Sea.

The analysis of mean annual volumes of total river flows into the Black Sea Basin over more than 60 years showed as follows: 1943 was the most low-water year (Fig. 1). Specifically, total volume of river flow constituted only 69.5% of the long-term mean one and 46.6% of the maximum registered in 1941. Thus, hydrological conditions in 1943 were the most favorable for penetration of the Mediterranean horse mackerel *T. mediterraneus mediterraneus* into the Black Sea over a long period of time.

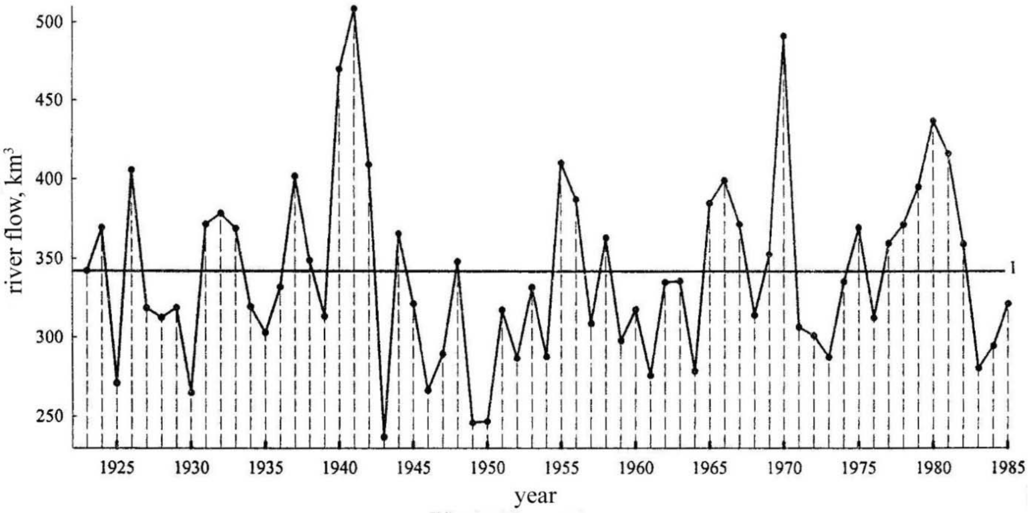

Fig. 1. Long-term dynamics of river flow in the Black Sea Basin (1, the long-term mean value of river flow [Gidrometeorologiya i gidrokhimiya morei SSSR, 1991])

Interestingly, the study of the age composition for the “large” horse mackerel population confirmed its appearance in the Black Sea in 1943 [Tikhonov, 1959]. We suggest as follows: in 1943, a mass migration of *T. mediterraneus mediterraneus* to the Black Sea occurred; it was accompanied by crossing of *T. mediterraneus mediterraneus* and *T. mediterraneus ponticus* and, accordingly, by formation of hybrids. A similar process of intraspecific hybridization of the Sea of Azov anchovy (*Engraulis encrasicolus maeoticus* Pusanov & Zeeb, 1926) and the Black Sea anchovy (*Engraulis encrasicolus ponticus* Alexandrov, 1927) resulted from *E. encrasicolus ponticus* penetration into the Black Sea in the post-glacial period [Zuyev, Skuratovskaya, 2023].

According to the law of maximum development of the heterosis effect, the first-generation (F_1) hybrids should highly exceed the subsequent ones in terms of viability, endurance, and productivity. To confirm, we carried out a comparative analysis of viability of different hybrid generations. We concentrated on indicators of viability providing the “large” horse mackerel an advantage in struggle for survival: its growth rate, maximum size, and population fecundity.

We suggested that *T. mediterraneus mediterraneus* penetrated from the Mediterranean Sea into the Black Sea in 1943, and its population included all age classes. The lifespan of the Mediterranean horse mackerel is 7–8 years, and its maturity occurs in the 2nd year [Dobrovolov, 1988]. Accordingly, the appearance of F_1 hybrids might be expected annually over 6 to 7 years (1943 to 1949–1950). With life expectancy of 17–18 years [Revina, Saf’yanova, 1966a, b; Saf’yanova, Revina, 1960], F_1 hybrids could live until 1967–1968. Considering the fact that F_1 hybrids reach maturity at the age of 4, the appearance of the second-generation (F_2) hybrids should have been expected no earlier than in 1947. So, only the first four “large” horse mackerel generations (those of 1943–1946) were regarded as F_1 hybrids. Starting from the generation born in 1947, the “large” horse mackerel population included F_1 and F_2 hybrids; since the early 1950s, it could cover representatives of more than two genetically different hybrid generations in which shares of F_1 and F_2 hybrids consistently decreased.

In accordance with the law of heterosis effect attenuation resulting from crossing of genetically different hybrid generations, there should have been a drop in viability of succeeding generations of the “large” horse mackerel. R. Shaverdov studied growth of the “large” form representing generations of the 1950s and concluded as follows: growth rates for the “large” and “small” horse mackerel were similar [1964]. However, by that time, the “large” horse mackerel was a totality of genetically different hybrid generations that varied significantly in viability, namely in growth rate. This is how, in our opinion, the “paradox of Shaverdov” can be explained.

According to the version of the hybrid origin of the “large” horse mackerel against the backdrop of the law of heterosis effect attenuation, starting from the generation of 1947 (the year when F_2 hybrid appeared in the population), the differentiation of specimens by viability could be observed. Therefore, certain attention should be paid to detection of the “middle” (“intermediate”) form differing from the “large” one by lower growth rate, smaller size of specimens, lower fecundity, a special type of population abundance dynamics, and even body shape (Fig. 2). Importantly, the “middle” form was initially registered in 1947, and this corresponded to the appearance of F_2 hybrids.

Fig. 2. Growth of different forms of the horse mackerel in the Black Sea: 1, the “large” horse mackerel; 2, the “middle” one; 3, the “small” one [Tikhonov, 1959]

Similar biological differentiation was found in generations of the “large” horse mackerel in 1957, 1958, 1962, and 1963 [Revina, Saf’yanova, 1966b]. Within each of them, two groups of specimens were determined. Those were similar in growth rate, maximum size, life expectancy, and age of maturity. However, in contrast to previous generations, those were significantly smaller and less abundant which fully complied with the law of heterosis effect attenuation.

Meanwhile, admitting the version of the hybrid origin, many other phenomena can be explained as well: a drop in fecundity in succeeding generations, dramatic reduction in commercial catches within a short period of time, “biological transformation” of the “large” horse mackerel into a “small” one, *etc.* The abovementioned facts result from the heterosis effect attenuation. Thus, the most abundant generations, those of 1945–1949, included mainly F_1 hybrids, with a very small share of F_2 ones in which the heterosis effect was most pronounced. A decline in population abundance of the “large” horse mackerel in the 1950s reflected a decrease in a share of fast-growing and larger F_1 hybrids with higher absolute fecundity and their replacement by smaller and, correspondingly, less fecund F_2 hybrids in which the heterosis effect was less pronounced.

At the same time, a decline in total abundance of F_1 hybrids and in fecundity and growth rate of F_2 hybrids resulted in a noticeable decrease in the population recruitment. Consequently, there was a sharp increase in the mean age of the “large” horse mackerel exploited population. Within 1953–1963, the mean age of commercially caught fish gradually rose from 4.7 to 12.9 years, and this led to a dramatic reduction in catches. In 1957, catches of the “large” form dropped by more than 3 times compared to those in previous year.

Nevertheless, a decrease in abundance of subsequent generations of hybrids is not the only reason for a decline in recruitment of the “large” horse mackerel population. Interestingly, in 1955 and 1956, very high spawning intensity and abundance of juveniles were recorded [Saf’yanova, Revina, 1960]. The second reason for disappearance of the “large” form is the occurrence of significant changes in its biology, since those result not only in a decrease of growth rate and size of specimens, but also in reduction in lifespan and fluctuations in the age of maturity, *i. e.*, a complete “biological transformation” of the “large” horse mackerel into a “small” one.

Naturally, the question arises whether the Mediterranean horse mackerel migration into the Black Sea and its hybridization with the Black Sea horse mackerel occurred throughout the whole history of the Mediterranean–Black Sea Basin, over the last 5–7 thousand years (since the last opening of the Bosphorus), or only once, in 1943. There is no definite answer. However, considering evidences for the “large” form appearance in various areas of the sea in different periods, it can be assumed as follows: processes of crossing might have occurred in the past, but their scale was obviously not so significant. The question could be answered properly after clarification of the taxonomic status of specimens. At the same time, the belonging of large specimens to *Trachurus trachurus* seems to be very doubtful: this species is sporadic in the Black Sea and is registered only in waters adjacent to the Bosphorus, while its occurrence off the coasts of Georgia and Crimea remains questionable [Svetovidov, 1959]. Literature data on the “large” horse mackerel confirm its appearance in various areas of the sea: off the Crimean and Caucasian coasts and off the coasts of Georgia, Turkey, and Romania.

It is noteworthy as follows. The registration of an 11-year-old horse mackerel specimen, 44 cm in length, in 1979 [Dobrovolov, Manolov, 1983] also could not clarify this issue, for it might belong to fast-growing F_2 hybrids related to the generation of 1968 or to hypothetical F_1 hybrids that might appear after a possible migration of the Mediterranean horse mackerel into the Black Sea during low-water years: 1949 and 1950.

In our conviction, the invasion of the Mediterranean horse mackerel into the Black Sea and its hybridization with the Black Sea horse mackerel are not accidental, but reflect the mediterranization. This process has intensified due to increasing anthropogenic load on the Black Sea ecosystem, ongoing disruption of freshwater balance because of the overregulation of river flows and sea salinization, and climate warming. The probability of the "large" horse mackerel invasion into the Black Sea may become higher in future.

Unfortunately, it is impossible to confirm or refute any version by molecular genetic methods due to the current absence of the "large" form in the Black Sea. Therefore, despite the existence of these versions, the origin of the "large" horse mackerel and reasons for its appearance in the Black Sea remain unexplained.

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ФЕНОМЕН ПОЯВЛЕНИЯ КРУПНОЙ СТАВРИДЫ В ЧЁРНОМ МОРЕ: ВЕРСИИ И ДОКАЗАТЕЛЬСТВА

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В статье обсуждаются литературные данные и результаты собственных исследований авторов, посвящённых объяснению феномена внезапного массового появления в Чёрном море в конце 1940-х гг. и столь же неожиданного исчезновения в середине 1960-х гг. крупной («гигантской») формы ставриды (род *Trachurus*, Carangidae) (КС). Данный феномен вызвал огромный интерес и привлёк внимание многих авторов как возможный источник новых сведений для познания процессов и путей адаптации организмов, а также проблем микроэволюции и видообразования. Проведён анализ разных подходов к изучению этого явления, включая выяснение происхождения и таксономического статуса КС. На их основе были предложены три взаимоисключающие версии. Согласно первой, КС является новым видом средиземноморского происхождения, согласно второй — экоморфой *Trachurus mediterraneus ponticus*, согласно третьей версии — гибридом *Trachurus mediterraneus mediterraneus* и *T. mediterraneus ponticus*. В результате анализа теплоустойчивости изолированных мышц и серологического исследования тканевых белков Ю. П. Алтухов с соавторами выделили КС в самостоятельный вид, имеющий средиземноморское (и даже, возможно, атлантическое) происхождение, и указали, что его массовое появление в Чёрном море произошло из-за внезапного расширения ареала вследствие резкого увеличения (взрыва) численности. Основные аргументы в пользу черноморского происхождения КС заключаются в том, что все этапы её жизненного цикла — размножение, рост молоди, нагул и зимовка взрослых особей — проходят в Чёрном море, а крупные экземпляры были известны и ранее. Принадлежность мелкой черноморской ставриды и КС к одному виду подтверждена результатами электрофоретических исследований белкового состава сыворотки крови этих форм. Версию гибридного происхождения КС первым предложил И. Доброволов после изучения электрофоретических спектров неспецифических мышечных эстераз *T. mediterraneus ponticus* и *T. mediterraneus mediterraneus*. В соответствии с нашими исследованиями, третья версия происхождения КС кажется более вероятной, чем другие. Проникновение *T. mediterraneus mediterraneus* в Чёрное море и её скрещивание с *T. mediterraneus ponticus* — это проявление процесса медитерранизации, усилившегося в результате возрастающего антропогенного воздействия на экосистему Азово-Черноморского бассейна, а также продолжающегося нарушения баланса пресных вод из-за зарегулирования речного стока и осолонения моря. Ни одна из трёх версий не может быть опровергнута или доказана молекулярно-генетическими методами ввиду отсутствия в настоящее время КС в Чёрном море. Таким образом, феномен появления крупной ставриды в Чёрном море остаётся необъяснённым.

Ключевые слова: черноморская крупная ставрида, версии, средиземноморское происхождение, черноморское происхождение, гибридное происхождение

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FACTORS AFFECTING THE REPRODUCTION OF *LITTORINA OBTUSATA* POPULATION (GASTROPODA: LITTORINIDAE) IN THE WHITE SEA

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Studying the factors that affect the population size is necessary for understanding the functioning of natural ecosystems and for planning of measures for environmental protection and management. Periwinkles of the genus *Littorina* are an important component of the intertidal ecosystems in the Northern Hemisphere, but the factors involved in their population dynamics are still poorly understood. This paper presents the data obtained during monitoring surveys of *Littorina obtusata* in 2001–2020. During this period, quasi-cyclic changes in periwinkles population density were observed around the average level, which is about 2.6 thousand ind.$\cdot$m^{-2}. A total of 67 variables characterizing the state of the studied *L. obtusata* population and the effect of key environmental factors were analyzed. Fluctuations in recruitment were found to account for 83% of observed changes in population density. Multiple regression analysis showed that 78% of changes in the reproduction rate of *L. obtusata* population were determined by four variables: density of individuals aged two years and older, biomass of a brown alga *Fucus vesiculosus*, precipitation in July, and biomass of a bivalve *Mytilus edulis*. The first variable reflects the rate of intraspecific competition, the second, the ability of a given habitat to support a mollusc population of a certain size, and the third, the effect of environmental factors. The connection between the reproduction rate of periwinkle population and bivalve biomass can be explained either by the existence of some common factor with which both variables are correlated, or by the presence of interspecific interactions between *M. edulis* and *L. obtusata*.

Keywords: *Littorina obtusata*, population size, fluctuations in abundance, environmental factors, intraspecific competition, interspecific competition, habitat capacity

Fluctuations in abundance have been identified in populations of a wide range of animals: mammals, birds, insects, and molluscs [Bachelet, 1986; Baltensweiler, 1964; Elton, 1942; Lack, 1966; MacLulich, 1937; Maximovich, Gerasimova, 2004]. They were found to be driven by changes in food abundance, the impact of predators and parasites, intraspecific competition, and genetic heterogeneity of a population [Hudson et al., 1998; Högstedt et al., 2005; Kozminsky, 2013, 2017, 2020; Maximovich, Gerasimova, 2004; Sinclair et al., 2003]. However, the causes of population fluctuations have not been established in all known cases, and the underlying mechanisms are far from being fully understood.

Analyzing the factors that mediate changes in population size is necessary for a better understanding of the functioning of natural ecosystems and for planning measures of environmental protection and management. Therefore, it is important to cover with relevant studies as wide a range of animals as possible.

A key component of littoral ecosystems of the seas of the Northern Hemisphere are gastropods of the genus *Littorina* A. Férussac, 1822. On the one hand, they consume microfouling and littoral macrophytes; on the other hand, they are food for coastal fish and waterfowl. *Littorina* are the first intermediate host for several trematode species [Galaktionov, Dobrovolsky, 1984; Granovitch et al., 2000; Sergievsky, 1985] that cause epizootics in waterfowl, in particular the common eider *Somateria mollissima* (Linnaeus, 1758) [Kulachkova, 1960, 1979]. Being common littoral species, these molluscs are also a promising object for assessing the state of the environment.

The factors determining the population dynamics of periwinkles are poorly understood, the same as the corresponding mechanisms. Only in a few cases, not only such factors were identified, but also their contribution to the population dynamics was assessed. Thus, in the course of long-term observations [Kozminsky, 2013, 2017, 2020], it was established as follows: one of the factors mediating changes in population density of *Littorina obtusata* (Linnaeus, 1758) is intraspecific competition for a resource – a brown alga *Fucus vesiculosus* Linnaeus, 1753 – under conditions of limited habitat capacity. Intraspecific competition for food resources with an increase in mortality and a decrease in the growth rate of molluscs was revealed in some other *Littorina* species as well: *L. unifasciata*, *L. plena*, and *L. littorea* [Branch, Branch, 1981; Chow, 1989; Petraitis, 2002]. A number of studies have shown that the dynamics of the periwinkle population may be affected by such factors, as temperature [Chow, 1989; Kozminsky et al., 2008] and seawater salinity [Rusanova, Khlebovich, 1967; Sokolova, 1997, 2000], although their contribution to the long-term dynamics of population has not been assessed. There are data on interspecific competition between cohabiting periwinkle species as a possible cause of fluctuations in their abundance [Kozminsky, 2020]. As shown in some cases, parasitic pressure from digenetic flukes can result in a significant drop in the population reproduction rate [Brown et al., 1988; Kohler, Wiley, 1992]. However, long-term investigations have not revealed the effect of infection on the reproduction rate of *Littorina* populations [Granovitch, Maximovich, 2013; Kozminsky, 2020; Sergievsky et al., 1997].

This paper provides data obtained during long-term (2001–2020) observations on *Littorina obtusata* population dynamics. During this period, quasi-cyclic changes in the periwinkle population density were observed around the average level of about 2.6 thousand ind.$\cdot$m^{-2}. The aim of our work was to identify factors governing the revealed fluctuations in abundance of this White Sea mollusc.

MATERIAL AND METHODS

The material for the study was sampled on the western spit of the Southern Bay of Ryazhkov Island (67°00′N, 32°34′E; Kandalaksha State Nature Reserve) located in the apex of Kandalaksha Bay of the White Sea. The Southern Bay is oriented toward Kandalaksha Bay exit and protected from the open sea by a number of islands; therefore, it is characterized by a moderate level of wave action.

The surveyed area of the littoral zone is bounded below by zero depth and above by a littoral bath lined with fine sand and strewn with small stones. The sediment in the surveyed area is chiefly fine and medium gravel, with an admixture of moderately silted sand. The littoral zone is covered with different-sized stones, up to boulders of 1.5 m. Most of the biotope, approximately to 20 m from zero depth, is the zone of *F. vesiculosus* growth. In the area of zero depth, this alga forms mixed thickets with *Ascophyllum nodosum*. The fauna of invertebrates in the surveyed habitat is typical for the rocky littoral of the White Sea [Naumov, Olenev, 1981]. In some spots, species characteristics of the silted littoral zone are found in small quantities as well (molluscs *Peringia ulvae* and *Macoma balthica* and a sea cucumber *Chiridota laevis*).

The material was sampled annually in 2001–2020 between 10 August and 6 September. Quantitative samples were taken from plots of 1/40 m^2 arranged in series of three along the transect at levels of 0, 5, 10, 15, 20, and 25 m. During sampling, macrophytes (if present) and the upper sediment layer were removed from the plots. The samples were transported to a laboratory; there, they were washed with freshwater using a sieve with a mesh diameter of 0.6 mm and quantitatively sorted. For each sample, we determined the wet weight of the brown algae *F. vesiculosus* and *A. nodosum* (by species, with an accuracy of ±0.5 g), filamentous algae[*] (with an accuracy of ±0.1 g), and mollusc species found: *Littorina saxatilis* (±1 mg), *L. obtusata* (±1 mg), *Mytilus edulis* (±0.5 g), and *P. ulvae* (±1 mg). Also, we counted the total number of registered periwinkles.

The total biomass of *L. obtusata* aged one year and older was determined by weighing molluscs. In the case of recruits (0+), due to their small size, the shell diameter–weight relationship was first constructed and then used to calculate the individual weight of specimens and to refine the total biomass. Notably, biomass of recruits is low (0.7% on average) compared to the total biomass of periwinkles aged one year and older.

For each *L. obtusata*, the maximum shell diameter (±0.1 mm) was recorded, and age was determined based on the annual rings on a shell [Kozminsky, 2006]. When indicating age, we used the following designations: recruits (individuals born during the current year), 0+; molluscs aged one year (born in the previous year), 1+; molluscs aged two years (born in the penultimate year), 2+; *etc.*

By dissecting the molluscs, we determined their sex, the state of the reproductive system (rudimentary, underdeveloped, normal, or reduced), and infection with trematode parthenitae.

The White Sea *L. obtusata* serve as the first intermediate host for a number of trematode species [Galaktionov, Dobrovolsky, 1984; Granovitch et al., 2000; Sergievsky, 1985]. All detected species of flukes cause complete parasitic castration [Galaktionov, 1993; Ganzha, Granovich, 2008; Granovitch, Sergiyevsky, 1990] and affect the reproduction of the host population to one degree or another. Since the purposes of this study required an assessment of only the total parasitic pressure on the reproduction of the periwinkle population, the prevalence of mollusc infection with all trematode species was used as the corresponding indicator.

Meteorological data on the state of the environment during the study period (air temperature, precipitation, and sunshine duration) from the nearby weather station Kandalaksha (No. 22217) were taken from the website of the All-Russian Research Institute of Hydrometeorological Information (http://meteo.ru/data). The values of the North Atlantic Oscillation index, NAO (annual, seasonal, and monthly) were obtained from the website of the US National Center for Atmospheric Research (https://climatedataguide.ucar.edu/climate-data/hurrell-north-atlantic-oscillation-nao-index-station-based).

During the work, four groups of variables were analyzed; those characterize the state of the studied *L. obtusata* population and its habitat conditions.

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[*] Approximately ¾ are *Cladophora* sp. and ¼ are *Stictyosiphon* sp.

The first group covers the indicators featuring the state of the *L. obtusata* population (10 pcs): biomass ($g \cdot m^{-2}$) (W_{LO}); density ($ind. \cdot m^{-2}$) of recruits (0+), molluscs aged one year (1+), molluscs aged two years (2+), molluscs aged two years (2+) and older, and molluscs aged three years (3+) and older (D_{LO0+}, D_{LO1+}, D_{LO2+}, $D_{LO\geq2+}$, and $D_{LO\geq3+}$, respectively); density of males with normally developed penis (D_{PN}) and mature uninfected females (D_{FRT}); the ratio of abundance of males to abundance of females among mature uninfected individuals ($R_{ML/FML}$); and the average shell diameter of mature uninfected females (mm) (X_D).

The second group includes indicators of the population state of the cohabiting species *L. saxatilis* (5 pcs): biomass ($g \cdot m^{-2}$) (W_{LS}); density ($ind. \cdot m^{-2}$) of recruits (0+), molluscs aged one year (1+), molluscs aged two years (2+), and molluscs aged three years (3+) and older (D_{LS0+}, D_{LS1+}, D_{LS2+}, and $D_{LS\geq3+}$, respectively).

The third group covers indicators characterizing the biotic habitat conditions of molluscs (6 pcs): biomass ($g \cdot m^{-2}$) of the brown algae *F. vesiculosus* (W_{FV}) and *A. nodosum* (W_{AN}), filamentous algae (W_{FA}), and molluscs *M. edulis* (W_{ME}) and *P. ulvae* (W_{PU}); prevalence of infection with trematode parthenitae (%) (PI).

The fourth group includes indicators featuring the environmental conditions during the study period (45 pcs): values of the North Atlantic Oscillation index for a year, by season, and for a month (NAO_{AN}, NAO_{JJA}, NAO_{JAS}, NAO_{ASO}, NAO_{JUN}, NAO_{JUL}, NAO_{AUG}, and NAO_{SEP}); air temperature (°C) average for a year, for a period of several months, and for a month (T_{AN}, $T_{V–IX}$, $T_{V–X}$, $T_{VI–IX}$, $T_{VI–X}$, $T_{XI–IV}$, $T_{XI–V}$, T_V, T_{VI}, T_{VII}, T_{VIII}, T_{IX}, and T_X); amount of precipitation (mm) for a year, in the cold and warm period, and for a month (H_{AN}, $H_{V–IX}$, $H_{V–X}$, $H_{VI–IX}$, $H_{VI–X}$, $H_{XI–IV}$, $H_{XI–V}$, H_V, H_{VI}, H_{VII}, H_{VIII}, H_{IX}, and H_X); and sunshine duration (h) for a year, for a period of several months, and for a month (DS_{AN}, $DS_{V–IX}$, $DS_{V–X}$, $DS_{VI–IX}$, $DS_{VI–X}$, DS_V, DS_{VI}, DS_{VII}, DS_{VIII}, DS_{IX}, and DS_X).

Generally accepted statistical methods were applied. When checking for normality, we used the Kolmogorov–Smirnov test (the Lilliefors probabilities were calculated [Lilliefors, 1967], as the parameters of the normal distribution were estimated based on sample data) and the Shapiro–Wilk test. Correlation analysis was applied for preliminary selection of data (filtering) for multiple regression analysis. Since almost all the considered indicators (with rare exceptions) were normally distributed ones, the Pearson correlation coefficient (R) was used to estimate the degree of relationship between them. The correlation of non-normally distributed variables with abundance of recruits was estimated by the Spearman's rank correlation coefficient (R_S). Multiple regression analysis was applied to identify factors affecting the reproduction of the *L. obtusata* population. All calculations were performed in Statistica 7.0.

RESULTS

During the study period, quasi-cyclic changes in *L. obtusata* population density were observed around the average level of 2,620 $ind. \cdot m^{-2}$ (Fig. 1A). Fluctuations in abundance of recruits accounted for 82.5% of the population changes ($R = 0.908$; $\alpha << 0.001$). Densities of periwinkles aged one year, periwinkles aged two years, and sexually mature molluscs (≥ 3+) generally followed them with a corresponding shift (Fig. 1B–E). Thus, revealing the factors that mediate changes in the *L. obtusata* population size comes down to analyzing the causes of fluctuations in abundance of recruits (D_{LO0+}).

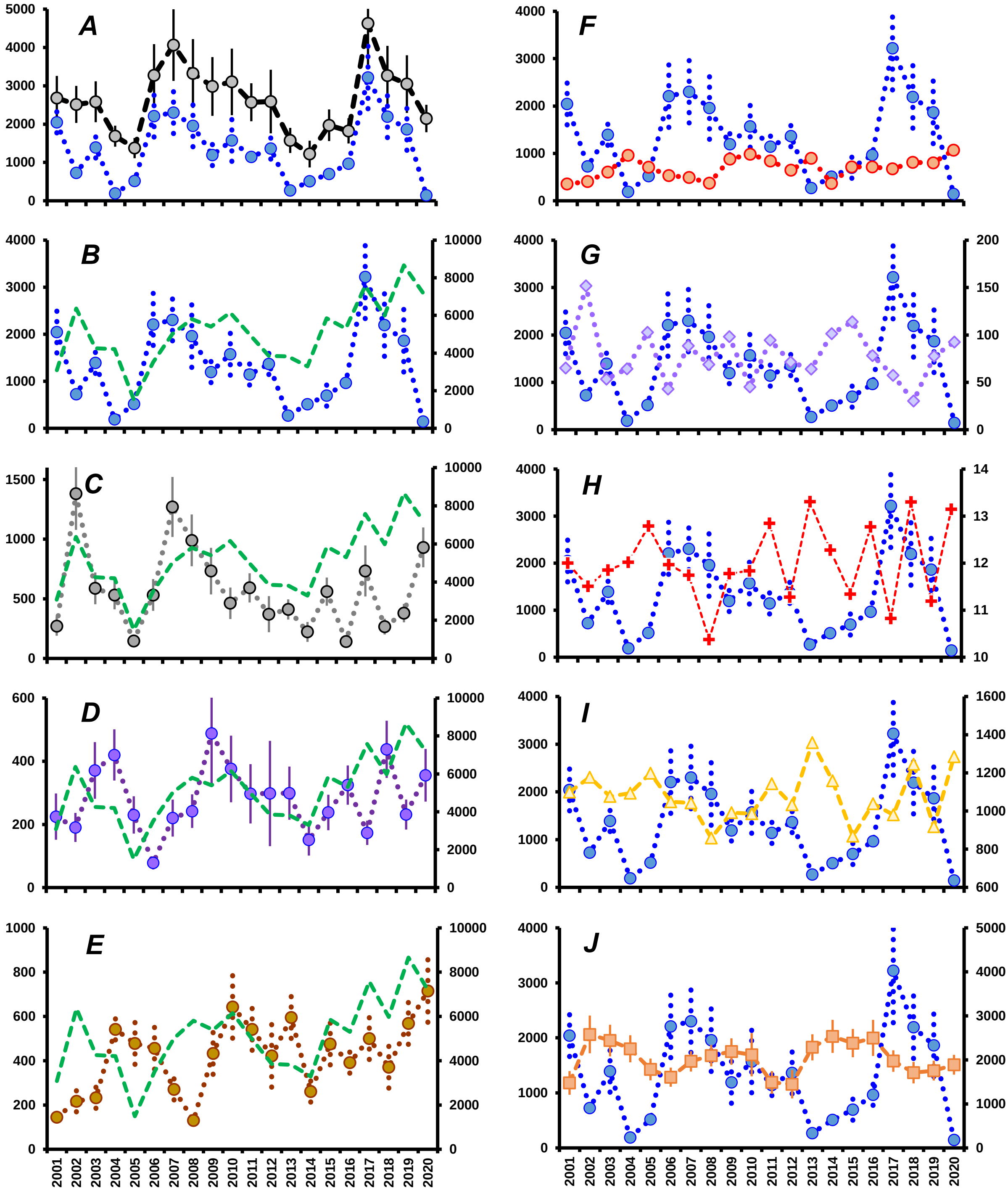

Fig. 1. Dynamics of changes in density of different age groups of *Littorina obtusata* (A–F) and environmental factors (G–J) during the study period. The dotted lines (○ marker) denote different age groups of molluscs: black, the entire population; blue, 0+; gray, 1+; purple, 2+; red, ≥ 2+; and brown, ≥ 3+. The dashed lines denote the variables: lilac, H_{VII} (♦); red, $T_{VI–IX}$ (+); yellow, $DS_{V–IX}$ (▲); orange, W_{ME} (■); and green, W_{FV} (no marker). Variables and age groups are explained in the text. On the abscissa axis, years are shown. On the left ordinate axis, the density of individuals is shown (ind.$\cdot$m^{-2}). On the right ordinate axis, the values of environmental factors are shown: biomass (g$\cdot$m^{-2}), precipitation (mm), temperature (°C), and sunshine duration (h). The standard errors of the mean are indicated

Normality test. The vast majority of variables were normally distributed. The exceptions were three indicators: density of *L. saxatilis* recruits (D_{LS0+}), *A. nodosum* biomass (W_{AN}), and sunshine duration in September (DS_{IX}).

Data filtration. At the first stage, by correlation analysis, we selected relevant indicators, *i. e.*, those noticeably correlated with the analyzed variable (D_{LO0+}). For certainty, we considered the values of correlation coefficients higher than or equal to |0.30| to be significant; this corresponds to the contribution of the analyzed indicators to the variability of the dependent variable of about 10% (notably, the values of the correlation coefficients higher than or equal to |0.45| were statistically reliable at the 5% confidence level). The correlation of NAO_{ASO} with the density of *L. obtusata* recruits was formally a little lower than the accepted critical level ($R = +0.27$); however, we retained this variable in the analysis as an integral indicator featuring weather conditions in the second half of the warm period. When testing the correlation level between abundance of *L. obtusata* recruits and three non-normally distributed variables (D_{LS0+}, W_{AN}, and DS_{IX}) with the Spearman's rank correlation coefficient, we found that it was lower than the accepted critical value, |0.30|. As a result, 18 variables were considered relevant: W_{LO}, $D_{LO\geq2+}$, $D_{LO\geq3+}$, D_{PN}, W_{FV}, W_{ME}, H_{VII}, H_{IX}, NAO_{JUL}, NAO_{ASO}, $DS_{V–IX}$, $DS_{V–X}$, T_{VI}, T_{IX}, $T_{VI–IX}$, $T_{VI–X}$, $T_{V–IX}$, and $T_{V–X}$.

Then, the relevant variables were checked for redundancy. The indicators significantly associated ($\alpha < 0.05$) with other variables selected for the analysis were excluded if they were less strongly correlated with the dependent variable (D_{LO0+}) or – with a similar strength of correlation – were less preferable for use in the analysis. T_{VI}, T_{IX}, $T_{VI–X}$, $T_{V–IX}$, and $T_{V–X}$ were excluded from further analysis, because they were significantly correlated with $T_{VI–IX}$. The latter, in turn, was excluded, as it was significantly correlated with the density of molluscs aged two years and older ($R = 0.45$; $\alpha = 0.049$). Out of four variables featuring the state of the *L. obtusata* population ($D_{LO\geq2+}$, $D_{LO\geq3+}$, W_{LO}, and D_{PN}), abundance of molluscs aged two years and older was most strongly associated with the density of recruits ($R = 0.35$; $\alpha = 0.133$). Three other variables were significantly correlated with this indicator ($R \geq 0.80$; $\alpha << 0.001$) and were excluded from the analysis. Sunshine duration within May–October ($DS_{V–X}$) was excluded, for it was noticeably correlated with $DS_{V–IX}$ ($R = 0.99$; $\alpha << 0.001$) – the variable more precisely reflecting the idea of a warm period. Finally, the amount of precipitation in September (H_{IX}) was significantly correlated with *F. vesiculosus* biomass ($R = 0.46$; $\alpha < 0.044$). As a result, seven indicators ($D_{LO\geq2+}$, W_{FV}, W_{ME}, H_{VII}, $DS_{V–IX}$, NAO_{JUL}, and NAO_{ASO}) were selected for further analysis; there was no significant relationship between them (Table 1).

Table 1. Pearson correlation coefficients between the variables selected for multiple regression analysis

D_{LO0+}	–							
H_{VII}	**−0.50**	–						
$DS_{V–IX}$	**−0.46**	0.04	–					
NAO_{JUL}	0.35	−0.23	0.19	–				
NAO_{ASO}	0.27	−0.24	−0.01	0.06	–			
$D_{LO\geq2+}$	−0.35	−0.20	0.24	−0.12	0.15	–		
W_{ME}	−0.41	0.37	−0.03	−0.03	−0.15	−0.07	–	
W_{FV}	0.30	0.03	−0.33	0.25	0.23	0.30	0.10	–
	D_{LO0+}	H_{VII}	$DS_{V–IX}$	NAO_{JUL}	NAO_{ASO}	$D_{LO\geq2+}$	W_{ME}	W_{FV}

Note: the coefficients at 5% confidence level are highlighted in bold. Variables are explained in the text.

Selection of the regression model. Since the correlation between the independent variables was low, we did not use variants of multiple regression analysis involving the regularization procedure to improve the stability of the model (ridge regression, lasso regression, and elastic net). We analyzed models with and without a non-zero intercept. In both cases, various methods of variable selection were used: forward stepwise selection of all variables, their backward stepwise selection, and forced stepwise selection. Models with a zero intercept were excluded due to their poor quality: a high standard error of the estimate (*SEE*), low tolerance of variables, and strong correlation between some regression coefficients and between residuals and independent variables.

Results of regression analysis: Factors affecting population reproduction. Analysis of regression models with a non-zero intercept and different numbers of variables showed that the overwhelming majority of models include two variables, H_{VII} and $D_{LO\geq2+}$. When adding to such a "basic" model the variables $DS_{V–IX}$, NAO_{JUL}, and NAO_{ASO} in various combinations, the corresponding regression coefficients did not significantly differ from zero. The highest values of the multiple correlation coefficient ($R = 0.881$) and the lowest standard error of the estimate ($SEE = 446.7$) were obtained for the equation involving four variables, $D_{LO\geq2+}$, H_{VII}, W_{FV}, and W_{ME}:

$$D_{LO0+} = -2.46 \times D_{LO\geq2+} + 0.26 \times W_{FV} - 15.63 \times H_{VII} - 0.73 \times W_{ME} + 4,363.37\ . \quad (1)$$

The plot corresponding to the model is given in Fig. 2. The obtained dependence allows us to explain 77.6% of the variability of the dependent variable. The value of F-criterion (the Fisher criterion) evidences for a high reliability of the approximation ($F(4,15) = 13.03$; $\alpha << 0.001$).

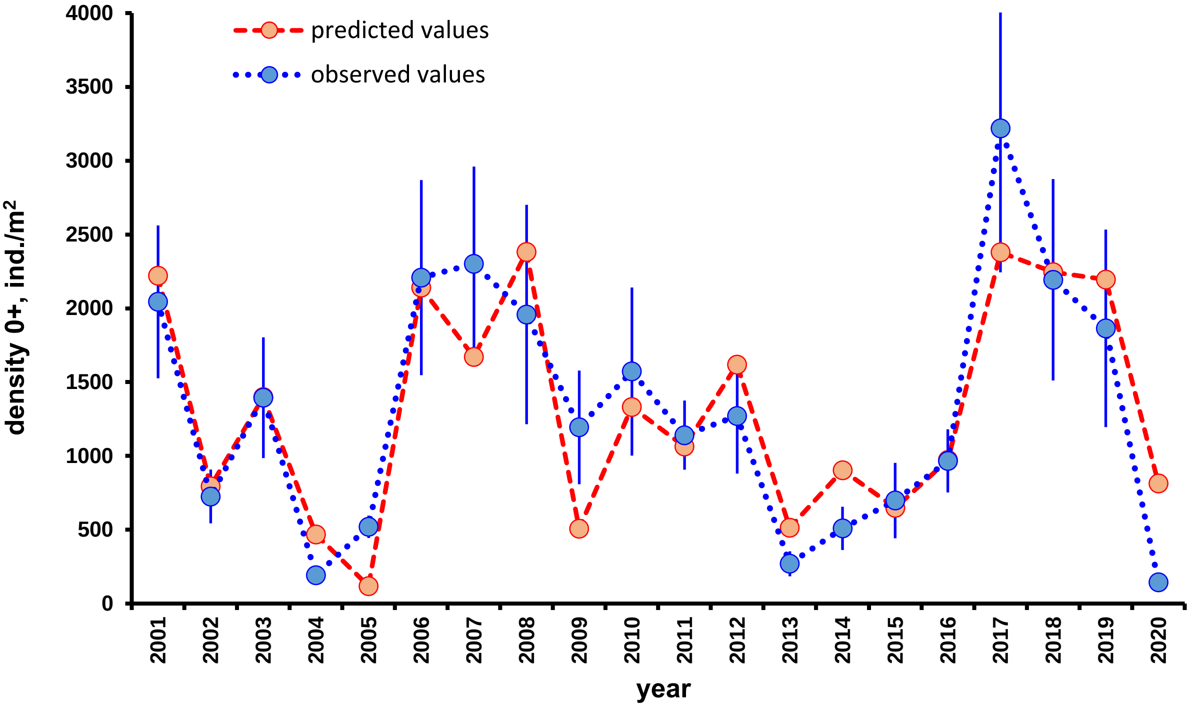

Fig. 2. The observed and predicted densities of *Littorina obtusata* yearlings. The standard errors of the mean are indicated

The analysis of the parameters (Table 2) evidences for a high quality of the model. All the main statistics were reliable at least at the 5% confidence level. The tolerance of all independent variables is quite high indicating a weak correlation between them. Apparently, the value of tolerance is affected by the presence of weak connections in two pairs of variables: $D_{LO\geq2+}$ and W_{FV} ($R = +0.30$) and H_{VII} and W_{ME} ($R = +0.37$). The correlation between the variables of different pairs is much weaker. Partial and semipartial correlations are high in all cases confirming the presence of a strong connection between the independent variables and a dependent one. The correlation between the regression coefficients is unreliable and does not exceed $|0.35|$. The analysis of the residuals showed a low level of correlation between them and the independent variables (R was about 10^{-7}). The residuals fit the straight line of the normal probability plot; the analysis of the distribution of the standardized residuals shows that they all are within $\pm2\sigma$. Both indicators evidence for the fact that the residuals are normally distributed. The Cook's distance is moderately significant for three points alone (2005, 2017, and 2020), but the Mahalanobis distances and the distribution of standardized residuals show that there is no reason to consider these points as outliers.

Thus, our results indicate that during the study period, the reproduction rate of the *L. obtusata* population was affected by four variables: $D_{LO\geq2+}$, W_{FV}, H_{VII}, and W_{ME}.

Table 2. The main parameters of the regression equation for the density of *Littorina obtusata* yearlings

	BETA	SE_{BETA}	B	SE_B	R_{PART}	R_{SPART}	T	$t(15)$	α
Int			4,363.37	706.18				6.18	0.0000
$D_{LO\geq2+}$	−0.6376	0.1313	−2.46	0.51	−0.782	−0.593	0.864	−4.86	0.0002
W_{FV}	0.5369	0.1292	0.26	0.06	0.731	0.507	0.892	4.15	0.0008
H_{VII}	−0.5265	0.1338	−15.63	3.97	−0.713	−0.480	0.832	−3.93	0.0013
W_{ME}	−0.3200	0.1318	−0.73	0.30	−0.531	−0.296	0.858	−2.43	0.0283

Note: BETA, regression equation coefficients calculated from standardized values of the variables; SE_{BETA}, standard errors of BETA; B, regression equation coefficients calculated from initial values of the variables; SE_B, standard errors of B; R_{PART}, partial correlations; R_{SPART}, semipartial correlations; T, tolerance; t, t-test value associated with the calculated statistics; α, corresponding probabilities; and Int, intercept. Variables are explained in the text.

Analysis of individual factors. As shown by the value of the standardized regression coefficient (BETA = −0.6376) (Table 2), the density of individuals aged two years and older has the greatest effect on fluctuations in abundance of *L. obtusata* recruits. These variables are in relationships close to antiphase ones ($R = -0.35$; $\alpha = 0.133$) (Fig. 1F).

The second most important factor (BETA = +0.5369) affecting abundance of recruits is biomass of a brown alga *F. vesiculosus*. Although the correlation between *F. vesiculosus* biomass and density of *L. obtusata* recruits is not reliable ($R = +0.30$; $\alpha = 0.203$), it is obvious that abundance of recruits in general follows abundance of this alga (Fig. 2B), that is, the higher the *F. vesiculosus* biomass, the more juveniles survive. Against this backdrop, numerous chaotic deviations are observed which seem to be governed by the fact that the survival of *L. obtusata* recruits is affected by many factors. The above-mentioned trend is disrupted only at the very end of the study period (in 2018–2020). Abundance of molluscs aged one year also changed almost synchronously with *F. vesiculosus* biomass ($R = +0.40$; $\alpha = 0.082$) (Fig. 1C). Abundance of *L. obtusata* in the age group 2+, as well as 3+ and older, followed *F. vesiculosus* biomass with some lag (Fig. 1D, E). This indicates that with a high abundance of *F. vesiculosus*, more juvenile *L. obtusata* survive. Between 2014 and 2020, against the backdrop of a rapid gain in *F. vesiculosus* biomass, the density of molluscs at the age of 1+, 2+, and 3+ and older also increased.

The third important factor (BETA = –0.5265) comparable in strength of effect with *F. vesiculosus* biomass, is the amount of precipitation in July (H_{VII}). This variable reflects the effect of abiotic environmental factors on the reproduction of the mollusc population. The density of recruits is in antiphase with the amount of precipitation ($R = -0.50$; $\alpha = 0.026$) (Fig. 1G). Notably, abundance of *L. obtusata* recruits is negatively correlated with air temperature ($T_{VI–IX}$) ($R = -0.47$; $\alpha = 0.036$) (Fig. 1H) and with sunshine duration ($DS_{V–IX}$) ($R = -0.46$; $\alpha = 0.043$) (Fig. 1I) during the warm period. Both characteristics are significantly related to each other ($R = 0.84$; $\alpha << 0.001$), but are not correlated with the amount of precipitation in July.

The last factor in terms of strength of the relationship with the density of *L. obtusata* recruits (BETA = –0.3200) is *M. edulis* biomass. The same as the amount of precipitation, this variable is in relationships close to antiphase ones (Fig. 1J) with the density of recruits ($R = -0.42$; $\alpha = 0.069$). With the exception of three cases, no reliable correlations were found between *M. edulis* biomass and most of the other variables considered. The first exception is the positive relationship ($R = +0.53$; $\alpha = 0.017$) between *M. edulis* abundance and the amount of precipitation in the warm period ($H_{VI–IX}$). The second one is the negative correlation between the mussel biomass and the prevalence of periwinkle infection with trematode parthenitae ($R = -0.59$; $\alpha = 0.006$). The third exception is a positive relationship between *M. edulis* abundance and *L. saxatilis* biomass ($R = +0.72$; $\alpha < 0.001$).

DISCUSSION

Long-term studies have revealed noticeable interannual fluctuations in the periwinkle population density with pronounced cyclicity [Kozminsky, 2013, 2017, 2020; Sergievsky et al., 1997]. Analyzing such fluctuations, S. Sergievsky *et al.* [1997] concluded that they are mediated by changes in the reproduction rate of the population. Our data are fully consistent with it: 82.5% of the registered fluctuations were governed by changes in abundance of recruits.

We have shown earlier [Kozminsky, 2013, 2017, 2020] that quasi-cyclic fluctuations in abundance are due to intraspecific competition between recruits and *L. obtusata* aged three years and older for a resource, *F. vesiculosus*, under conditions of limited habitat capacity. This brown alga is the main source of food for *L. obtusata* (both by itself and due to microfouling) and a substrate for habitation and oviposition [Beskupskaya, 1963; Matveeva, 1974]. Abundance of younger *L. obtusata* (0+ and 1+) follows *F. vesiculosus* abundance; therefore, this alga is a key resource limiting the periwinkle population size. The occurrence of intraspecific competition is evidenced by the existence of relationships close to antiphase ones between abundance of recruits and abundance and biomass of mature *L. obtusata* [Kozminsky, 2013, 2017, 2020].

The data obtained in the course of this study are in good agreement with the previous observations. The key role of *F. vesiculosus* as a resource limiting the mollusc population density is confirmed by coordinated changes in the periwinkle abundance and abundance of individual age groups (Fig. 1B–D). This is also indicated by the fact that *L. obtusata* biomass and *F. vesiculosus* biomass were reliably correlated ($R = +0.55$; $\alpha = 0.013$). The occurrence of intraspecific competition is confirmed by the presence of relationships close to antiphase ones between abundance of *L. obtusata* recruits and density of large molluscs – those aged two years and older (Fig. 1F). Interestingly, in this study, in contrast to earlier investigations [Kozminsky, 2013, 2017, 2020], the strongest negative correlation was revealed between densities of recruits and *L. obtusata* aged two years and older, not between recruits and mature individuals (≥ 3+). Apparently, this means that abundance of just large periwinkles is more important, than abundance of mature ones. Another detail worth noting is a rise (since 2014) in densities of molluscs aged 1+, 2+, 3+ and older against the backdrop of a sharp increase in *F. vesiculosus* biomass. These processes seem to reflect a gain in habitat capacity. Abundance of recruits also rose during this time (until 2018), but then dropped (clearly, due to an increase in density of large *L. obtusata*).

Thus, the first variable in the equation (1), abundance of molluscs aged two years and older, reflects the rate of intraspecific competition. The second one, *F. vesiculosus* biomass, reflects habitat capacity, *i. e.*, its ability to maintain *L. obtusata* population of a certain size.

The most important factor for marine animals is salinity. The zone of optimal salinity for the White Sea *L. obtusata* is limited by 34–36‰ from above and by 14–16‰ from below. The lower boundary of the transitional low salinity zone (at which some molluscs still remain active) is 6–8‰ [Berger, 1976b]. The same as in most marine animals [Berger, 1986; Kinne, 1971; Remane, Schlieper, 1972], in periwinkles, individuals in the early stages of ontogenesis are the most vulnerable to effects of unfavorable environmental factors. In a closely related species, *L. saxatilis*, the level of perinatal and juvenile mortality was found to rise under conditions of low salinity [Sokolova, 1997, 2000]. *L. obtusata* tolerance to a decrease in salinity varies throughout the year and peaks in spring and summer [Berger, 1976b].

The relationship between the amount of precipitation and the reproduction rate of the *L. obtusata* population seems to be mediated by salinity fluctuations during precipitation. The presence of a relationship between the amount of precipitation and salinity (level of desalination) is confirmed by data of V. Khaitov [2008; 2009; 2010]. The mean salinity in the summer of 2007, 2008, and 2009 in the Southern Bay of Ryazhkov Island was 18.8, 19.9, and 20.2‰, respectively, while the amount of precipitation in the period from November of the previous year to August of the current year (according to the weather station in Kandalaksha) was 561, 514, and 501 mm. Apparently, the effect of precipitation on *L. obtusata* juveniles is chiefly due to desalination of the surface layer of seawater immediately at the time of precipitation. Mass oviposition of *L. obtusata* occurs in late May–early July. The embryonic development of *L. obtusata* lasts about a month; accordingly, most juveniles hatch in late June–early July [Kozminsky, 2006; Kuznetzov, 1960; Matveeva, 1974]. It is logical to assume that *L. obtusata* juveniles are most sensitive to desalination immediately after hatching, and this seems to explain the negative correlation between the density of *L. obtusata* recruits and the amount of precipitation in July.

Thus, the third variable in the equation (1) characterizes the effect of environmental factors (in this case, precipitation) on the reproduction of the periwinkle population.

As shown by the obtained results, abundance of *L. obtusata* recruits also negatively correlates with temperature and sunshine duration during the warm period. Accordingly, the survival rate of juveniles should be lower in a warm and sunny summer. This conclusion is supported by both literature data and our own observations. Thus, as shown for *Littorina plena* [Chow, 1989], high temperature can be the cause of mass mortality of molluscs. With a rise in water temperature to values exceeding +22 °C, an increase in mortality of juveniles and frequency of embryonic malformations are observed in *L. obtusata* [Kozminsky et al., 2008]. Temperature is reliably correlated with sunshine duration. Obviously, in clear weather, air heats up more, and this results in more heating of the surface layer of seawater and surface of substrates during low tide. Both can govern a rise in mortality of juveniles. At the same time, sunshine duration seems to be significant on its own: in clear, sunny weather, the risk of death of fish juveniles due to desiccation increases.

However, none of the above-mentioned variables was involved in the obtained equation (1). Most likely, this is explained by the fact as follows: for *L. obtusata*, seawater salinity is more important than the possibility of overheating or desiccation. At the same time, both factors can come to the fore with a shift in conditions of the population's existence. Values of the North Atlantic Oscillation index (NAO_{JUL} and NAO_{ASO}) were also not included in the equation (1). This seemed to happen, because these are integral indicators that latently "take into account" both precipitation and temperature; accordingly, the connection with them should be weaker.

The occurrence of a relationship between *M. edulis* biomass and the reproduction rate of the *L. obtusata* population is unexpected. It can be explained in two ways: the relationship can be governed by the presence of some common factor which both variables are correlated with and by the occurrence of interspecific interactions between *M. edulis* and *L. obtusata*.

In our opinion, out of the variables considered in this study, the only real candidate for the role of a common factor affecting both the reproduction of the *L. obtusata* population and *M. edulis* abundance is the amount of precipitation correlated with both the reproduction of the periwinkle population and *M. edulis* biomass. The negative relationship between the amount of precipitation and the reproduction rate of the mollusc population was discussed earlier. The positive correlation between the amount of precipitation and *M. edulis* biomass can be explained by a gain in the amount of biogenic elements entering Kandalaksha Bay with freshwater runoff from the mainland with an increase in the amount of precipitation, and, accordingly, a more rapid development of phyto- and zooplankton (the food for *M. edulis*). Moreover, we cannot rule out the occurrence of some common factor not taken into account in this study.

Competitive interactions between different mollusc species are quite common, although their occurrence seems to be species-specific, and interspecific competition has not been detected in many cases [Watz, Nyqvist, 2022]. Interspecific competition can result in separation of ecological niches of cohabiting mollusc species [Golikov et al., 2020; Kimura, Chiba, 2010], an increase in mortality [Baker, 2021; Espinosa et al., 2006], and changes in feeding intensity [Smallridge, Kirby, 1988], growth rate [Aguilera, Navarrete, 2012; Campbell et al., 2015; Yamada, Mansour, 1987], individual fecundity, and the reproduction rate of a population [Rollo, 1983].

Prerequisites for the emergence of competitive interactions between *M. edulis* and *L. obtusata* do exist. Both species use macrophytes in general and *Fucus* in particular as a substrate for habitation. For *L. obtusata*, *F. vesiculosus* is the main substrate for habitation, feeding, and oviposition. In the case of *M. edulis*, it is just one of possible substrates for habitation; however, as our data show, even if only 5% of mussels inhabit *Fucus*, their biomass is already equivalent to that of *L. obtusata*. Competitive relationships between periwinkles and mussels can be indirect: in the case of a significant reduction in the area of the substrate suitable for habitation of *L. obtusata* recruits. Anyway, the presence of direct competition associated with *M. edulis* release of some biologically active substances (repellents or metabolites) seems to be more realistic. In this case, not only mussels inhabiting *Fucus*, but also molluscs attached to the underlying substrate may participate in the competition. This assumption is consistent with literature data: it has been shown, in particular, that mussels are capable of displacing both other molluscs and crustaceans from the substrate [Zolotnitsky, 2011].

Thus, the fourth component of the equation (1), *M. edulis* biomass, may evidence for the presence of interspecific competition or result from the occurrence of a common factor which both the density of *L. obtusata* recruits and the mussel biomass are correlated with. Additional studies are required to verify the assumptions made.

Notably, it would be logical to expect the presence of interspecific competitive interactions with a closely related cohabiting mollusc, *L. saxatilis*. However, we found no significant relationships between *L. saxatilis* abundance (biomass or abundance of different age groups) and the reproduction of the *L. obtusata* population. *L. saxatilis* abundance is not "included" in the final equation, either as an alternative to *M. edulis* biomass or in addition to the mussel abundance. Interestingly, the absence of a significant effect of *L. saxatilis* on the reproduction of the *L. obtusata* population does not exclude the possibility of the opposite effect, since interspecific competitive relationships are not necessarily symmetrical [Aguilera, Navarrete, 2012; Espinosa et al., 2006].

The reproduction of the mollusc population may be affected by parasites. Ten species of trematodes parasitize the White Sea *Littorina* representatives. As already mentioned, all the detected fluke species cause complete parasitic castration of molluscs [Galaktionov, 1993; Ganzha, Granovich, 2008; Granovitch, Sergiyevsky, 1990] and, therefore, to one degree or another affect the reproductive potential of a population. As shown in a number of cases, parasitic pressure leads to a pronounced decrease in the reproduction rate of the host population [Brown et al., 1988; Kohler, Wiley, 1992]. However, other cases are known – when the impact of parasites was not noticeable at the population level [Kube et al., 2006]. In our study, we found no significant relationship between abundance of recruits and the prevalence of infestation of the population ($R = +0.028$; $\alpha = 0.906$). Since the overall prevalence of infection was relatively low (4 to 15% in different years), this can be explained by a low infestation rate. Similarly, S. Sergievsky *et al.* [1997] recorded no effect of infestation on the reproduction rate of the *L. obtusata* population in the habitat we surveyed, although the prevalence of infestation in the period of their research (1982–1995) was significantly higher (20–60%). A. Granovich and A. Maximovich [2013] also did not register any effect of infection on the reproduction of the population; however, they reported a negative correlation between the host population density in the current year and the prevalence of trematode infection in the previous year mediated by the death of infected middle-aged individuals. In addition, a positive relationship was revealed between the prevalence of trematode infection in the previous year and abundance of *L. obtusata* aged one year in the current year. The latter result is in agreement with our observations on the occurrence of intraspecific competition between adult and juvenile periwinkles [Kozminsky, 2013, 2017, 2020; this study]. Most likely, the positive correlation found by the above-mentioned authors is explained by the release of additional resources (a brown alga *F. vesiculosus*) when adult infected individuals die: this mediates survival of more juveniles.

Conclusion. Most of the studied variables were excluded from the analysis due to their low relevance or strong correlation with other variables. Apparently, a set of variables selected for multiple regression analysis or included in the final dependence (1) is not random: all of them reflect habitat conditions of molluscs (biotic and abiotic ones) and can be somehow associated with the reproduction rate of *Littorina obtusata* population. Specifically, *Fucus vesiculosus* biomass evidences for high abundance of the key resource limiting *L. obtusata* population size. The density of molluscs aged two years and older characterizes the rate of intraspecific competition between recruits and large individuals. A bivalve *Mytilus edulis* is one of the most common littoral species using *Fucus*, the same as the periwinkles do, as a substrate for habitation; due to it, interspecific competition is possible between them. Four climate variables (H_{VII}, $DS_{V–IX}$, NAO_{JUL}, and NAO_{ASO}) feature habitat conditions for juveniles during their first year of life. Out of them, the key role is played by the amount of precipitation which is involved in the final regression equation (1) and characterizes level of desalination of the surface layer of seawater.

The obtained equation (1) has a clear biological meaning. It shows as follows: during the study period, the reproduction of the *L. obtusata* population was governed by both intrapopulation factors and environmental factors (biotic and abiotic ones). Obviously, the reproduction of the *L. obtusata* population should be affected by a significantly larger number of factors than we revealed during the study. The level of effect and the set of factors may change with shifts in conditions of the population's existence: other factors may come to the fore.

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ФАКТОРЫ, ВЛИЯЮЩИЕ НА ВОСПРОИЗВОДСТВО ПОПУЛЯЦИИ МОЛЛЮСКОВ *LITTORINA OBTUSATA* (GASTROPODA: LITTORINIDAE) В БЕЛОМ МОРЕ

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Изучение факторов, влияющих на изменения численности популяций, необходимо для понимания функционирования природных экосистем и планирования мероприятий по охране и менеджменту окружающей среды. Важным компонентом литоральных экосистем морей Северного полушария являются моллюски рода *Littorina*, однако факторы, определяющие динамику их численности, исследованы недостаточно. В настоящей работе представлены данные, полученные в ходе наблюдений за популяционной динамикой *Littorina obtusata* с 2001 по 2020 г. В этот период отмечены квазициклические изменения плотности популяции литторин вокруг среднего уровня, составляющего около 2,6 тыс. экз.·м$^{-2}$. Проанализировано 67 переменных, характеризующих состояние изученной популяции моллюсков и воздействие на неё ключевых биотических и экологических факторов. Показано, что изменения плотности популяции на 83 % обусловлены флуктуациями численности сеголеток. Множественный регрессионный анализ продемонстрировал, что уровень воспроизводства популяции *L. obtusata* на 78 % определялся флуктуациями четырёх переменных — плотности особей в возрасте двух лет и старше, биомассы водорослей *Fucus vesiculosus*, количества осадков в июле и биомассы двустворок *Mytilus edulis*. Первая переменная отражает уровень внутривидовой конкуренции, вторая — способность данного местообитания к поддержанию популяции моллюсков определённой численности, а третья — воздействие факторов окружающей среды. Наличие связи между уровнем воспроизводства популяции литторин и биомассой двустворок может быть объяснено либо существованием некоего общего фактора, с которым коррелированы обе переменные, либо наличием межвидовых взаимодействий между *M. edulis* и *L. obtusata*.

Ключевые слова: *Littorina obtusata*, численность популяции, флуктуации численности, биотические и абиотические факторы, внутривидовая конкуренция, межвидовая конкуренция, ёмкость местообитания

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CULTURAL STUDY OF MICROMYCETES FROM DEEP-SEA BOTTOM SEDIMENTS OF THE ADRIATIC SEA

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For the first time, taxonomic composition of fungi and features of structure of their complexes were identified for horizons down to 30 cm below the water–bottom boundary in deep-sea bottom sediments of the Adriatic Sea. A 0–30-cm core of bottom sediments was sampled on 18.10.2007 from aboard the RV “Palagruža” (Croatia) with a column sampler of a QUEST 4000 remotely operated vehicle at a 1,020-m depth (41°43′13″N, 17°34′19″E). The sample of gray silt was cut into 30 sections, each 1 cm thick. Fungi were isolated on Czapek agar and Sabouraud agar, two replicates on each medium, under aerobic conditions, at +18 °C, with chloramphenicol 3% alcohol solution added (1 mL *per* 1 L of a medium). Abundance of fungal colony-forming units (CFU) was calculated *per* 1 g of dry sediment. Sixteen taxa were found; 12 were identified down to the species level, and 4, to the genus level. The taxa were assigned to 13 genera, 8 families, 7 orders, and 5 classes of the phyla Ascomycota and Basidiomycota; sterile mycelium was identified as well. Fungi were not recorded in a horizon of 0–1 cm. Maximum abundance of fungi was 4,300 $\text{CFU}\cdot\text{g}^{-1}$ dry sediment (a horizon of 6–7 cm). Maximum number of taxa, 6, was revealed for a horizon of 14–15 cm. In the sample studied, 87.5% of fungal species belonged to Ascomycota. Based on literature data, we compiled a list of fungal species known for various sediment horizons of the Atlantic, Indian, and Pacific oceans and the Black Sea. This list was used to calculate two indices, Δ^+ (average taxonomic distinctness index, AvTD) and Λ^+ (variation in taxonomic distinctness index, VarTD), and to plot graphs. The analysis showed the similarity of mycobiota structure of the water basins as evidenced by values of the indices within the 95% confidence interval. Close values of the indices are due to the prevalence of fungal species representing the classes Dothideomycetes, Eurotiomycetes, Sordariomycetes, and Saccharomycetes; those account for 73.13% (the Indian Ocean) to 87.50% (the Black and Adriatic seas) of the species composition.

Keywords: deep-sea bottom sediments, underlying horizons, marine fungi, taxonomic distinctness indices

Micromycetes inhabit various environments – marine, freshwater, and terrestrial ones – and maintain viability under extreme conditions. In a number of works, fungal diversity in deep-sea bottom sediments at depths from several centimeters to 1,884 m below the seabed was studied using culture-dependent and molecular methods [Florio Furno et al., 2022; Jebaraj et al., 2010; Rojas-Jimenez et al., 2020; Rédou et al., 2015; Xu et al., 2018]. As a rule, fungi isolated from different horizons of bottom sediments are well-known cosmopolitans capable of adapting to conditions of deep-sea habitats [Damare et al., 2006; Rédou et al., 2015; Wang et al., 2019; Zhang et al., 2014]. For example, a yeast *Rhodotorula mucilaginosa* (A. Jörgensen) F. C. Harrison, 1927 contains an antifreeze protein that plays a key role in deep-sea adaptation [Rédou et al., 2015; Zhang et al., 2015].

Due to their structural and functional diversity, deep-sea fungi inhabiting marine sediments at depths of more than 1,000 m from the bottom surface have become an important source of industrial, agricultural, and nutraceutical compounds. To date, over 180 biologically active secondary metabolites obtained from deep-sea fungi have been described in literature. These include compounds with antitumor, antimicrobial, antifungal, antiprotozoal, and antiviral activities [Wang et al., 2015].

In sediments of the Mediterranean submarine canyons (Tricase and Crotone) at depths of 200–1,000 m, 1,742 fungal OUT (operational taxonomic units) were found belonging to all currently known fungal phyla. Out of them, representatives of the phylum Ascomycota dominated, with known genera accounting for only 36% [Barone et al., 2018].

The Adriatic Sea is a part of the Mediterranean Sea. This semi-enclosed sea with an area of 138,600 km^2 is located between the Apennine and Balkan peninsulas. The mean depth is 252 m, and the maximum one is 1,230 m. The marine biodiversity of the Adriatic Sea is determined by many biogeographical, ecological, geological, and anthropogenic factors. Over a long period of time, it has undergone significant changes both due to enrichment with endogenous substances and due to anthropogenic load: hydrocarbon production, heavy shipping traffic, intensive fishing and aquaculture, and pollution with plastic and other economic waste [Lipej et al., 2022].

We did not find available data on mycobiota of bottom sediments of the South Adriatic Basin of the Adriatic Sea; so, our studies are relevant. The aim of this work is to identify the taxonomic composition and structural features of mycocomplexes from different horizons of the Adriatic Sea sediments.

MATERIAL AND METHODS

A 0–30-cm core of bottom sediments was sampled on 18.10.2007 from aboard the RV "Palagruža" (Croatia) at a 1,020-m depth (41°43′13″N, 17°34′19″E). Water salinity at this site was 34‰. The sampling was carried out by D. Sc., Prof. S. Gulin. The bottom sediments were sampled in the South Adriatic Basin area with a column sampler of a QUEST 4000 remotely operated vehicle. The sample of gray silt was cut into 30 sections, each 1 cm thick (0–1, 1–2, 2–3… 29–30 cm). These sediment samples were placed in sterile Petri dishes and stored in a freezer at −18 °C for a month until laboratory processing.

Fungi were isolated by sediment suspension seeding on Czapek agar and Sabouraud agar prepared with artificial seawater, two replicates on each medium. Specifically, 1 mL of sediment suspension in a 1 : 10 dilution was added to a Petri dish and filled with molten medium cooled to approximately +45 °C, with chloramphenicol 3% alcohol solution added (1 mL *per* 1 L of a medium) to suppress bacterial growth. Dishes with the material were incubated in a thermostat at +18 °C under aerobic conditions for a month. Pure cultures were isolated from grown colonies and used to identify the fungi; some isolates were identified down to the genus level.

The number of fungal embryos was calculated *per* 1 g of dry sediment by the formula:

$$A = a \times b \times c / d \ ,$$

where A is the mean abundance of fungal colony-forming units *per* 1 g of dry sediment ($\text{CFU} \cdot \text{g}^{-1}$);

a is the mean number of fungal colonies in Petri dishes (CFU);

b is the dilution from which the inoculation was made;

c is the weight of wet sediment, g;

d is the weight of dry sediment, g [Metody, 1982].

From the sediment sample, two identical samples were made. One of them was dried to a constant weight at +105 °C in a drying oven.

Micromycetes were identified by morphological and cultural features according to [Atlas of Clinical Fungi, 2000; Bilai, Koval, 1988]. All fungal names were checked in the electronic database Index Fungorum [2024].

The data were processed using MS Office Excel and a software package PRIMER 5.2.8. The species similarity of mycocomplexes was calculated using the Bray–Curtis coefficient based on the presence/absence of a taxon according to its frequency of occurrence relative to the total number of samples (Similarity function). The Shannon index of mycocomplexes by sediment horizons was determined based on the number of taxa and their abundance (DIVERSE).

Based on literature data, the authors compiled a list of marine micromycete species isolated from different horizons of bottom sediments of the Indian Ocean (down to 4.7 m) [Damare et al., 2006; Raghukumar, Raghukumar, 1998; Raghukumar et al., 2010; Xu et al., 2018; Zhang et al., 2014], the Pacific Ocean (down to 1,884 m) [Keeler, 2021; Rédou et al., 2015; Xu et al., 2014], the Atlantic Ocean (down to 5 m) [Keeler, 2021], and the Black Sea (down to 0.40 m) [Kopytina et al., 2024; Zaitsev, Polikarpov, 2008]. In the absence of species identification, but the genus of a micromycete indicated by other author, the name of the genus was included in the list with a designation sp. 1. The list consists of 182 names of taxa isolated by seeding or identified by genetic analysis techniques (accuracy is up to 98%).

The list was used to calculate indices of the taxonomic composition of fungal complexes in order to plot graphs of taxonomic indices for each considered area. The indices were determined based on data on the presence or absence of a species taking into account family relationships between species (from species to phylum) and the number of species in each community. Δ^+ is the average taxonomic distinctness index, AvTD; Λ^+ is the variation in taxonomic distinctness index, VarTD. Those show the similarity/dissimilarity in the species structure of mycocomplexes (TAXDTEST). The graphs of these indices provide a statistical justification for the structure relationship within the community. Specifically, Δ^+ is the average length of links in a series of species of the taxonomic tree, and this index reflects vertical links (number of families, orders, *etc.*). Λ^+ is the dispersion of paired lengths of related links, and this index reproduces the horizontal proportion of the community (the number of taxa at each level of the hierarchical tree) [Clarke et al., 2014].

The percentage of the abundance of fungi from different phyla was calculated as the ratio of the total abundance of taxa within the phylum to the total abundance of sediments isolated from samples. The frequency of occurrence of taxa was determined by taking the number of samples (30) as 100%.

RESULTS

For the first time, deep-sea bottom sediments below the water–bottom boundary down to 30 cm were studied layer by layer in the Adriatic Sea. Twelve fungal taxa were identified down to the species level, and four, down to the genus level. Micromycetes were assigned to the phyla Ascomycota and Basidiomycota. Sterile mycelium was also isolated. Most species were grouped in the phylum Ascomycota; in terms of the number of representatives, its classes Sordariomycetes (5 species), Dothideomycetes (4), and Eurotiomycetes (4) were the prevailing ones.

In the sediments studied, all species of micromycetes were represented by terrestrial cosmopolitan species.

The frequency of occurrence of taxa varied from 3.3% (*Alternaria tenuissima* and *Cladosporium sphaerospermum*) to 56.7% (*Metschnikowia* sp. 1). In 18 sediment horizons, 1–2 taxa were identified. Information on the systematic affiliation of micromycetes, their distribution in sediment horizons, and abundance is provided in Table 1.

Table 1. Species composition, abundance, and distribution of fungi in sediments of the Adriatic Sea (0–30-cm layers)

Species	Sediment horizon, cm	Abundance, CFU·g^{-1} dry sediment (min–max)
Phylum Ascomycota, class Dothideomycetes		
Alternaria chartarum Preuss, 1851	8–9, 11–12, 14–15, 24–25	30–180
Al. tenuissima (Kunze) Wiltshire, 1933	18–19	90
Cladosporium sphaerospermum Penzig, 1882	11–12	30
Stemphylium sarciniforme (Cavara) Wiltshire, 1938	4–5, 11–12, 13–14, 14–15, 20–21, 24–25	35–100
Class Eurotiomycetes		
Aspergillus flavus Link, 1809	17–18, 27–28	40–100
As. fumigatus Fresenius, 1863	16–17, 19–20	40–80
Penicillium chrysogenum Thom, 1910	9–10, 14–15, 15–16, 17–18, 24–25	30–90
Penicillium sp. 1	5–6, 6–7, 17–18, 27–28	30–440
Class Sordariomycetes		
Acremonium sp. 1	20–21, 21–22, 28–29, 29–30	16–96
Amesia atrobrunnea (L. M. Ames) X. Wei Wang et Samson, 2016	6–7, 13–14, 15–16	30–487
Botryotrichum murorum (Corda) X. Wei Wang et Samson, 2016	4–5, 5–6, 6–7, 13–14	30–792
Chaetomium elongatum Czerepanova, 1962	6–7, 13–14	80–860
Stachybotrys chartarum (Ehrenberg) S. Hughes, 1958	1–2, 14–15, 20–21	30–41
Class Saccharomycetes		
Metschnikowia sp. 1	2–3, 3–4, 6–7, 8–9, 10–11, 11–12, 12–13, 14–15, 17–18, 18–19, 19–20, 20–21, 22–23, 23–24, 25–26, 27–28, 29–30	90–1,720
Phylum Basidiomycota, class Tremellomycetes		
Trichosporon asteroides (Rischin) M. Ota, 1926	9–10, 26–27	60–90
Class Malasseziomycetes		
Malassezia sp. 1	1–2, 5–6, 16–17, 21–22, 28–29	150–300
Unidentified taxa		
Mycelia sterilia 1	2–3, 7–8, 14–15	30–70
Mycelia sterilia 2	25–26	240

Fungi were not found in a horizon of 0–1 cm. The maximum number of taxa, 6, was revealed in a layer of 14–15 cm. Only sterile mycelium was recorded in a layer of 7–8 cm. The similarity of the fungal species composition by horizons varied from 0.0 (no common taxa were registered) to 100.0% (1 species was identified, *Metschnikowia* sp. 1) (sediment horizons of 3–4, 10–11, 12–13, 22–23, and 23–24 cm).

The abundance of fungi fluctuated from 0 (a layer of 0–1 cm) to 4,300 CFU·g^{-1} dry sediment (a layer of 6–7 cm). The Shannon diversity index value was 0 to 1.55. H'($\log_e$) = 0 in case of isolation of 1 species or absence of species (7 sediment horizons) (Table 2).

Table 2. Indicators of fungi abundance and taxonomic diversity in deep-sea sediment horizons of the Adriatic Sea (0–30 cm)

Sediment horizon, cm	Number of taxa	Abundance, CFU·g^{-1} dry sediment	Shannon index, H'($\log_e$)
0–1	0	0	0.000
1–2	2	330	0.305
2–3	2	240	0.604
3–4	1	160	0.000
4–5	2	200	0.693
5–6	3	420	0.759
6–7	5	4,300	1.480
7–8	1	30	0.000
8–9	2	300	0.637
9–10	2	90	0.637
10–11	1	400	0.000
11–12	4	570	1.094
12–13	1	100	0.000
13–14	4	240	1.330
14–15	6	380	1.550
15–16	2	120	0.562
16–17	2	220	0.474
17–18	4	400	1.321
18–19	2	220	0.677
19–20	2	420	0.451
20–21	4	574	0.895
21–22	2	178	0.416
22–23	1	480	0.000
23–24	1	360	0.000
24–25	3	120	1.040
25–26	2	640	0.662
26–27	2	160	0.685
27–28	3	190	0.910
28–29	2	250	0.673
29–30	2	176	0.305

In sediment samples, representatives of the phylum Ascomycota dominated in terms of the number of species and abundance: 88.9 and 89.64%, respectively.

No pattern was revealed in distribution of the number of fungal taxa and abundance across sediment horizons. This can be explained by the small number of samples studied and certain limitations in isolating microscopic fungi with culture-dependent methods applied.

DISCUSSION

Many studies on deep-sea sediments of the World Ocean provide taxonomic ranks at the level of families, orders, classes, and phyla; in some cases, only a genus of fungi is indicated. This creates difficulties when comparing the species composition in water bodies. We compiled a list of 182 fungal species assigned to 93 genera, 56 families, 31 orders, and 13 classes from the phyla Ascomycota, Basidiomycota, and Mucoromycota. The indicators of taxonomic richness and diversity of fungal complexes in underlying sediment horizons of the Adriatic and Black seas and the Atlantic, Indian, and Pacific oceans are presented in Table 3.

Table 3. Indices of taxonomic richness and diversity of fungal complexes from deep-sea sediments of the Adriatic and Black seas and Atlantic, Indian, and Pacific oceans (g, genus; f, family; s, species)

Water body	Number of taxa					Proportions		
	species	genus	family	order	class	g/f	s/f	s/g
Phylum Ascomycota								
The Adriatic Sea	15	12	8	6	4	1.50	1.76	1.25
The Black Sea	21	10	8	7	4	1.25	2.62	2.1
The Atlantic Ocean	34	15	12	11	5	1.25	2.83	2.27
The Indian Ocean	68	37	23	12	6	1.61	2.96	1.84
The Pacific Ocean	63	39	24	15	6	1.63	2.63	1.62
Phylum Basidiomycota								
The Adriatic Sea	2	2	2	2	2	1.00	1.00	1.00
The Black Sea	2	2	2	2	2	1.00	1.00	1.00
The Atlantic Ocean	4	4	3	3	4	1.33	1.33	1.00
The Indian Ocean	18	15	10	8	6	1.50	1.80	1.20
The Pacific Ocean	16	12	8	7	3	1.50	2.00	1.33
Phylum Mucoromycota								
The Adriatic Sea	0	0	0	0	0	0	0	0
The Black Sea	1	1	1	1	1	1.00	1.00	1.00
The Atlantic Ocean	0	0	0	0	0	0	0	0
The Indian Ocean	0	0	0	0	0	0	0	0
The Pacific Ocean	1	1	1	1	1	1.00	1.00	1.00

The similarity of the species composition of fungi isolated from sediments of the Adriatic Sea and the Indian Ocean is 5.8% (common taxa are *Penicillium chrysogenum*, *Cladosporium sphaerospermum*, and yeasts of the genus *Malassezia*); the Atlantic Ocean, 10.9% (*Alternaria tenuissima*, *C. sphaerospermum*, and *P. chrysogenum*); the Pacific Ocean, 18.56% (*Aspergillus fumigatus*, *As. flavus*, *C. sphaerospermum*, *P. chrysogenum*, *Stachybotrys chartarum*, and species of the genera *Acremonium*, *Metschnikowia*, and *Meyerozyma*); and the Black Sea, 34.2% (*Al. tenuissima*, *As. fumigatus*, *Botryotrichum murorum*, *C. sphaerospermum*, *S. chartarum*, and yeasts of the genera *Metschnikowia* and *Malassezia*). Seven species of micromycetes and representatives of four genera isolated from bottom sediment samples of the Adriatic Sea were found in other areas of the World Ocean as well [Edgcomb et al., 2002; Kiel Reese et al., 2021; Kopytina et al., 2024; Pachiadaki et al., 2016; Rojas-Jimenez et al., 2020; Rédou et al., 2014, 2015; Xu et al., 2018, 2019; Zhang et al., 2014; Zhou et al., 2021].

Deep-sea sediments of the Black Sea are contaminated with hydrogen sulfide. However, some species of yeast and mycelial fungi easily adapt to permanent or temporary anoxia using oxygen from the material they have contaminated, as they are facultative anaerobes [Kurakov et al., 2008, 2011].

The graphs of the taxonomic indices Δ^+ and Λ^+ reflect the general pattern of the vertical and horizontal distribution of lower taxa (species and genus) by higher taxonomic ranks in five water basins (Fig. 1A, B).

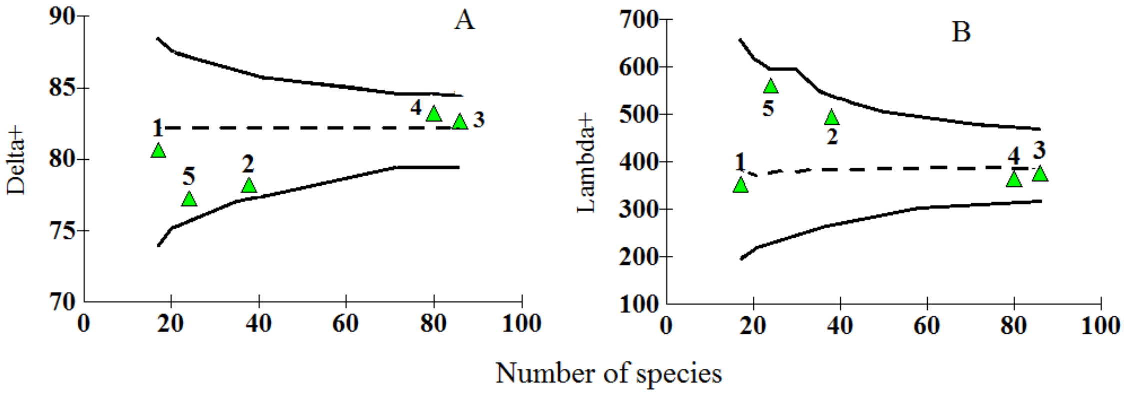

Fig. 1. Values of taxonomic indices Δ^+ (A) and Λ^+ (B) for fungal complexes in underlying sediment horizons in different water bodies calculated according to the general list of species from these water bodies (1, the Adriatic Sea; 2, the Atlantic Ocean; 3, the Indian Ocean; 4, the Pacific Ocean; 5, the Black Sea). Solid lines denote the 95% probability funnel; dashed line denotes the mean calculated value of the index

In Fig. 1A, the values of Δ^+ are included in the probability funnel. Therefore, the structures of mycocomplexes are similar and do not differ statistically significantly. Distribution of lower taxa by higher ranks corresponds to an average one, as evidenced by the index values (77.23–82.66). Despite the fact that in the water basins analyzed, different numbers of higher taxa were found (2–3 phyla and 7–12 classes), the overall structure remained proportional, and similar index values were governed by the presence of fungal species of the classes Dothideomycetes, Eurotiomycetes, Sordariomycetes, Saccharomycetes, and Malasseziomycetes. Those accounted for 71.25% (the Pacific Ocean) to 94.12% (the Adriatic Sea) of the species composition.

In Fig. 1B, the values of Λ^+ are within the confidence funnel of the calculated average expected value. Consequently, in complexes of underlying layers of deep-sea bottom sediments of the Adriatic and Black seas, as well as the Atlantic, Pacific, and Indian oceans, a high similarity of the taxonomic structure was revealed (with species distribution by higher taxonomic ranks taken into account). This fact is confirmed by s/g proportions (see Table 3): the values vary within 1.21–2.00.

Studies carried out using culture-dependent and transcriptomic methods have shown as follows. In underlying sediments of the World Ocean, fungi from the phyla Ascomycota and Basidiomycota dominate: 43–80 and 3–20%, respectively. Unidentified fungal species account for 2.4–64%; representatives of the phylum Zygomycota, 0.55–1.5%; and representatives of the phylum Chytridiomycota, 0.8% [Barone et al., 2018; Florio Furno et al., 2022; Jebaraj et al., 2010; Rojas-Jimenez et al., 2020; Vargas-Gastélum, Riquelme, 2020; Xu et al., 2018, 2019; Zhang et al., 2016]. Taxa from the classes Eurotiomycetes, Sordariomycetes, Dothideomycetes, and Saccharomycetes (Ascomycota), as well as Tremellomycetes and Malasseziomycetes (Basidiomycota), often occur in various horizons of deep-sea sediments. Representatives of the above-listed classes were isolated in sediment samples from the Adriatic Sea. In this study, 87.5% of the species belonged to the phylum Ascomycota, and 12.5%, to the phylum Basidiomycota. The similarity of mycocomplexes is confirmed by the graphs of the taxonomic indices.

Species of the genera *Penicillium* and *Aspergillus* prevail in water and bottom sediments of the Adriatic Sea; *Cladosporium*, *Alternaria*, and *Chaetomium* representatives are found there as well [Muntañola-Cvetković, Ristanović, 1980; Ristanović et al., 1975]. It suggests that fungi of these genera are typical inhabitants of the Adriatic Sea.

Conclusion. In the Adriatic Sea sediment core sampled down to 30 cm, 16 fungal taxa from the phyla Ascomycota and Basidiomycota were identified; Mycelia sterilia was also found. In terms of the number of representatives, three classes of the phylum Ascomycota dominated: Sordariomycetes (5 species), Dothideomycetes (4), and Eurotiomycetes (4). The results obtained are consistent with literature data on micromycetes from deep-sea sediments, *inter alia* from underlying horizons of other areas of the World Ocean. Analysis of the taxonomic structure of mycocomplexes from underlying layers of deep-sea sediments of the Adriatic and Black seas, as well as the Atlantic, Pacific, and Indian oceans, was performed applying the taxonomic indices Δ^+ and Λ^+. It showed the similarity of their structure, as evidenced by the values of the indices included in the 95% confidence funnel. Similar values of the indices are governed by the dominance of fungal species from the classes Dothideomycetes, Eurotiomycetes, Sordariomycetes, and Saccharomycetes accounting for 73.13% (the Indian Ocean) to 87.50% of the species composition (the Black and Adriatic seas).

Deep-sea micromycetes are not investigated properly. So, further detailed and coordinated studies of their biological diversity, quantitative characteristics, ecological role, and adaptive mechanisms to different habitat conditions in various areas of the World Ocean are required.

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КУЛЬТУРАЛЬНОЕ ИССЛЕДОВАНИЕ МИКРОМИЦЕТОВ ИЗ ГЛУБОКОВОДНЫХ ДОННЫХ ОТЛОЖЕНИЙ АДРИАТИЧЕСКОГО МОРЯ

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Впервые выявлены таксономический состав и особенности структуры комплексов грибов с горизонтов до 30 см ниже границы вода — дно в глубоководных донных отложениях Адриатического моря. Керн донных отложений 0–30 см был взят 18.10.2007 с борта НИС Palagruža (Хорватия) с помощью колончатого пробоотборника подводного телеробота QUEST 4000 на глубине 1020 м в точке с координатами 41°43′13″N, 17°34′19″E. Пробу серого ила разрезали на 30 частей толщиной в 1 см. Грибы выделяли на агаризованные среды Чапека и Сабуро, по две повторности на каждой среде, в аэробных условиях, при температуре +18 °C, с добавлением 3%-ного спиртового раствора хлорамфеникола (1 мл·л$^{-1}$ среды). Количество грибных колониеобразующих единиц (КОЕ) рассчитывали на 1 г сухого осадка. Обнаружили 16 таксонов; 12 определили до вида, 4 — до рода. Таксоны отнесены к 13 родам, 8 семействам, 7 порядкам, 5 классам отделов Ascomycota и Basidiomycota; также выделен стерильный мицелий. В горизонте осадка 0–1 см грибы не выявлены. Максимальная численность грибов составляла 4300 КОЕ·г$^{-1}$ сухого осадка (горизонт 6–7 см). Максимальное количество таксонов, 6, обнаружено в горизонте 14–15 см. В этом исследовании к отделу Ascomycota относились 87,5 % видов. По литературным данным составлены списки видов грибов из подстилающих горизонтов осадков Атлантического, Индийского и Тихого океанов и Чёрного моря, на основании которых вычислены значения таксономических индексов Δ^+ (average taxonomic distinctness index, AvTD) и Λ^+ (variation in taxonomic distinctness index, VarTD) и построены их графики. Анализ показал общую структуру микобиот водоёмов, о чём свидетельствуют значения индексов, входящие в 95%-ную доверительную воронку. Близкие значения индексов обусловлены доминированием видов грибов из классов Dothideomycetes, Eurotiomycetes, Sordariomycetes и Saccharomycetes, составляющих от 73,13 % (Индийский океан) до 87,50 (Чёрное и Адриатическое моря) от видового состава.

Ключевые слова: глубоководные донные отложения, подстилающие слои, морские грибы, таксономические индексы

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MACROZOOBENTHOS OF THE SUSUYA RIVER ESTUARY (SAKHALIN ISLAND): I. HYDROLOGICAL CHARACTERISTICS OF THE ESTUARY, SPECIES COMPOSITION AND DISTRIBUTION OF MACROZOOBENTHOS

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The paper provides description of species composition of macrozoobenthos, its structure, quantitative indicators, and features of distribution along the riverbed of the Susuya River estuary (Sakhalin Island). By hydrological characteristics and macrobenthos parameters, the main biotopic zones of the estuary are distinguished: the mouth (polyhaline zone), the lower estuary polyhaline–mesohaline zone, the middle estuary oligohaline zone, the upper estuary δ-chorohaline zone, and the freshwater zone. In total, 58 species of bottom hydrobionts were found in the river estuary. Malacostraca, 22 species, forms the basis of the species composition. Amphibiotic insects account for 15 species; Polychaeta, 9 species; and Mollusca, 7 species. Other groups are represented by 1–2 species. Each identified zone features its own, specific composition of bottom hydrobionts. Species richness decreases from the mouth with the sea salinity of water (30 species) to the α-chorohaline boundary (12 species) which corresponds to provisions of the theory of critical salinity. The value increases to 20 species when moving to the freshwater zone of the riverbed. The density of macrobenthos rises from (476 ± 59) ind.·m^{-2} at the river mouth to (6,653 ± 915) ind.·m^{-2} in the center of the lower estuary. The minimum density, (653 ± 72) ind.·m^{-2}, characterizes the α-chorohalinicum, while the maximum one, (3,529 ± 336) ind.·m^{-2}, is confined to the upper estuary. The basis of macrozoobenthos density is formed by polychaetes, gastropods, and amphipods in the lower estuary; by polychaetes, amphipods, and oligochaetes in the middle estuary; and by oligochaetes and dipterans in the upper estuary. Four areas with high biomass were registered: the lower estuary, (51.2 ± 5.7) g·m^{-2}; the middle estuary, (190.5 ± 41.2) g·m^{-2}; the upper estuary, (397.5 ± 82.0) g·m^{-2}; and the riffle separating the estuary from the freshwater zone of the riverbed, (23.4 ± 2.78) g·m^{-2}. At the river mouth, decapods form the basis of macrozoobenthos biomass. Upstream, the main contributors are bivalves, chiefly *Macoma balthica*, and gastropods, mostly *Fluviocingula nipponica*. In the middle estuary oligohaline zone, bivalves form the basis of macrozoobenthos biomass; those are represented almost by *Corbicula japonica* alone. At the boundary of the oligohaline zone and upper estuary, Diptera species (Chironomidae) predominate. On the freshwater riffle, decapods become the main group again; those are represented by a crab *Eriocheir japonica* alone. The boundaries between the mouth and lower estuary zone, α-chorohalinicum and δ-chorohalinicum, are clearly distinguished by macrobenthos abundance and structural indices. The boundary between the upper estuary oligohaline zone and freshwater zone extends for several hundred meters along the estuary.

Keywords: estuary, salinity, macrozoobenthos, density, biomass, Sakhalin Island

An estuary is a semi-enclosed water body; it is a part of the river mouth area characterized by active processes of mixing of river water and seawater [Mikhailov et al., 2009; Pritchard, 1952]. During such a mixing, several transition zones are formed along the estuary bed which are critical for hydrobionts; these are so-called chorohalinicums [Aladin, 1988; Aladin, Plotnikov, 2013; Khlebovich, 1974, 1989]. Passages through chorohalinicums lead to a shift in species composition of benthic communities, their structure, trophic characteristics, and production indicators [Burkovsky, Stolyarov, 1995; Burkovsky et al., 2002; Kolpakov, 2018; Labay et al., 2022; Stolyarov, 2011, 2015, 2019a, b; Stolyarov, Burkovsky, 2018]. Estuaries are spots of concentration of specific brackish-water fauna. There, unique communities develop, and many species of fish and invertebrates feed and spawn. Estuary ecosystems are characterized by increased productivity [Kolpakov, 2018; Saf'yanov, 1987].

The estuary macrozoobenthos of Sakhalin Island, benthic communities, and their characteristics have been poorly studied [Labay et al., 2022; Safronov et al., 2000; Watercourses of Sakhalin Island, 2015]. In the most investigated estuary, that of the Manuy River, typical of most small rivers of the island, macrozoobenthos is greatly depleted compared to that of estuaries of other rivers in the Russian Far East; it lacks a clear horizontal division into zones by salinity [Labay et al., 2022]. In September 2022, the Susuya River estuary was surveyed. It is full-size compared to estuaries of small rivers on Sakhalin Island. The material of the study formed the basis of this work.

The aim of the work is to describe the main patterns of macrobenthos variability, its structure, and trophic characteristics along the salinity gradient in the full-size estuary of the Susuya River on Sakhalin Island.

MATERIAL AND METHODS

The Susuya River estuary was surveyed in September 2022 (Fig. 1, Table 1). Ten benthic transects were completed. The number of stations on the transects varied depending on the riverbed width. Thus, on transects 1–4, where the riverbed width exceeded 40 m, sampling was carried out at seven stations; on the remaining transects, with the riverbed width of less than 30 m, at five stations (near each bank at the water's edge, as well as on the fairway and on its sides). Three macrobenthos samples were taken at each station. A total of 174 samples were taken.

At depths of less than 0.3 m on pebble-gravel sediment, sampling was carried out with a folding benthometer (0.12 m^2); at greater depths on soft sediment, with a lightweight model of the small Petersen bottom grab (0.025 m^2) [Metodicheskie rekomendatsii, 2003; Rukovodstvo, 1983]. The samples were washed, fixed with 4% neutralized formalin, and labeled.

The initial analysis of the samples and species identification were carried out under laboratory conditions. The samples were washed through sieves with different mesh sizes, with the last sieve with a mesh of no more than 1 mm. After washing and identification, hydrobionts were counted, dried on filter paper until a wet spot disappeared, and weighed on electronic scales with an accuracy of tenths of a milligram. Subsequently, the quantitative data were recalculated *per* m^2.

In parallel with benthos sampling, water salinity (psu) and temperature (°C) were measured at the surface and at the bottom of the fairway with a Horiba U-5000G multi-parameter water quality checker.

The names of species and supraspecific taxa were checked on World Register of Marine Species website [2023]. The names of sediment types are given in accordance with tables E.1, E.2, and E.3 of the State Standard 25100-2011 “Soils. Classification (with Amendments).”

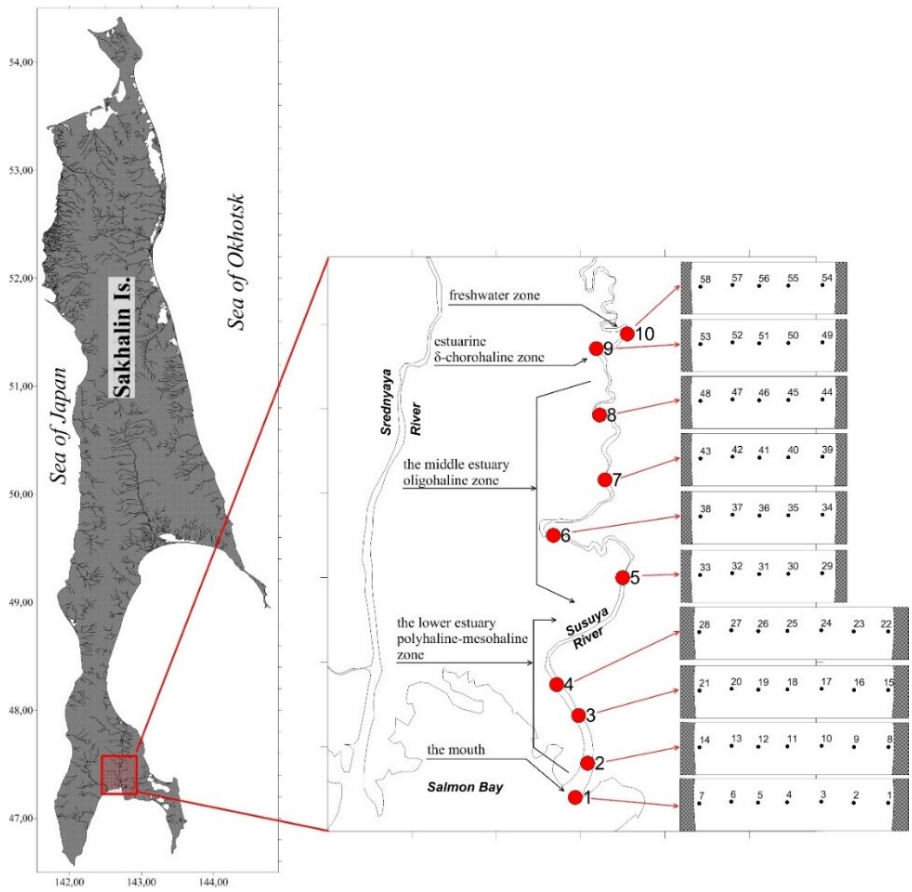

Fig. 1. Schematic map of the study area; benthos sampling sections are shown with rectangles

Table 1. Collected data

Transect No.	Distance from the mouth, km	Depth, m	Width, m	Salinity, psu	Number of stations
1	0	0	60–70	15.2	7
		0.7		22.3	
2	0.4	0	50–60	18.1	7
		1.35		21.7	
3	1.01	0	50–60	21.7	7
		2		21.7	
4	1.43	0	40–50	24.8	7
		2.1		25	
5	3.10	0	25–30	6.6	5
		0.6		6.6	
6	4.65	0	25–30	3.8	5
		1.4		11.7	
7	5.94	0	25–27	0.6	5
		1.8		7.6	
8	6.95	0	18–20		5
		0.8			
9	7.98	0	12–15		5
		1.6			
10	8.50	0	10–12	0.1	5
		0.15		0.1	

For comparison and analysis, the Shannon diversity index (entropy index) (I, bit·specimen^{-1}) was used [Shannon, 1948; Shannon, Weaver, 1949], separately for density (I_N) and biomass (I_B). Also, the ABC method (abundance/biomass comparison method) was applied [Warwick, 1986] according to the ABC index (I_{ABC}, %) [Meire, Dereu, 1990].

The expected number of species was estimated with the Chao-2 species richness extrapolation algorithm [Chao, Chiu, 2016; Petrov, Nevrova, 2012].

The study area. The Susuya River flows into Lososey Bay (the Aniva Bay, the Sea of Okhotsk), is 83 km long, and has a catchment area of 823 km^2 [Resursy, 1963]. Yuzhno-Sakhalinsk, a large administrative and industrial center, is located in the middle area of the river; this city is a source of water pollution with iron, zinc, nitrogen and phosphate groups of biogenic substances, oil products, and phenols [Chayko, 2009, 2014, 2015a, b]. In the vicinity of the Mitsulevka village, the riverbed is blocked by a dam and flows through a canal into the riverbed of the Srednyaya River. The canal was dug in 1906–1945 [Makeev, 2020; Resursy, 1964]. To date, the lower part of the riverbed, including the estuary, is isolated from the rest of the river system. This isolated part extends from the Mitsulevka River confluence to the river mouth and has a catchment area of 725 km^2 [Makeev, 2020].

According to the classification of V. Mikhailov *et al.* [2009], the Susuya River estuary is a riverbed estuary with a mouth widening (a, lower parts of riverbeds). The estuary length up to the freshwater area is almost 8.5 km (according to our data). In the upper estuary, the riverbed is 10–12 m wide; towards the mouth, the width increases to 60–70 m (our data).

The vertical distribution of water salinity in the river estuary is shown in Fig. 2. Seawater with salinity of more than 22 psu extends up to 1.5 km from the mouth. The measurements were carried out during the time interval between high and low tide; importantly, at high tide, the boundary of waters with sea salinity can occur further up the estuary. This salinity barrier separates the polyhaline estuary zone (filled with seawater) and mesohaline one (brackish-water) (the boundaries of waters with different salinity are given according to [Aladin, 1988; Aladin, Plotnikov, 2013; Khlebovich, 1974, 1989]). Brackish water with salinity from 5–7 psu (the α-chorohaline boundary) to 22–26 psu (the β-chorohaline boundary) fills the estuary for more than 3 km from the mouth. Below the 6th km and to 3 km, throughout the entire water area of the estuary reach, there is a vertical salinity gradient. The lower layer corresponds to the α-chorohaline boundary, while the narrow upper layer is freshened (oligohaline). A similar vertical distribution of salinity is revealed for typical estuaries of Sakhalin Island rivers [Labay et al., 2022]. From the 6th km upstream, an oligohaline zone is noted for about 1 km. At a distance of approximately 7 km from the mouth, it is limited by the δ-chorohalinicum (0.5–2.0 psu) which extends upward to the last river riffle (transect 10). Above the riffle, the river water is fresh (about 0 psu).

In terms of distribution of water salinity, five estuary zones are distinguished: the mouth filled with seawater (polyhaline zone); the lower estuary polyhaline–mesohaline zone; the middle estuary oligohaline zone; the upper estuary δ-chorohaline zone, and the freshwater zone. The middle estuary zone is the longest: it extends along the riverbed for almost 4 km. The lower and upper estuary zones are shorter, 1–2 km each. The obtained scheme is similar to the typification of river estuaries developed by N. Kolpakov [2018]. In contrast to the typical estuaries of Sakhalin Island, there is a clearly defined lower estuary zone, about 1.5 km long, filled along its entire vertical with water with sea salinity (in other estuaries, seawater penetrates no further than 100–200 m from the mouth) [Labay et al., 2022]. Apparently, this phenomenon can be explained by redistribution of the river flow in the middle reach into the riverbed of the Srednyaya River [Makeev, 2020; Resursy, 1964]. As a result, with the same size of the estuary bed, the volume of river runoff has significantly decreased, and this mediated a noticeable penetration of seawater up the riverbed at the present time.

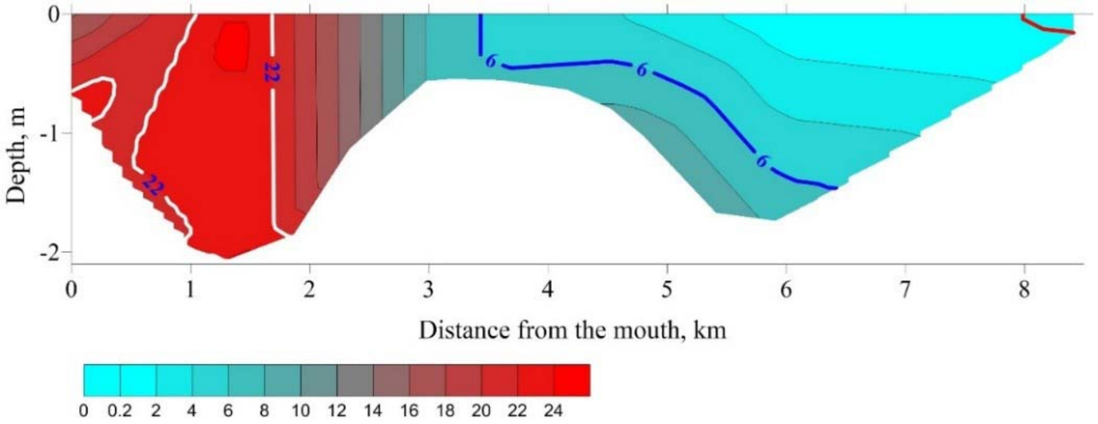

Fig. 2. Vertical distribution of water salinity along the Susuya River estuary on 15.09.2022

At the mouth, the bottom is covered with fine sand with silt and clay, and in the fairway, with sand (about 80%) with large pebbles. Upstream, in the middle and lower estuary zones, the river banks are clayey or sandy-silty. In the mouth reach, organic silt and clay occur; less often, silt is registered. At the boundary between the lower estuary and middle estuary, the bottom sediment is represented by sands of various sizes with pebbles and silts. In the upper estuary, on the reach, the banks are silty, while with increasing depth, the bottom sediment changes to silts. The river riffle which separates the freshwater zone of the riverbed and the δ-chorohaline zone, is pebble-gravel with an admixture of sand.

During the study period, the current was expressed only in the riffle (transect 10): there, the current velocity varied by stations within 0.15–0.38 $m \cdot s^{-1}$. Already on transect 9, the value did not exceed 0.01 $m \cdot s^{-1}$. On other transects, there was no unidirectional current.

On transects 2 and 3, thickets of macrophytes, chiefly *Zostera* ones, were noted along the coast. There, aggregations of dead macrophytes were also observed, probably brought in by tide.

RESULTS AND DISCUSSION

The river estuary is inhabited by brackish-water and marine fish species. These are the saffron cod *Eleginus gracilis* (Tilesius, 1810); smelts of the genus *Hypomesus*, Japanese ones *H. japonicus* (Brevoort, 1856) and *H. nipponensis* McAllister, 1963, as well as the pond one *H. olidus* (Pallas, 1814); a three-spined stickleback *Gasterosteus nipponicus* (Higuchi, Sakai et Goto, 2014); nine-spined sticklebacks, *Pungitius pungitius* (Linnaeus, 1758) and *P. sinensis* (Guechenot, 1869); a goby *Gymnogobius urotaenia* (Hilgendorf, 1879); a sculpin *Megalocottus taeniopterus* (Kner, 1868); the starry flounder *Platichthys stellatus* (Pallas, 1787); the eastern viviparous blenny *Zoarces elongatus* (Kner, 1868); and *Brachyopsis segaliensis* (Tilesius, 1809). In the early XX century, the Sakhalin sturgeon *Acipenser mikadoi* Hilgendorf, 1892 was abundant in the estuary; to date, this species has completely disappeared [Makeev, 2020; Shmidt, 1905].

Distribution of macrobenthos. Earlier, unique species of benthic invertebrates were discovered in the Susuya River estuary, absent from the typical estuaries of Sakhalin Island rivers. There, a crab *Deiratonotus cristatum* (De Man, 1895) and an amphipod *Melita shimizui sakhalinensis* Labay, 2016 were described for the first time in Russia [Labai, 2004; Labay, 2016, 2021; Marin, 2017].

The species list of the macrobenthos of the Susuya River estuary includes 58 species of benthic invertebrates and cyclostomes (Table 2). The expected number of species Chao-2 was 65. Accordingly, 89% of the possible maximum number of species were found in the samples. The most represented group is Malacostraca, 22 species (amphipods, 11 species). A significant difference from other estuaries of the island is the developed fauna of decapods, 7 species [Labay, 2011, 2021; Labay et al., 2022; Watercourses of Sakhalin Island, 2015]. Insects, mostly chironomids, cover 15 species. Polychaetes are diverse as well, 9 species. Molluscs are represented by 7 species (3 species of gastropods and 4 species of bivalves), and this makes the Susuya River estuary more similar to other estuaries of large and medium rivers in the Russian Far East [Kolpakov, 2018; Komendantov, Orlova, 2003; Watercourses of Sakhalin Island, 2015]. Other groups include 1–2 species each. A distinctive feature of the Susuya River estuary is the noticeable representation of typical lagoon brackish-water and marine species absent from other estuaries of the island: *Fluviocingula nipponica* Kuroda & Habe, 1954, *Assiminea lutea* A. Adams, 1861, *Batillaria attramentaria* (G. B. Sowerby II, 1855), *Macoma balthica* (Linnaeus, 1758), *Ampithoe lacertosa* Spence Bate, 1858, *Eogammarus possjeticus* (Tzvetkova, 1967), *Hemigrapsus takanoi* Asakura & Watanabe, 2005, and *Upogebia major* (De Haan, 1841) [Labay, 2015; Reservoirs of Sakhalin Island, 2014].

Table 2. The species composition of the Susuya River estuary

No.	Species	Transect No.									
		1	2	3	4	5	6	7	8	9	10
	Phylum Nemertea										
1	Nemertea indet.	+	+	+	+	+	+	+	−	−	+
	Phylum Annelida										
	Class Polychaeta										
2	Polychaeta indet.	−	+	−	−	−	−	−	−	−	−
	Order Phyllodocida										
3	agg. *Eteone flava* (Fabricius, 1780)	+	+	+	+	+	+	−	−	−	−
4	*Glycera capitata* Örsted, 1843	+	−	−	−	−	−	−	−	−	−
5	*Goniada maculata* Örsted, 1843	+	+	+	+	−	−	−	−	−	−
6	*Hediste japonica* (Izuka, 1908)	+	+	+	+	+	+	+	+	+	+
	Order Spionida										
7	*Aonides oxycephala* (Sars, 1862)	−	−	−	−	+	−	−	−	−	−
8	*Polydora* indet.	+	+	+	−	−	−	−	−	−	−
	Infraclass Scolecida										
9	Capitellidae indet.	+	+	+	+	+	+	+	+	−	−
10	*Ophelia limacina* (Rathke, 1843)	+	−	−	−	−	−	−	−	−	−
	Class Clitellata										
	Subclass Oligochaeta										

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No.	Species	Transect No.									
		1	2	3	4	5	6	7	8	9	10
	Order Tubificida										
11	*Limnodrilus hoffmeisteri* f. *typica* Claparède, 1862	–	+	+	+	+	+	+	+	+	+
12	*Tubifex tubifex* (O. F. Müller, 1773)	–	–	–	–	–	–	–	–	+	+
	Subclass Hirudinea										
13	Hirudinea indet.	+	–	–	–	–	–	–	–	–	–
	Phylum Mollusca										
	Class Gastropoda										
	Order Littorinimorpha										
14	*Fluviocingula nipponica* Kuroda & Habe, 1954	+	+	+	+	+	–	–	–	–	–
15	*Assiminea lutea* A. Adams, 1861	+	+	+	+	+	+	+	–	–	–
	Order Caenogastropoda										
16	*Batillaria attramentaria* (G. B. Sowerby II, 1855)	+	+	–	+	–	–	–	–	–	–
	Class Bivalvia										
	Order Cardiida										
17	*Macoma balthica* (Linnaeus, 1758)	+	+	+	+	–	–	–	+	–	–
	Order Nuculanida										
18	*Nuculana pernula* (O. F. Müller, 1779)	+	+	–	–	–	–	–	–	–	–
	Order Venerida										
19	*Corbicula japonica* Prime, 1864	+	+	–	+	+	+	+	+	–	+
	Superorder Anomalodesmata										
20	*Exolaternula liautaudi* (Mittre, 1844)	+	+	+	+	–	–	–	–	–	–
	Phylum Arthropoda										
	Subphylum Crustacea										
	Superclass Multicrustacea										
	Class Malacostraca										
	Order Cumacea										
21	*Bodotria parva* Calman, 1907	+	+	–	–	–	–	–	–	–	–
	Order Amphipoda										
22	*Ampithoe lacertosa* Spence Bate, 1858	+	+	+	+	–	–	–	–	–	–
23	*Caprella algaceus* Vassilenko, 1967	+	+	–	–	–	–	–	–	–	–
24	*Crassicorophium crassicorne* (Bruzelius, 1859)	+	–	–	+	–	–	–	–	–	–
25	*Eogammarus kygi* (Derzhavin, 1923)	–	–	–	–	–	–	–	–	–	+
26	*Eogammarus possjeticus* (Tzvetkova, 1967)	+	–	–	–	–	–	–	–	–	–
27	*Eogammarus tiuschovi* (Derzhavin, 1927)	+	+	+	+	+	–	–	–	–	–
28	*Ischyrocerus elongatus* Gurjanova, 1938	+	–	–	–	–	–	–	–	–	–
29	*Kamaka derzhavini* Gurjanova, 1951	+	+	+	+	–	–	–	–	–	–
30	*Kamaka kuthae* Derzhavin, 1923	–	–	–	–	–	+	+	+	–	–
31	*Melita shimizui sakhalinensis* Labay, 2016	+	+	–	+	–	–	–	–	–	–
32	*Melita* sp.	–	–	–	–	+	+	+	–	–	+
	Order Isopoda										
33	*Gnorimosphaeroma kurilense* Kussakin, 1974	–	–	–	–	–	–	–	–	–	+
34	*Gnorimosphaeroma ovatum* (Gurjanova, 1933)	+	+	+	+	+	+	–	–	–	–

Continued on the next page…

No.	Species	Transect No.									
		1	2	3	4	5	6	7	8	9	10
	Order Mysida										
35	*Neomysis awatschensis* (Brandt, 1851)	+	+	+	+	+	+	+	−	+	+
	Order Decapoda										
36	*Crangon amurensis* Bražnikov, 1907	+	+	−	−	+	+	+	−	−	−
37	*Deiratonotus cristatum* (De Man, 1895)	−	+	+	−	+	+	−	−	−	−
38	*Eriocheir japonica* (De Haan, 1835)	+	−	−	−	−	−	−	+	−	+
39	*Hemigrapsus longitarsis* (Miers, 1879)	−	−	+	−	−	−	−	−	−	−
40	*Hemigrapsus takanoi* Asakura & Watanabe, 2005	+	−	−	−	−	−	−	−	−	−
41	*Palaemonetes sinensis* (Sollaud, 1911)	−	−	−	−	−	−	−	+	−	−
42	*Upogebia major* (De Haan, 1841)	−	+	+	+	−	−	−	−	−	−
	Superclass Allotriocarida										
	Class Hexapoda										
	Order Diptera										
43	Ceratopogonidae indet. (larv.)	−	−	−	−	−	−	−	−	−	+
44	*Chironomus (Lobochironomus) dorsalis* (Meigen, 1818) (larv.)	−	−	−	−	−	−	−	+	+	+
	Chironomus (Lobochironomus) dorsalis (Meigen, 1818) (pupa)	−	−	−	−	−	−	−	−	+	+
45	*Chironomus salinarius* Kieffer, 1915 (larv.)	−	−	−	+	−	−	+	+	−	−
46	*Cladotanytarsus* gr. *mancus* (Walker, 1856) (larv.)	−	+	−	+	−	−	−	−	−	−
47	*Dicrotendipes* indet. (larv.)	−	−	−	−	−	−	−	−	−	+
48	*Glyptotendipes cauliginellus* (Kieffer, 1913) (larv.)	−	−	−	−	−	−	−	+	+	+
49	*Glyptotendipes* gr. *gripekoveni* (Kieffer, 1913) (larv.)	−	−	−	−	−	−	−	−	−	+
50	*Glyptotendipes* gr. *paripes* (Edwards, 1929) (larv.)	−	−	−	−	−	−	−	−	+	−
51	*Paratendipes albimanus* (Meigen, 1804) (larv.)	−	−	−	−	−	−	−	−	+	+
52	*Polypedilum* indet. (pupa)	−	−	−	−	−	−	−	+	−	−
53	*Polypedilum (Tripodura) scalaenum* (Schrank, 1803) (larv.)	−	−	−	−	−	−	−	+	−	−
54	*Psectrocladius* gr. *zetterstedti* Brundin, 1949 (larv.)	−	−	−	−	−	−	−	−	−	+
55	*Sergentia baueri* Wulker, Kiknadze & Kerkis, 1999 (larv.)	−	−	−	−	−	−	−	+	+	−
	Sergentia baueri Wulker, Kiknadze & Kerkis, 1999 (pupa)	−	−	−	−	−	−	−	−	+	−
56	*Stictochironomus pictulus* (Meigen, 1830) (larv.)	−	−	+	+	+	+	+	+	+	+
57	*Trissopelopia longimana* (Staeger, 1839) (larv.)	−	−	−	−	−	−	−	+	+	+
	Phylum Chordata										
	Infraphylum Agnatha										
	Class Petromyzonti										
	Order Petromyzontiformes										
58	*Lethenteron reissneri* (Dybowski, 1869)	−	−	−	−	−	−	−	+	−	−
Total number of species		30	27	20	23	16	14	12	17	12	20
Expected number of species Chao-2		37	41	20	27	22	17	13	18	15	25

Species richness changes much with distance from the mouth: from 30 species *per* section on transect 1 at the river mouth to 12 species on transect 7 with water corresponding to the α-chorohaline boundary at the bottom and to 20 species *per* section on transect 10 at the transition to the freshwater area of the riverbed (Fig. 3A). A drop in the indicator value is recorded from the mouth transect with sea salinity to the α-chorohaline boundary, and this corresponds to provisions of the theory of critical salinity [Khlebovich, 1974, 1989]. The expected number of species Chao-2 was also the highest in the area of effect of polyhaline (marine) water on transects 1 and 2, while the number of species found in this part of the riverbed was 66–81% of the expected one (Table 2). In the brackish-water (mesohaline), oligohaline, and δ-chorohaline zones of the estuary, the indicator varied within 73–100% of the expected number of species with a minimum on transect 7. In the freshwater part of the estuary, Chao-2 increased and was equal to 25 species (80% of species were registered).

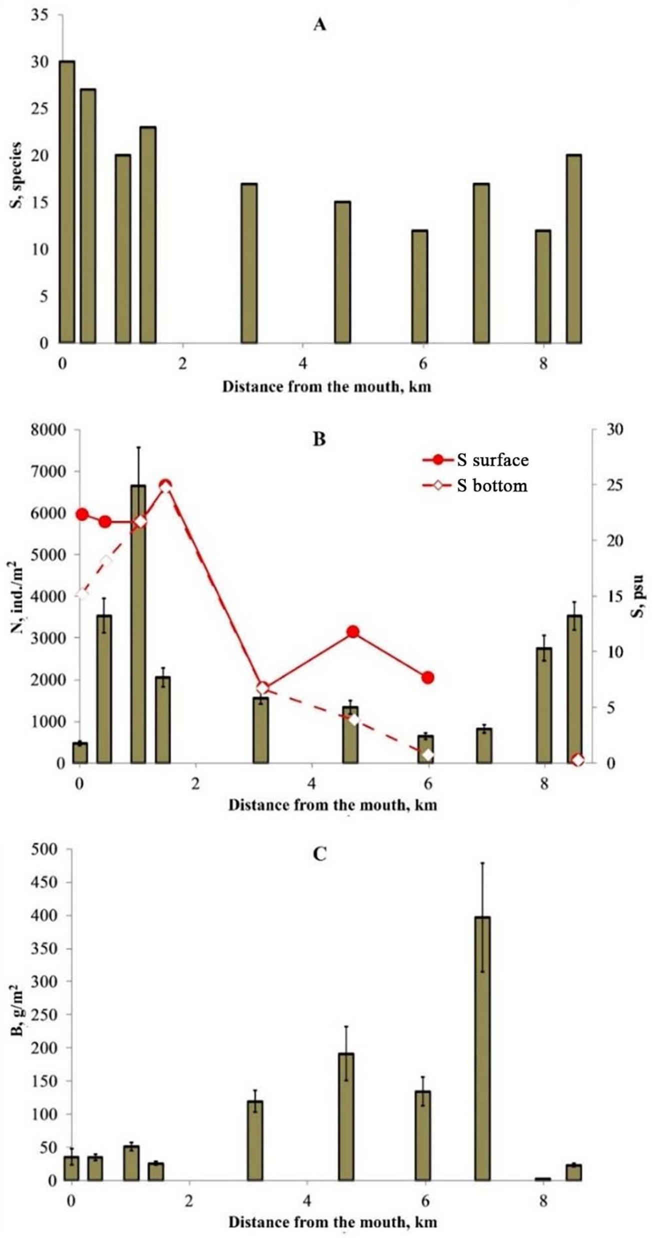

Fig. 3. Variability of macrozoobenthos abundance along the Susuya River estuary: A, number of species *per* section; B, total density and salinity (S); C, total biomass (error bars correspond to the error of the mean)

In the lower estuary zone, both estuary-specific hydrobionts and coastal-marine and lagoon species were revealed. The first ones cover a bivalve *Exolaternula liautaudi* (Mittre, 1844) and amphipods *Kamaka derzhavini* Gurjanova, 1951 and *M. shimizui sakhalinensis* [Labay, 2021]. The second ones include polychaetes *Eteone flava* (Fabricius, 1780), *Glycera capitata* Örsted, 1843, *Goniada maculata* Örsted, 1843, and *Ophelia limacina* (Rathke, 1843); gastropods *F. nipponica*, *As. lutea*, and *B. attramentaria*; bivalves *M. balthica* and *Nuculana pernula* (O. F. Müller, 1779); a cumacean *Bodotria parva* Calman, 1907; amphipods *Am. lacertosa*, *Caprella algaceus* Vassilenko, 1967, *Crassicorophium crassicorne* (Bruzelius, 1859), *Eo. possjeticus*, and *Ischyrocerus elongatus* Gurjanova, 1938; crabs *Hemigrapsus longitarsis* (Miers, 1879) and *H. takanoi*; and *Up. major*.

Typical representatives of the brackish-water fauna of Sakhalin in the middle estuary zone cover an isopod *Gnorimosphaeroma ovatum* (Gurjanova, 1933) and a crab *D. cristatum* [Labay, 2015, 2021; Reservoirs of Sakhalin Island, 2014; Watercourses of Sakhalin Island, 2015].

Indicators of oligohaline waters are a polychaete *Hediste japonica* (Izuka, 1908), a bivalve *Corbicula japonica* Prime, 1864, amphipods *Melita* sp., and a shrimp *Palaemonetes sinensis* (Sollaud, 1911) [Labay, 2021; Watercourses of Sakhalin Island, 2015]. The first one was also registered in the brackish-water zone of the estuary.

Several species were euryhaline and occurred in all the zones. Those are a polychaete *H. japonica*, an oligochaete *Limnodrilus hoffmeisteri* f. *typica* Claparède, 1862, a mysid *Neomysis awatschensis* (Brandt, 1851), a sand shrimp *Crangon amurensis* Bražnikov, 1907, and a mitten crab *Eriocheir japonica* (De Haan, 1835).

From the river mouth (transect 1) to the center of the lower estuary (transect 3), an increase in macrobenthos density is recorded, from (476 ± 59) to (6,653 ± 915) ind.·m^{-2} (Fig. 3B). In the middle estuary zone, the value drops sharply, to (653 ± 72) ind.·m^{-2} on transect 7 near the α-chorohaline boundary. In the upper estuary zone, the density rises again: to a maximum of (3,529 ± 336) ind.·m^{-2} on transect 10.

In the lower estuary zone, the indicator is formed by polychaetes (7.7–47.4%), gastropods (11.4–50.0%), and amphipods (21.2–67.8%). Out of polychaetes, the highest density is revealed for *H. japonica* and Capitellidae indet.; out of gastropods, for *F. nipponica*; and out of amphipods, for *K. derzhavini*, *M. shimizui sakhalinensis*, and *Eogammarus tiuschovi* (Derzhavin, 1927).

In the middle estuary zone, the main contributors to the total density are polychaetes, 45.7–63.7%; amphipods account for 7.7–16.1%; and the role of oligochaetes (0.6–29.7%) and isopods (up to 11.0%) increases. The density of polychaetes is formed by *H. japonica* and Capitellidae indet.; oligochaetes, by *L. hoffmeisteri*; amphipods, by *Melita* sp.; and isopods, by *Gn. ovatum*.

On the transects of the upper estuary zone, oligochaetes (9.9–49.7%) and dipterans (23.3–89.7%) prevail in density. The most significant species of oligochaetes is *L. hoffmeisteri*; the key species of dipterans are chironomids *Glyptotendipes cauliginellus* (Kieffer, 1913), *Paratendipes albimanus* (Meigen, 1804), *Sergentia baueri* Wulker, Kiknadze & Kerkis, 1999, *Stictochironomus pictulus* (Meigen, 1830), and *Trissopelopia longimana* (Staeger, 1839).

Four peaks are observed in the distribution of the total biomass throughout the estuary (Fig. 3C). The first one characterizes the lower estuary zone: transect 3, (51.2 ± 5.7) g·m^{-2}. The second peak is confined to the middle estuary zone: transect 6, (190.5 ± 41.2) g·m^{-2}. The third one is recorded in the upper estuary: transect 8, (397.5 ± 82.0) g·m^{-2}. The fourth peak is noted at the riffle separating the estuary from the freshwater zone of the riverbed: (23.4 ± 2.78) g·m^{-2}. The area of critically low biomass is revealed on transect 9: (2.67 ± 0.261) g·m^{-2}.

The contribution of different macrozoobenthos groups to the total biomass along the estuary is shown in Fig. 4. At the river mouth, in terms of biomass, decapods prevail (95.5%) represented chiefly by crabs *H. takanoi* and *Er. japonica* (Table 3).

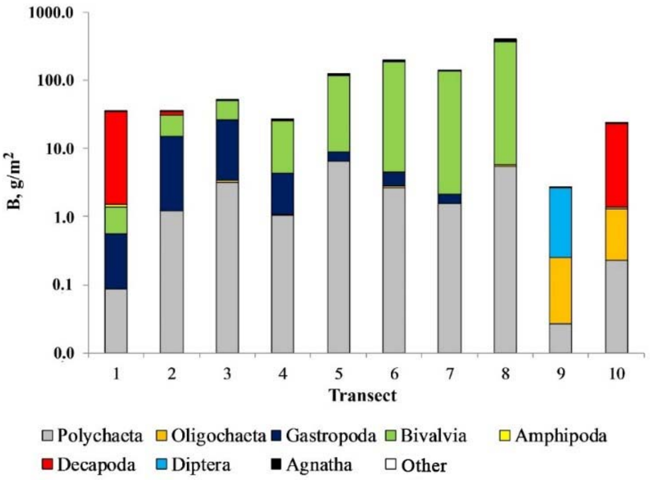

Fig. 4. The biomass of the main taxonomic groups of macrozoobenthos

Table 3. Characteristics of salinity zones (S) and changes in macrozoobenthos along the Susuya River estuary

Indicator	Zone				
	mouth (transect 1)	lower estuary polyhaline–mesohaline (transects 2–4)	middle estuary oligohaline (transects 5–8)	upper estuary δ-chorohaline (transect 9)	freshwater (transect 10)
$S_{surface}$, psu	15.2	18.1–24.8	0.6–6.6	0.2	0.1
S_{bottom}, psu	22.3	21.7–25	6.6–11.7	1	0.1
Number of species *per* transect	30	20–27	12–17	12	20
Chao-2	37	20–41	13–22	15	25
Abundance (N), ind.·m^{-2}	476 ± 59	2,051–6,653	653–1,561	2,760 ± 306	3,529 ± 336
Biomass (B), g·m^{-2}	35.7 ± 12.6	26.2–51.2	119.3–397.5	2.67 ± 0.26	23.4 ± 2.78
Key density groups	Amphipoda, Gastropoda	Gastropoda, Polychaeta, Amphipoda	Polychaeta, Amphipoda, Oligochaeta, Diptera, Isopoda	Diptera, Oligochaeta	Oligochaeta, Diptera
Key biomass groups	Decapoda	Bivalvia, Gastropoda	Bivalvia, Polychaeta	Diptera, Oligochaeta	Decapoda
Key species	*Eriocheir japonica*, *Hemigrapsus takanoi*	*Fluviocingula nipponica*, *Macoma balthica*, *Hediste japonica*	*Corbicula japonica*, *Hediste japonica*	*Chironomus dorsalis*, *Sergentia baueri*, *Glyptotendipes cauliginellus*, *Limnodrilus hoffmeisteri*	*Eriocheir japonica*, *Limnodrilus hoffmeisteri*, *Stictochironomus pictulus*
I_N, bit·specimen^{-1}	1.97	1.77–2.05	1.24–1.86	1.57	1.35
I_B, bit·specimen^{-1}	0.71	0.85–1.62	0.12–0.42	1.54	0.40
I_{ABC}, %	39.3	14.6–25.7	14.5–35.9	15.6	11.8

Upstream, there is a shift in prevailing taxa. Specifically, on transects 2–4, the key contributors are bivalves, mostly *M. balthica* (42.5–81.4%), and gastropods, mainly *F. nipponica* (12.2–45.1%); less significant contributors are polychaetes (3.4–6.2%) and decapods (0.1–11.3%). On transects 5–8, in the middle estuary oligohaline zone, the biomass is formed by bivalves represented almost by *C. japonica* alone (90.8–98.1%). On transect 9 indicating the transition from the upper estuary oligohaline zone to the freshwater one, the greatest contribution (90.0%) is that of dipterans (chironomids). On transect 10, on the freshwater riffle, the main contributors are again decapods represented by *Er. japonica* alone (91.7%).

The distribution of areas critical for bottom hydrobionts along the river estuary is estimated by the Shannon diversity index (Fig. 5A). For typical bottom communities, where the biomass is chiefly concentrated in several key species, *I* values in terms of biomass are always lower than those in terms of density [Labay et al., 2022]. The ratio of *I* values changes to the opposite at critical points indicating the transition from one type of community to another. In the Susuya River estuary, index ratios close to critical ones characterize transect 2 (the pre-estuary) and transect 9 (the border of the upper estuary oligohaline zone and the freshwater area). More critical points are revealed in the distribution of the ABC index (Fig. 5B): its values are low on transects 2 (β-chorohalinicum), 5 (α-chorohalinicum), and 9–10 (δ-chorohalinicum). The location of the lower critical (boundary) point on transect 2 is natural and characterizes the boundary between the lower estuary zone and the marine area of the mouth. The upper critical point (transect 10) is observed 520 m upstream than it is recorded by the total biomass and *I* (transect 9). Thus, by biological indicators, the boundary between the upper estuary oligohaline zone and the freshwater area of the riverbed extends for several hundred meters. A decrease in I_{ABC} on transect 5 evidences for its proximity to the α-chorohaline boundary in terms of salinity.

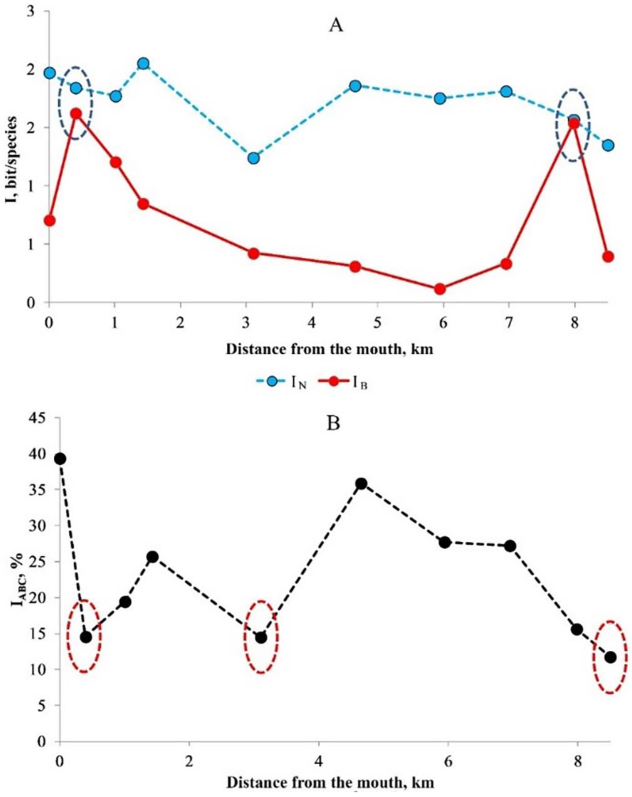

Fig. 5. Variability of the Shannon–Wiener diversity index *I* (A) and ABC index I_{ABC} (B) in macrobenthos along the Susuya River estuary

Conclusion. In the Susuya River estuary, five zones are clearly distinguished by the hydrological regime and by distribution of macrobenthos and main communities: the mouth, the lower estuary polyhaline–mesohaline zone, the middle estuary oligohaline zone, the upper estuary δ-chorohaline zone, and the freshwater zone. In the middle estuary zone, a vertical salinity gradient from 0.8 to 7.0 (11.0) psu is recorded. The river estuary is limited from above by a pebble-gravel riffle with freshwater.

The species composition of macrozoobenthos covers 58 species. Each of the identified zones has its own composition of hydrobionts.

An increase in the density of macrozoobenthos is registered from the river mouth to the lower estuary zone, with a subsequent drop in the value at the α-chorohaline boundary. In the upper estuary zone, the specific abundance sharply rises to a maximum at the freshwater riffle. In the lower estuary zone, the indicator is formed mostly by polychaetes, gastropods, and amphipods. In the middle estuary zone, the main contribution to the total density is made by polychaetes and amphipods; the role of oligochaetes and isopods increases as well. On the transects of the upper estuary, oligochaetes and dipterans are most significant in density.

Four peaks are observed in the distribution of the total biomass along the estuary: in the lower estuary, in the middle estuary zone, in the upper estuary oligohaline zone, and on the riffle separating the estuary from the freshwater area of the riverbed. The area of critically low biomass is confined to the δ-chorohalinicum in the upper estuary zone. In the river mouth, decapods are the most significant in terms of biomass. In the lower estuary, the main contributors to the total density are bivalves, mainly *Macoma balthica*, and gastropods, mostly *Fluviocingula nipponica* (with a smaller contribution of polychaetes and decapods). In the middle estuary oligohaline zone, the basis of the indicator is formed by bivalves represented almost by *Corbicula japonica* alone. In the δ-chorohalinicum, the key contributors are dipterans (chironomids). In the freshwater riffle, decapods become the main group again; those are represented by *Eriocheir japonica* alone.

Based on abundance of macrobenthos and structural indices, the boundaries between the mouth and the lower estuary zone are clearly distinguished, α-chorohalinicum and δ-chorohalinicum. The boundary between the upper estuary oligohaline zone and freshwater zone extends for several hundred meters along the Susuya River estuary.

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МАКРОЗООБЕНТОС ЭСТУАРИЯ РЕКИ СУСУЯ (ОСТРОВ САХАЛИН): I. ГИДРОЛОГИЧЕСКАЯ ХАРАКТЕРИСТИКА ЭСТУАРИЯ, ВИДОВОЙ СОСТАВ И РАСПРЕДЕЛЕНИЕ МАКРОЗООБЕНТОСА

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Описаны видовой состав, структура, количественные показатели и особенности распределения вдоль русла реки макрозообентоса эстуария реки Сусуя (остров Сахалин). По гидрологическим характеристикам и показателям макробентоса выделены основные биотопические зоны эстуария: устье (полигалинная), нижнеэстуарная полигалинно-мезогалинная, среднеэстуарная олигогалинная, верхнеэстуарная δ-хорогалинная и пресноводная. В эстуарии реки обнаружены 58 видов донных гидробионтов. Основу видового состава формируют высшие раки — 22 вида. К амфибиотическим насекомым относятся 15 видов, к полихетам — 9, к моллюскам — 7. Прочие группы представлены 1–2 видами. Каждой из выделенных зон соответствует специфический состав донных гидробионтов. Видовое богатство снижается от устьевого разреза с морской солёностью воды (30 видов) до α-хорогалинной границы (12 видов), что соответствует положениям теории критической солёности. При переходе в пресноводную часть русла значение возрастает до 20 видов. Плотность макробентоса увеличивается от (476 ± 59) экз.·м$^{-2}$ в устье реки до (6653 ± 915) экз.·м$^{-2}$ в центре нижней части эстуария. Минимум плотности, (653 ± 72) экз.·м$^{-2}$, характеризует α-хорогалинную границу, а максимум, (3529 ± 336) экз.·м$^{-2}$, — верхнеэстуарную зону. В нижней части эстуария основу плотности формируют полихеты, гастроподы и амфиподы; в среднеэстуарной зоне — полихеты, амфиподы и олигохеты; в верхнеэстуарной зоне — олигохеты и двукрылые насекомые. Отмечены четыре участка с высокой биомассой макрозообентоса: нижняя часть эстуария, (51,2 ± 5,7) $г \cdot м^{-2}$; среднеэстуарная зона, (190,5 ± 41,2) $г \cdot м^{-2}$; верхняя часть, (397,5 ± 82,0) $г \cdot м^{-2}$; перекат, отграничивающий эстуарий от пресноводной части русла, (23,4 ± 2,78) $г \cdot м^{-2}$. В устье реки основу биомассы формируют десятиногие раки. Выше по течению самый существенный вклад вносят двустворчатые моллюски, преимущественно *Macoma balthica*, и гастроподы, в основном *Fluviocingula nipponica*. В средней олигогалинной зоне наиболее значимы двустворчатые моллюски, представленные почти исключительно *Corbicula japonica*. На границе олигогалинной и верхнеэстуарной зон преобладают двукрылые насекомые (хирономиды). На пресноводном перекате главной группой опять становятся десятиногие раки, представленные исключительно крабом *Eriocheir japonica*. По показателям обилия макробентоса и структурным индексам чётко выделяются границы между устьем и нижнеэстуарной зоной, α-хорогалиникум и δ-хорогалиникум. Граница между верхнеэстуарной олигогалинной и пресноводной зонами имеет протяжённость несколько сот метров вдоль эстуария.

Ключевые слова: эстуарий, солёность, макрозообентос, плотность, биомасса, остров Сахалин

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SPECIFICS OF SAMPLE PREPARATION OF *ARTHROSPIRA (SPIRULINA) PLATENSIS* CULTURE IN THE STUDY OF ASSOCIATED MICROFLORA BY FLOW CYTOMETRY AND SCANNING ELECTRON MICROSCOPY

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In biotechnological research, it is important to control quantitative characteristics of associated microflora in algal cultures. With the aim of more complete detection and detailed study of associated microflora in *Arthrospira (Spirulina) platensis* culture, we applied methods of physical and chemical preparation of samples and subsequent investigation of a suspension by flow cytometry after cell staining with SYBR Green I fluorochrome and scanning electron microscopy. As shown, optimal sample preparation options were exposure to a reagent (sodium pyrophosphate or methanol), suspension stirring, ultrasonic treatment, centrifugation, and additional washing procedures. A mean of (27.1 ± 3.9) % of "potential" cumulative bacterial abundance was initially extracted from a cyanobacterial culture. During the following three washes, abundance of microorganisms increased significantly and averaged (88.9 ± 6.3) % (paired *t*-test; $p < 0.05$). Further, abundance of microorganisms in the sediment remained insignificant, 6–11%, and could be neglected. As shown, bacterial abundance at different stages of *A. platensis* cultivation changed from 6.7×10^6 to 1.7×10^8 cells·mL^{-1}. The morphological structure of associated microflora in *A. platensis* at the stationary phase was dominated by large rod-shaped cells (67.2%); the proportion of spiral forms was almost twice as low (30.2%); and spherical forms were even less common (2.6%). The mean bacterial cell volume was (0.16 ± 0.02) μm^3, and biomass was 0.022–0.025 g·L^{-1}. The values obtained for indicators of associated microflora are comparable to those provided in literature for *A. platensis* and other algal cultures. The proposed methods of treatment of *A. platensis* suspension boosted the efficiency of bacterial separation, facilitated removal of fragments of cyanobacterial trichomes, detritus, and other particles in samples, and provided an opportunity to study associated microflora by flow cytometry and scanning electron microscopy.

Keywords: Cyanobacteria, *Arthrospira (Spirulina) platensis*, associated microflora, bacteria, microorganisms, bacterial abundance, bacterial biomass, sample preparation methods, cell desorption, physical and chemical treatment, ultrasonic treatment, fluorescence microscopy, scanning electron microscopy, flow cytometry

In experimental setups and in open pools, associated microflora is always present when growing algacultures. Microorganisms can affect the viability and biochemical parameters of microalgae and cyanobacteria [Borisova, 1996; Kublanovskaya, 2019; Tarhova, 2005]. The relationships between heterotrophic microorganisms and algal culture are diverse: from inhibition to stimulation of bacterial growth by algae/cyanobacteria, and *vice versa* [Ashen, Goff, 2000; Ignatenko, Nemtseva, 2012;

Kublanovskaya, 2019; Ramanan et al., 2015; Sapp et al., 2007; Seymour et al., 2017]. Control of quantitative characteristics of associated microflora is an important issue in biotechnological research, since the biomass obtained can be further used for production of biologically active substances, food supplements, *etc.* [Falquet, Hurni, 2006; Jung et al., 2022; Kalenik et al., 2019].

It is known that flow cytometry, scanning electron microscopy, and fluorescence microscopy are successfully used in algological and microbiological research. For most microalgae with the size less than 30 µm (*e. g.*, *Chlorella vulgaris*, *Dunaliella salina*, *Isochrysis galbana*, *Phaeodactylum tricornutum*, *Platymonas viridis*, *Scenedesmus obliquus*, *Tetraselmis viridis*, and *Thalassiosira weissflogii*), various parameters of autotrophic microalgae (abundance, granularity, pigment fluorescence intensity, and so on) are analyzed directly in the culture suspension without any sample preparation using flow cytometry [Rauen et al., 2011; Solomonova et al., 2024; *etc.*]. In the same subsamples, after staining with fluorochromes, studying of the associated heterotrophic microflora is also possible [Danger et al., 2007; Rauen et al., 2011].

Considering that *Arthrospira (Spirulina) platensis* trichomes reach a length of 50–300 µm, the use of flow cytometry for investigating the native suspension of cyanobacteria is impossible. However, preliminary sample preparation, aimed at separating bacterial cells from *A. platensis* trichomes or non-biological particles, makes cytometric or electron microscopic studies of the associated microflora possible [Kharchuk et al., 2022].

When studying bacteria from soil samples, bottom sediments, and macrophyte thalli, preliminary physical and chemical treatment of samples is generally used [Danovaro et al., 2001; Kallmeyer et al., 2008; Lunau et al., 2005; Pugovkin, 2016; Zvyagintsev, 1970]. This methodological approach ensures desorption and release of microorganism cells from particles of various nature which greatly complicate identification of bacterial cells independently of method of their registration [Danovaro et al., 2001; Kallmeyer et al., 2008; Morono et al., 2009; Weinbauer et al., 1998].

The most commonly used chemical reagents are various surfactants: non-ionic (Tween 80) and ionic (sodium pyrophosphate) [Danovaro, Middelboe, 2010; Danovaro et al., 2001; Frischer, Danforth, 2000]. The use of methanol which destroys polysaccharide exopolymers that hold microorganism cells on organic or inorganic particles is also known [Kallmeyer et al., 2008; Lunau et al., 2005]. In addition to chemical treatment of samples, subsequent mechanical treatment is proposed: stirring, ultrasonic treatment, and centrifugation [Kallmeyer et al., 2008; Lindahl, Bakken, 1995]. It is stated that this approach allows, for example, to increase the abundance of registered bacteria in marine sediments by several orders of magnitude and to determine abundance of epiphytic bacteria on *Macrocystis integrifolia* thalli, whereas standard methods failed to do so [Velji, Albright, 1986]. In this case, it is necessary to use the correct processing mode in order to extract maximum abundance of microorganisms from a sample, not to destroy them, and not to reduce bacterial abundance during their subsequent quantitative count [Pugovkin, 2016]. In literature, we did not find any works on using a similar methodological approach in the study of microflora associated with *A. platensis* culture.

Therefore, the aim of this work was to analyze known methods of physical and chemical sample preparation for effective desorption and subsequent release of bacterial cells and to test them in the study of *Arthrospira platensis* suspension, as well as to determine quantitative indicators and morphological characteristics of microflora associated with the cyanobacteria culture using flow cytometry and scanning electron microscopy.

MATERIAL AND METHODS

Plant material. The object of the study was an algologically pure non-axenic culture of *Arthrospira (Spirulina) platensis* (Nordstedt) Gomont (strain IBSS-31) from the collection of IBSS Department of Biotechnology and Phytoresources [batch cultivation, temperature +20…+21 °C, continuous illumination, light intensity on the surface of the culture medium 5 klx (lux meter Yu-116, Russia)].

At the first stage (development of the method of preliminary sample preparation of cyanobacterial suspension for cytometry and microscopy), samples were taken from *A. platensis* collection culture daily for a week.

At the second stage, abundance of associated microflora was studied at various stages of *A. platensis* cultivation. At the stationary phase, the morphological structure of the associate was analyzed in detail: linear sizes of bacteria were determined, and their biomass was calculated.

To reduce the initial bacterial contamination during *A. platensis* preparation for the experiment and further, sterile dishes, consumables, and solutions were used: Zarrouk medium for culturing cyanobacteria [Faucher et al., 1979] and distilled water for washing. *A. platensis* trichomes (a paste) were concentrated by filtration *via* gauze (100–105 PE) and then washed three times with distilled water (in a ratio of 1 : 3) and two times with a nutrient medium. For maximum removal of associated microflora settling on mucous sheaths of cyanobacteria, the paste was placed in test tubes with a nutrient medium and centrifuged for 5 min at 3,000 rpm (centrifuge OPN-3 UKhL 42, Russia), the supernatant was removed, and the nutrient solution was again added to the sediment. This procedure was repeated three times.

The *A. platensis* paste purified in this way (2 mL) was transferred to glass flasks (volume of 0.5 dm^3, medium volume of 0.3 dm^3, and layer height of 5 cm) and grown by a batch culture technique at a temperature of +20…+25 °C on a luminostat (continuous illumination and light intensity on the culture medium surface of 10 klx). The optical density of cyanobacteria was monitored daily. Sampling for assessing the associated microflora was carried out once a week covering all stages of *A. platensis* growth. The experiment lasted for 35 days.

Preparation of *A. platensis* suspension for micoscopic and cytometric studies. When studying the associated microflora, all reagents were filtered *via* a filter with a pore size of 0.2 µm. Sterile 2-mL and 15-mL test tubes were used as glassware. An aliquot of *A. platensis* suspension (5–10 mL) was fixed with glutaraldehyde to a final concentration of 2.5% and stored in a refrigerator at +4 °C for no more than two weeks.

Preparation of *A. platensis* suspension without chemical treatment. To separate bacteria from *A. platensis* trichomes, 2 mL of the fixed suspension were thoroughly mixed and centrifuged (Microspin FV-2400, Biosan, Latvia) for 5 min at 3,000 rpm; the initial total abundance of bacteria (start) was determined in the supernatant using flow cytometry after preliminary staining with SYBR Green I fluorochrome (see below). Then, a series of washes of the entire sediment volume was performed (see below).

Treatment of *A. platensis* suspension with sodium pyrophosphate ($Na_4P_2O_7$). A 50 mM sodium pyrophosphate solution was added to a test tube with fixed *A. platensis* to a final concentration of 5 mM in a sample, and this mixture was incubated for 15 min in the dark at room temperature [Danovaro, Middelboe, 2010; Danovaro et al., 2001]. After incubation, for more complete desorption and separation of bacteria from a non-biological fraction and *A. platensis* trichomes, all samples were mixed for 10–15 s with a vortex (Microspin FV-2400) and then processed for 15 min at 0 °C in an ultrasonic unit (Unitra Unima 01SZTYN UM-4, VA140, V220, 50 Hz). For the final release of bacteria from the sediment, samples were centrifuged (5 min at 3,000 rpm), and the initial total abundance of bacteria (start) was determined in the supernatant using flow cytometry after staining with SYBR Green I fluorochrome. The remaining supernatant was poured off, and a series of washes was performed for the entire sediment 3–10 times (see below).

Treatment of *A. platensis* suspension with methanol (CH_3OH). Methanol was added to a test tube with fixed *A. platensis* to a final concentration of 10% in a sample and mixed for 10–15 s with a vortex. Then, samples were kept in an ultrasonic unit for 15 min at +35 °C, mixed again, and centrifuged (5 min at 3,000 rpm) [Kallmeyer et al., 2008; Lunau et al., 2005]. Similar to the treatment of a sample with sodium pyrophosphate, an aliquot of the supernatant (start) was stained with a fluorochrome and studied in a flow cytometer; a series of washes of the sediment were performed (see below).

A series of washing procedures (both for samples without chemical treatment and samples exposed to sodium pyrophosphate or methanol). For more complete release of remaining microorganisms, 1 mL of sterile Zarrouk medium was added to the entire volume of the sediment [after aliquot sampling for the initial determination of abundance (start)], resuspended with a vortex, and centrifuged again for 5 min at 3,000 rpm. An aliquot of the supernatant was stained with SYBR Green I fluorochrome, and bacterial abundance was determined by flow cytometry (see methodology of staining with fluorochrome and cytometric measurements below). The washing procedure was repeated 3–10 times.

Cytometric studies. Bacteria were stained with SYBR Green I fluorochrome (×1,000, Maclin, China) according to the protocols [Marie et al., 1997; Noble, Fuhrman, 1998]. The fluorochrome solution was prepared at a rate of 10 μL of the stock solution *per* 1 mL of sterile Milli-Q water and stored at −20 °C. Each sample was stained by adding 10 μL of this solution *per* 1 mL of sample and incubated in the dark for 40 min right before measurements. Samples were analyzed on a MACSQuant Analyzer (Miltenyi Biotec, Germany) equipped with three lasers (405, 488, and 635 nm) at “Spectrometry and Chromatography” core facility (IBSS). Bacterial abundance was determined using cell population gating on 2-parameter forward scatter (FS) cytograms and SYBR Green I fluorescence in the green area of the spectrum (FL1 channel, 525 nm) on dimensionless logarithmic scales. FSC Express 7 Research Edition software was used for data processing.

Calculation of bacterial abundance. When calculating total bacterial abundance, volumes of solutions were taken into account: a fixative and introduced reagents. Abundances of microorganisms at the start and after a series of washes were summed up. Based on data obtained, cumulative curves were plotted; the results obtained after 10 washes were taken as 100% of cells desorbed from *A. platensis* suspension.

Microscopic studies. To prepare microscopic slides, various options were tested: only stirring and centrifugation of *A. platensis* suspension (without chemical treatment); with treatment of the suspension with sodium pyrophosphate or methanol followed by complex mechanical treatment (stirring, ultrasonic treatment, and centrifugation).

For fluorescence microscopy (hereinafter FM), 0.5–1 mL of *A. platensis* supernatant was placed in a filter unit and stained with DAPI fluorochrome (4’,6-diamidino-2-phenylindole, Servicebio, China). The concentration of the working fluorochrome solution was 2 $\mu g \cdot mL^{-1}$; 50 μL of dye *per* 1 mL of the supernatant were added to a sample, and it was stained for 2 min in the dark [Hoff, 1988]. After staining, the sample was concentrated on a polycarbonate filter with a pore diameter of 0.2 μm (manufactured by Joint Institute for Nuclear Research, Dubna, Russia); the vacuum during filtration did not exceed 0.2 atm. To reduce autofluorescence, filters were pre-stained for 24 h with irgalan black (2 g in 0.2% acetic acid) [Hobbie et al., 1977]. The material was studied under an Olympus CX43 microscope (Japan), magnification ×1,000, with excitation in the ultraviolet area of the spectrum (358 nm) and emission in the blue one (461 nm).

To prepare samples for scanning electron microscopy (hereinafter SEM), 0.5–1 mL of *A. platensis* supernatant was concentrated onto a polycarbonate filter with a pore diameter of 0.2 μm (manufactured by Joint Institute for Nuclear Research). Then, samples were dehydrated in a series of ethanol dilutions: 20, 30, 50, 75, 96, and 100% [Bratbak, 1993]. A Leica EM CPD300 critical point dryer (Germany) was used to dry the samples at the critical point (1.5–2.5 h). A Leica EM ACE200 vacuum coater (Germany) was used for sputter coating (Au/Pd, 0.5–1.0 min). Samples were viewed under a Hitachi SU3500 scanning electron microscope (Japan), magnification ×4,000.

Morphological studies. Determination of cell sizes and calculation of bacterial biomass. Sizes of bacteria (according to SEM data) were established using ImageJ 1.50i software [National Institutes of Health, the USA, Java 1.6.0_20 (32-bit)]. A total of 475 cells were measured, their linear sizes were registered, and contribution of each morphological group of bacteria to abundance was assessed. The volume of cocci was calculated applying a formula for the volume of a sphere ($V_{sph} = 1 / 6\pi d^3$), where d is the diameter of cocci. The volume of rods and curved forms was determined by a formula for the volume of a cylinder ($V_{cyl} = 1 / 4\pi d^2 h$), where d is the width, and h is the length [Potapova, Korolevskaya, 1991; Romanenko, Dobrynin, 1973]. When calculating the volume of bacterial cells, a cell "shrinkage" conversion factor of 1.6 was introduced [Potapova, Korolevskaya, 1991; Sazhin et al., 1987]. Carbon content in bacterial cells was taken as 11% of raw biomass [Troitsky, Sorokin, 1967].

The biomass was calculated:

a) taking into account bacterial abundance (according to flow cytometry data) and the average cell volume (according to SEM data);
b) taking into account bacterial abundance (according to flow cytometry data) and a conversion factor of 2.0×10^{-14} g C·cell^{-1} [Lee, Furman, 1987].

Statistical analysis was carried out in Statistica package (Data Analysis Software System), version 10 (StatSoft, Inc., https://www.statsoft.com/). Graphs were plotted in SigmaPlot 10.0 (Systat Software, Inc.), Grapher 8 (Golden Software, Inc., https://www.goldensoftware.com/), and Surfer 11 (Golden Software, Inc.).

RESULTS

A series of preliminary experiments was carried out to determine the efficiency of bacterial separation from detritus particles and cyanobacterial cells:

- without chemical treatment (only centrifugation and stirring of *A. platensis* suspension);
- with treatment of the suspension with chemical reagents (sodium pyrophosphate or methanol) and subsequent complex mechanical treatment which involves stirring, ultrasonic treatment, and centrifugation.

In each case, a series of 10 washing procedures was additionally carried out. Bacterial abundance was registered by flow cytometry after sample staining with SYBR Green I fluorochrome.

The experiment showed as follows: both without chemical treatment and after exposure to sodium pyrophosphate or methanol, 14.8 to 45.9% of bacterial cells [(27.1 ± 9.0) % on average] were initially washed out of the culture suspension (start). After a series of 3 washes, abundance of microorganisms significantly increased (paired *t*-test; $p < 0.05$) to 82.0–94.3% [(88.9 ± 6.3) % on average] (Fig. 1A–C).

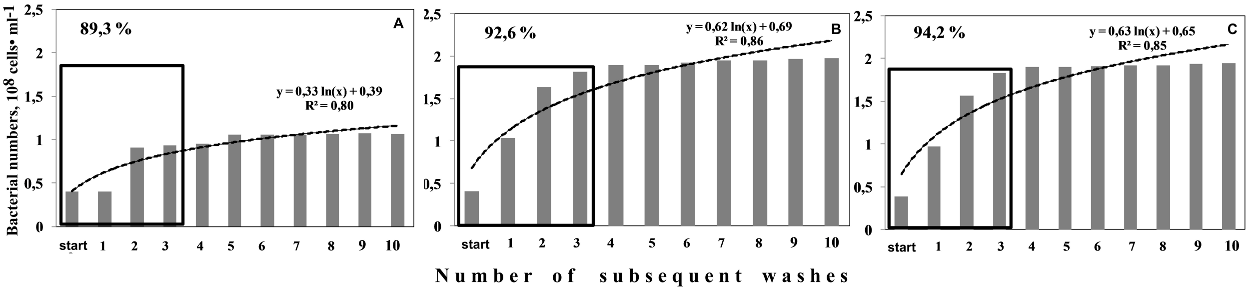

Fig. 1. Initial bacterial abundance (start) and abundance after washes (1–10) in *Arthrospira platensis* suspension after staining with SYBR Green I and cell counting by flow cytometry (*A. platensis* collection culture at the stationary phase, batch cultivation): A, without chemical treatment (stirring and centrifugation only); B, after exposure to methanol, stirring, ultrasonic treatment, and centrifugation; C, after exposure to sodium pyrophosphate, stirring, ultrasonic treatment, and centrifugation

With a gain in the number of washes to 4–10, bacteria continued to wash out of the sediment, but the difference between the "potential" cumulative cell abundance during subsequent washes was non-significant (paired *t*-test; $p > 0.05$). Thus, without using a chemical reagent (only stirring and centrifugation), another 11% of associated bacteria were extracted from *A. platensis* suspension. When using sodium pyrophosphate or methanol and carrying out subsequent complex mechanical treatment (stirring, ultrasonic treatment, and centrifugation), another 6–7% of microorganisms were washed out. Of course, even after 10 washing procedures, not all bacteria were washed out of the sediment, and some remained associated with particles. However, their abundance was insignificant and fairly constant, and this could be neglected.

In general, in this preliminary series of experiments, the total abundance of bacteria in *A. platensis* suspension without chemical treatment (centrifugation only) averaged $(1.24 \pm 0.21) \times 10^8$ cells·mL^{-1}, while when using sodium pyrophosphate or methanol, ultrasonic treatment, and centrifugation, the value increased to $(2.10 \pm 0.50) \times 10^8$ cells·mL^{-1}. Thus, with preliminary physical and chemical sample preparation, the efficiency of cell release was on average 1.8 times higher. Subsequently (at the second stage), we used a protocol prescribing exposure to a reagent (methanol), stirring, ultrasonic treatment, and centrifugation.

At the second stage, we tested physical and chemical treatment (exposure to methanol, stirring, ultrasonic treatment, and centrifugation) of *A. platensis* at various stages of cultivation with determining abundance of associated microflora by flow cytometry. The number of associated bacteria at the beginning of the experiment was 6.7×10^6 cells·mL^{-1}. As the culture grew, abundance of bacteria gradually rose by 18–25 times, up to values 1.5×10^8 to 2.2×10^8 cells·mL^{-1}, averaging $(0.9 \pm 0.6) \times 10^8$ cells·mL^{-1} (Fig. 2).

Cytometric measurements were monitored by microscopic studies which pursued two goals. By FM, we checked that the supernatant (after sample preparation) contained bacteria, and that they got into a cytometer capillary. By SEM, we detailed the morphological structure of the bacterial associate. We showed as follows: without chemical treatment of the cyanobacterial suspension (in the case of centrifugation only), fragments of *A. platensis* trichomes often got onto microscopic slides. This complicated the visualization of bacterial cells (in the case of SEM) and made it extremely difficult to view a slide (in the case of FM) due to a high autofluorescence of cyanobacteria and a rapid fading of the field of view.

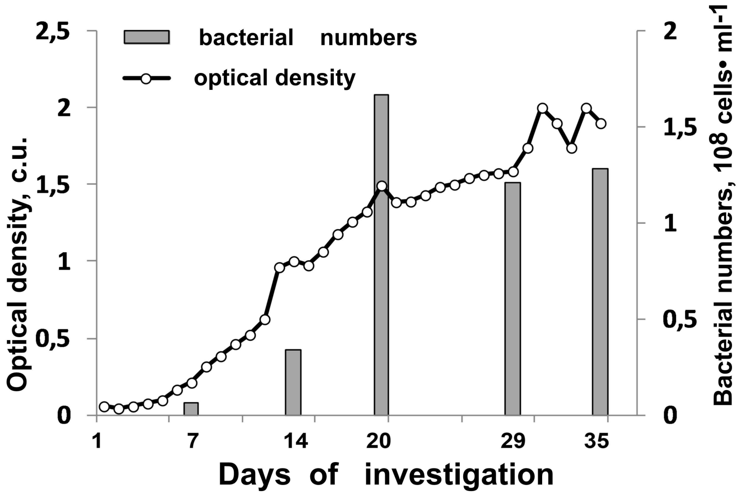

Fig. 2. Abundance of associated microflora at different stages of *Arthrospira platensis* cultivation after preliminary physical and chemical treatment of suspension (exposure to methanol, stirring, ultrasonic treatment, and centrifugation) followed by staining with SYBR Green I and cell counting by flow cytometry

Slides after preliminary complex preparation (exposure to methanol, stirring, ultrasonic treatment, and centrifugation) were the "cleanest" from cyanobacterial trichomes. Fig. 3A clearly shows that rods predominated in microflora from *A. platensis* suspension at the stationary phase. By SEM, the morphological structure of associated microflora was studied in detail, and the prevalence of rod-shaped bacteria and the presence of curved forms and cocci were confirmed [Laboratory Workbook, 2020; Schlegel, 1987] (Fig. 3B).

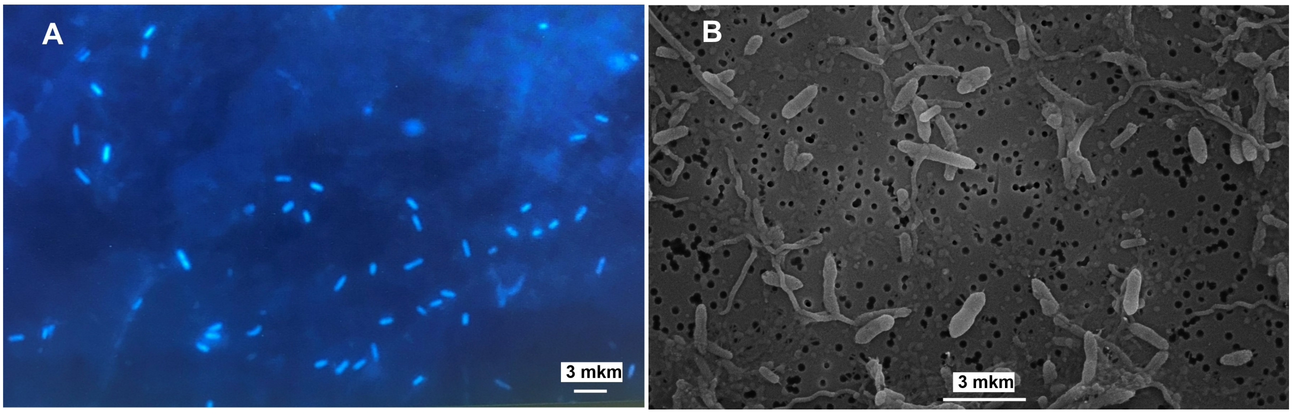

Fig. 3. Microflora associated with *Arthrospira platensis* culture (stationary phase, batch cultivation) after physical and chemical treatment (exposure to methanol, stirring, ultrasonic treatment, and centrifugation): A, according to fluorescence microscopy data after staining with DAPI fluorochrome; B, according to scanning electron microscopy data

In associated microbial community of *A. platensis* at the stationary phase, large rod-shaped cells dominated (67.2% of the total abundance of counted cells), with the volume of (0.12 ± 0.01) μm^3. The cell length varied within 0.46–7.18 μm, and the width, within 0.11–0.94 μm. The contribution of curved forms (spirilla and vibrio) accounted for 30.2%. Their volume was (0.07 ± 0.01) μm^3; length was 0.11–11.04 μm; and width was 0.05–0.33 μm. Even less common (2.6% of cases) were rounded forms, with the diameter of 0.19–0.55 μm and volume of (0.03 ± 0.01) μm^3 (Table 1, Fig. 3A, B). On average, the volume of a bacterial cell calculated from measurements of 475 cells was (0.10 ± 0.01) μm^3 and varied 0.001 to 1.03 μm^3.

Table 1. Morphological parameters of microflora associated with *Arthrospira platensis* culture (stationary phase, batch cultivation) according to scanning electron microscopy data

Morphotype	% of occurrence	Dimensions, μm		Volume, μm^3
		length/diameter	width	
Rods	67.2	1.35 ± 0.07 0.46–7.18	0.31 ± 0.01 0.11–0.94	0.12 ± 0.01 0.04–1.03
Curved forms	30.2	4.76 ± 0.39 0.11–11.04	0.13 ± 0.009 0.05–0.33	0.07 ± 0.01 0.004–0.35
Cocci	2.6	0.36 ± 0.05 0.19–0.55	–	0.03 ± 0.01 0.0004–0.09

Note: above the line, mean ± standard deviation; under the line, range (min–max).

Considering the fact that sample preparation for SEM was quite "rigid" (treatment with fixative, dehydration in ethanol, drying at the critical point, and sputter coating), we used the cell "shrinkage" conversion factor of 1.6 [Potapova, Korolevskaya, 1991; Sazhin et al., 1987], as mentioned above. Thus, the obtained average volume of a bacterial cell in the cyanobacterial culture, (0.10 ± 0.009) μm^3, was corrected to (0.16 ± 0.02) μm^3. Taking into account carbon content in bacterial cells (11% of raw biomass) [Troitsky, Sorokin, 1967] and the average bacterial abundance determined by flow cytometry [$(1.39 \pm 0.28) \times 10^8$ $cells \cdot mL^{-1}$], biomass of microflora associated with *A. platensis* culture was 0.022 $g \cdot L^{-1}$. Also, biomass was calculated using the conversion factor of 2.0×10^{-14} $g\,C \cdot cell^{-1}$ [Lee, Furman, 1987] and the same bacterial abundance. In this case, biomass of bacteria associated with *A. platensis* culture was 0.025 $g \cdot L^{-1}$.

DISCUSSION

According to various manuals on *A. platensis* growing, non-axenic cultures usually contain 3×10^4 to 1.6×10^6 $cells \cdot mL^{-1}$ (Table 2) [Falquet, Hurni, 2006; Khadad et al., 1990; Tarhova, 2005; Wu, Pond, 1981]. However, those studies were carried out using classical microbiological methods of bacteria seeding on nutrient media, but it is known that not all bacteria are capable of growing on media [ZoBell, 1943].

Therefore, in this study, a more accurate and up-to-date technique was used for recording abundance of microorganisms: flow cytometry after staining with bright SYBR Green I fluorochrome. Notably, the efficiency of bacterial release was governed by preliminary physical and chemical sample preparation and a series of washes. This is consistent with the opinion of authors who use similar methods when analyzing abundance of microorganisms in samples containing a large amount of particles of various nature. Thus, for bottom sediments, the efficiency of bacterial release from the sediment at the start and after three washes was 60–95% of the "potential" cumulative abundance of microorganisms [Danovaro, Middelboe, 2010; Rylkova et al., 2019; Siem-Jørgensen et al., 2008]. For microflora associated with *A. platensis* culture (this study), similar values were obtained: 82.0–94.3%. Washing procedures slightly increased the sample treatment time but ensured the most accurate cell counting.

Table 2. Abundance of bacteria associated with different non-axenic algal cultures (mean values are given with confidence intervals)

Algal culture	Bacterial abundance, cells·mL^{-1}	Method	References
Arthrospira platensis	from 3×10^4 to 6×10^5	plating on nutrient media	Tarhova, 2005; Khadad et al., 1990; Falquet, Hurni, 2006; Wu, Pond, 1981
Various strains of *Arthrospira platensis*	from 2.1×10^6 to 1.3×10^7	staining with DAPI, FM	Shiraishi, 2015
Scenedesmus obliquus	from 1.1×10^7 to 1.5×10^7	staining with DAPI, FM	Danger et al., 2007
Pavlova lutheri, *Platymonas suecica*	$(5.1 \pm 3.6) \times 10^6$, $(1.1 \pm 0.4) \times 10^7$	staining with acridine orange, FM	Nicolas et al., 1989
Chlorella vulgaris, *Platymonas viridis*, *Dunaliella salina*, *Isochrysis galbana*, *Thalassiosira weissflogii*	from 10^5 to 10^7	staining with SYBR Green I, FC	Rauen et al., 2011
Arthrospira platensis at various stages of cultivation	from 6.7×10^6 to 1.7×10^8, $(0.9 \pm 0.6) \times 10^8$	exposure to methanol, US, CF; staining with SYBR Green I, FC, SEM	this study

Note: FM, fluorescence microscopy; FC, flow cytometry; US, ultrasonic treatment; CF, centrifugation; and SEM, scanning electron microscopy.

The quantitative characteristics of associated microflora obtained at various stages of the cyanobacteria cultivation, 6.7×10^6 to 1.7×10^8 cells·mL^{-1}, are consistent with data provided in literature for algal cultures when registering bacterial cells under a fluorescence microscope or in a flow cytometer (Table 2). It is known that for various strains of *A. platensis*, abundance of associated bacteria was 2.1×10^6 to 1.3×10^7 cells·mL^{-1} (when studied by FM after staining with DAPI) [Shiraishi, 2015]. For *S. obliquus* (FM, staining with DAPI), abundance of microorganisms varied 1.1×10^7 to 1.5×10^7 cells·mL^{-1} [Danger et al., 2007]. In *Pavlova lutheri* and *Platymonas suecica* cultures, the values were $(5.1 \pm 3.6) \times 10^6$ and $(1.1 \pm 0.4) \times 10^7$ cells·mL^{-1}, respectively (FM, staining with acridine orange) [Nicolas et al., 1989]. Based on flow cytometry data obtained after staining with SYBR Green I, T. Rauen *et al.* [2011] reported as follows: abundance of microflora associated with cultures of *C. vulgaris*, *P. viridis*, *D. salina*, *I. galbana*, and *T. weissflogii* was 10^5 to 10^7 cells·mL^{-1}.

Thus, the used approach of preliminary physical and chemical preparation of suspension (exposure to methanol, stirring, ultrasonic treatment, and centrifugation) is quite applicable for studying microflora associated with *A. platensis* when recording bacterial abundance by flow cytometry after staining with SYBR Green I.

As known, to calculate biomass of microflora, in addition to abundance of microorganisms, it is necessary to know their volume (therefore, linear sizes of cells) or to use conversion factors frequently proposed in literature, for example, those within 1.7×10^{-14} to 2.0×10^{-14} g C·cell^{-1} [Alongi, 1988; Lee, Furman, 1987].

It turned out that used methods of preliminary sample preparation (exposure to methanol, stirring, ultrasonic treatment, and centrifugation) of the cyanobacterial suspension significantly improved the quality of microscopic slides. However, it should be taken into account as follows: for any microscopy, bacterial cells undergo additional processing procedures. In the case of light microscopy and FM, in addition to fixation, several procedures are carried out (sample staining, its filtration, and drying), and each stage can lead to deformation and compression of cells. As known, after fixation with formalin and staining with erythrosine, bacterial cells "shrink" by 1.3–2.5 times (1.6 times on average) [Potapova, Korolevskaya, 1991; Sazhin et al., 1987; Troitsky, Sorokin, 1967]. The use of fluorochrome dyes and FM (when there is no stage of a slide drying) minimized the distortion of linear sizes, but there was a disadvantage: the rapid fading of the background (in the case of staining with acridine orange). Besides, the volume of bacteria stained with DAPI was 59% of the volume of cells stained with acridine orange [Posch et al., 2001; Suzuki et al., 1993]. It should be noted that measurements and determination of the morphological affiliation of bacteria are often at the limit of the resolving power of optical microscopes.

SEM that can magnify images more than 1,000 times noticeably reduces the errors related to cell measurements and determination of their morphotypes. However, a rather more "rigid" sample preparation for a scanning electron microscope (dehydration in ethanol/acetone, subsequent drying at the critical point, and sputter coating) can also lead to cell deformation. When studied by SEM, "true" linear sizes of bacteria are known to be underestimated by 11–37% (by 24% on average) compared to those analyzed by FM [Fuhrman, 1981]. In this case, the average volume of a bacterial cell should be corrected – increased by 2.5 times: it is the upper limit of the "shrinkage" conversion factor proposed by Russian researchers [Potapova, Korolevskaya, 1991; Sazhin et al., 1987; Troitsky, Sorokin, 1967].

In this study, when calculating cell volume and biomass of microorganisms, we used the average value of the "shrinkage" conversion factor of 1.6 [Potapova, Korolevskaya, 1991; Sazhin et al., 1987] and the value of carbon content in bacterial cells of 11% of raw biomass [Troitsky, Sorokin, 1967]. Interestingly, biomass of microflora associated with *A. platensis* culture established in this way (0.022 $g \cdot L^{-1}$) was close to biomass determined using the conversion factor of 2.0×10^{-14} $g\,C \cdot cell^{-1}$ (0.025 $g \cdot L^{-1}$) [Lee, Furman, 1987].

As we have previously indicated for marine bacterioplankton [Rylkova, Polikarpov, 2021], a detailed study of the morphological structure of the microbial community requires determination of linear sizes of bacterial cells. However, in the absence of data on direct cell measurements, when calculating biomass, conversion factors can be used [Alongi, 1988; Lee, Furman, 1987].

Conclusions:

1. When determining abundance of microflora associated with *Arthrospira (Spirulina) platensis*, it is proposed to use complex physical and chemical sample preparation of the culture suspension (methanol treatment, stirring, ultrasonic treatment, and centrifugation). On average, (27.1 ± 3.9) % of "potential" cumulative bacterial abundance was initially extracted from the cyanobacterial culture. During the following three washes, abundance of microorganisms significantly increased to an average of (88.9 ± 6.3) % (paired *t*-test; $p < 0.05$). Then, in the sediment consisting of *A. platensis* trichomes, abundance of microorganisms remained insignificant, 6–7%, and could be neglected.
2. An optimal registration method for determining abundance of bacteria associated with *A. platensis* culture was flow cytometry after sample staining with bright SYBR Green I fluorochrome. Abundance of bacterial associates at different stages of *A. platensis* growth varied 6.7×10^6 to 1.7×10^8 $cells \cdot mL^{-1}$ and averaged $(0.9 \pm 0.6) \times 10^8$ $cells \cdot mL^{-1}$. These values are comparable with abundance of bacteria associated with other non-axenic algal cultures.

3. According to scanning electron microscopy data, the community of associated microflora of *A. platensis* at the stationary phase was dominated by large rod-shaped cells (67.2%); the contribution of spiral forms was 30.2%; and spherical forms accounted for 2.6%. The mean volume of a bacterial cell, with a cell "shrinkage" conversion factor taken into account, was $(0.16 \pm 0.02)\ \mu m^3$.
4. Biomass of microorganisms in *A. platensis* culture based on "true" cell measurements and using a conversion factor of 2.0×10^{-14} g C·cell^{-1} was 0.02 and 0.025 g·L^{-1}, respectively. To calculate biomass of bacteria, in the absence of data on linear sizes of cells, it is entirely acceptable to use known conversion factors.

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ОСОБЕННОСТИ ПРЕДВАРИТЕЛЬНОЙ ПРОБОПОДГОТОВКИ КУЛЬТУРЫ *ARTHROSPIRA (SPIRULINA) PLATENSIS* ПРИ ИССЛЕДОВАНИИ АССОЦИИРОВАННОЙ С НЕЙ МИКРОФЛОРЫ МЕТОДАМИ ПРОТОЧНОЙ ЦИТОМЕТРИИ И СКАНИРУЮЩЕЙ ЭЛЕКТРОННОЙ МИКРОСКОПИИ

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Контроль количественных характеристик ассоциированной с альгокультурами микрофлоры важен в биотехнологических исследованиях. С целью более полного выявления и детального анализа сопутствующей микрофлоры в культуре *Arthrospira (Spirulina) platensis* использованы методы физико-химической пробоподготовки образцов для последующего изучения суспензии с помощью проточной цитометрии после окраски клеток флуорохромом SYBR Green I и сканирующей электронной микроскопии. Показано, что оптимальными вариантами пробоподготовки являлись применение химического реагента (пирофосфата натрия или метанола), перемешивание суспензии, ультразвуковая обработка, центрифугирование и дополнительные промывочные процедуры. При этом из культуры цианобактерий изначально извлекалось в среднем (27,1 ± 3,9) % «потенциальной» кумулятивной численности бактерий. При последующих трёх промывках количество микроорганизмов достоверно увеличивалось в среднем до (88,9 ± 6,3) % (парный *t*-тест; $p < 0{,}05$). Далее в осадке количество микроорганизмов оставалось незначительным, 6–11 %, и этим можно было пренебречь. Показано, что численность бактерий на различных этапах культивирования *A. platensis* изменялась от $6{,}7 \times 10^6$ до $1{,}7 \times 10^8$ кл.·мл$^{-1}$. В морфологической структуре ассоциированной микрофлоры (на стационарной стадии роста *A. platensis*) доминировали крупные палочковидные клетки (67,2 %), доля извитых форм была почти вдвое ниже (30,2 %), ещё реже встречались округлые формы (2,6 %). Средний объём бактериальной клетки составлял (0,16 ± 0,02) мкм^3, биомасса — 0,022–0,025 г·л$^{-1}$. Полученные величины показателей сопутствующей микрофлоры сопоставимы с данными, приведёнными в литературе для *A. platensis* и других альгокультур. Предложенные методы обработки суспензии *A. platensis* существенно повышали эффективность высвобождения бактерий, способствовали очищению пробы от фрагментов трихом цианобактерий, детрита и прочих частиц и обеспечивали возможность исследования ассоциированной микрофлоры с использованием проточной цитометрии и сканирующей электронной микроскопии.

Ключевые слова: цианобактерии, *Arthrospira (Spirulina) platensis*, ассоциированная микрофлора, бактерии, микроорганизмы, численность бактерий, биомасса бактерий, методы пробоподготовки, десорбция клеток, физико-химическая обработка, ультразвуковая обработка, люминесцентная микроскопия, сканирующая электронная микроскопия, проточная цитометрия

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TAXONOMIC COMPOSITION AND LONG-TERM DYNAMICS OF MEROPLANKTON ABUNDANCE IN COASTAL WATERS OF THE TAMAN PENINSULA (THE KERCH STRAIT, BLACK SEA)

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At the study site between the Taman Bay and the Bugazsky Liman, larvae of benthic invertebrates representing 47 taxa were identified: Polychaeta, 14; Cirripedia, 4; Phoronida, 1; Decapoda, 10; Bivalvia, 13; Gastropoda, 3; and Hydrozoa, 2. The highest meroplankton density (up to 30.6×10^{3} ind.·m^{-3}) against the backdrop of the lowest taxonomic diversity (17 taxa) was recorded in the semi-closed Taman Bay affected by the Sea of Azov waters. In June and July, meroplankton was chiefly represented by bivalves *Cerastoderma* sp. in the Taman Bay; by *Cerastoderma* sp. and *Spisula subtruncata* in the Tuzla Spit–Panagiya Cape; and by *S. subtruncata* and *Mytilaster lineatus* in the Zhelezny Rog Cape–Bugazsky Liman. Despite heavy shipping traffic and significant anthropogenic pollution of the study site, meroplankton density ensured sufficient reproductive potential of the area. In the seaport of Taman, a summer–autumn peak in abundance was registered (13×10^{3} to 15.4×10^{3} ind.·m^{-3}) driven by mass release of larvae of bivalves *C. gallina* and *M. lineatus*; it is characteristic of the Black Sea waters. In the Kerch Strait, larval density of previously rare decapod *Upogebia pusilla* increased (0.24×10^{3} ind.·m^{-3}), and this confirms the necessity of further study of meroplankton biodiversity in the Taman Peninsula coastal waters.

Keywords: meroplankton, Taman Bay, Tuzla Spit–Panagiya Cape, Zhelezny Rog Cape–Bugazsky Liman, port of Taman

The Kerch Strait and adjacent coastal waters of the Black Sea are the zone of the heaviest shipping traffic and very intensive fishing [Fashchuk, Petrenko, 2008]. Transport-related pollution due to cargo transfers, oil spills, port operations in Taman, dumping of substratum, *etc.* leads to sedimentation, contamination of bottom sediments, and restructuring of zoobenthic taxonomic composition and diversity in the strait area and adjacent coastal waters of the Black Sea [Eremeev et al., 2008; Fashchuk et al., 2012; Golovkina, Nabozhenko, 2012]. As known, taxonomic composition, density, and abundance of benthic animal larvae (meroplankton) are among the main indicators of the state of benthos [Lisitskaya, 2017, 2018; Selifonova, 2012; Selifonova, Samyshev, 2022]. In 2018, autumn meroplankton community was investigated in the Kerch Strait and adjacent coastal waters of the Black Sea [Remizova, Teyubova, 2021]. However, there was no long-term monitoring of dynamics and changes in the meroplankton structure/composition and its current state in this area.

The aim of this study is to analyze the taxonomic composition and seasonal dynamics of meroplankton abundance in the Kerch Strait and adjacent Taman coastal waters of the Black Sea in a long-term perspective.

MATERIAL AND METHODS

The study area. The Taman Peninsula is located in the west of the Kuban Plain and washed by the Black Sea, Sea of Azov, and Kerch Strait connecting them (Fig. 1). The maximum depths at the entrance to the strait from the Black Sea exceed 18 m. In the strait, on the borderline of the Black Sea and Sea of Azov, there is a frontal zone with noticeable salinity gradients (from 11‰ in the north to 17‰ in the south) [Eremeev et al., 2003; Samyshev, 2004]. It is the area of specific distribution of planktonic and benthic communities.

The Taman Bay occupies the eastern coast of the Kerch Strait between the Chushka and Tuzla spits. It juts into the mainland for 16 km (width at the entrance is 8 km, and depth is up to 5 m). The Panagiya Cape is situated on the borderline of the Black Sea and Sea of Azov, on the Kerch Strait coast. This cape, about 30 m high, is a reef built by mosses. It has a much-indented coastline and is characterized by occurrence of numerous bays, coastal spits, estuaries, and mud volcanoes. The Zhelezny Rog Cape occupies the southern coast of the Taman Peninsula. The cape stretches for 1.3 km and is located at an altitude of 65 m above sea level. On the cape, there is the open-type port of Taman, with free water exchange with the open sea. The port is a transshipment point for land and sea transportation.

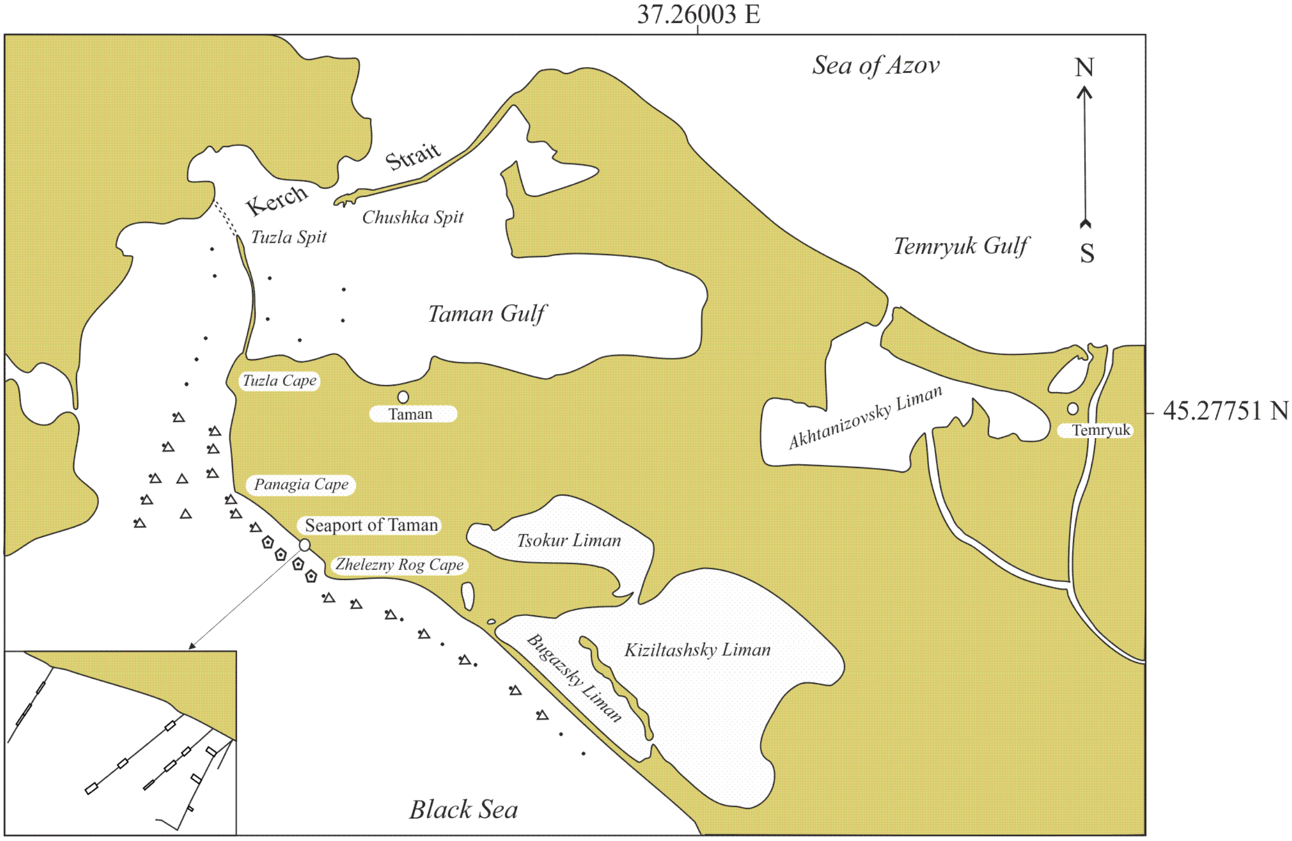

Fig. 1. The scheme of zooplankton sampling stations in the study area. Sampling stations are indicated by points (2010) and triangles (2015); sampling spots in the seaport of Taman are indicated by pentagons (2013–2014 and 2018–2021)

Sampling and laboratory studies. Zooplankton was sampled in June–July 2010 and 2015 in the Kerch Strait and Taman coastal waters of the Black Sea (see Fig. 1). In 2013–2014 and 2018–2021, a full-season monitoring was carried out in the port of Taman area. Depths of sampling varied from 3 m (the Taman Bay) to 16 m (the seaport of Taman). During sampling, the sea surface temperature ranged from +8.5 °C (December 2018) to +26.5 °C (August 2019) (see Fig. 2). Meroplankton (larvae of benthic organisms > 100–500 µm) was sampled throughout the water column with a medium-sized Juday net with an opening diameter of 25 cm (mesh size of 110 and 120 µm) by total catch. The material was fixed in 2–4% neutral formaldehyde and processed in a laboratory by a conventional procedure. Zooplankton abundance was calculated taking into account the catch power of the net [Shushkina, Vinogradov, 2002; Sorokin, Sorokin, 2010]. Standard plankton nets mostly have mesh size of > 150–200 µm and thus significantly undercatch small hydrobionts, 0.15–0.25 mm wide (2–10 times, depending on zooplankton composition and kind of a net). To calibrate our net, we filtered 50 L of water sampled with a bucket through a 40-µm mesh. The main literature sources used to identify major zooplanktonic species and families were those of K. Zakhvatkina [1972], V. Chukhchin [1984], Yu. Makarov [2004], O. Poltarukha and O. Korn [2008], and V. Surugiu *et al.* [2023]. Systematic classification and nomenclature of zooplankton species are given according to WoRMS [2024].

RESULTS AND DISCUSSION

Meroplankton of the Kerch Strait and adjacent Taman coastal waters of the Black Sea comprised 47 taxa: Polychaeta, 14 species; Cirripedia, 4; Phoronida, 1; Decapoda, 10; Bivalvia, 13; Gastropoda, 3; and Hydrozoa, 2 (Table 1). In the seaport of Taman, the maximum number of taxa was recorded, 36–37. In the Taman Bay, Tuzla Spit–Panagiya Cape, and Zhelezny Rog Cape–Bugazsky Liman, we registered 17, 25, and 26–33 species, respectively.

Table 1. Taxonomic composition of meroplankton of the Kerch Strait and adjacent Taman coastal waters of the Black Sea: TB, Taman Bay; TS–PC, Tuzla Spit–Panagiya Cape; ZRC–BL, Zhelezny Rog Cape–Bugazsky Liman; TSP, the seaport of Taman area; +, taxon was found; ++, common taxon; +++, mass taxon

Taxon	Kerch Strait			Taman coastal waters of the Black Sea			
	TB	TS–PC		ZRC–BL		TSP	
	2010	2010	2015	2010	2015	2013–2014	2018–2021
POLYCHAETA							
Nephthys hombergii Savigny in Lamarck, 1818	++	+	+	+	+	++	++
Harmothoe spp.						+	+
Alitta succinea (Leuckart, 1847)		+	+	+	+	+	+
Platynereis dumerilii (Audouin & Milne Edwards, 1833)		+	+		+		
Perinereis cultrifera (Grube, 1840)					+	+	
Hediste diversicolor (O. F. Müller, 1776)					+	+	+
Malacoceros fuliginosus (Claparède, 1868)		+	+		+	++	++
Microspio mecznikowiana (Claparède, 1869)		+	+	+	+	+	+
Spio filicornis (O. F. Müller, 1776)		+	+	+	+	+	+
Polydora cornuta Bosc, 1802	++	+	+		+	++	++

Continued on the next page…

Taxon	Kerch Strait			Taman coastal waters of the Black Sea			
	TB	TS–PC		ZRC–BL		TSP	
	2010	2010	2015	2010	2015	2013–2014	2018–2021
Prionospio spp.		+	+	+	+	+	+
Pygospio elegans Claparède, 1863	+	+					
Capitella capitata capitata (Fabricius, 1780)						+	+
Heteromastus filiformis (Claparède, 1864)						+	+
PHORONIDA							
Phoronis euxinicola Selys-Longchamps, 1907	++	+	+	+	+	+	+
CIRRIPEDIA							
Amphibalanus improvisus (Darwin, 1854)	++	++	++	+	+	++	++
Amphibalanus eburneus (Gould, 1841)				+	+	+	+
Verruca spengleri Darwin, 1854			+	+	+	+	+
Chthamalus sp.		+	+	+	+	+	+
DECAPODA							
Palaemon elegans Rathke, 1836						+	+
Upogebia pusilla (Petagna, 1792)	++	++	++	+	+	+	++
Diogenes pugilator (Roux, 1829)	+	+	+	+	+	++	++
Pisidia bluteli (Risso, 1816)				+	+		+
Xantho poressa (Olivi, 1792)				+	+	+	+
Eriphia verrucosa (Forskål, 1775)					+		+
Pilumnus spinulosus Kessler, 1861				+	+	+	+
Rhithropanopeus harrisii (Gould, 1841)	+	+	+	+	+	+	+
Liocarcinus vernalis (Risso, 1827)		+	+	+	+	++	++
Carcinus aestuarii Nardo, 1847					+	+	+
BIVALVIA							
Anadara kagoshimensis (Tokunaga, 1906)						+	+
Mytilus galloprovincialis Lamarck, 1819	+	+		+	+	+	+
Mytilaster lineatus (Gmelin, 1791)	+	++	++	+++	+++	+++	+++
Abra segmentum (Récluz, 1843)	+	+	+	+	+		
Spisula subtruncata (da Costa, 1778)		+++	+++	+++	+++	+++	+++
Chamelea gallina (Linnaeus, 1758)						+++	+++
Cerastoderma sp.	+++	+++	+++				
Cardiidae gen. sp.				+	++		
Teredo navalis Linnaeus, 1758						+	+
Mya arenaria Linnaeus, 1758	+	+	+				
GASTROPODA							
Bittium reticulatum (da Costa, 1778)	++	+	+	++	++	++	++
Rapana venosa (Valenciennes, 1846)				+	+	++	++
Parthenina terebellum (R. A. Philippi, 1844)	+	+	+	+	+		
HYDROZOA							
Sarsia tubulosa (M. Sars, 1835)			+		+	+	++
Blackfordia virginica Mayer, 1910	+	+	+	+		+	+

The Taman Bay (Kerch Strait). In June–July 2010, 17 taxa were found in meroplankton: Polychaeta, 3; Phoronida, 1; Cirripedia, 2; Decapoda, 3; Bivalvia, 5; Gastropoda, 2; and Hydrozoa, 1. During the study period, the total abundance of larvae of benthic invertebrates reached the maximum, $(30.6 \pm 0.5) \times 10^3$ ind.·m^{-3} (Table 2). Meroplankton mostly consisted of larvae of bivalves *Cerastoderma* sp. (72.2% of the total abundance of meroplankton). Low values were registered for larvae of polychaetes *Nephthys hombergii* and *Polydora cornuta*, a gastropod *Bittium reticulatum*, a barnacle *Amphibalanus improvisus*, a decapod *Upogebia pusilla*, a phoronid *Phoronis euxinicola*, and others. It is noteworthy as follows: high density of *P. euxinicola* larvae was observed only in this area and in the Anapa Bay [$(0.3 \pm 0.01) \times 10^3$ ind.·m^{-3} and 0.2×10^3 ind.·m^{-3}, respectively], while these larvae are usually rare in summer meroplankton in the northeastern Black Sea [Selifonova, 2012; Selifonova, Samyshev, 2022] and Crimean coastal waters [Lisitskaya, 2017, 2018].

Table 2. Meroplankton abundance ($\times 10^3$ ind.·m^{-3}) in the Kerch Strait and adjacent Taman coastal waters of the Black Sea (June–July 2010 and 2015). Designations are the same as in Table 1

Taxon	Kerch Strait			Taman coastal waters of the Black Sea	
	TB	TS–PC		ZRC–BL	
	2010	2010	2015	2010	2015
Polychaeta	3 ± 0.05	0.78 ± 0.05	0.66 ± 0.09	0.06 ± 0.01	0.07 ± 0.02
Phoronida	0.3 ± 0.01	0.003 ± 0.004	0.01 ± 0.03	0	0.1 ± 0.01
Cirripedia	1.3 ± 0.05	0.3 ± 0.01	0.9 ± 0.01	0.006 ± 0.001	0.2 ± 0.02
Decapoda	0.76 ± 0.03	0.14 ± 0.01	0.3 ± 0.02	0.016 ± 0.01	0.1 ± 0.03
Bivalvia	22.1 ± 0.1	10.3 ± 0.01	15.4 ± 0.05	6.7 ± 0.2	10.5 ± 0.08
Gastropoda	2.9 ± 0.01	1.5 ± 0.05	2 ± 0.06	1.4 ± 0.04	1.9 ± 0.3
Hydrozoa	0.1 ± 0.02	0.007 ± 0.01	0.005 ± 0.01	0	0.02 ± 0.08
Meroplankton share in the total abundance of zooplankton, %	45.5	62	65	34.8	25.2

The Tuzla Spit–Panagiya Cape (Kerch Strait). Species composition of larvae of benthic invertebrates was richer there than in the Taman Bay. In June–July 2010, a total of 25 taxa were recorded in meroplankton: Polychaeta, 9; Phoronida, 1; Cirripedia, 2; Decapoda, 4; Bivalvia, 6; Gastropoda, 2; and Hydrozoa, 1. However, the total abundance of meroplankton was twice lower than in the Taman Bay, while its share in zooplankton reached 62%. Meroplankton was formed chiefly by larvae of bivalves *Spisula subtruncata* and *Cerastoderma* sp. (79.2% of the total abundance of meroplankton). From June to mid-July, larvae of *U. pusilla* (Decapoda), *A. improvisus* (Cirripedia), and *Mytilaster lineatus* (Bivalvia) were common in plankton. Interestingly, 80% of decapod larvae were *U. pusilla* ones; this hydrobiont, the Mediterranean mud shrimp, used to be a protected and rare species in several countries of the Black Sea region [Revkov et al., 2019]. Adult individuals of this species inhabit burrows on soil bottom sediments of coastal zones of the Black Sea (from the water's edge down to 45 m) and Sea of Azov. In 1960s, the communities dominated by *U. pusilla* and a bivalve *Pitar rudis* were registered in the Caucasian coastal waters at depths of 24–30 m [Kiseleva, Slavina, 1965, 1966]. Since the late 1980s, there is a significant drop in *U. pusilla* abundance both off the Caucasian and Crimean coasts. A depressive period in the development of *U. pusilla* population was associated with the Black Sea eutrophication which affected coastal zones the most [Revkov et al., 2019; Zaika, 2011; Zaitsev, 1992]. Nowadays, *U. pusilla* abundance increases off the Crimean coast [Revkov et al., 2019]. Therefore, in the northeastern

Black Sea, a rise in this species abundance is possible. It is confirmed by findings of an adult *U. pusilla* specimen in July 2022 at a 49-m depth in Adler vicinity (an oral communication of PhD N. Bulysheva, Southern Scientific Centre of the Russian Academy of Sciences) and its larvae in the Tuzla Spit–Panagiya Cape (our study).

In 2015, larvae of benthic invertebrates of 25 taxa were recorded in the Tuzla Spit–Panagiya Cape: Polychaeta, 8; Phoronida, 1; Cirripedia, 3; Decapoda, 4; Bivalvia, 5; Gastropoda, 2; and Hydrozoa, 2. The total abundance of meroplankton during the study period averaged $(19.3 \pm 0.3) \times 10^3$ ind.·m^{-3}. The value was 1.5 times higher than in 2010. Meroplankton contribution to total zooplankton by density averaged 65%. Larvae of bivalves were the most abundant (79.7% of the total abundance of meroplankton) mainly determining the dynamics of the total density of meroplankton. The key contributors to a summer density peak were larvae of *S. subtruncata* and *Cerastoderma* sp. (80% of the total abundance of bivalves). In 2010 and 2015, we registered maximum density of *U. pusilla* larvae: $(0.11 \pm 0.01) \times 10^3$ and $(0.24 \pm 0.02) \times 10^3$ ind.·m^{-3}, respectively.

The Zhelezny Rog Cape–Bugazsky Liman (Taman coastal waters of the Black Sea). In June–July 2010, larvae of benthic invertebrates comprised 26 taxa: Polychaeta, 5; Phoronida, 1; Cirripedia, 4; Decapoda, 7; Bivalvia, 5; Gastropoda, 3; and Hydrozoa, 1. The total abundance of meroplankton was at the minimum, $(8.1 \pm 1.6) \times 10^3$ ind.·m^{-3}; this value was 3.5 times lower than in the Taman Bay. Meroplankton share in the total abundance of zooplankton was 34.8%. Summer meroplankton was represented mostly by Bivalvia larvae (81.7% of the total abundance of meroplankton). In larvae of bivalves, *S. subtruncata* and *M. lineatus* dominated accounting for 81.7% of meroplankton. The most common meroplankton species was a gastropod *B. reticulatum*.

In 2015, meroplankton of the Zhelezny Rog Cape–Bugazsky Liman comprised 33 taxa: Polychaeta, 10; Phoronida, 1; Cirripedia, 4; Decapoda, 9; Bivalvia, 5; Gastropoda, 3; and Hydrozoa, 1. The total abundance of meroplankton was of 12.7×10^3 ind.·m^{-3}, 1.5 times higher than in 2010. Its share in zooplankton did not exceed 25.2%. Meroplankton mostly consisted of larvae of bivalves *S. subtruncata* and *M. lineatus*. Abundance of larvae of bivalves in total meroplankton reached 82.6%. We noted elevated concentrations of larvae of a non-identified bivalve Cardiidae gen. sp., gastropod *B. reticulatum*, barnacle *A. improvisus*, and decapod *Liocarcinus vernalis*.

The port of Taman area (Taman coastal waters of the Black Sea). In 2013–2014, larvae of benthic invertebrates representing 36 taxa were identified in the seaport area: Polychaeta, 13; Phoronida, 1; Cirripedia, 4; Decapoda, 8; Bivalvia, 6; Gastropoda, 2; and Hydrozoa, 2. Meroplankton abundance averaged $(5.5 \pm 2.1) \times 10^3$ ind.·m^{-3}. A noticeable decrease in meroplankton abundance was observed at low temperatures, +6…+12 °C (Fig. 2).

Meroplankton abundance had the lowest values in March and November: 0.09×10^3 to 0.37×10^3 ind.·m^{-3}. In these months, water temperature in the port of Taman area did not exceed +10…+14 °C. However, meroplankton share in zooplankton was high, for the basis of zooplankton was formed by larvae of a barnacle *A. improvisus*. Its share in the total abundance of meroplankton reached 94.5–100%. In summer and early autumn, with water warming up to +22.6…+26.5 °C, abundance of larvae of benthic invertebrates in zooplankton naturally rose. In July, a density peak (13×10^3 ind.·m^{-3}) was ensured by larvae of a bivalve *M. lineatus* (80% of the total abundance of meroplankton). Meroplankton contribution to total zooplankton was 67%. In August, water temperature reached +26.7 °C, and we recorded elevated density of larvae of bivalves *C. gallina* and *M. lineatus*: up to 6.6×10^3 ind.·m^{-3} in total (77.4% of the total abundance of meroplankton).

In 2018–2021, larvae of benthic invertebrates of 37 taxa were registered in the seaport of Taman area: Polychaeta, 12; Phoronida, 1; Cirripedia, 4; Decapoda, 10; Bivalvia, 6; Gastropoda, 2; and Hydrozoa, 2. Long-term mean abundance of meroplankton, $(10 \pm 4.67) \times 10^3$ ind.·m^{-3}, differed much from that of 2013–2014; it was on average 3.5 times higher than in previous years [Remizova, Teyubova, 2021]. Larvae of bivalves made a significant contribution to a larval pool in 2018–2021 (80% of the total abundance of zooplankton). In the dynamics of meroplankton abundance, three density peaks were determined: in July (12.9×10^3 ind.·m^{-3}), August (8.5×10^3 ind.·m^{-3}), and September (9.9×10^3 to 15.4×10^3 ind.·m^{-3}) (see Fig. 2). Larvae of bivalves prevailed in summer and early autumn (76–93% of the total abundance of meroplankton). In June, with a rise in water temperature up to +24.8 °C, meroplankton abundance reached 1.9×10^3 ind.·m^{-3}. In the seaport area, larvae of bivalves *S. subtruncata* and *M. lineatus* dominated ensuring 57.8% of the total abundance of meroplankton. Along with larvae of these species, larvae of a gastropod *B. reticulatum* were recorded in small abundance (26.3%), as well as larvae of polychaetes (5.2%), barnacles (6.3%), *etc.* Meroplankton share in zooplankton reached 55%. In July, meroplankton was abundant largely due to larvae of a bivalve *M. lineatus* (9.9×10^3 ind.·m^{-3}), a gastropod *B. reticulatum* (1.2×10^3 ind.·m^{-3}), a barnacle *A. improvisus* (0.8×10^3 ind.·m^{-3}), polychaetes *P. cornuta* and *N. hombergii* (0.9×10^3 ind.·m^{-3} in total), and decapods *U. pusilla*, *Diogenes pugilator*, and *Brachynotus sexdentatus* (0.1×10^3 ind.·m^{-3} in total). Meroplankton contribution to the total abundance of zooplankton increased to a mean of 74.8%. In August, we established a rise in meroplankton density due to mass release of *C. gallina* and *M. lineatus* larvae (9.9×10^3 ind.·m^{-3}). Along with the latter, significant densities of planktonic larvae were observed for a barnacle *A. improvisus*, gastropods *B. reticulatum* and *Rapana venosa*, a polychaete *Malacoceros fuliginosus*, and others. An autumn generation of larvae of benthic invertebrates was represented chiefly by those of bivalves *C. gallina* and *M. lineatus* (80.8–93.9%). From late autumn to early spring, meroplankton mostly consisted of *A. improvisus* larvae (91.1–94.7%).

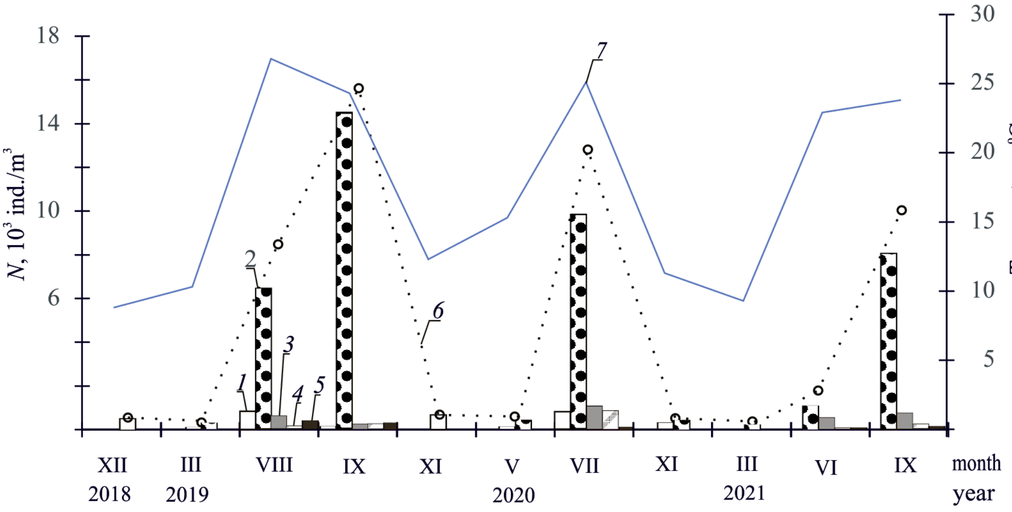

Fig. 2. Dynamics of meroplankton abundance (N, left axis) and water temperature (right axis) in the seaport of Taman area (2018–2021): 1, barnacles; 2, bivalves; 3, gastropods; 4, polychaetes; 5, decapods; 6, total meroplankton; 7, temperature

Hence, larvae of benthic invertebrates of the Kerch Strait and Taman coastal waters of the Black Sea are characterized by higher taxonomic richness, in contrast to meroplankton of the northern Kerch Strait in the area of the seaport Kavkaz and Sea of Azov [Selifonova, 2008; Selifonova, Samyshev, 2022].

However, taxonomic composition registered (47 taxa) is less diverse in comparison with that for other areas of the Black Sea. Specifically, at the well-studied sites, the southwestern Crimea and northeastern Black Sea, researchers recorded 63 and 73 taxa, respectively [Lisitskaya, 2018; Selifonova, 2012]. In the Kerch Strait and Taman coastal waters of the Black Sea, the list of dominant meroplankton species in summer season consisted of larvae of bivalves, and it differed from lists for polluted bays and harbors of the northeastern Black Sea and southwestern Crimea. In June–July, bivalves were represented mostly by *Cerastoderma* sp. in the Taman Bay; *Cerastoderma* sp. and *S. subtruncata* in the Tuzla Spit–Panagiya Cape; and *S. subtruncata* and *M. lineatus* in the Zhelezny Rog Cape–Bugazsky Liman. Pretty high density of meroplankton, up to 30.6×10^3 ind.·m^{-3}, against the backdrop of the lowest taxonomic diversity (17 taxa) was noted only in the semi-closed Taman Bay affected by the Sea of Azov waters. Similar early-summer peaks of meroplankton density were observed for the Sea of Azov [Selifonova, 2008]. As already mentioned, high density of phoronid larvae was reported only for this area and the Anapa Bay, whereas usually these larvae rarely occur in meroplankton [Selifonova, 2012]. In the Tuzla Spit–Panagiya Cape, larvae of decapods were represented mainly by *U. pusilla* which, as noted above, used to be a rare and even protected species in several countries of the Black Sea region. To date, abundance of its larvae increased to 0.24×10^3 ind.·m^{-3}. Meroplankton contribution to the total abundance of zooplankton was the most noticeable there accounting for 62–65%.

In spite of heavy shipping traffic and anthropogenic pollution, meroplankton density at the study site is characterized by sufficient reproductive potential. In the seaport of Taman area, a summer–autumn peak in abundance was recorded (13×10^3 to 15.4×10^3 ind.·m^{-3}) governed by mass release of larvae of bivalves *C. gallina* and *M. lineatus* which is typical for the Black Sea waters. Larvae of a barnacle *A. improvisus* prevailed from late autumn to early spring.

The results obtained provide an insight into ecological state of marine pelagic and benthic communities in the Kerch Strait and Taman coastal waters of the Black Sea. An increase in larval density of *U. pusilla*, a previously rare decapod, was registered for the Kerch Strait, and it confirms expediency and necessity of further study of meroplankton biodiversity in the Taman Peninsula coastal waters.

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ТАКСОНОМИЧЕСКИЙ СОСТАВ И МНОГОЛЕТНЯЯ ДИНАМИКА ЧИСЛЕННОСТИ МЕРОПЛАНКТОНА В ПРИБРЕЖНЫХ ВОДАХ ТАМАНСКОГО ПОЛУОСТРОВА (КЕРЧЕНСКИЙ ПРОЛИВ, ЧЁРНОЕ МОРЕ)

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В исследуемом районе от Таманского залива до Бугазского лимана идентифицировано 47 таксонов личинок донных беспозвоночных: Polychaeta — 14, Cirripedia — 4, Phoronida — 1, Decapoda — 10, Bivalvia — 13, Gastropoda — 3, Hydrozoa — 2. В полузакрытом Таманском заливе, находящемся под влиянием вод Азовского моря, отмечена самая высокая плотность меропланктона (до 30,6×10^3 экз.·$м^{-3}$) при низком таксономическом разнообразии (17 таксонов). В Таманском заливе в июне и июле меропланктон был представлен преимущественно двустворчатыми моллюсками *Cerastoderma* sp.; в районе косы Тузла и мыса Панагия — *Cerastoderma* sp. и *Spisula subtruncata*; в районе мыса Железный Рог и Бугазского лимана — *S. subtruncata* и *Mytilaster lineatus*. Несмотря на интенсификацию судоходства и значительное антропогенное загрязнение исследуемого полигона, высокая плотность меропланктона обеспечивала достаточный репродуктивный потенциал района. В акватории морского порта Тамань зарегистрирован летне-осенний пик численности (от 13×10^3 до 15,4×10^3 экз.·$м^{-3}$), обусловленный массовым выходом личинок двустворчатых моллюсков *C. gallina* и *M. lineatus*, что характерно для вод Чёрного моря. Увеличение в Керченском проливе плотности личинок ранее редкого вида десятиногих ракообразных *Upogebia pusilla* (0,24×10^3 экз.·$м^{-3}$) подтверждает целесообразность и необходимость дальнейшего изучения биоразнообразия меропланктона в прибрежных водах Таманского полуострова.

Ключевые слова: меропланктон, Таманский залив, коса Тузла — мыс Панагия, мыс Железный Рог — Бугазский лиман, порт Тамань

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NOTES

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NEW RECORDS OF COPEPODS *OITHONA NANA* AND *ACARTIA MARGALEFI* FROM THE BLACK SEA OFF THE COAST OF THE CRIMEA

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Oithona nana and *Acartia margalefi*, native Black Sea copepods which were not recorded in coastal waters of the Crimea in 1990–2000, were registered in 2020 and 2023 in the course of local zooplankton research along the southern coast of the Crimea, inshore Gurzuf, and during the 126th and 127th cruises of the RV "Professor Vodyanitsky." This short communication provides data on location of the finding sites and presents photographs of these two species.

Keywords: copepods, new findings, native species, Black Sea

Since the mid-1970s, changes occurred in the nature and degree of anthropogenic load on the Black Sea ecosystem. Since the 1990s, significant climate change was observed associated with general warming and a slow increase in mean annual temperatures of the Black Sea surface layer by 1–2 °C over recent decades [Artamonov et al., 2005]. Under these conditions, new species for the region were recorded in the pelagic zone: the warty comb jelly *Mnemiopsis leidyi* A. Agassiz, 1865 and copepods *Acartia tonsa* Dana, 1849 and *Oithona davisae* Ferrari F. D. & Orsi, 1984 [Temnykh, Nishida, 2012; Zagorodnyaya et al., 2003]. During the same period, native copepods, previously abundant *Oithona nana* Giesbrecht, 1893 and common in coastal waters *Acartia margalefi* Alcaraz, 1976 (*Acartia clausi* "small form" [Belmonte, Mazzocchi, 1997]), practically disappeared from plankton [Gubanova et al., 2014; Zagorodnyaya, Shadrin, 1999; Zagorodnyaya, Skryabin, 1995]. Increased anthropogenic load combined with *M. leidyi* press on biota (the warty comb jelly feeds on zooplankton) resulted in the undermining of the food supply not only for pelagic fish, but also for several demersal fish due to a sharp drop in abundance of copepods and pelagic larvae of bottom animals. This had adverse consequences for the entire Black Sea ecosystem [Modern Condition of Biological Diversity, 2003].

In the early 2000s, a decrease in anthropogenic load due to industrial stagnation in the Black Sea countries and the invasion of an active consumer of the warty comb jelly – the predatory comb jelly *Beroe ovata* Bruguière, 1789 – governed certain changes in the Black Sea biota: the diversity of various taxonomic groups of both zoo- and ichthyoplankton and benthos increased, and the total abundance of hydrobionts and individual species rose as well [Biological Resources, 2011]. In this regard, the facts of the finding of adult copepods *O. nana* and *A. margalefi* in coastal waters of the Crimea in 2020 and 2023 are of great interest.

MATERIAL AND METHODS

The reported material is based on zooplankton samples taken from a boat at six stations off the southern coast of the Crimea (SCC) in the Gurzuf area in December 2020 and during research cruises of the RV "Professor Vodyanitsky" in March 2023 (the 126th cruise, 55 stations) and June 2023 (the 127th cruise, 37 stations) (Fig. 1). Zooplankton was sampled with a Juday net, Ø = 36, gauze with a mesh of 112 µm (at stations off the SCC) and 145 µm (in the open sea), with total vertical tows; their length depended on depth at a station. The samples were fixed with 4% neutral formalin. The taxonomic and quantitative composition of zooplankton was studied. Organisms were identified, measured, and counted under an MBS-9 microscope in a Bogorov chamber by the standard counting-portion technique [Kozhova, Melnik, 1978]; rare and/or large organisms were examined in the entire sample. Species names of hydrobionts follow the World of Copepods database [2024].

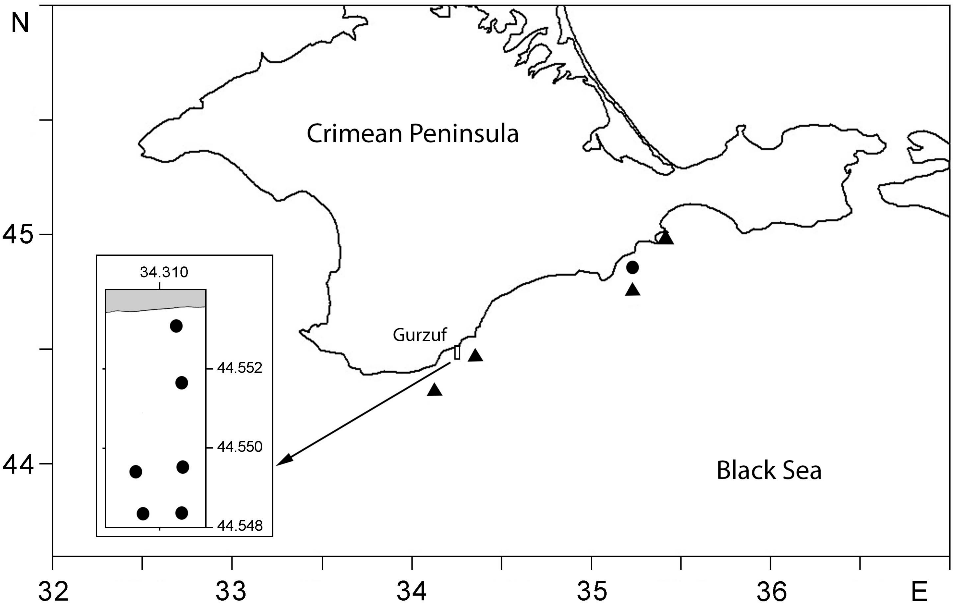

Fig. 1. Location of stations where *Oithona nana* (●) and *Acartia margalefi* (▲) were found. The arrow indicates the study site near Gurzuf

RESULTS AND DISCUSSION

A native copepod *O. nana* in fairly high abundance (380 $\text{ind.}\cdot\text{m}^{-3}$) was found along with an invasive copepod *O. davisae* off the SCC, inshore Gurzuf, at six coastal stations in the material sampled in December 2020. The share of *O. nana* in samples averaged 10% of the total copepod abundance; females (Fig. 2A–C), males, and late copepodites were registered. Two years later, in March 2023, sexually mature females of *O. nana* (4 ind.) were recorded off the coast of the Crimea in the material of the 126th cruise of the RV "Professor Vodyanitsky" (see Fig. 1).

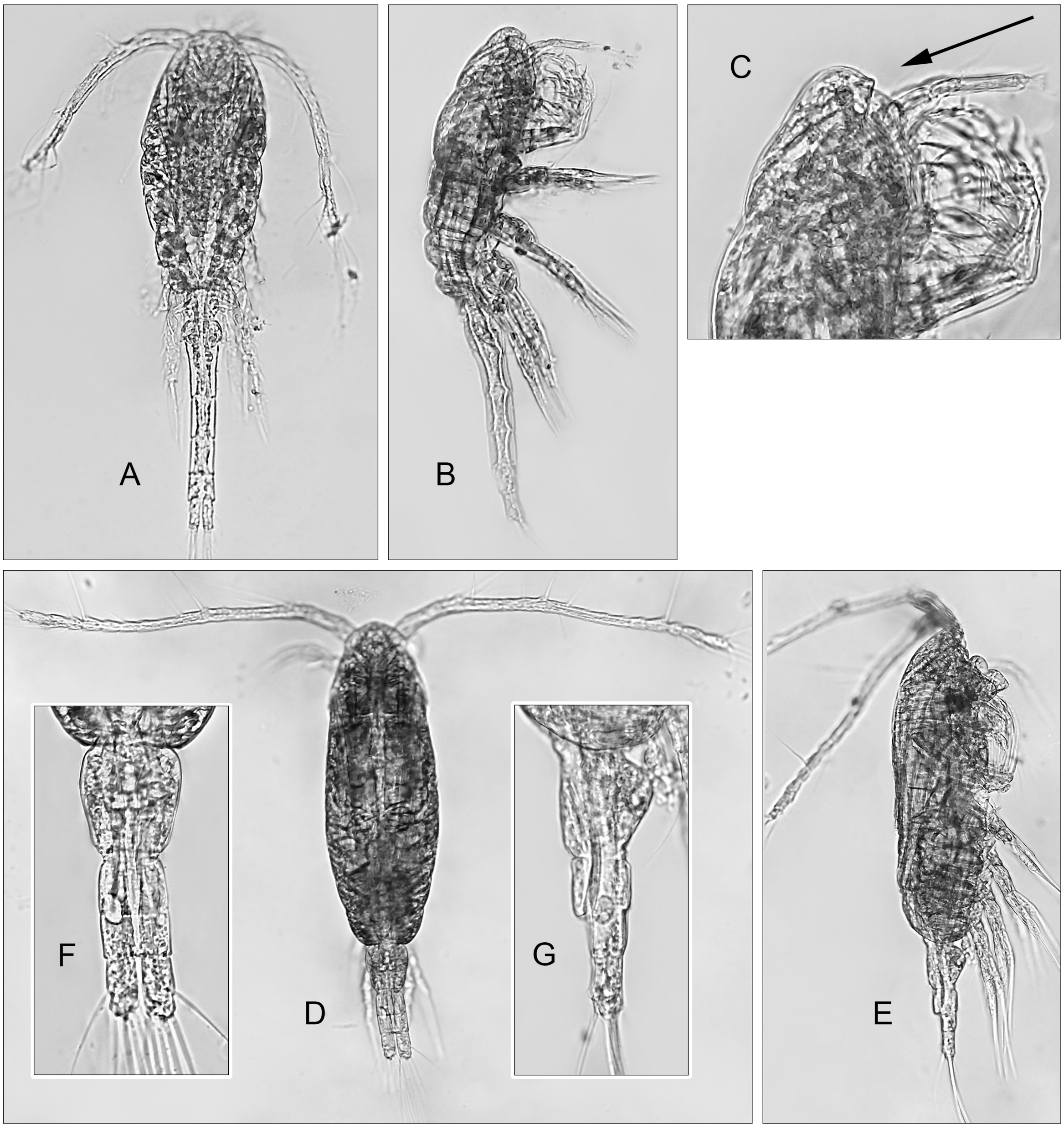

Fig. 2. *Oithona nana*, female, total length of 0.56 mm, southern coast of the Crimea near Gurzuf, December 2020 (A, dorsal view; B, lateral view; C, anterior head, lateral view, the arrow indicates the absence of a rostrum). *Acartia margalefi*, female, total length of 0.62 mm, June 2023 (D, dorsal view; E, lateral view; F, urosome, dorsal view; G, urosome, lateral view)

Mature females (Fig. 2D–G) and males of *A. margalefi* (a total of 28 ind.) were noted in coastal waters of the Crimea in samples from four stations in the material of the 127th cruise of the RV "Professor Vodyanitsky" (June 2023) (see Fig. 1).

Our findings do not allow to assess the current state of populations of these species off the coast of the Crimea. Apparently, their records are associated with a drop in anthropogenic load on biota and a decrease in *M. leidyi* press on the Black Sea plankton, on copepods in particular. In this regard, it is advisable to continue monitoring the taxonomic composition of zooplankton: this will help to better understand the biodiversity of pelagic communities, describe the spatial and temporal dynamics, and reveal trends in its formation in the region.

This work was carried out within the framework of IBSS state research assignment "Biodiversity as the basis for the sustainable functioning of marine ecosystems, criteria and scientific principles for its conservation" (No. 124022400148-4) and "Transformation of the structure and functions of marine pelagic ecosystems under the anthropogenic load and climate change" (No. 124030400057-4).

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НОВЫЕ НАХОДКИ КОПЕПОД *OITHONA NANA* И *ACARTIA MARGALEFI* В ЧЁРНОМ МОРЕ У БЕРЕГОВ КРЫМА

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Черноморские аборигенные виды копепод *Oithona nana* и *Acartia margalefi*, которых на протяжении 1990–2000 гг. не регистрировали у берегов Крыма, были обнаружены в 2020 и 2023 гг. у Южного берега Крыма, в прибрежной зоне возле Гурзуфа, и в морских экспедициях на НИС «Профессор Водяницкий» (126-й и 127-й рейсы). Представлены фотографии найденных копепод и места их обнаружения.

Ключевые слова: копеподы, новые находки, аборигенные виды, Чёрное море

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MACROPHYTOBENTHIC SPECIES NEW TO THE CAPE MARTYAN NATURE RESERVE (THE CRIMEA, BLACK SEA)

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In the epiphyton of communities formed by representatives of *Cystoseira* s. l., three species of macroalgae were identified in spring samples in 2019 which are new for the water area of the Cape Martyan nature reserve (southern coast of the Crimea, Black Sea). Those are *Ulva compressa* L., *Pylaiella littoralis* (L.) Kjellm., and *Phaeostroma bertholdii* Kuck. With these findings taken into account, 166 species of macrophytes are recorded for the reserve which is about 38% of the Black Sea macroflora. It is one of the key refugia of algae diversity and phytodiversity off the coast of the Crimea and in the Sea of Azov–Black Sea Basin as a whole.

Keywords: Black Sea, southern coast of the Crimea, Cape Martyan nature reserve, macrophytobenthos, new species

Since 1973, the Cape Martyan nature reserve has been functioning on the southern coast of the Crimea (since 2015, it is a natural park). There, land and coastal-marine biotopes of the Mediterranean type are preserved on the northern border of their distribution. In order to clarify the provisions of composition and structure of macrophytobenthos of the territorial-aquatic specially protected natural area and to optimize nature management within the boundaries of protected and recreational areas of the southern coast of the Crimea, hydrobotanical monitoring is carried out in the reserve.

Macrophytes were sampled in spring (17.05.2019) in the depth range (h) 0–8 m at a distance (l) up to 300 m from the shore (44°30′20.3″N, 34°14′40.4″E at the spot where the profile intersects the water's edge) in accordance with the generally accepted hydrobotanical technique [Kalugina-Gutnik, 1975]. The nomenclature and taxonomy of macrophytes are given after [AlgaeBase, 2024]; ecological and floristic characteristics, after [Kalugina-Gutnik, 1975]. General distribution is indicated after [AlgaeBase, 2024], with clarifications for the Caspian Sea according to [Zinova, 1967]. Distribution along the coast of the Crimea within boundaries of hydrobotanical regions (hereinafter HBR) is given after [Kalugina-Gutnik, 1975], with some additions if the species were later registered within new HBR.

The following macroalgae are recorded for the first time in the Cape Martyan nature reserve.

Ulva compressa Linnaeus, 1753 (Ulvales F. F. Blackman et Tansley, 1902, Ulvaceae J. V. Lamouroux ex Dumortier, 1822). In sublittoral zone, h = 8 m, l = 200…300 m. Annual, cosmopolitan, oligosaprobic, brackish-marine species. General distribution: shores of all oceans, including subpolar and tropical regions, inland seas (*inter alia* the Mediterranean Basin), and oceanic islands. Distribution along the coast of the Crimea is wide (HBR No. 3–8, 16), but the species is relatively rare, usually occurs in small abundance, and sometimes is abundant in pseudolittoral zone [Kalugina-Gutnik, 1975].

Pylaiella littoralis (Linnaeus) Kjellman, nom. cons. 1872 (Ectocarpales Bessey, 1907, Acinetosporaceae G. Hamel ex J. Feldmann, 1937). In sublittoral zone, h = 1 m, l = 30 m. Seasonal winter, arctic-boreal, mesosaprobic, brackish-marine species. General distribution: shores of all oceans, including subpolar and tropical regions, inland seas (*inter alia* the Mediterranean Basin), and oceanic islands; the Caspian Sea. Distribution along the coast of the Crimea is quite wide (HBR No. 4, 6–8), but the species is relatively rare and usually occurs in small abundance [Kalugina-Gutnik, 1975; Kalugina-Gutnik, Kostenko, 1981; Maslov et al., 1996; Sadogurskiy, 1996; Shiroyan, 2022].

Phaeostroma bertholdii Kuckuck, 1895 (Ectocarpales Bessey, 1907, Chordariaceae Greville, 1830). In sublittoral zone, h = 5 m, l = 150 m. Lower boreal, mesosaprobic, marine species; vegetation period is not established. General distribution: the Mediterranean and Black seas; the Caspian Sea. Distribution along the coast of the Crimea is limited; the species is rare (HBR No. 3, 5, 7) and occurs in small abundance [Kalugina-Gutnik, 1975; Sadogurskiy, 2013; Sadogursky, 2009].

All the listed species were found in the epiphyton of communities formed by representatives of the genus *Cystoseira* s. l. As a rule, they do not occur in noticeable aggregations and have small-sized thalli usually identified under a microscope; to a certain extent, this fact governs their relatively rare detection. With these findings taken into account, 166 species of macrophytes are registered for the Cape Martyan nature reserve – about 38% of the Black Sea macroflora [Minicheva et al., 2014]. It is one of the key refugia of algae diversity and phytodiversity off the coast of the Crimea and in the Sea of Azov–Black Sea Basin as a whole.

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ВИДЫ МАКРОФИТОБЕНТОСА, НОВЫЕ ДЛЯ ЗАПОВЕДНИКА «МЫС МАРТЬЯН» (КРЫМ, ЧЁРНОЕ МОРЕ)

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В эпифитоне сообществ, формируемых представителями *Cystoseira* s. l., в весенних сборах 2019 г. выявлено три вида макроводорослей, новых для прибрежно-морской акватории заповедника «Мыс Мартьян» (Южный берег Крыма, Чёрное море): *Ulva compressa* L., *Pylaiella littoralis* (L.) Kjellm. и *Phaeostroma bertholdii* Kuck. С учётом этих находок в акватории заповедника насчитывается 166 видов макрофитов, что составляет около 38 % макрофлоры Чёрного моря. Это один из ключевых рефугиумов альгофиторазнообразия у берегов Крыма и в Азово-Черноморском бассейне в целом.

Ключевые слова: Чёрное море, Южный берег Крыма, заповедник «Мыс Мартьян», макрофитобентос, новые виды

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THE NEW DATA ON THE FOOD SPECTRUM OF *SARPA SALPA* (LINNAEUS, 1758) (SPARIDAE) IN THE BLACK SEA

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The first data on the food spectrum of the Mediterranean immigrant *Sarpa salpa* (Linnaeus, 1758) in the Sevastopol coastal area (the southwestern Crimea) are presented. Consumed species were identified in food bolus of one mature *S. salpa* specimen caught off the Kruglaya Bay in August 2023. As shown, the food spectrum covered 13 species of macroalgae. Out of them, red (Rhodophyta) and brown algae (Ochrophyta) predominated by diversity and mass, while the contribution of green algae (Chlorophyta) was minimum. To assess the effect of *S. salpa* (the species that feeds on phytobenthos) on macrophytobenthos communities and coastal ecosystems, regular monitoring of its population in the Black Sea area is required.

Keywords: *Sarpa salpa*, nutrition, macroalgae, Crimea, Black Sea

The Mediterranean invader *Sarpa salpa* (Linnaeus, 1758) was first recorded in the Black Sea in 1937 [Maiorova, Marti, 1938]. Until the XXI century, single specimens of this species were registered off the coasts of Bulgaria, Romania, Ukraine, Russia, Georgia, and Turkey [Guchmanidze, Boltachev, 2017; Öztürk, 2021]. Since 2001, it has become more common off the coasts of the Caucasus and Crimea, both singly and in schools up to a hundred individuals [Guchmanidze, Boltachev, 2017]. The natural range of *S. salpa*, an obligate benthic and pelagic phytophage, covers the eastern Atlantic Ocean and the western Indian Ocean [Carpenter, 2016]. This species is an object of commercial fishing and mariculture in the Mediterranean Sea [Şahinyilmaz, Yigit, 2015].

Previously, information on *S. salpa* diet in the Black Sea was provided based on a study of two individuals caught more than 70 years ago, with the identification of three genera of algae [Georgiev, 1954; Maiorova, Marti, 1938]. Therefore, the aim of the work was to obtain data on the salpa food spectrum and feeding characteristics in the coastal zone of the Sevastopol region (the southwestern Crimea).

MATERIAL AND METHODS

The composition of the food of an adult sexually mature specimen of *S. salpa*, a hermaphrodite, was studied. The fish was caught by a spearfisherman on 29.08.2023 at the entrance capes of the Kruglaya Bay, Sevastopol (44°60′73.51″N, 33°43′83.84″E) during the daytime at a depth of 19 m and at a water temperature in the bottom layer of +26 °C. The salpa occurred among a school of large *Diplodus puntazzo* (Walbaum, 1792).

The just-caught fish was identified using the guides [Carpenter, 2016; FishBase, 2024; Svetovidov, 1964; Vasil'eva, 2007]. Length–weight indicators were determined according to I. Pravdin [1966]. The general index of gastrointestinal tract filling (I, ‱) was calculated by the formula:

$$I = (m/M) \times 10,000\,,$$

where m is the weight of the food bolus, g;

M is the weight of the fish, g [Rukovodstvo, 1961].

The fish and food items were weighted on an AXIS ADG500C electronic scale with an accuracy of 0.001 g. Mollusc species were identified according to [Opredelitel' fauny, 1972], and their names were updated following WoRMS [2024]. Macroalgae in the food bolus were identified according to [Zinova, 1967], and the species names were indicated taking into account nomenclatural changes [AlgaeBase, 2024].

Water temperature and depth were determined using a dive computer Suunto D4f (Suunto Oy, Finland) with an accuracy up to 1 °C and up to 1 m, respectively.

Information on macrophytobenthos of the Kruglaya Bay coast was obtained by us earlier (in the summer of 2017 and 2018). Sampling was carried out by the standard hydrobotanical technique [Kalugina-Gutnik, 1975]: a 25 × 25 cm recording frame was laid in four replicates at depths of 0.5, 1, 3, 5, 10, and 15–17 m (down to the lower boundary of the phytal zone). When processing samples, we determined the species composition of macrophytes and projective coverage, as well as biomass and abundance of cenosis-forming and mass species, with the contribution of epiphytic algae taken into account. Specific caloric content of algae was indicated according to B. Alexandrov [2001].

RESULTS AND DISCUSSION

Characteristics of the *Sarpa salpa* specimen and its habitat. The length–weight indicators of the *S. salpa* we examined (total length of 485 mm and weight of 1,700 g) (Fig. 1) are comparable to the maximum known for this species [Carpenter, 2016]. The index of gastrointestinal tract filling was 177‱.

The ichthyofauna of the Kruglaya Bay includes 42 to 57 species [Boltachev, Karpova, 2012; Hetman, 2017], and its high diversity is driven by environmental conditions and biotopic features. The coastal slope in the area where the salpa was found is characterized by a heap of stones and large boulders. Stony-block and rocky biotopes feature an *Ericaria–Gongolaria* phytocenosis (*Ericaria crinita* + *Gongolaria barbata* – *Cladostephus spongiosus* – *Ellisolandia elongata*), with a projective coverage of 70–90% and a high diversity of red algae (Rhodophyta), which is typical for this community in the Black Sea [Kalugina-Gutnik, 1975]. The highest biomass was recorded for brown algae (Ochrophyta): the values varied within 173–4,300 g·m^{-2}. For red algae (Rhodophyta), biomass varied within 64–1,682 g·m^{-2}, while for green algae (Chlorophyta), it did not exceed 12 g·m^{-2} [Cernysheva, 2019].

Phyllophora crispa phytocenosis (depth of 10–15 m) is confined to biotopes of shell rock and silty sands. Mosaic groups of *Zostera* seagrasses were observed only in certain areas of the Kruglaya Bay [Kovardakov, Prazukin, 2011].

Fig. 1. *Sarpa salpa* caught off the Kruglaya Bay, Sevastopol (29.08.2023)

Macroalgae of the food spectrum and feeding strategy. For the *S. salpa* examined, the food bolus weight was 30 g, and the wet weight of macrophytes *per* 1 kg of fish weight was 18 g. It is 1.4–1.7 times lower than values for this size–age group of salps in the Mediterranean Basin [Goldenberg, Erzini, 2014].

We found 13 species of macroalgae in the food bolus, including 2 Chlorophyta, 5 Ochrophyta, and 6 Rhodophyta (Table 1). The contribution of brown and red algae to the weight of the bolus reached 43.6 and 56.1%, respectively, while that of green algae did not exceed 0.3%.

In the *S. salpa* food bolus, the main component of red macroalgae was *Vertebrata subulifera* epiphytizing on *Ericaria* thallus (Table 1). A brown alga *E. crinita* occurred chiefly in the form of apical parts of branches with receptacles. The contribution of a green alga *Ulva rigida* and a calcified red alga *Corallina* sp. was minimum (Table 1). Representatives of the macrophyte epifauna – molluscs and a hydroid *Aglaophenia pluma* (Linnaeus, 1758) – were noted in insignificant quantities.

The features of the food spectrum composition that we revealed are consistent with the data on *S. salpa* diet in the Mediterranean Sea [Carpenter, 2016; Verlaque, 1990]. Thus, out of 138 identified species of macroalgae, a half also belonged to red algae, and the proportion of brown algae was similar to that obtained by us: 46.4 and 43.6%, respectively [Franco et al., 2015; Verlaque, 1990]. It should be noted that earlier, during the first catches of the salpa in the Black Sea, red algae were not found in its food [Georgiev, 1954; Maiorova, Marti, 1938].

The confinement of the salpa to the coastal zone of the Kruglaya Bay may be due to high biomass values of the dominant *Ericaria*–*Gongolaria* phytocenosis (687–4,611 $g \cdot m^{-2}$) featuring a significant contribution of *Ericaria* (up to 70–90%) and epiphytes (5–24%). The epiphytic synusium was dominated by *V. subulifera*, *Ectocarpus siliculosus*, *Sphacelaria cirrosa*, *Antithamnion cruciatum*, and *Chondria* representatives [Cernysheva, 2019] found in the salpa food (Table 1). The lower layers of phytocenoses at the entrance capes of the Kruglaya Bay are characterized by occurrence of the following lithophytes: *C. spongiosus*, *Carradoriella elongata* (Hudson) Savoie & G. W. Saunders, 2019, *Cladophoropsis membranacea* (Bang ex C. Agardh) Børgesen, 1905, *Gelidium crinale* (Hare ex Turner) Gaillon, 1828, *Gelidium spinosum* (S. G. Gmelin) P. C. Silva, 1996, *Osmundea pinnatifida* (Hudson)

Stackhouse, 1809, *Phyllophora crispa* (Hudson) P. S. Dixon, 1964, *Zanardinia typus* (Nardo) P. C. Silva, 2000, and other finely branched sciaphilous and soft coralline algae, also registered in the salpa food spectrum in the Mediterranean Sea [Cernysheva, 2019; Verlaque, 1990].

Table 1. Species composition, weight, and caloric content of food bolus components of *Sarpa salpa* (the Kruglaya Bay, 29.08.2023)

Taxon	Weight, g	Contribution, %	Specific caloric content, kJ·g^{-1}
Chlorophyta			
Lychaete echinus (Biasoletto) M. J. Wynne, 2017 (ep.)	0.05	0.17	18.92 / 25.26
Ulva rigida C. Agardh, 1823	0.03	0.1	13.79 / 17.37
Ochrophyta			
Cladostephus spongiosus (Hudson) C. Agardh, 1817	0.5	1.7	21.74 / 23.50*
Ectocarpus siliculosus (Dillwyn) Lyngbye, 1819 (ep.)	1.2	4.0	19.27 / 26.28
Ericaria crinita (Duby) Molinari & Guiry, 2020	10.3	34.3	16.46 / 18.00*
Sphacelaria cirrosa (Roth) C. Agardh, 1824 (ep.)	0.3	1.0	21.74 / 23.50*
Zanhlineardinia typus (Nardo) P. C. Silva, 2000	0.8	2.6	18.70 / 20.62*
Rhodophyta			
Antithamnion cruciatum (C. Agardh) Nägeli, 1847 (ep.)	0.05	0.17	20.46 / 22.94*
Chondria capillaris (Hudson) M. J. Wynne, 1991 (ep.)	1.4	4.67	16.99 / 18.61*
Chondria dasyphylla (Woodward) C. Agardh, 1817 (ep.)	0.8	2.7	16.99 / 18.61*
Corallina sp.	0.04	0.13	–
Laurencia obtusa (Hudson) J. V. Lamouroux, 1813 (ep.)	0.07	0.23	15.94 / 18.82
Vertebrata subulifera (C. Agardh) Kuntze, 1891 (ep.)	14.5	48.25	20.59 / 22.38
Mollusca			
Bittium reticulatum (da Costa, 1778)	0.041	0.01	–
Tricolia pullus (Linnaeus, 1758)	0.0028	0.009	–
Mytilidae gen. sp.	0.0003	0.01	–
In total	**30.08**	100.00	

Note: ep., an epiphyte. Specific caloric content is given according to B. Alexandrov [2001]: before the line, in terms of dry weight (DW); after the line, in terms of ash-free dry weight (AFDW). *, values for closely related species are provided. A dash denotes no data.

Since caloric content of red algae (Rhodophyta) in the food bolus of the studied specimen did not differ much, their predominance in the salpa diet could be associated with features of their chemical composition, *inter alia* their higher protein content (30–40%) compared to that for species of Ochrophyta (5–11%) and Chlorophyta (up to 20%) [Sukhoveeva, Podkorytova, 2006]. Moreover, red algae accumulate significant amounts of polysaccharides (up to 50–80%) and polyunsaturated fatty acids (eicosapentaenoic and arachidonic ones) occurring in fish tissues. The predominance of Rhodophyta in the *S. salpa* diet could be also governed by the fact that they were epiphytes (Table 1) with a high specific surface area the value of which positively correlates with their caloric content [Alexandrov, 2001].

The revealed features of the structure and distribution of macrophytobenthos in the coastal zone at the entrance capes of the Kruglaya Bay are important for assessing *S. salpa* feeding. The finding of an adult specimen in a warm layer (+26 °C) at a depth of 19 m may evidence for migrations from "pasture feeding" sites in dense macrophyte thickets at depths of 3–7 m to deeper layers, including the thermocline. According to mean long-term data [Troshchenko et al., 2019], in the Kruglaya Bay area, the core of the thermocline coincides with a depth of the salpa record. Such an adaptive strategy may also be associated with the rhythm of *S. salpa* feeding and the duration of digestion of polysaccharides that predominate in its food [Buñuel et al., 2020]. At the same time, fish can move hundreds of meters away from the coast without wasting much energy, and this resource is limited in species feeding on phytobenthos, especially with a carbohydrate type of nutrition [Verlaque, 1990].

Conclusion. For the first time, macroalgae were registered in the diet of the Black Sea *Sarpa salpa*; 13 species were identified. Red and brown algae accounted for 85% of species, and their contribution to the weight of the salpa food bolus was 99.7%. Regular monitoring of the state of *S. salpa* population in the Black Sea is required, especially under global climate change and processes of biota mediterranization: it will help in assessing the scale of the salpa effect on key cenosis-forming macrophyte species.

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НОВЫЕ СВЕДЕНИЯ О ПИЩЕВОМ СПЕКТРЕ *SARPA SALPA* (LINNAEUS, 1758) (SPARIDAE) В ЧЁРНОМ МОРЕ

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Представлены сведения о питании средиземноморского иммигранта *Sarpa salpa* (Linnaeus, 1758) в прибрежной зоне региона Севастополя (Юго-Западный Крым). Видовой состав компонентов пищевого комка определён по данным изучения одного экземпляра *S. salpa* — взрослой особи, отловленной на взморье бухты Круглая в августе 2023 г. Показано, что спектр питания сальпы охватывал 13 видов макроводорослей. Среди них по разнообразию и массе преобладали представители красных (Rhodophyta) и бурых водорослей (Ochrophyta), а вклад зелёных (Chlorophyta) был минимальным. Для оценки воздействия *S. salpa* — облигатного и активного фитобентофага — на сообщества макрофитобентоса и прибрежные экосистемы необходим регулярный мониторинг состояния популяции этого вида в бассейне Чёрного моря.

Ключевые слова: *Sarpa salpa*, питание, макроводоросли, Крым, Чёрное море

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