
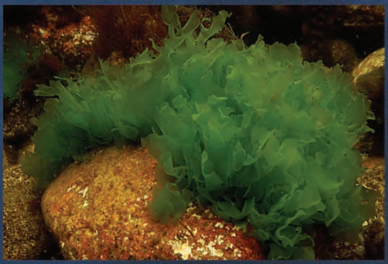


PROCEEDINGS
of the Russia–China Bilateral Symposium on

Marine Ecosystems under the Global Change in the Northwestern Pacific

Vladivostok, Russia, October 8–9, 2012



A.V. Zhirmunsky Institute of Marine Biology,
Far Eastern Branch of the Russian Academy of Sciences

Institute of Oceanology,
Chinese Academy of Sciences

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The Proceedings of the Russia-China Bilateral Symposium *Marine Ecosystems under the Global Change in the Northwestern Pacific* held in the A.V. Zhirmunsky Institute of Marine Biology FEB RAS, Vladivostok, Russia, on October 8–9, 2012 contain extended abstracts and papers dealing with state of marine ecosystems, communities and biotic changes in the northwestern Pacific. The symposium is the third one jointly organized by the IMB FEB RAS and the Institute of Oceanology CAS (two previous meetings were held in Qingdao in 2007 and 2010).

Editor

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Preface

A.V. Zhirmunsky Institute of Marine Biology, Far Eastern Branch of the Russian Academy of Sciences (Vladivostok) and the Institute of Oceanology, Chinese Academy of Sciences (Qingdao) developed a collaboration in the field of marine biology, ecology and biodiversity since 2006 when the Agreement on Cooperation was signed between these research institutes. Both organizations play an important role in the global change studies in the Northwestern Pacific since their establishment (IMB was founded in 1970, and the IOCAS – in 1950) as related to climatic impact on marine ecosystems, biodiversity changes, biological invasions, etc. For that reason, we decided to convene the third Russia-China bilateral symposium under the theme “***Marine Ecosystems under the Global Change in the Northwestern Pacific***” in October 2012 in Vladivostok, Russia. More than 40 scientists would participate in this meeting including 12 Chinese marine biologists.

Two previous workshops held in Qingdao, China in 2007 and 2010 were highly successful in terms of mutual cooperation, exchange by ideas and research findings and discussions between scientists of two countries studying the same ocean (see: K.A. Lutaenko (Ed.). *Biodiversity of the Marginal Seas of the Northwestern Pacific Ocean: Proceedings of the Workshop, Institute of Oceanology CAS, Qingdao, China, November 21–23, 2007.* Qingdao: IOCAS, 2007. 105 pp; Proceedings of China-Russia Bilateral Symposium on “*Comparison on Marine Biodiversity in the Northwest Pacific Ocean*”, 10–11 October 2010, Qingdao, China. Qingdao: IOCAS, 2010, 234 pp.). The Asia-Pacific region is becoming an important part of the world in terms of economy and changing environment. In Asia, due to rapid growth of population and industrial activities during dramatic economic development over the past 30 years, human pressure on marine ecosystems increased tremendously, especially taking into account that about half of the world population lives in Asia and the Pacific region and about 60% of which exists on or near the coasts. Thus, the importance of understanding the changes of coastal zones and oceans in Earth system modifications cannot be overestimated. We hope that regular Russian-Chinese meetings and other joint activities in marine biology would contribute to more understanding of the global change in the Asia-Pacific region and help to develop close collaboration between scientists of two countries.

Conveners
October 2012

Changes in flora of benthic green algae (Chlorophyta) of Hainan Island during the period from 1933 to 2009

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Hainan Island is located in the subtropical northern periphery of the Indo-Pacific Ocean in the South China Sea area (19°00′ N, 109°30′ E). The island has monsoon tropical climate, the average annual temperatures are 22–28°C. Annual rainfall ranges between 1.500 mm and 2.000 mm. On average, more than 300 days of a year are sunny, typhoons are common from July to September. The annual mean sea surface temperature (SST) is 26.0°C, with an average seasonal range of 12.1°C (Sun et al., 2005). Annual SST maximum (30.8°C) and minimum (18.7°C) commonly occur in July and January, respectively. Sea surface salinity (SSS) in the South China Sea as a whole fluctuates between 33.3 and 34.0‰. The southwest coast of the island has the tidal current velocity above 1.5 m/s, it has a maximum current velocity as high as 2.0–3.0 m/s. The mean tidal range is mostly less than 1.5 m (Yuan et al., 1995).

The highest biodiversity of main coastal ecosystems of Hainan Island (coral reefs, seagrass beds) was recorded between the 1950s and 1960s (Gurjanova, 1959) but it was destroyed severely in the following 20 years. It has been estimated that as much as 80% of the fringing reefs along the coastline of Hainan Island are damaged because of the unreasonable human activities (Hutching, Wu, 1987).

We suppose that changes in environmental conditions during the last 40 years in Hainan Island with partial degradation of coral reefs in shallow waters could lead to changes in algal species composition and the dominance of some species green algae (Chlorophyta), known as indices of the seawater pollution by dissolved organic and inorganic nitrogen and phosphorus (Titlyanov et al., 2011a). To prove our suppositions, we analyzed checklist of green algae of Hainan Island (Titlyanov et al., 2011b) composed on the basis of literature data of the Chinese phycologists from 1935 to 1985, materials of the joint German-Chinese expeditions in 1990, 1992 and sampling by Eduard Titlyanov and Tamara Titlyanova in 2008 and 2009.

Materials and Methods

The first collections of marine plants were made by Tseng with colleagues mainly in 1933–1935 (Tseng, 1936; Titlyanov et al., 2011b). In the frame of the German-Chinese cooperation, two expeditions in 1990 and 1992 took place to Hainan Island and the herbarium was placed at Marine Biological Institute of Helgoland in Hamburg, herbarium samples were identified by the authors. Anatomical studies were made using slides prepared from pre-soaked herbarium material and sectioned by hand with a razor blade. In October of 2008 and April 2009, marine plants were collected by T. Titlyanova, E. Titlyanov and Li Xiu Bao in the intertidal and upper subtidal zones only in Sanya Bay (Hainan Island) and identified by T. Titlyanova and E. Titlyanov using slides prepared from fresh material.

Results and Discussion

Algal species composition in Hainan Island in 1930–1960 and 1990–1992

104 taxa of green algae have been collected and identified in the Hainan Island from 1933 to 2009. Bryopsidales (38 species or 37% of total species number) and Cladophorales – 26 species (25%) predominated in the Hainan flora. From these orders the most algal numbers were presented by 24 species (23%) of Cladophoraceae, 14 species (13%) of Caulerpaceae, 11 species (11%) of Codiaceae and 12 species (12%) of Ulvales.

During the period from 1933 to 1985, 67 species of green algae were collected and identified. During the German-Chinese expeditions in 1990 and 1992, a total of 60 species of green algae were found. On the basis of the checklist of green algae collected and identified during 75 years in Hainan (Titlyanov et al., 2011b) and comparison with the flora of Taiwan Island numbering 99 species of green algae (Lewis, Norris, 1987), Fiji Islands (136 species) (South, Skelton, 2003), Dampier Archipelago in western Australia (54 species) (Huisman, Borowitska, 2003), we can say about rich biodiversity of flora of Hainan Island.

Collections at the beginning of last century were very rich in Caulerpaceae (18% of total number) and Codiaceae (16%). Collections of 1990–1992 were rich in Cladophoraceae (24% of total number) and Ulvellaceae+Ulvaceae (22%), Caulerpaceae were presented by only 8% and Codiaceae – by 5%. During the 1990 and 1992, 8 species of *Cladophora*, 6 species of *Ulva* were found in Hainan for the first time. However there were not found 9 species of *Caulerpa* and 6 species of *Codium* that were recorded earlier by Tseng (1936).

Some literature data point out that underwater ecosystems of Hainan Island in 1930–1940 were not subjected to anthropogenic pressing, although coral reefs, mangrove and seagrass meadows of Hainan Island were exploited (fishing, sampling of mollusks and edible algae) (Gurjanova, 1959). During the subsequent years, underwater ecosystems were subjected to destructive action of explosions (dynamite fishing, lime production, building). Since 1980 up to the present, intensive development of pond mariculture (mostly of shrimps and fish) on shallow coastal platform pollutes the sea by the mariculture waste. We suppose that significant changes in environment occurred by the 1990 (by the time of the German-Chinese expedition): free substrate and space (non-occupied by benthic organisms) were increased; areas with living corals and other animals were decreased, organic and inorganic sediments, concentrations of dissolved organic nitrogen and phosphorus in seawater was increased. Such changes in environment led to: (1) occupation of freely formed substrate by benthic algae with the dominance of green algae in the communities; development of epiphytic algae and decline in growth rates of frondose marine plants; changes in the structure of algal communities: complete replacement of communities, the appearance of new dominant species (filamentous, fine filamentous, finely branched and fine blade-like algae); the disappearance of frondose, fleshy and other forms of algae that have low ratio of surface area to volume of thalli.

Analysis of checklist (Titlyanov et al., 2011b) shows that some of the supposed changes in the structure of algal communities occurred in the intertidal and upper subtidal zones of Hainan Island during the period from 1930 to 1990. New species of green algae such as *Acrochaete* spp. (epiphytes and endophytes), *Ulva* spp. (tube-like and filamentous forms), *Cladophora* spp. (finely filamentous), *Rhizoclonium riparium* and *Chaetomorpha minima* (filamentous), *Phyllodictyon anastomosans* (netlike blade), *Valonia ventricosa* (coenocytic, coarse sack-like), *Bryopsis australiensis* (bushy, filamentous), *Caulerpella ambigua* and *Pedobesia ryukyuensis* (finely filamentous) occurred in communities. Probably these species came from near-by areas because the majority of them were found earlier in the Asian-Pacific region, except *Acrochaete leptochaete* (found in Europe, Atlantic Islands, North America and Africa), *Chaetomorpha minima* (recorded for North, Central and South America, Caribbean Islands, Indian Ocean Islands), *Cladophora laetevirens* (Europe, South America, Caribbean Islands, Indian Ocean Islands, South-west Asia).

Changes in the structure of algal communities in Sanya Bay during the last 19 years

From 1990, tourism became to develop in Sanya Bay that strengthened pressing on coral reef ecosystem by building hotels and swimming pools, by urban waste, scuba-diving tourism, fishing, hunting and navigation.

As was shown by our investigations (Titlyanov et al., 2011a), the main sources of dissolved inorganic nitrogen (DIN) and orthophosphates in Sanya Bay are farms cultivating fish, shrimps and lobsters and also urban waste. By the 2008–2009, DIN threshold concentration increased 3 times and orthophosphates 10 times. However the comparison of algal green flora of 1990, 1992 with that of 2008–2009 in Sanya Bay did not reveal any serious changes in species composition. Only 5 new species for Hainan were found in 2008–2009 (*Ulothrix implexa*, *Chaetomorpha ligustica*, *Parvocaulis clavatus*, *P. exiguus* and *Penicillus sibogae*) and all these species inhabit the Asian-Pacific region. Based on these data, we can suppose that during the last 19 years insignificant changes in Sanya Bay occurred only by the appearance of new filamentous green species in algal communities.

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The joint Russian-German deep-sea research projects SoJaBio (Sea of Japan Biodiversity Studies) and KuramBio (Kurile Kamchatka Deep-Sea Biodiversity)

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In 2007 in Vladivostok a Memorandum of Understanding was signed by IMB, ZMH (Zoological Museum, Hamburg) and DZMB (German Centre for Marine Biodiversity Research, Senckenberg, Wilhelmshaven), which included the exchange of scientists and the planning and realization of joint deep-sea expeditions in the north-west Pacific. The first Russian-German expedition SoJaBio (Sea of Japan Biodiversity Studies) onboard of the R/V *Akademik M.A. Lavrentyev* (51st cruise) to the slope and deep-sea basin of the Sea of Japan took place in the summer 2010. The German-Russian expedition with RV *Sonne* was performed during 21.07.–7.09.2012 in the slope of the Kurile-Kamchatka Trench (KKT) and adjacent abyssal plain, (223 expedition of RV *Sonne*). Aims of the both expeditions were to investigate the biodiversity and community patterns of the benthic meio-, macro- and megafauna in these two areas of the Northwestern Pacific. Almost all of the SoJaBio material has been already studied and analysed. Using methods of community analyses, the selected meio-, macro-, and megabenthic communities of the KKT area will be characterised and compared with regard to their composition and diversity. The fauna of the geologically older abyssal plain of the KKT region will be compared to the fauna of the semi-enclosed and younger Sea of Japan.

Results

After a few deep-sea Russian biological investigations in the Sea of Japan in the last century the deep-sea fauna of the Sea of Japan was characterized as scanty (about 30 species of invertebrates), consisting of cold-adapted eurybathic rather than true deep-sea species (Derjugin, 1939; Zenkevich, 1963; Nishimura, 1983; Tyler, 2002). This was attributed to its isolation from the oceanic abyssal and the relatively young age of the basin. The SoJaBio expedition was designed to study the biodiversity of organisms of all size classes and the environment in a semi-enclosed comparatively young deep-sea basin isolated from adjacent deep-sea basins, an area that is characterised by quite a high productivity and influenced by anthropogenic impact.

For biologists, the Sea of Japan might be a model of a young ocean in terms of studying the colonization processes of deep-sea environments, the development of a new deep-sea ecosystem at the early stages of its evolution. Throughout its history the Sea of Japan has been and still is connected to the Pacific Ocean through shallow straits less than 150 m depth, the deepest Japan Basin reaching a depth about 3800 m. Water masses filling the deep-sea basin are formed in the north of the sea due to immersion of cold dense waters during winter. In contrast to the recent anoxic history of the Sea (~30,000–18,000 years ago the sea level was about 130 m lower than now, and the deep basin was completely blocked), post-glacial oceanographic conditions in the deep-sea basin, are optimal for deep-sea biota. The water below 500 m is characterized by an abnormally high level of dissolved oxygen, and it is homogenous in temperature and salinity (Zenkevich, 1963; Kobayashi, 1985; Min, Warner, 2005; Talley et al., 2006). One of the topographic peculiarities of the Sea of Japan is a narrow continental shelf and a steep slope that goes down to 3000 m near the coast line (Dobrovolski, Zalogin, 1982). This prevents sedimentation on the slope and provides a direct and fast delivery of organic matter from the shore to the floor of the deep basin.

Four transects with 19 stations at different depths (roughly in 500, 1000, 1500, 2500, 3300 and 3600 m) were sampled (Fig. 1). About 300 underwater pictures and video sequences were taken to

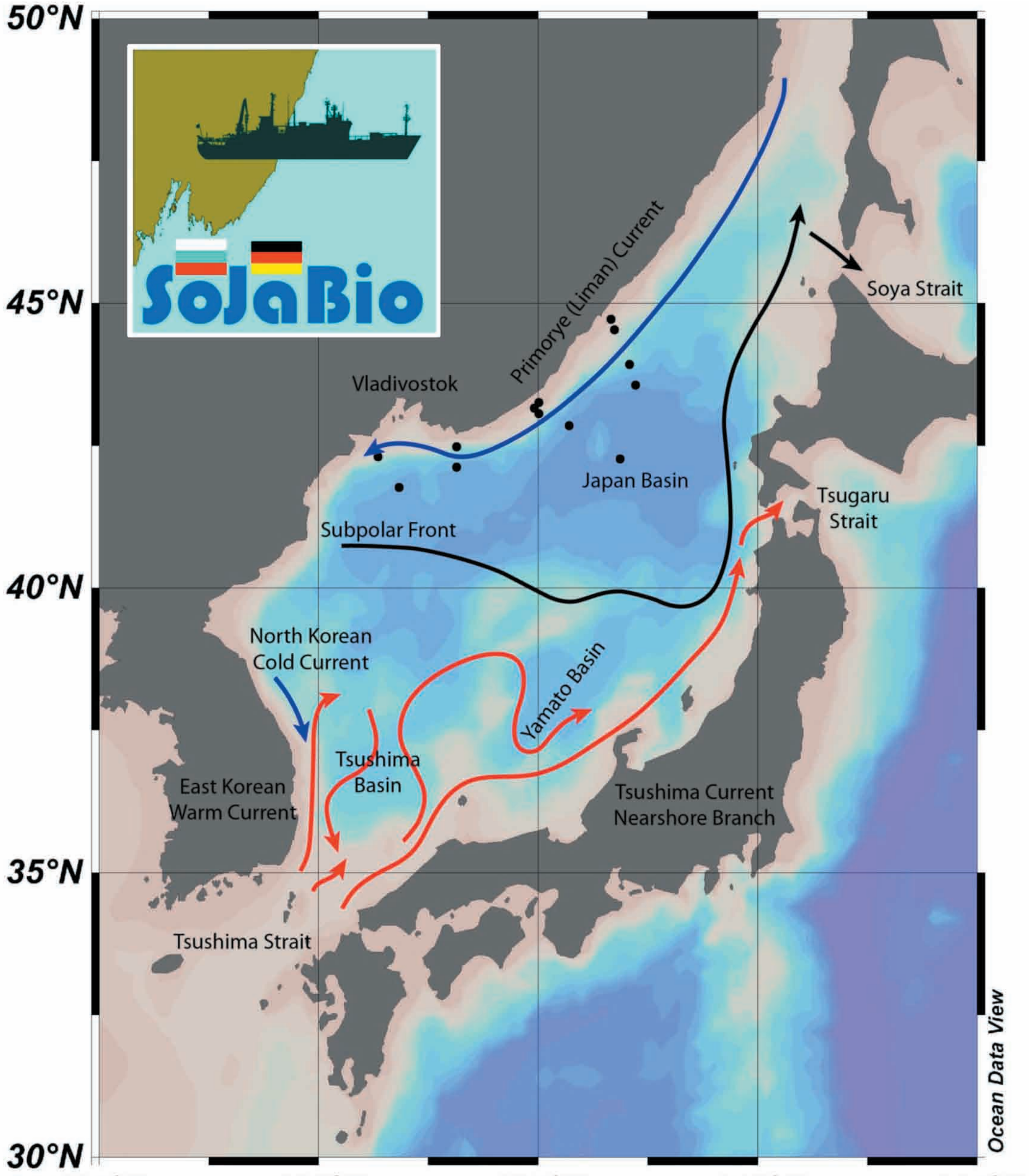

Fig. 1. Sampling station of the R/V *Akademik M.A. Lavrentyev* (51st cruise) during SoJaBio expedition in 2010.

document the sampling behavior of the C-EBS, the bottom topography, sediment characteristics, food availability, and input of organic matter as well as abundance of megafaunal animals.

On the basis of the SoJaBio expedition the list of organisms inhabiting the Sea of Japan deeper than 455 m has been extended significantly. The preliminary estimation of the collected species of benthic organisms have already given the impressive results: more than 600 species were sampled in the Sea of Japan and about one third of these are new for science, some species being recorded the first time for the Sea or for the northwest Pacific. The analyses of the the meio- and macrofauna and a few surveys of the dominant faunal elements, the descriptions of 3 new genera, 16 new species and redescriptions and detailed studies of more than 30 poorly known species of Tanaida, Isopoda, Nematoda, Nemertina, Hirudinea, Gastropoda, Hydrozoa and Anthozoa based on the new SoJaBio collections are presented in the volume of the Deep-Sea Research Part II (Malyutina, Brandt, 2012). The results confirm that the deep-sea fauna of the Sea of Japan consists mainly of eurybathic species. However, almost all studied taxa include few true abyssal species which already successfully colonized the young deep-sea environments of the Sea of Japan.

The results will be compared with the biodiversity of an open, non-isolated deep-sea area of the Pacific close to the Kurile-Kamchatka Trench on base of the new samples of the benthos of all sizes collected with the same gears using a standardised sampling methodology during the KuramBio expedition. The expedition was successfully fulfilled in the August–September 2012 (Fig. 2).

The future comparison the SoJaBio results with the new material from the KKT area will be important and interesting. Biodiversity and productivity in the northwestern deep sea of the Pacific were already investigated in the last century during expeditions onboard the R/V *Vityaz* in 1949,

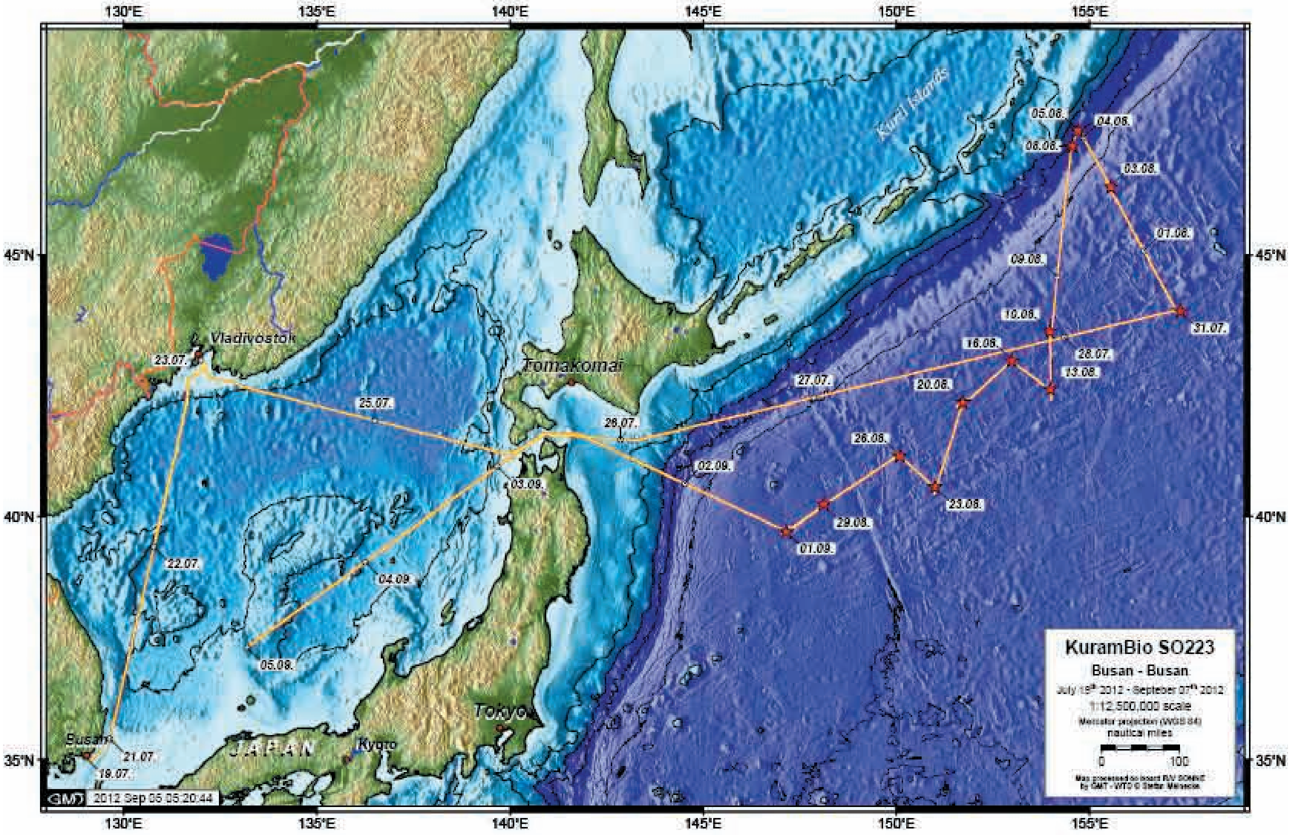

Fig. 2. Route of the expedition of the R/V *Sonne* and sampling stations in 2012.

1953 and 1966 (Belyaev, 1983, 1989; Belyaev, Vilenkin, 1983). The fauna found in the adjacent Kurile-Kamchatka Trench has been described in many publications, for example data on 660 species were published into two volumes of the transactions P.P. Shirshov Institute of Oceanology, USSR Academy of Sciences (1970, 1971). Isopoda was one of the dominant taxa in species richness (more than 100 species) in the deep-water macrobenthos (Birstein, 1963, 1970, 1971). Nevertheless, Birstein (1971) noticed that major fractions of the fauna were probably not sampled due to inappropriate sampling gear and methods used for sieving and washing; and thus the true deep-sea biodiversity was only partly studied. The preliminary results of the KuramBio expedition have already showed that the new methods of sample treatment and more effective gears, which were deployed successfully during the expedition, helped to discover a higher biodiversity and collected more species than known at present.

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Study of the intertidal biota of the Far Eastern seas of Russia

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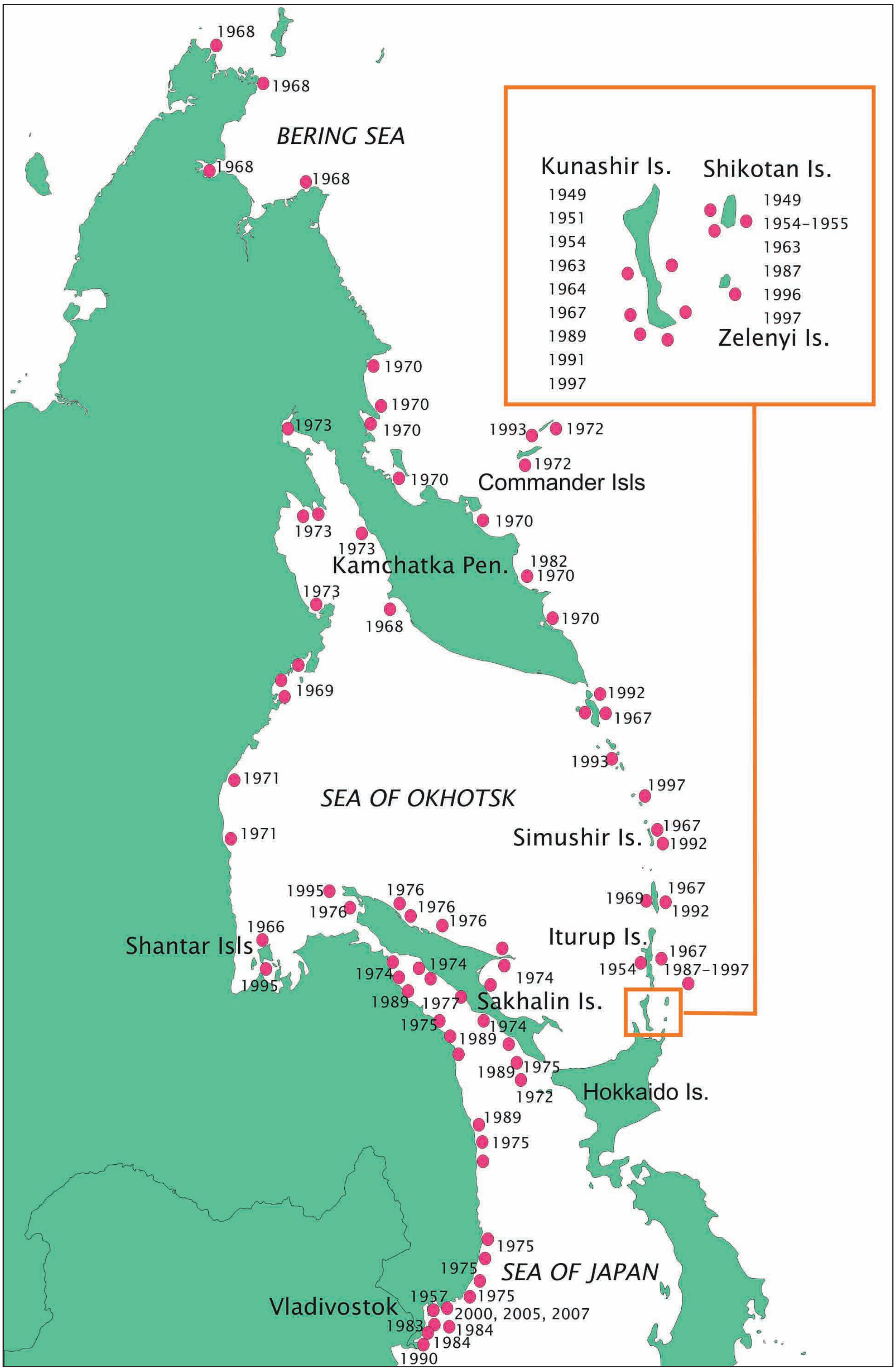
The earliest systematic studies of the intertidal biota and communities in Russia were conducted in the intertidal zone of Kola Bay, western and eastern Murman in the late 1920s by E.F. Gurjanova, I.G. Zachs, and P.V. Ushakov (Zachs, 1929; Gurianova et al., 1930a, b). At further hydrobiological surveys in the Russian Far Eastern seas, these scientists continued their research in the intertidal zone of the Sea of Okhotsk, Commander Islands, south-western Sakhalin and Peter the Great Bay of the Sea of Japan. Since the establishment of the Institute of Marine Biology FEB RAS (1966), systematic surveys of the intertidal zones of the Far Eastern seas were performed under the leadership of Academician O.G. Kussakin and are being continued by his followers at present (see figure).

Much of the expedition materials were published in a series of papers on the ecology of intertidal communities in various editions. More than 75 specialists participated in the materials' processing, i.e., in identification of different systematic groups of phyto- and zoobenthos. The main goal of inventorying of flora and fauna was completed in 1997 with publication of "The List of Animals, Plants and Fungi of the Intertidal Zone of the Far Eastern Seas of Russia", containing 2772 names of species, subspecies, forms and varieties and covering the intertidal biota from cyanobacteria to mammals (Kussakin et al., 1997).

A wide geographical coverage of the Far Eastern seas with intertidal research of the intertidal zone and detailed surveys permitted some generalizations. It is now recognized that the studied marine biota differs from biotas of the Barents and the White seas in terms of systematics on the species level and in zonal-biogeographical respect on the level of regions, despite bionomical similarities among them. It has been found that the transition zone between Holarctic and Sino-Indian brackish-water areas includes desalinated plots of Shantar Islands, Tugursky Bay, Amur estuary, lagoons of the north and north-eastern Sakhalin and the eastern coast of Sakhalin Island up to the northern part of the Patience Bay (Zaliv Terpeniya).

The study of macrobentos of the intertidal zone of Shikotan Island (Kuril Islands) has shown on materials of the expedition of 1987 (16 stations in the intertidal zone, 226 species) that the basic principle underlying the bionomical classification of Murman intertidal zone (the degree of connection of intertidal sites with the open sea) (Gurianova et al., 1930a, b) is also suitable for conditions of Shikotan Island. There is a common regularity that the species diversity of the macrobenthos in the intertidal zone of the island also increases from the freshened top of the bays towards open sea capes. We have called the found regularity "gradient of biodiversity" and made an attempt to trace it in the intertidal zone of Peter the Great Bay (Sea of Japan) (Ivanova, Tsurpalo, 2007). A comparison of the species diversity of macrobenthos of Russky Island with that of the estuarine zone of the top of Amursky Bay, on the one hand, and islands of the Far-Eastern State Biospheric Marine Reserve located in the open part of Peter the Great Bay, on the other hand, has shown a regular increase in the number of species of macrobenthos from freshened plots towards the open sea shores.



Map of intertidal expeditions carried out by the Institute of Marine Biology FEB RAS and the Far Eastern State University.

It is known for a long time that marine biota decreases in richness at desalination and then changes to a poor brackish-water biota (Remane, 1934; Berger et al.,1995; Chesunov, 2006; Ivanova et al., 2009). However, biodiversity and salinity gradients do not always coincide in their directions.

It is shown that salinity gradient of biodiversity has an unusual pattern in the White Sea (Berger et al., 1995). The largest number of benthic species is found in the waters of the White Sea with salinity of 26 to 29‰. Both decrease and increase in salinity beyond this range causes a drastic impoverishment of the fauna. Apparently, the salinity gradient of biodiversity in the White Sea enters into interaction with the latitude gradient, which, in its turn, is determined mainly by thermal factor, and reduction of the species number of the White Sea benthos at higher (over 29‰) salinity is due to the influence of very specific conditions, primarily the effect of low temperature. This is confirmed by the research of Fedyakov, who showed that the influence of temperature on several characteristics of settlements of the White Sea mollusks is significantly much greater than the impact of salinity (Fedyakov, 1986).

In some cases, reduction of the number of benthic species in lower salinity is aggravated by impact of anthropogenic factors.

As to the latitudinal gradient in the intertidal zone of the Far Eastern seas of Russia, this gradient is, according to our data, particularly evident on the Kuril Islands chain: 352 macrobenthic species were found at Northern Kuril, 475 at the Middle Kuril, and 794 were identified at the Southern Kuril Islands. In the northernmost area of study (Anadyr Bay and Saint Lawrence Bay in the Bering Sea), 264 species were found in the intertidal zone, and 717 species were found in the Southernmost area (Peter the Great Bay, Sea of Japan). Thus, there is a trend of increasing biodiversity at the species level from high to low latitudes, as it is generally observed in the intertidal zone of the far-eastern seas of Russia and also in some specific regions.

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Planktonic ciliates in the Yellow Sea and East China Sea, their distribution in relation to hydrography

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Planktonic ciliates are important components of microzooplankton assemblages. They are the trophic link between the microbial food web and metazoans. As primary consumers of pico- and nano-sized producers, and important food sources of metazoan zooplankton and fish larvae, ciliates play an important role in carbon and energy fluxes in pelagic marine systems.

The temperate marginal seas experience two kinds of seasonal changes annually: seasonal stratification in warm seasons and intrusion of oceanic water in cold seasons. Influence of seasonal stratification and oceanic water intrusion on phytoplankton is well studied, while their influence on microzooplankton (ciliates, dinoflagellates, flagellates) is less known.

Yellow Sea (YS) and East China Sea (EAS) are marginal seas in the west boundary currents. The hydrography of YS and EAS has an obvious seasonal change. In summer, the Yellow Sea Cold Bottom water occupied in the central YS. In winter, Yellow Sea Warm current intrudes into the central YS. In summer, EAS is influenced by Changjiang River diluted water (CDW). In winter, CDW shrank, and the Kuroshio intrudes into the shelf area. We introduce the influence of Yellow Sea Cold Bottom Water, Yellow Sea Warm Current, Changjiang River diluted water and Kuroshio on the distribution of planktonic ciliates abundance.

In the sea area around Zhangzidao Island, northern YS, monthly investigation was carried out in July 2009 through to June 2010. The transect was at the edge of Yellow Sea Cold Bottom Water. In winter, water was well mixed with thermal front at nearshore and offshore stations. Planktonic ciliates mainly distributed in the nearshore area. In summer, Yellow Sea Cold Bottom Water showed up in the offshore stations with low ciliated abundance.

In the southern YS, eight cruises were carried out in two transects in April 2006 through to August 2007. During the cold half of year, higher ciliate abundance occurred in coastal areas and the thermal fronts than in the Yellow Sea Warm Current. During the warm half of the year, there is less ciliates in the Yellow Sea Cold Bottom Water, while higher ciliate abundance occurred in the vicinity of the tidal front (Figs. 1, 2).

In the EAS, the distribution of planktonic ciliate was influenced by CDW. In summer, higher abundance of planktonic ciliates appeared in CDW with salinity lower than 32 (Fig. 3A). In winter, CDW was confined in a narrow area along the coast. Higher abundance occurred in the shelf water (SW) with salinity of 32–34.5. The Kuroshio water (KW) with salinity >34.5 has low ciliate abundance (Fig. 3B).

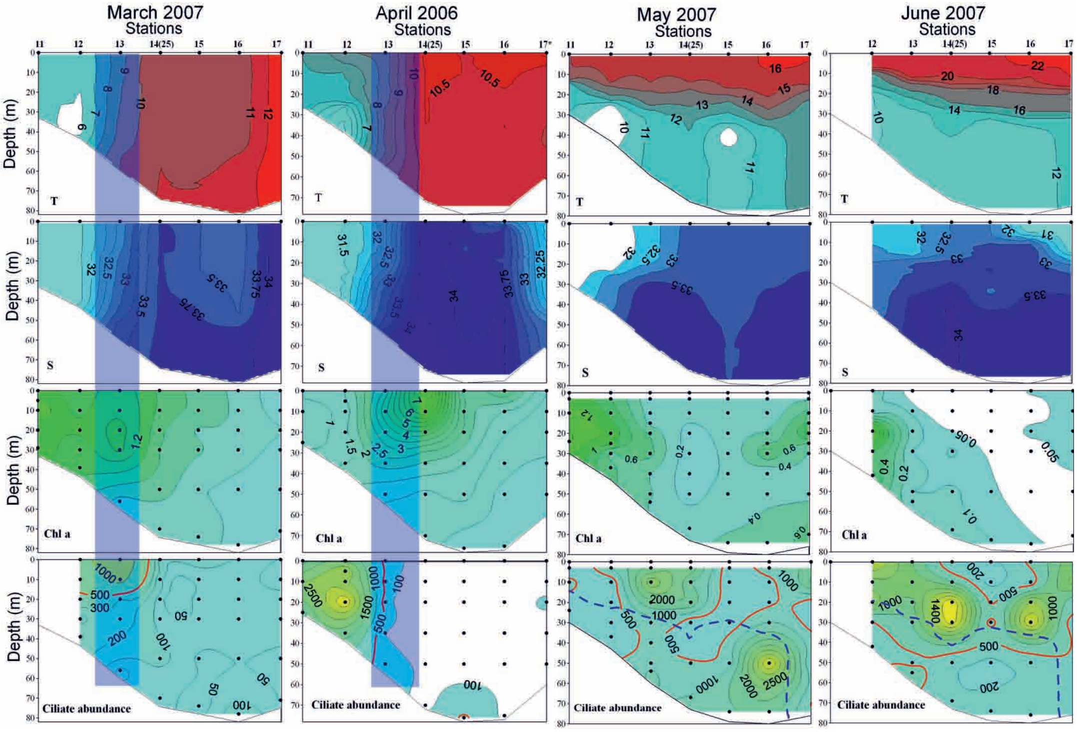

Fig. 1. Vertical distribution of temperature (°C), salinity, Chl *a* (µg L^{-1}) and ciliate abundance (ind. L^{-1}) along Transect 1 in March, April, May and June. The vertical shaded column showed the position of fronts. Dashed line was the 12 °C contour line.

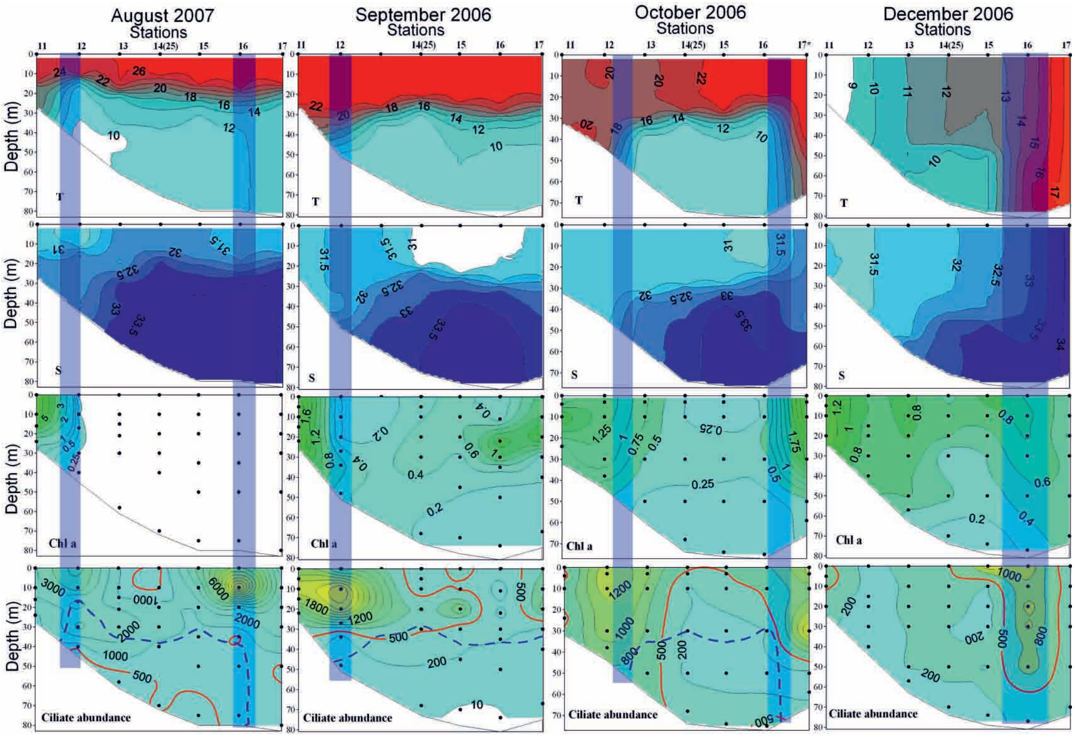

Fig. 2. Vertical distribution of temperature (°C), salinity, Chl *a* (µg L^{-1}) and ciliate abundance (ind. L^{-1}) along Transect 1 in August, September, October and December. The vertical shaded column showed the position of fronts. Dashed line was the 12°C contour line.

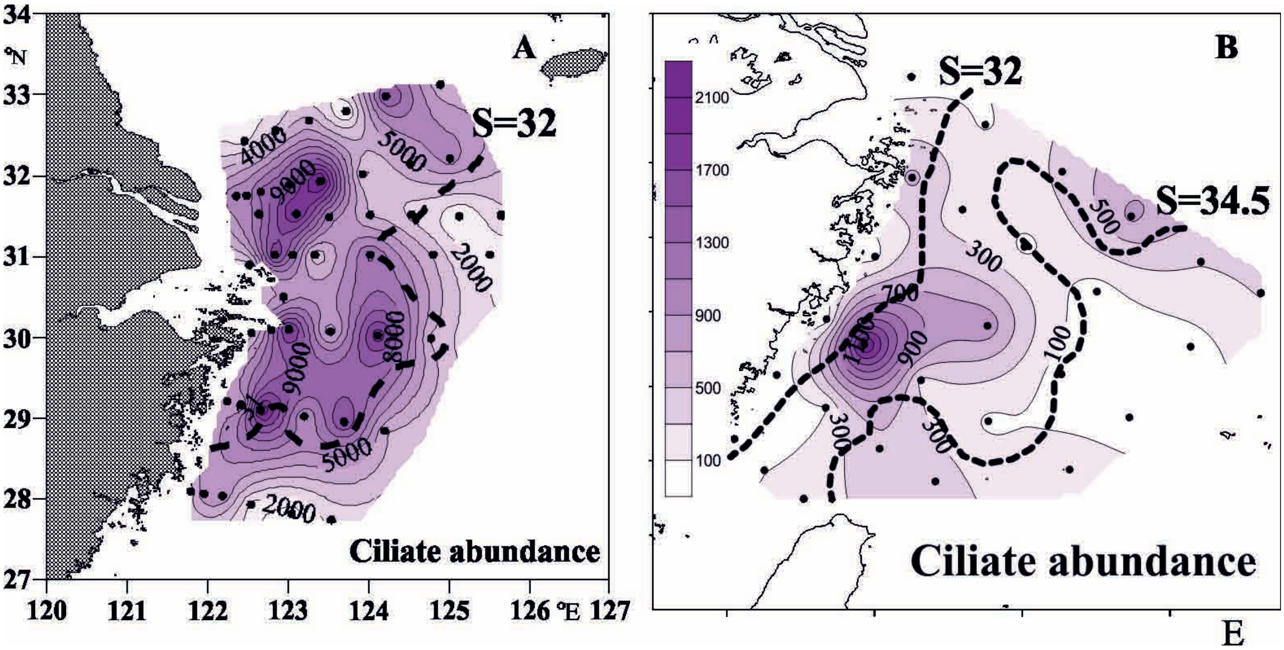

Fig. 3. Horizontal distribution of surface ciliate abundance (ind. L^{-1}) in summer 2011 (**A**) and winter 2007 (**B**). The dash lines were isohaline of 32 as the boundary of the CDW and SW; dashed isohalines of 34.5 indicate the boundary of the SW and KW.

We found a new tintinnid species, *Tintinnopsis yantzensis*, in the mouth area of Changjiang River. The prominent characteristic of this species is the duck-bill shape at the aboral end of the lorica, which is different from all other known species. The lorica measures 150–170 micron in length and opens at both ends (Fig. 4). It occurred in waters with salinity <30, mainly in the mouth area west of 122.7° W.

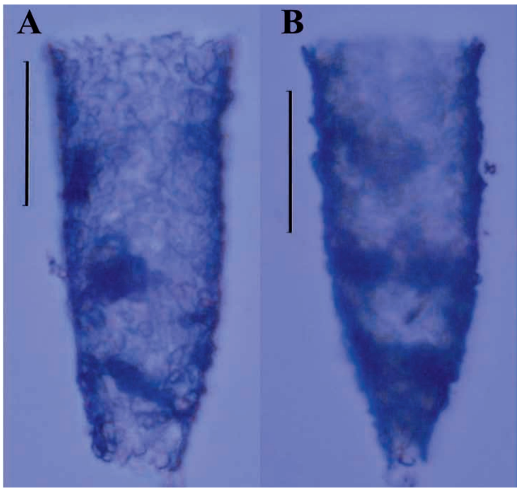

Fig. 4. Photos of *Tintinnopsis yangtzensis*. **A**, Front view; **B**, Side view.

Ecological conditions of Amursky and Ussuriysky bays of the Sea of Japan and of the rivers flowing into them

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Polluted rivers and waste water flowing into the coastal zone are the main sources of pollutants entering the sea. Estuaries and marine bays with rivers flowing into them are subjected to great anthropogenic influence. Concurrently these areas are defined by significant variation of primary abiotic factors, largely of temperature and salinity. Such situations require careful study of the biota's response to pollution, depending on the fluctuation of natural physicochemical parameters of the environment. In this case, it is possible to find reliable information on possible physiological alterations in organisms as the result of combined effects of natural and anthropogenic factors.

The composition of bottom sediments in water bodies is highly influenced by anthropogenic factors. Large quantities of contaminants (heavy metals, surfactants and supertoxins) have accumulated on the bottom. Concentrations of chemical substances in bottom sediments exceed their concentration in the water column by several orders of magnitude.

Some chemical compounds can modify their properties as a result of chemical and metabolic transformation (e.g., the methylation of mercury) and become more dangerous for hydrobionts. On the contrary, other compounds lose their biological activity through microbiological metabolism (e.g., some petroleum hydrocarbons).

We estimated the ecological condition of rivers entering Amursky and Ussuriysky bays of the Sea of Japan using macroinvertebrates as biological indicators. Bioassessment of the marine waters was based on sea urchin embryo biotest (Kobayashi, 1974, 1981, 1990) of both sea water and bottom sediments from different areas of Peter Great Bay of the Sea of Japan.

Our bioassessment analyses provided reliable estimates of water quality, in both freshwater (Fig. 1) and marine (Fig. 2) environments. Analyses using bioindicators were able to distinguish between clean and polluted conditions and could detect zones of transformation of contaminants. The most polluted areas were Murav'inaya Bay and Shkotovka and Sukhodol estuaries of Ussuriysky Bay and the estuary of Razdo'naya River of Amursky Bay. This indicates that there is significant domestic and industrial pollution flowing into Amursky and Ussuriysky bays from terrestrial sources

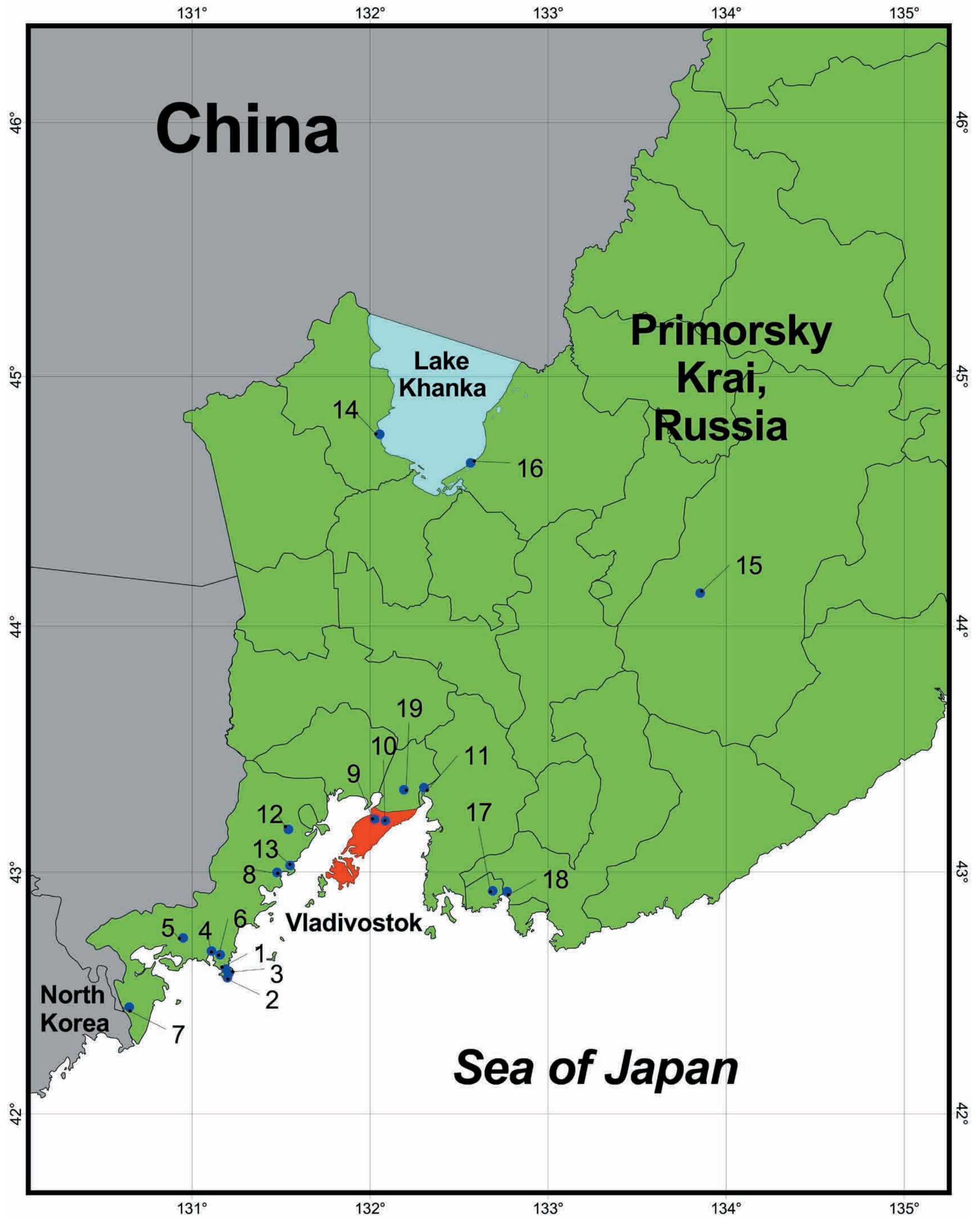

Fig. 1. Points on rivers where macroinvertebrates were collected.

(Figs. 3, 4). The cleanest water was in Narva River, its estuary, and adjacent Amursky Bay, where the Narva River discharges (Fig. 3).

We conclude that bottom sediments are more toxic than those in the water column. In estuaries where river flow is mixed with salt water, chemical transformation of pollutants occurs. Toxicity is particularly high in these areas.

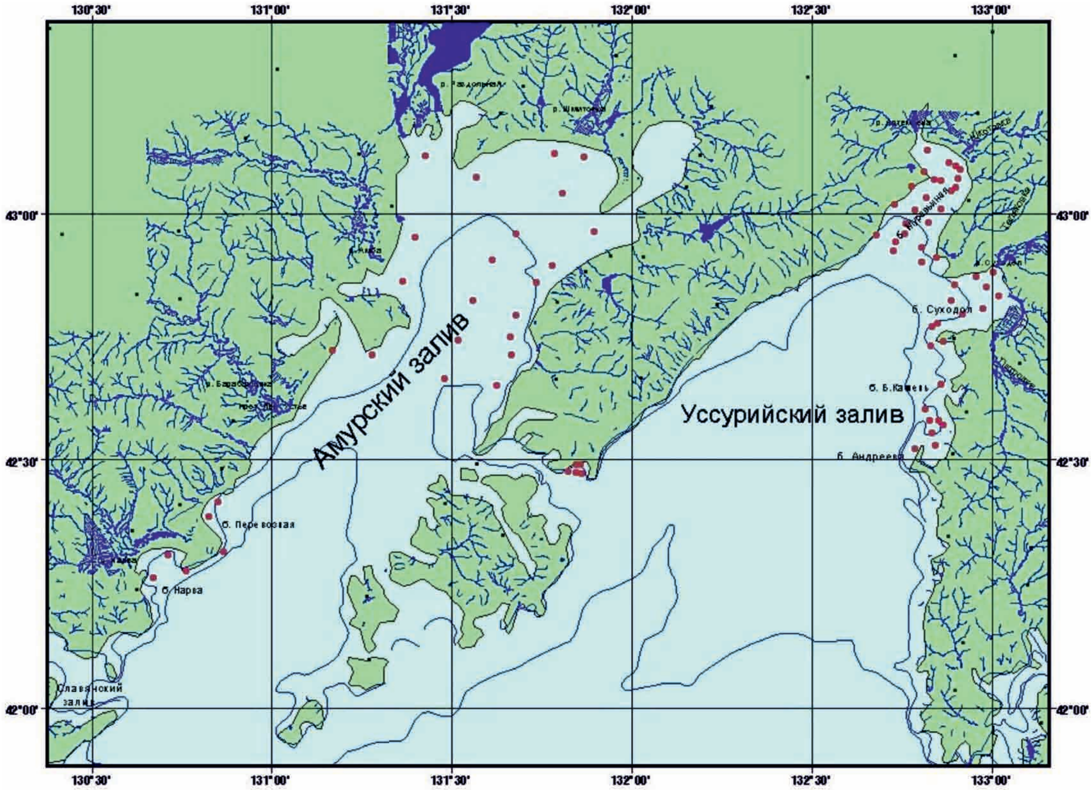

Fig. 2. Points on marine area where sea water and bottom sediments were tested.

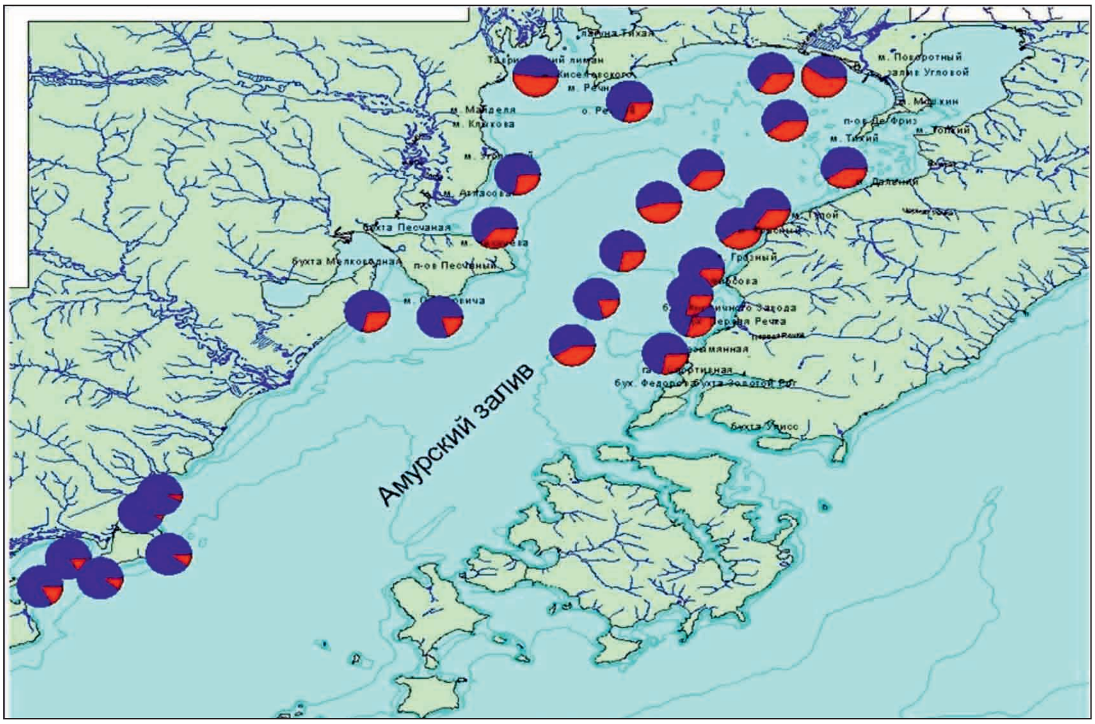

Fig. 3. Result of bioassessment analyses of bottom sediments in Amursky Bay. Blue – normal larvae on pluteus stages, red – misshapen larvae. The estuary of Razdolnaya River is most polluted, and estuary of Narva River is most clean.

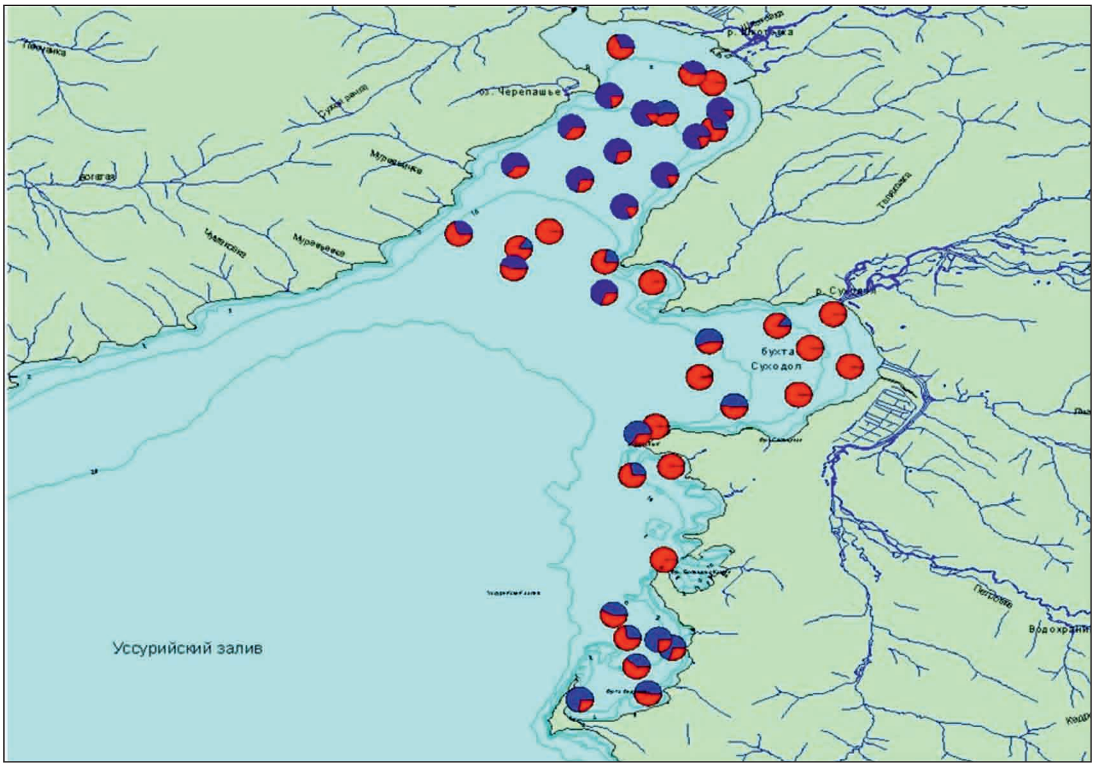

Fig. 4. Result of bioassessment analyses of bottom sediments in Ussuriysky Bay. Blue – normal larvae on pluteus stages, red – misshapen larvae. The estuary of Sukhodol River is most polluted.

Using bioassessment and biotesting we were able to obtain an overview of the spread of pollutants in the water cycle of Ussuriysky and Amursky bays and the open marine zone of Peter the Great Bay.

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Soft bottom macrobenthos of the Far Eastern Marine Reserve

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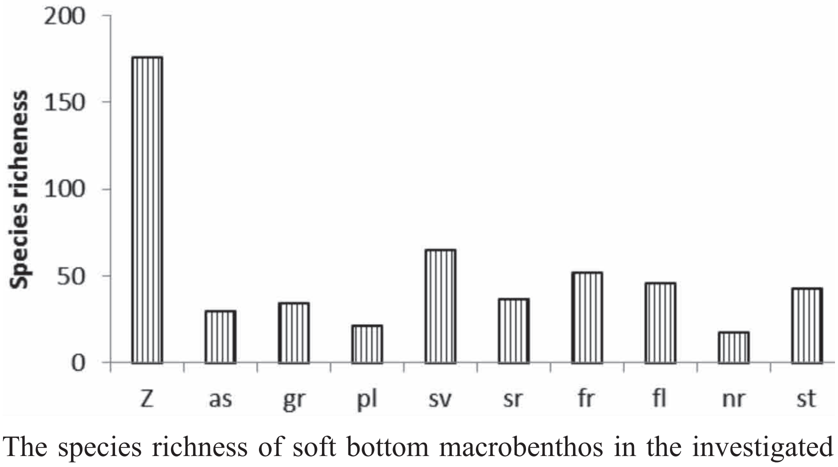
In 2006–2008, a hydrobiological survey of soft bottom macrobenthos was performed at 182 stations in nine localities of the reserve and in surrounding areas. Sampling was carried out from June to September from the R/V *Professor Nasonov* and R/V *Vnimatelny*. Samples were collected using a Van Veen bottom grab (0.25 m^2) from depths to 40 m; and for counts of macropbenthos, using a 5 m^2 frame and scuba. The coefficient of diversity for soft bottoms was determined by the formula $E = (m + c)/r$, where m is the number of species of mollusks and crustaceans; r, polychaetes registered in this study (Udalov et al., 2006).

Soft bottom substrates are the most common type in subtidal bays and in areas around islands of the reserve. They are mainly silty, to a greater or lesser extent, with fine sandstone and small and medium-sized psammites in the central parts of the bays; siltstones and silty coastal parts of psammites are at depths greater than 10 m; areas with mixed substrates containing inclusions of small and medium-sized gravel and small amounts of shell debris are found at depths of 5–7 m. A total of 176 species of soft-bottom subtidal macrobenthos were identified (see figure). The greatest diversity was found for Polychaeta (67 species) and Mollusca (48 species).

Based on the frequency of occurrence, the most common were the starfish *Patiria pectinifera* (occurred everywhere), the bivalve *Acila insignis* (77.7%), and the ophiuran *Ophiura sarsi* (66.6%); the isopod *Gnorimosphaeroma ova* (55.5%), crustaceans with no more than 40%, and polychaetes, though generally found in all areas but rarely made up more than 30% of the total mass of the sample (see Table). Maximum biomass of echinoderms (64.426 g/m^2), polychaetes (13.662 g/m^2), and the bivalve *A. insignis* (2.207 g/m^2) was found in well-sorted, slightly muddy sand at Furughelm Island at 21 m depth. The population density of soft bottom macrobenthos varied from 14 to 925 ind/m^2. Based on population density at most stations, polychaetes were from 14 to 90%. The dominant



The species richness of soft bottom macrobenthos in the investigated localities of the Reserve and in surrounding areas. Z, the total number of species; as, Astafyeva; gr, Gorshkova; pl, Bolshoi-Pelis; sv, Sivuchya; fr, Furughelma Island; fl, Falshivyi; nr, Nerpichya; st, Stenina.

Frequency of occurrence of species and taxonomic groups

Taxon	Occurrence, %	Biomass, g/m^2	Density, ind/m^2
Acila insignis	77.7	2.308±2.8	5.75±9.1
Ophiura sarsi	66.6	0.515±0.7	7.6±12.7
Polychaeta	30	0.593±0.2	22.25±32.1
Echinodermata	100	14.996±24.7	3.5±5.4

species of soft-bottom macrobenthos in the surveyed area were the bivalve *A. insignis*, the ophiuran *O. sarsi*, and the polychaete *S. armiger.* These species widely distributed in the south-western part of Peter the Great Bay were reported to be predominant in Possjet Bay (Klimova, 1980) and were previously recorded in waters of the Far Eastern Marine Biosphere Nature Reserve (Ozolinsh, 2002).

The diversity factor for soft-bottom subtidal areas of the reserve is 1.02. This is somewhat higher, as compared to soft bottom areas of dominance in northern latitudes (in the area of Spitsbergen and the North Sea), and closer to the values of this index for bays and coves of the South China Sea and the north-east coast of Australia, as well as Troitsa Bay (0.9) in Peter the Great Bay (Galysheva, Khristoforova, 2008).

Optimal abiotic conditions for existence of invertebrate fauna apparently exist in Sivuchya Bay, 43% of bivalves and 50.1% of polychaetes were found in this locality. The high percentage (58.8%) of echinoderms in the area of Cape Ostrovok Falshyvyi is probably due to the presence of less silty sand with an admixture of shell debris.

The most likely factors determining the distribution of soft-bottom benthos is the type of sediments and depth/temperature. All macrobenthic fauna exhibited an aggregated microdistribution. Calcaneal mats of polychaetes *S. armiger*, *Onuphis shirikishinaiensis* and ophiuroids *O. sarsi* (from several square meters to several tens of square meters) lead to the formation of aggregation of crustaceans and polychaete species in these mats.

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Spatio-temporal variations in planktonic and benthic communities in hypoxic zone of the Yangtze Estuary and East China Sea

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With increasing eutrophication and consequent algal blooms, the hypoxic zones in the Yangtze Estuary and East China Sea have spread dramatically over the last few decades. Recently much more attention has been paid to the ecosystems in these areas due to frequent jellyfish blooms. However, the role of microplankton, microbenthos and meiobenthos as well as their relationships with jellyfish bloom remains unexplored.

We analyzed the distribution and changes of pelagic and benthic diatoms, ciliates and heterotrophic microflagellates as well as meiobenthos at three representative stations (L1: 122.4° N 32.0° E, M1: 122.5° N 31.5° E, PN2: 123.0° N 30.8° E) in the Yangtze Estuary hypoxic zone in April (spring) and August (summer) 2011 (Fig. 1). Diatoms dominated the biomass of pelagic phototrophs in April, while in August the dominators were replaced by phototrophic dinoflagellates. The abundance of pelagic and benthic diatoms was very high and both in the 10^3 ind/cm^3 order of magnitude in April (Fig. 2), while the biomass of benthic diatoms was about one to two orders magnitude higher per unit volume than that of pelagic diatoms. Rapidly sinking diatoms, which were dominated by *Melosira* spp. and reached the sea floor relatively intact without being ingested by zooplankton, were very abundant in the surface 0–2 cm sediments (up to 6.7×10^5 ind/cm^3). In contrast to that of benthic diatoms, the biomass of benthic ciliates, HMF and meiobenthos was much higher in coastal region than in offshore area.

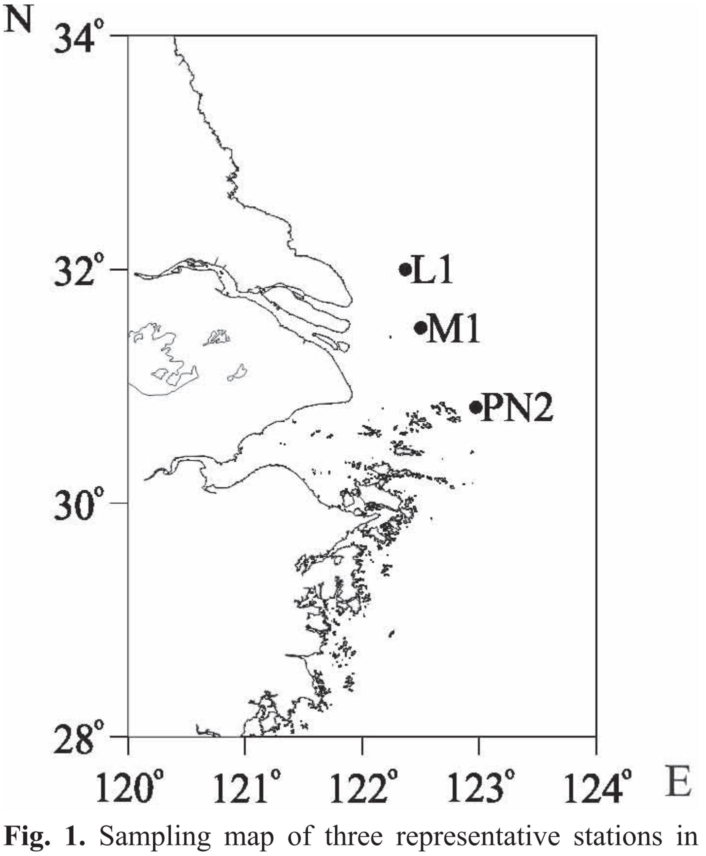

Fig. 1. Sampling map of three representative stations in the hypoxic zone of the Yangtze (Changjiang) Estuary, East China Sea.

The pelagic ciliate abundance ranged from 370 to 1780 ind/L in April, and slightly increased at the offshore station PN2 but sharply decreased at station L1 and M1 in August. We could not determine whether the decrease was due to the occurrence of jellyfish. The benthic ciliate was more abundant, about one order magnitude higher per unit volume than that of pelagic ones, and the biomass was up to two orders magnitude higher. The benthic ciliate abundance in the upper 8 cm of sediments were about 30 times higher and the biomass was 40 times higher than those in the water column of upper 30 m of the Yangtze River Estuary and East China Sea. Both, the abundance and biomass of benthic ciliate increased by 2–5 times in August compared to those in April (Fig. 3). Except that at station PN2, the species richness of pelagic ciliates was much lower in August than

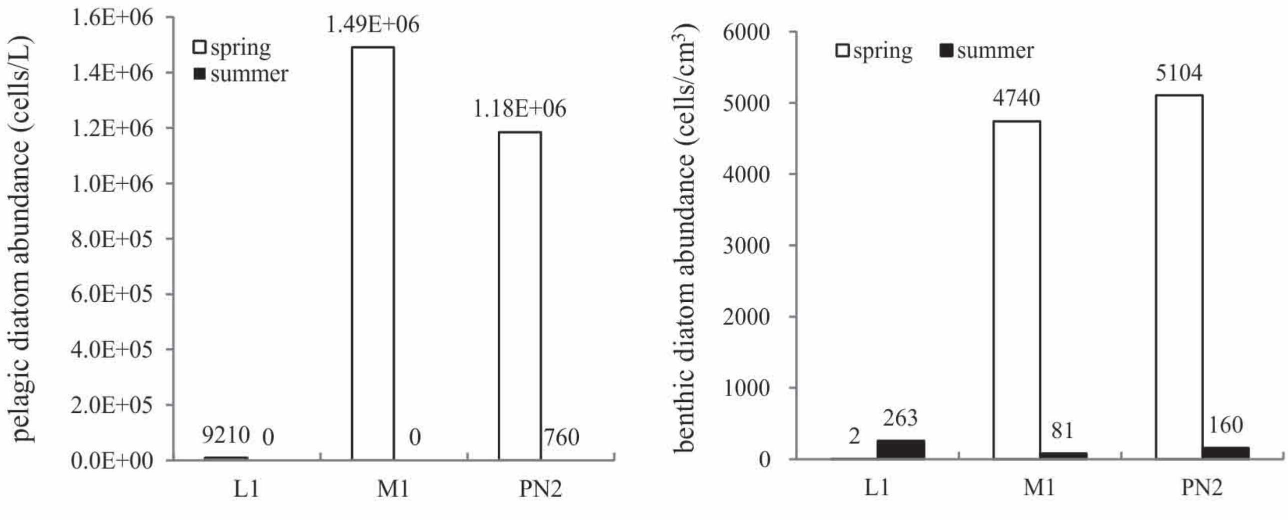

Fig. 2. Pelagic and benthic diatom abundance at stations L1, M1 and PN2 in spring (April) and summer (August).

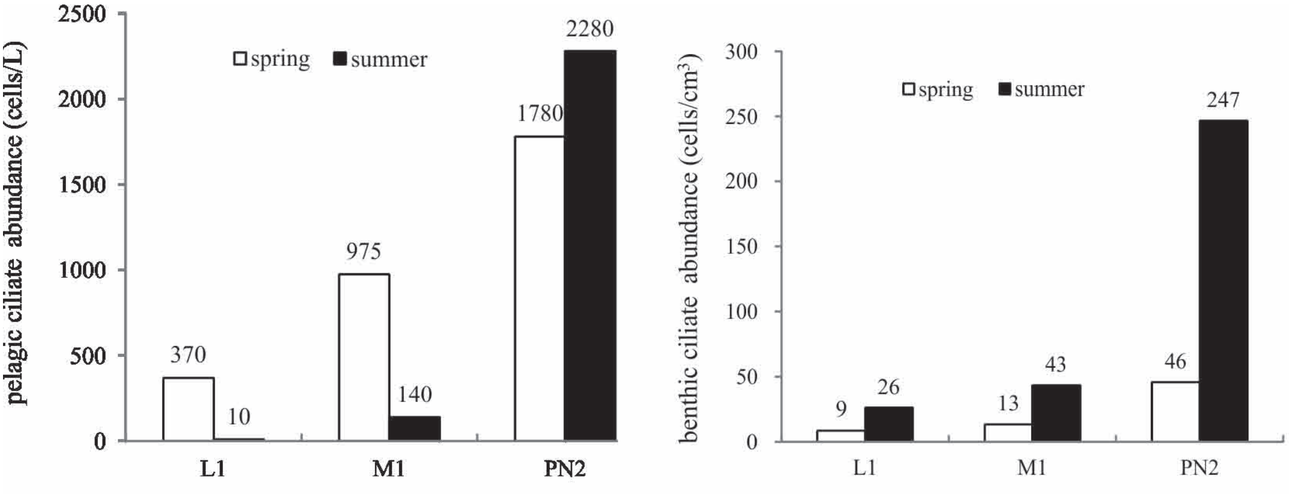

Fig. 3. Pelagic and benthic ciliate abundance at stations L1, M1 and PN2 in spring and summer.

in April, while that of benthic ciliates increased at all stations (Fig. 4). Both the morphological (Ludox-QPS) method and DGGE technique detected the highest diversity of benthic ciliates at station PN2 and the lowest diversity at station M1.

Heterotrophic microflagellates were hardly detected in the pelagic ecosystem, but were very abundant in sediments with abundance exceeding that of the total benthic ciliates and meiobenthos. Like ciliates, meiobenthos standing crops were much higher in August than in April (Fig. 5). Although the biomass of ciliates was lower than that of meiobenthos, their production was up to 3 times higher than that of meiobenthos. The relative turnover rate of heterotrophic microflagellates might even be much higher due to their small size.

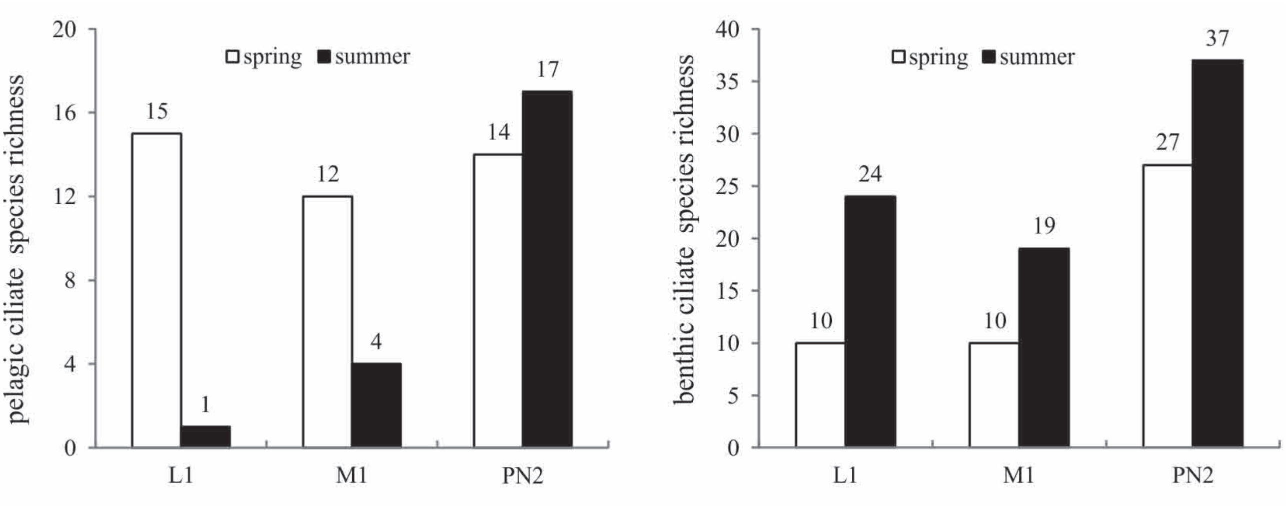

Fig. 4. Pelagic and benthic ciliate species richness at stations L1, M1 and PN2 in spring and summer.

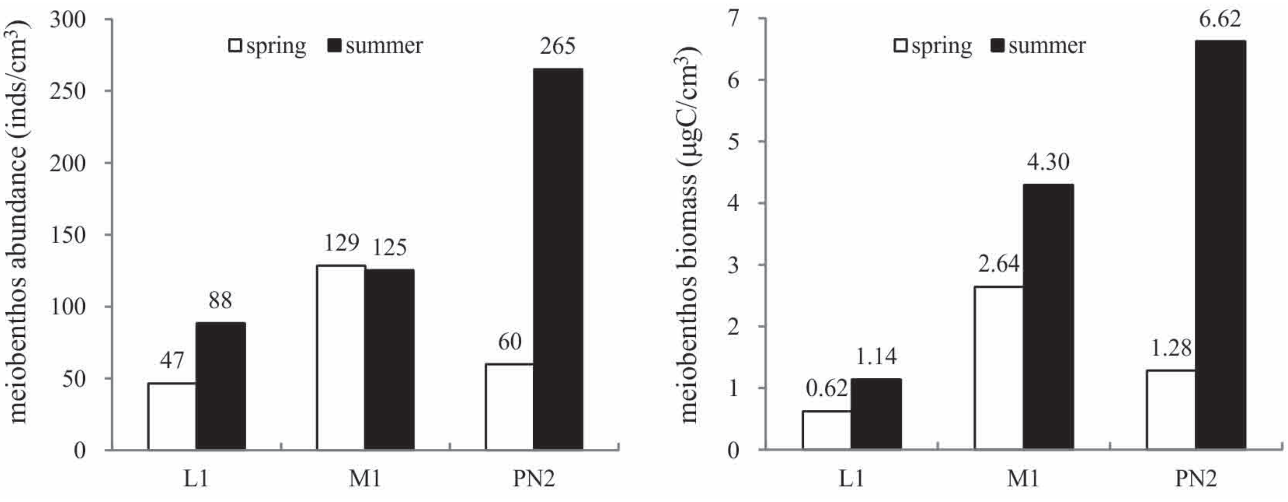

Fig. 5. Meiobenthos abundance and biomass at stations L1, M1 and PN2 in spring and summer.

These results underscore the quantitative importance of benthic versus planktonic communities and show that the ecosystem in the Yangtze Estuary hypoxic zone can be dominated by benthic phototrophs, protozoa and meiofauna despite of low oxygen levels in summer. Such an environment might provide a constant food supply for the polyp stage of jellyfish and create ideal conditions for jellyfish to flourish.

Acknowledgements

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The study of deep-sea meiofauna in north-western part of the Sea of Japan

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The past decades have shown a significant increase in the knowledge of the distribution of deep-sea meiofauna. Most of these studies, however, have focused on a few areas of North and Central Atlantic Ocean, Western Mediterranean, the Arctic and the Weddell Sea. As for the Russian sector of the Sea of Japan, no study on deep-sea meiofauna has been carried out.

The purpose of the investigation was: (1) to document the composition, abundance and structure of the meiofauna in selected area; (2) to describe the distribution of meiobenthos density depending on sediment structure and some environmental variables.

Materials and Methods

Samples were collected from 11 August to 5 September 2010 during the SoJaBio expedition in the Sea of Japan (RV “Akademik M.V. Lavrentyev”, Russian Academy of Science). Sampling stations are shown in Fig. 1. The samples were taken along four transects (A, B, C, D). The transect A includes four stations (A1, A2, A6, A7) (Fig. 1). Water depths ranged between 450 to 3370 m. The transect B includes five stations (B1, B4, B5, B6, B7), water depths ranged between 495 to 3666 m. The transect C includes only two stations (Fig. 1) with water depth from 2700 to 3426 m, and transect D was most southern with two stations D1 and D2, water depths ranged between 2698 to 3358 m.

The sediment samples were collected using with a multicorer Barnett. For the meiofauna analyses the overlying water and the first 5 cm of sediment were taken. Each cylinder has an inner diameter of 10 cm, consequently a surface of 78.54 cm^2 and a height of 5 cm result in a volume of 392.7 cm^3. In total, 164 samples of meiofauna were taken during the SoJaBio expedition.

For the meiobenthic studies, the upper 5 cm sediment layer from cores was fixed with approximately 5% formalin. All samples underwent further processing upon return to the laboratory. Sediment samples were sieved through sieves with 1000 µm and 40 µm mesh sizes. Meiofaunal animals (only metazoan) were identified and counted to higher taxa under a microscope after staining with Rose Bengal.

Grain size analysis was carried out by combination of two methods. First of all, the sediments were dried in the oven at 105°C for 24 h and weigh it. The sediment particles >0.5 mm were sieved on a series of standard sieves, meshes of which decrease at regular size intervals. The fractions <0.5 mm were present as water suspension and were analyzed using a laser particle size analyzer Analysette 22 (FRITSCH firm). Finally, sediment fractions are normalized to 100% of the initial total amount of the sediment sample.

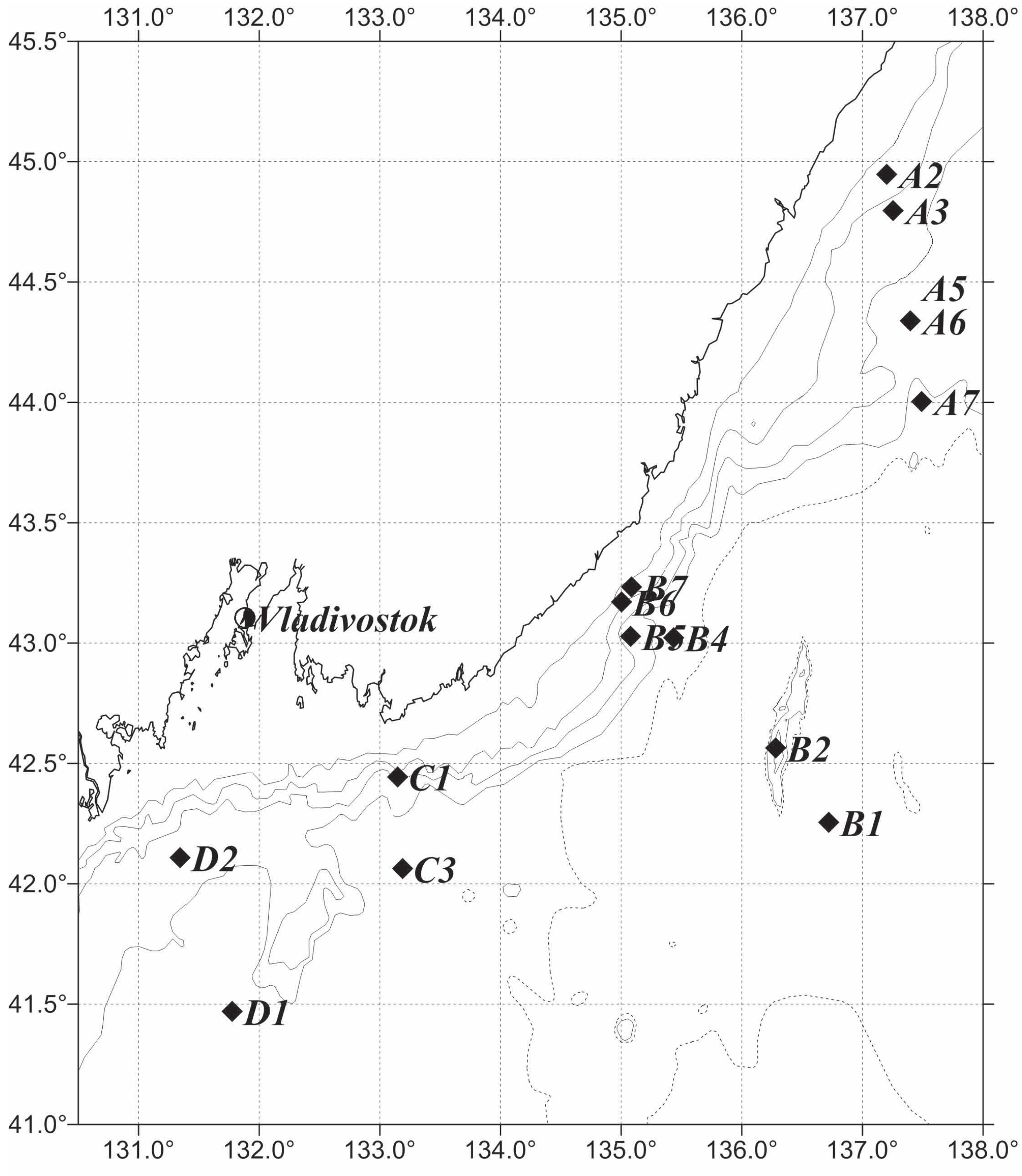

Fig. 1. A schematic map of sampling area and station locations in north-western part of Sea of Japan.

Results

The sediments in the north-western part of the Sea of Japan were presented by the mixed silts. Grain size was characterised by the dominance of the silt-clay fraction at most investigated stations.

Transect "A". The shallowest station A2 (depth 459–488 m) was characterized by silted mixed sand sediments with an admixture of clay. Median particle diameter ranged from 0.24 mm at 459-m depth to 0.005 mm at 3367-m depth. The organic matter content in deposits was the lowest – 0.9%.

The average density of meiofauna at station A2 was 125.75±40.1 ind/10 cm^2. The taxonomic composition of meiobenthos consisted of 18 groups (a class, order) (Fig. 2a). Nematodes were the numerically dominant taxon representing 55.9% of the total meiofauna abundance. Harpacticoid copepods were the second most abundant taxon, representing at most 13% of total abundance. Polychaetes dominated in pseudomeiobenthos with 14.5% (Fig. 2a).

At station A3 (depth 1584 m) the sediment has been presented by the mixed silt. The organic matter content was high enough. A station was characterized by much lower densities of meiobenthos (80.43±6.54 ind/10 cm^2) compared to the previous station. A total of 18 different groups were found (Fig. 2a). Nematodes were the most abundant and their contribution to total meiofaunal density made 69.5%, the second taxon were Harpacticoids accounted for 12% of total density. Polychaetes dominated in pseudomeiobenthos – 6.7%.

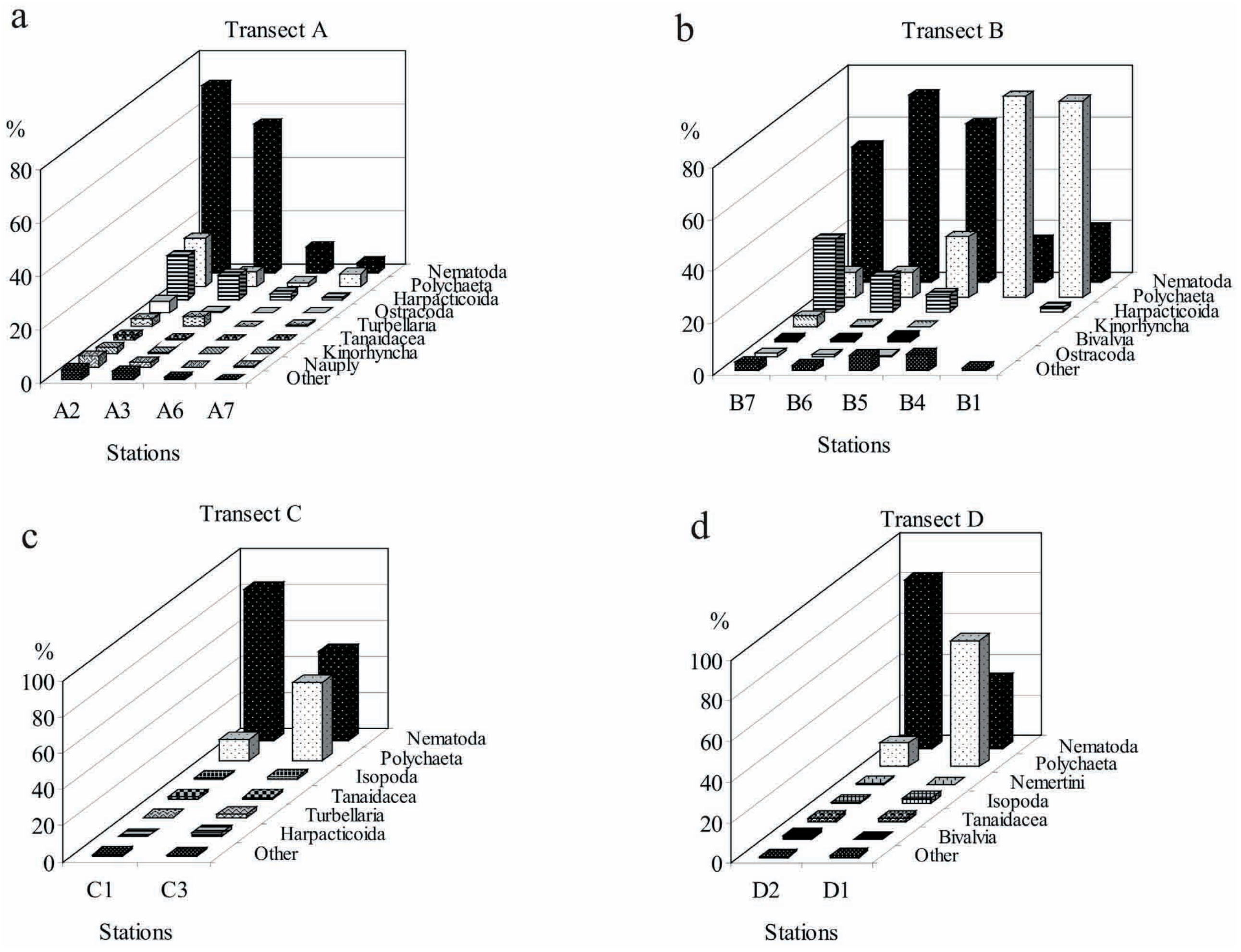

Fig. 2. The percentage of major meiobenthic groups at transects A, B, C, and D. **a**. Transects A. Other groups: Halacarida, Hydrozoa, Amphipoda, Cumacea, Gastropoda, Ophiuroidea, Sipuncula, Tanaidacea, Priapulida and Asteroidea. **b**. Transects B. Other groups: Ostracoda, Turbellaria, Hydrozoa, Amphipoda, Cumacea, Gastropoda, Ophiuroidea, Isopoda, Priopulida, Sipuncula, Nemertini, Echinoidea and Asteroidea. **c**. Transects C. Other groups: Ostracoda, Bivalvia, Amphipoda, Cumacea, Ophiuroidea and Nemertini. **d**. Transects C. Other groups: Harpacticoida, Amphipoda, Cumacea and Ophiuroidea.

At the station A6 (depth 2511m) the average density of meiobenthos has strongly decreased (14.5±5.8 ind/10 cm^2). Taxonomic meiobenthos structure was significantly reduced and consisted of 8 groups. The structure of meiofaunal assemblage is illustrated in Fig. 2a. Dominated nematodes – 66.8%, Harpacticoids have made 15.9% from total density of meiobenthos. In pseudomeiobenthos dominated Polychaeta – 10.3%. The sediments from the deepest-water station A7 (depth 3367 m) were represented by heterogenous silt. Meiobenthos abundance was lowest at the station A7 and has made 10.55±2.5 ind/10 cm^2. Taxonomic structure was poor and consisted of only 6 groups (Fig. 2a). Dominated nematodes 36.9%, other taxa were, in order of importance, harpacticoids, nauplii, turbellarians. Polychaetes were the dominant group in pseudomeiobenthos (45.7%).

Transect "B". The shallowest station B7 (depth 483–495 m) was characterized by silted mixed sand sediments with an admixture of clay. The average density of meiofauna at station B7 was 177.42±14.3 ind/10 cm^2. The taxonomic composition of meiobenthos consisted of 19 groups. The structure of meiofaunal assemblage is illustrated in Fig. 2b. Nematodes were the numerically dominant taxon representing 52.5% of the total meiofauna abundance. Harpacticoid copepods were the second most abundant taxon, representing at most 28.2% of total abundance, in pseudomeiobenthos dominated polychaetes – 9.62%.

The station B6 (depth 1040 m) was characterized by silt sediments with an admixture of sand and clay. The average density of meiobenthos has made 103.02±11.1 ind/10 cm^2. A total of 18 major groups were found (Fig. 2b). Nematodes were the numerically dominant taxon representing 72.3% of the total meiofauna abundance, harpacticoid copepods have made – 13.8%, in pseudomeiobenthos dominated Polychaetes – 9.7%. At the station B5 (depth 2666 m) the sediment has been presented by the mixed silt with clay. The average density of meiobenthos has strongly decreased and made 28.42±4.55 ind/10 cm^2. The taxonomic composition of meiobenthos consisted of 18 groups (Fig. 2b). Dominated nematodes 61.3%, the second group in density were harpacticoid copepods – 6.9%, in pseudomeiobenthos prevailed Polychaeta constituting of 23.4% of the total abundance. At the station B4 (depth 3381 m) the sediment was close in structure to the sediment from the station B5. The organic matter content in a sediment was the highest – 2.54%. The average density of meiobenthos was low and made 8.82±0.80 ind/10 cm^2. Taxonomic meiobenthos structure was poor and totaled only 6 groups (Fig. 2b). Dominated polychaetes 77.4%, in eumeiobenthos dominated nematodes 15.9%. At the deepest-water station B1 (depth 3666m) the sediment has been presented by the mixed silt with an impurity of clay. The average density of meiobenthos was the lowest and has made 4.80±1.17 ind/10 cm^2. Taxonomic meiobenthos structure was poor and totaled only 6 groups (Fig. 2b). Dominated polychaetes – 75.8%, nematodes have made 21%.

Transect "C". At the station C1 (depth 2781m) a sediment has been presented by the mixed silt with an impurity of sand and clay. The average density of meiobenthos has made 42.73±7.54 ind/10 cm^2. A total of 11 different meiofaunal taxa were found (Fig. 2c). Nematodes were the numerical dominant, representing 84.1% of the total abundance, polychaetes were the second most abundant taxon, representing at most 11.8% of total abundance. At the station C3, which is located deeper (depth 3426 m), the sediment has been presented by the mixed silt with an impurity of clay. The concentration of organic matter was higher, than at the station C1 and has made 2.28%. The average density of meiobenthos was low: 14.66±2.86 ind/10 cm^2. Taxonomic structure of meiobenthos totaled 10 groups (Fig. 2c). Dominated nematodes 49.6%, and polychaetes 43%.

Transect "D". At the station D2 (depth 2698 m) the sediment has been presented by the mixed silt with an impurity of sand and clay. The average density of meiobenthos has made 21.24±1.60 ind/10 cm^2. The structure of meiofaunal community is illustrated in Fig. 2d. Taxonomic structure of meiobenthos totaled 10 groups. Dominated polychaetes 61.9%, nematodes has made 33.3%. At the deeper-water station D1 (depth 3358 m) the sediment has been presented by the mixed silt with an impurity of clay. The average density of meiobenthos was low and has made 12.89±2.51 ind/10 cm^2. Taxonomic meiobenthos structure totaled 11 groups (Fig. 2d). Dominated nematodes 83%, polychaetes have made 12%.

Discussion

Taxonomic meiobenthos structure is the plentiful in the whole area. Overall at four locations in the northwestern part of the Sea of Japan, 20 taxa of meiofauna were encountered. At the depths to 1500 m 18 to 19 taxons are detected. With increase in depth the number of groups was considerably reduced. On the sampling locations with the depth over 3360 m 6 groups only were found. The meiobenthic communities in the north-western part of the Sea of Japan appears to be influenced by water depth. Meiofaunal densities in studied locations of the northwestern part of Sea of Japan were very low. Our data is comparable with the data from Eastern Mediterranean only (Danovaro et al., 1995 a, b, 2008). Total meiofaunal density was low and decreases with the depth increase**.** As it is known, with the depth increase, various environment factors change too, such as sediment structure, the organic carbon content in sediment, salinity, the oxygen concentration in a benthonic layer of water, etc. One of the environment factors, influencing structure and distribution of the deep-water meiofauna, is granulometric structure of sediments (Eckman, 1983; Grassle, Maciolek, 1992).

With increase in depth the quantity of particles of the small size in sediment increased in our study. The correlation analysis has revealed dependence between total meiobenthos density and nematode density with the average size of particles (Spearman rank correlation coefficient has made 0.60±0.09, $p<0.001$ and 0.59±0.07, $p=0.005$ accordingly).

However sediment structure and heterogeneity could not be invoked to explain large differences in the distribution of meiofaunal assemblages (Gambi, Danovaro, 2006). The structure and distribution of organisms depends not only on sediment structure, but also on the organic carbon content (Vincx et al., 1994; Flach et al., 2002). In the present research the high concentration of organic carbon has been noted in the sediments. In the present research the high concentration of organic carbon has been noted in the sediments (0.9–2.54%). The slight negative correlation was detected between the density of meiobenthos and nematode and the concentration of organic carbon in the sediments (Spearman rank correlation coefficient is equal – 0.48±0.01, $p=0.05$ and – 0.41±0.09, $p=0.015$ accordingly). Such correlation between meiobenthos density and organic carbon content shows that deep-water meiofauna, living in extreme oligotrophic environment, is more dependent on quality, than quantity of organic matter (Danovaro et al., 1995a; Shirayama, Kojima, 1994; Gambi, Danovaro, 2006.)

Acknowledgements

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Long-term monitoring of phytoplankton in coastal waters off Vladivostok City

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Amursky Bay is the second order bay in the north-western part of the Sea of Japan. It is located in the north-western part of Peter the Great Bay. There are two main sources of nutrients into Amursky Bay; these are Vladivostok industrial and municipal waste waters and runoff of Razdolnaya River. Annual river-runoff is about 2.5 km^3, this is one of the most important stressor for the coastal phytoplankton (Vaschenko, 2000; Grigoryeva, 2008). The coastal waters of Vladivostok are characterized by high concentrations of nitrates and nitrites, as well as great phytoplankton primary production, the eutrophic level of increased from the early 1980s (Tkalin et al., 1993). Studies of phytoplankton in Amursky Bay were initiated in the 1920s (Skvortzow, 1931; Kisselew, 1934, 1935). Essential information regarding phytoplankton species composition in this area has been published by Konovalova (1972), Stonik, Orlova (2002), Shevchenko et al. (2004), Orlova et al. (2009).

The aim of this study was to determine long-term changes in phytoplankton community composition in coastal waters off Vladivostok during 1991–2011.

Materials and Methods

Phytoplankton samples were collected at the long-term monitoring station (43º11′58″ N, 131º55′09″ E) 2–4 times per month from June until October 1991, in August and October 1992, from January until December 1993, as well as from January 1996 until May 1998, from May 1999 until April 2000 and from October 2004 until December 2011. Water was sampled from a depth of 0.5 m using 4 L Niskin bottle. Water samples were preserved in a Lugol's solution with sodium acetate and buffered formaldehyde. Phytoplankton samples were concentrated following the sedimentation method of Utermöhl (1958) or by a reverse filtration, using a 2 µm Nuclepore membrane as described by Sukhanova (1983). The routine observation and identification of microalgae cells were made using an Olympus BX 41 light microscope, operating with brightfield optics.

Results and Discussion

During the monitoring period 1991–2011 the overall composition of phytoplankton has changed. Six dominant phytoplankton groups were found on the monitoring station in Amursky Bay (see figure). The winter community was relatively stable. Diatoms belonging to the genus *Thalassiosira* and *Chaetoceros* frequently been observed to be abundant during winter in both the past (Konovalova, 1972) and the present study. After the initial outburst of diatoms, different phytoplankton groups have been major component to the total density. For instance, in 1996 and 2010 spring phytoplankton peak was formed Raphydophytes, in 2008 major component of spring phytoplankton was Cryptophytes, in 2011 the greatest values of microalgal densities were formed Chlorophytes and Euglenophytes. Diatoms and Dinoflagellates have been the major phytoplankton groups in the summer 1990s. The occurrence and density of the dominant species have varied during the summer monitoring period. *Skeletonema costatum* complex, *Chaetoceros curvisetus* and *Thalassiosira mala* were found in high density during 1991–1998. The first record of the dinoflagellate *Prorocentrum minimum* in Amursky Bay is from 1991 (Stonik, 1994). *Dactyliosolen fragilissimus, Thalassionema nitzschioides, Chaetoceros salsugineus* were abundant in 2000s. The diatom *S. costatum* complex, an indicator of organic pollution (Yamada et al., 1980), accounted up to 95% of the total phytoplankton density ($12.7*10^6$ cells/L) during the summer bloom period in 1996. In the 2000s, a tendency has arisen for a decrease in density of *S. costatum* complex*,* compared with the 1996. During the summer in 2010, 2011 *S. costatum* complex was not dominated in phytoplankton of Amursky Bay. The long-term, declining trend in *Skeletonema* abundance during the past 30 years was observed in Narragansett Bay, USA. Authors proved that changes in weather patterns were closely associated with long-term pattern of *Skeletonema* abundance and annual pattern (Borkman, Smayda, 2009). Besides of Diatoms and Dinoflagellates, flagellates have been dominant group of phytoplankton in the summer 2000s. Euglenophytes formed peak of phytoplankton density in 2005, Raphydophytes pronounced of the phytoplankton bloom in 2010 (see figure). It's well known that significant increase in the density of the flagellate component of phytoplankton has been observed in other eutrophic waters. The monitoring data on phytoplankton composition and long-term data on dominant species shown changes that are more complicated to correlate with the climate change combined with human activities, primarily through nutrient runoff.

In summary, following long-term trends in the phytoplankton composition of Amursky Bay were: 1) total density of phytoplankton increased from 1990s to 2000s; 2) the density of the diatom *S. costatum* complex decreased from 1990s to 2000s; 3) the density of the flagellates component of the phytoplankton increased since 2000s; 4. the new bloom-forming species were observed.

Acknowledgments

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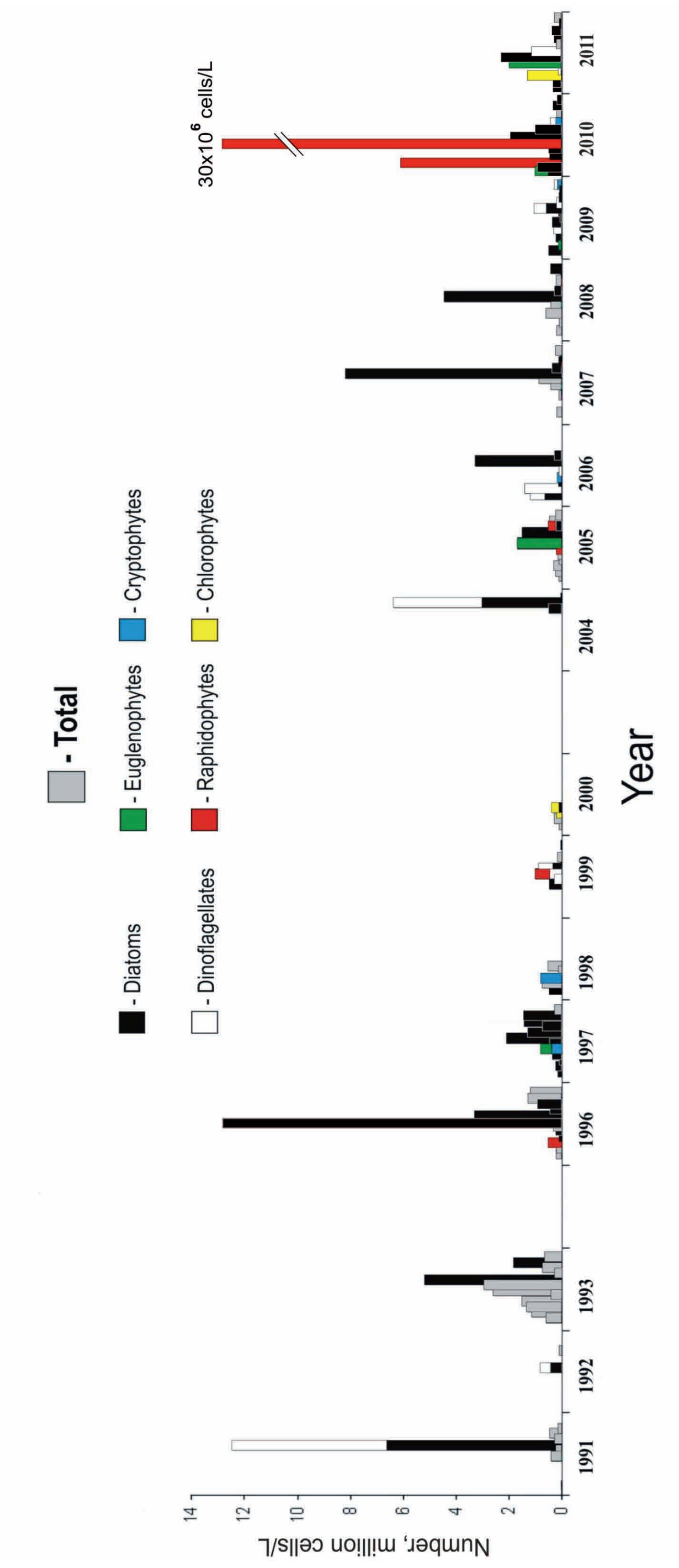

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Resting stages of planktonic microalgae in recent marine sediments from the east coast of Russia with special emphasis on toxic species

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Reliance on a resting stage to withstand periods of unfavorable environmental conditions is a well-known life history strategy in planktonic microalgae. During the last four decades, resting stages from recent marine sediments have been studied in many areas of the world: on the east coast of the United States, in Japan, Australia and New Zealand, in Europe, China, India, among other locations (Wall, Dale, 1968; Baldwin, 1987; Bolch, Hallegraeff, 1990; Qi et al., 1996). Such a broad interest was due to the importance of microalgal resting stages in coastal marine ecosystems. Resting stages have a major role in the biodiversity conservation and distribution of microalgal species, provide an “inoculum” for annual seasonal outbreaks of phytoplankton, and initiate toxic “red tides”. Surveys of resting stages can provide an early warning of the presence and abundance of toxic species in a body of water and elucidate the mechanisms responsible for the appearance of toxic algal blooms, among them the spore transport via ship ballast waters and/or currents. Microalgal cysts and spores are widely used as bioindicators of the hydrological regime, ecological features, and the development or presence of utrophication in marine environments (Dale et al., 1999). The resting stage survey reported here was prompted by questions about harmful algal blooms (HABs) previously recorded on the Russian Pacific coast (Konovalova, 1999). Recent investigations of HAB events on the east coast of Russia have shown an increase in species diversity and expansion of potentially toxic species over the last several decades (Orlova et al., 1998, 2008; Konovalova, 1999).

Materials and Methods

Surface sediment samples were collected between September 1999 and October 2011 from 54 stations along the Russian Pacific coast. Samples were collected by divers using glass or aluminum coring tubes, or by grab. Samples were stored in the dark at 4°C. A total of 181 samples were analyzed and used for germination experiments. Sediment processing generally followed the traditional methods for study dinoflagellate cysts (Anderson et al., 1982; Matsuoka et al., 1989). The total numbers of resting cells were counted in Sedgwick–Rafter counting chambers using an Olympus BX 40 (Tokyo, Japan) light microscope (LM) at a total magnification of 200. Only live cells were counted. Individual cysts were used for germination experiments, in order to identify the species characteristics of both cyst and motile stages.

Results and Discussion

A total of sixty-one morphological forms of resting stages represented by dinoflagellate and raphidophyte cysts and diatom spores and resting cells were recorded in the sediment samples. Cysts and resting cells of eight potentially toxic species and noxious bloom-forming species we found in surface sediments in the study area: *Alexandrium tamarense*, *Alexandrium* cf. *minutum*, *Alexandrium* sp., *Cochlodinium* cf. *polykrikoides*, *Gymnodinium* cf. *catenatum*, *P. reticulatum*, *Heterosigma* cf. *akashiwo* and *Pseudo-nitzschia* sp. Their total density varied from 0 to 5994 cells/cm^3.

Dinoflagellates of the genera *Alexandrium* and *Gymnodinium* are well-known producers of paralytic toxins (saxitoxin and its analogues), and *Protoceratium reticulatum* is a yessotoxin producing species. Diatoms of the genus *Pseudo-nitzschia* are producers of domoic acid, which causes amnesic shellfish poisoning of humans, which disturbs the function of the central nervous system. Cysts of toxic species of the genus *Alexandrium* were widespread in the surface sediments of the investigated area (the Bering Sea, Kamchatka, Sakhalin Island and Primorye) their total density varied from 0 to 5994 cells/cm^3. *Gymnodinium* cf. *catenatum* was found only in south Primorye. According to Matsuoka and Fukuyo (1994), both cysts and motile forms of *G. catenatum* are widely distributed in the plankton and surface sediments of the Japanese coast, including along the west coast of the Sea of Japan, where the maximum surface water temperature is above 25°C. The coastal waters of south Primorye are characterized by summer surface water temperatures above 28°C. The presence of *G. catenatum* in the plankton of the study area is doubtful. It is quite possible that cysts observed in recent sediments are actually fossilized cysts. Cysts of potentially toxic species *P. reticulatum* (a yessotoxin producing species) were widespread in the surface sediments of the investigated area (the Bering Sea, Kamchatka, Sakhalin Island and Primorye). Their total density was not exceeds 1200 cells/cm^3. Resting cells of the diatom *Pseudo-nitzschia* sp. were found only in Peter the Great Bay (Primorye) with densities from 10 to 44 cells/cm^3. Particular attention in the study of surface sediments was given to resting stages of noxious bloom-forming microalgae.

Raphidophytes belonging to the genus *Heterosigma* produce ichthyotoxins, cytotoxins, and neurotoxins; the blooms of these species have caused mass mortalities in cultured fish and have inflicted serious damage to aquaculture farms in Southeast Asia and Canada. Cyst density of the raphidophyte *Heterosigma* cf. *akashiwo* was dominant in Primorye coastal waters (4676 cells/cm^3). Our results are in agreement with phytoplankton survey data.

Cysts of *Cochlodinium* cf. *polykrikoides* occurred at three study locations (Peter the Great Bay, Aniva Bay, Avachinskaya Guba Inlet) but were not numerous. Blooms associated with the abundant development of this dinoflagellate have caused mass mortalities in cultivated fish and mollusks in coastal waters and have inflicted tremendous annual damage to aquaculture farms.

Data on composition and distribution of resting stages showed that potentially toxic species were found at all study locations over a much wider area than was previously known from planktonic observations. These results suggest that additional cyst surveys should be conducted in areas of the eastern Russian coastline not yet investigated, and that the potential for PSP toxicity as a result of blooms of toxic species may be more widespread than previously documented.

Conclusions and Summary

Data on the qualitative and quantitative composition of resting stages of planktonic microalgae in recent marine sediments collected from 54 stations along the eastern coast of Russia over the period 1999–2011 are presented. A total of sixty one morphological forms of resting stages represented by dinoflagellate and raphidophyte cysts and diatom spores and resting cells were recorded in the sediment samples. This study revealed cysts of the potentially toxic species *Alexandrium tamarense*, *A.* cf. *minutum*, *Alexandrium* sp., *Gymnodinium catenatum* (PSP toxin producers), and *Protoceratium reticulatum* (yessotoxin producer); resting cells of *Pseudo-nitzschia* sp.(potential producer of domoic acid) and cysts of bloom-forming species *Cochlodinium* cf. *polykrikoides* and *Heterosigma* cf. *akashiwo.* Ellipsoidal *Alexandrium tamarense* type cysts were widely distributed and dominated many localities in the study area. These data suggest that additional cyst surveys should be conducted in areas of the eastern Russian coastline not yet investigated, and that the potential for paralytic shellfish poisoning toxicity as a result of blooms of toxic species may be more widespread than previously documented.

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Spatial and temporal change of the size fractionated phytoplankton in the Yellow Sea and East China Sea

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Chlorophyll *a*, size fractionated chlorophyll *a* and primary productivity were studied during two expeditions in the Yellow and East China Seas during the spring and summer of 2011 in order to assess their spatial and seasonal variability as well as controlling factors.

Horizontal distribution patterns of chlorophyll *a* size structure in the Yellow and East China Seas in the spring of 2011 are shown in Fig. 1. The concentrations of micro-, nano- and pico-fractionated chlorophyll *a* at the sea surface were 0–4.36, 0.02–2.27, 0–2.66 mg/m^3, which gave chlorophyll *a* levels of 55.4%, 30.8% and 13.8%. The micro- and nano-phytoplankton dominated.

The horizontal distribution patterns of chlorophyll *a* at the 10 m, 30 m and bottom layers were similar to that of the surface, but the concentrations were slightly lower. The highest value areas for the different water depths of micro-phytoplankton distribution were in the center of Yellow Sea and outside of the Changjiang River estuary. The highest value areas for nano- and pico-phytoplankton were in the west of the South Yellow Sea and southeast of the Changjiang River estuary.

Horizontal distribution patterns of chlorophyll *a* size structure in the Yellow and East China Seas in the summer are shown in Fig. 2. The fractionated chlorophyll *a* concentration from large to small in the sea surface ranged from 0–6.78, 0–2.59, 0–0.86mg/m^3, respectively. The average chlorophyll *a* concentrations were 0.50, 0.24 and 0.07 mg/m^3, contributing to the chlorophyll *a* at 61.8%, 30.1% and 8.1%. The micro- and nano-phytoplankton still dominated.

The horizontal distribution pattern of chlorophyll *a* concentrations at the 10 m layer was similar to that of the surface. The Changjiang River Estuary and adjacent waters were dominated by micro-phytoplankton, while the highest value areas for nano- and pico-phytoplankton were mainly in the south Yellow Sea, especially in coastal waters. Although the concentration of total chlorophyll *a* in the southeast of the surveyed area was not high, it was mainly contributed to by nano- and pico-phytoplankton. The concentration of fractionated chlorophyll *a* at the 30 m layer and the bottom layer was significantly lower than that at the surface and subsurface, but the contribution of nano- and pico-phytoplankton was significantly higher than at the surface.

The changes in temperatures, salinities and nutrients affected by different water masses in spring and summer were the major factors leading to changes in the phytoplankton size structure in the Yellow and East China seas.

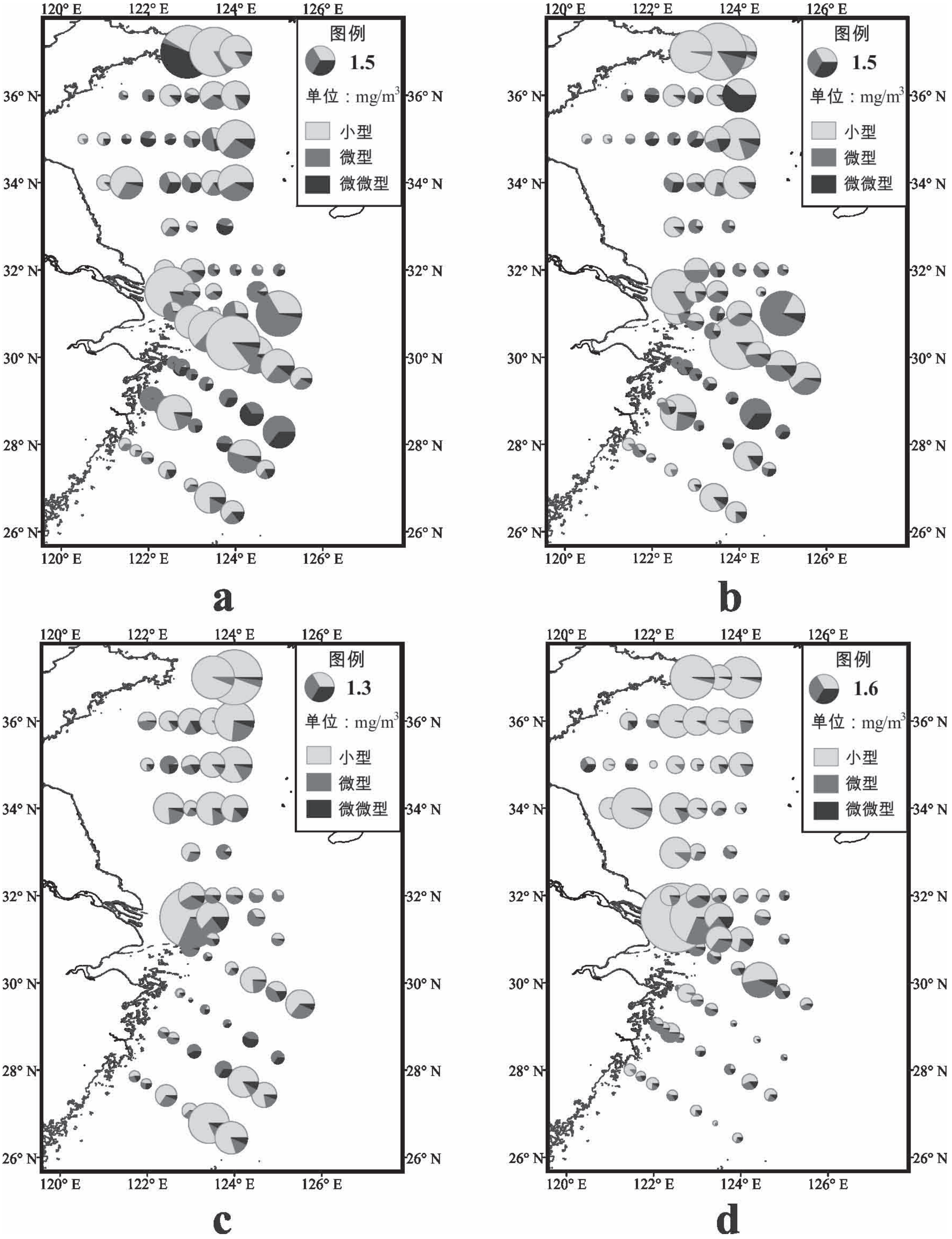

Fig. 1. Spatial pattern of the chl *a* size structure in the Yellow and the East China seas in the spring of 2011: (**a**) surface, (**b**) 10 m layer, (**c**) 30 m layer, (**d**) bottom.

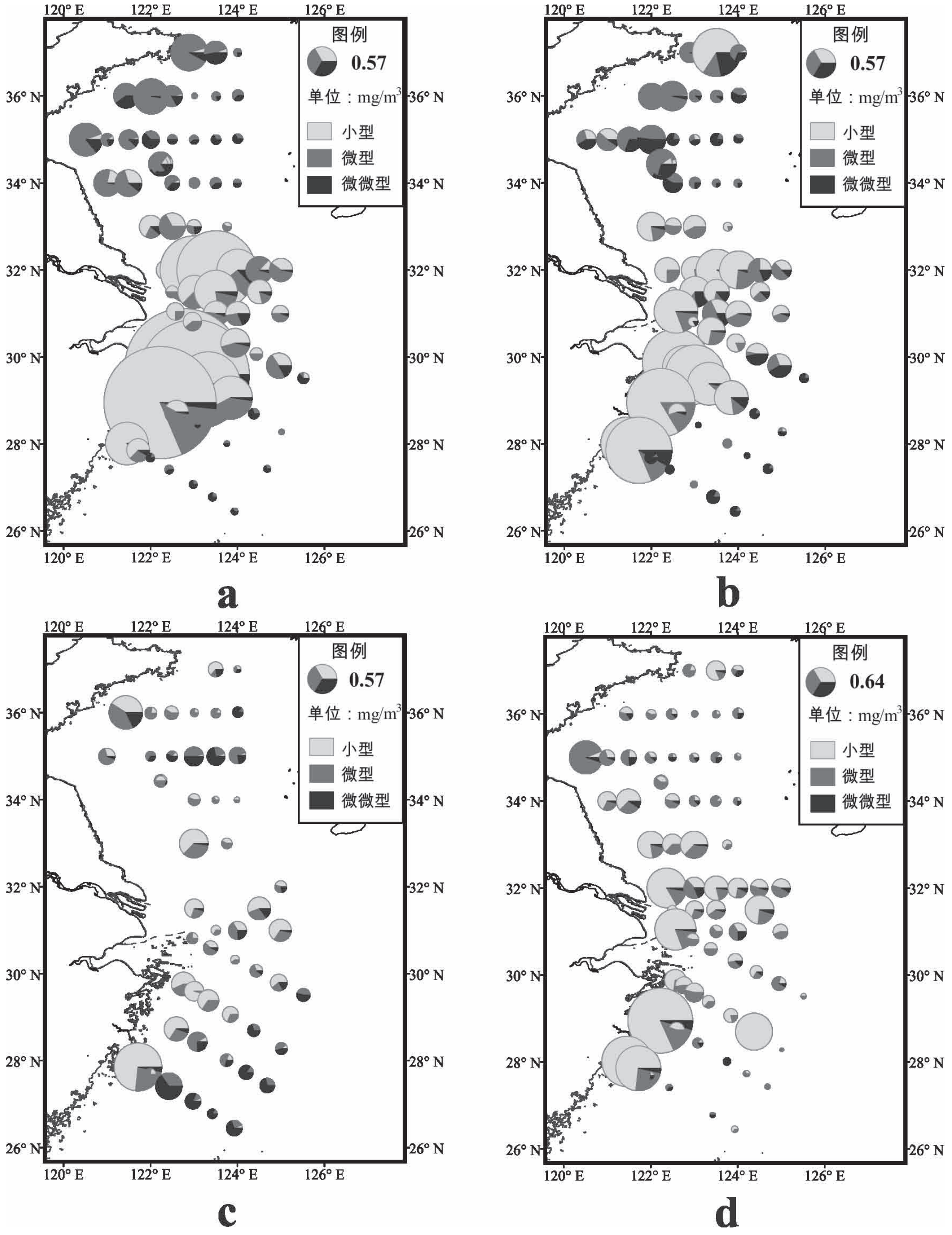

Fig. 2. Spatial pattern of the chl *a* size structure in the Yellow and the East China Seas in the summer of 2011: (**a**) surface, (**b**) 10 m layer, (**c**) 30 m layer, (**d**) bottom.

Intertidal macrophytes on the Sea of Okhotsk coast of Shikotan Island (southern Kuril Islands)

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Kuril Islands, Russia's largest archipelago, are situated in seismically active region of World Ocean. Kuril Islands bound between Okhotsk Sea and Pacific Ocean. This archipelago is composed of two ridges. Lesser Kuril Ridge stretches at 120 km southeastward of Greater Kuril Ridge. It consists of six islands and two groups of minor rocks. Shikotan is the largest island of Lesser Kuril Ridge (Fig. 1). There are nine bays and a lot of small creeks on its coast, because Shikotan shoreline is very incised. Due to upwelling, both cold-water and warm-water species inhabit this area. Being zone of the great biological productivity, shallow waters of Southern Kuril Islands attracts the attention of many investigators.

The littoral, or intertidal zone, is permanently influenced by air and water environments. Due to there are the most daily and seasonal variation of temperature, salinity and others factors on the littoral (Kussakin, 1977). Hydrological regime of shallow waters of Shikotan is abruptly changed with season. In summer island's seashore has filled by strongly warmed and salty masses of Sea of Japan, translating here with Soya Current. In winter cold water masses of Oyashio Current washes the coastal area (Bobkov, 2004). Therefore a salinity of coastal waters is varied from 12–16‰ in spring up to 29–33‰ in summer (Kussakin, 1978). This blending of warm and cold currents has resulted in an abundance of marine life.

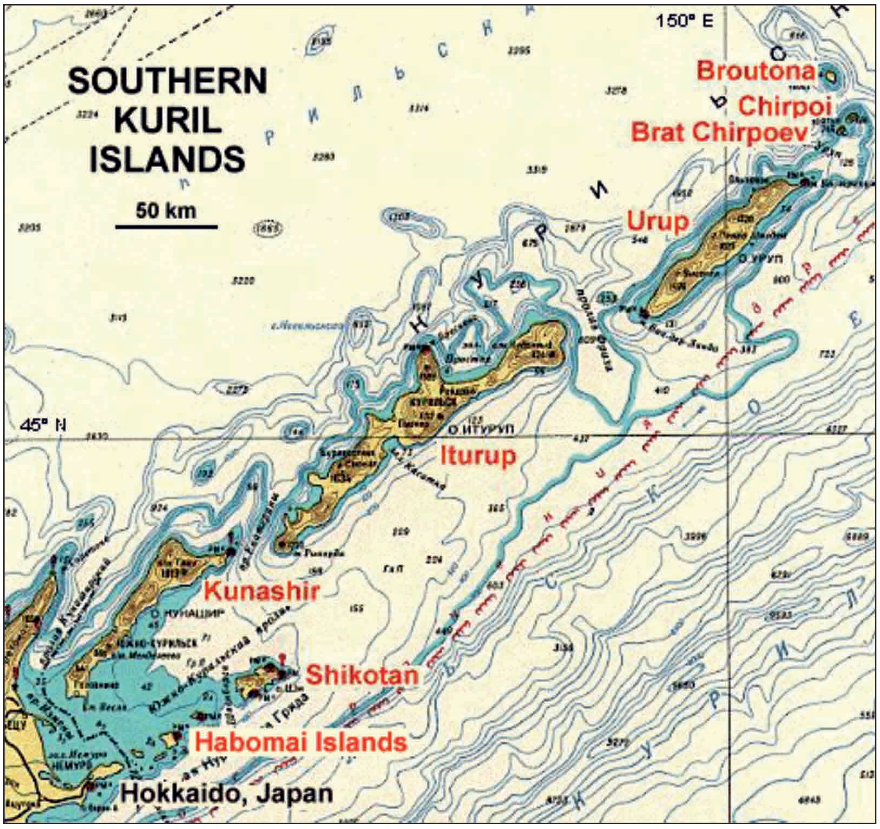

Fig. 1. The map of study area and adjacent regions.

The present study is devoted to describing and analysing of taxonomic and biogeographic composition of littoral flora on the Sea of Okhotsk coast of Shikotan Island.

Materials and Methods

We used material collected by the intertidal expedition of the Institute of Marine Biology in 1987. Macrophytes were sampled in four bays on the Sea of Okhotsk coast of Shikotan Island. There are Otradnaya Bay and Krabovaya Bay on the northwestern coast, and Galtsev Bay and Dolphin Bay on the southwestern one. Samples were collected and treated by the methods according to the standard procedure of chorological researches in the littolal (Kussakin et al., 1974). Phycological material was collected on the littoral fringe during low tides and herborized.

Material was sectioned freehand with razor blades, placed in a drop of fresh water on the slides and examined using the light microscope. The sections were studied uncoloured or stained with Lugol's solution or aniline dyes. The processing of collections was conducted at A.V. Zhirmunsky Institute of Marine Biology (Vladivostok, Russia).

Zonal-biogeographic composition of intertidal macroflora was analysed by means of phytogeographical and biogeographical systems of Louisa P. Perestenko (Perestenko, 1982), Alexander I. Kafanov and Valery A. Kudryashov (Kafanov, Kudryashov, 2000).

Results and Discussion

As a result of examination of algal collections, we found 70 species of macroalgae and 5 species of higher plants. These are Rhodophyta, 33 species (44% of the total species number); Ochrophyta (class Phaeophyceae), 23 species (30%); Chlorophyta, 11 species (19%) and Tracheophyta, 5 species (7%). Most of species identified in the study are widely distributed in the northwestern Pacific (Guiry, Guiry, 2012). The common green algae of Shikotan are species of *Ulva*, *Acrosiphonia* and *Monostroma*. The predominant red algae are species of *Halosaccion*, *Corallina*, *Porphyra* and *Mazzaella* (Fig. 2). Brown algal species of *Alaria*, *Fucus*, *Saccharina* and *Analipus*, as well as seagrass *Phyllospadix iwatensis* are predominant or characteristic in the intertidal communities (Fig. 3). Macrophyte biomass in studied island' coast can reach 27 kg/m^2.

Zonal-geographic analysis shows that intertidal floras' composition on the northwestern coast of Shikotan is similar on a number of wide boreal and boreal-arctic species. The difference is that the low boreal species number in Krabovaya Bay is 1.5 times more than one in Otradnaya Bay. Due to water salinity variation in areas of the southwestern coast the littoral floras' composition of bays is very differed. Galtsev Bay is typical marine area. So its flora is determined by wide boreal species (50% of the total). The peculiar feature of Galtsev Bay' flora is that boreal-arctic species number is 2 times more than ones in Otradnaya and Krabovaya Bays. Zonal-geographic floras' composition of freshened Dolphin Bay is characteristical. This flora if formed by widespread species (44%), boreal species (34%) and subtopic-boreal and tropic-boreal species (22%).

Zonal-biogeographic analysis suggests the boreal character of intertidal flora of the Sea of Okhotsk Shikotan Island' coast because of the predominance of boreal (61%) and widespread (24%) species (Fig. 4). Studied flora composition is characterized by presence of many eurybiontic species of brown and green algae.

The predominance of the red algae is a characteristic feature of typical marine floras (Perestenko, 1994; Shin et al., 2008). Our study shows that red algae prevailed on the species number predominantly occur in open areas of the littoral. Brown algae and eelgrasses dominate on biomass usually formed tickets in protected marine and freshened areas of island' coast.

In previous studies of the littoral fauna of Kuril Islands it was found that geographic distribution of species correlate with summer temperature regime of coastal waters. The bulk of fauna is

Fig. 2. View of *Ulva* (left), *Halosaccion* (centre) and *Corallina* (right).

Fig. 3. View of *Saccharina* (left) и *Phyllospadix* (right).

constituted by species widely distributed in Pacific or World Ocean. The wide boreal species number is maximal for the Iturup Island fauna (54.1% on the Sea of Okhotsk coast и 62.7% on the pacific coast). One is minimal for most warm-water Kunashir Island fauna (38.4% on the Sea of Okhotsk coast). The warm-water species number appropriately decreases from southern areas (57.1% for Kunashir) to northern areas (0.8% for Simushir). The distribution of cold-water species is in controversy (Kussakin, 1976).

In a whole, biogeographic structure of littoral flora of Shikotan shows an affinity with that of Southern Kurils. It was found that in typical marine areas (Galtsev Bay, Krabovaya Bay and Otradnaya Bay) the bulk of macrophytic flora is constituted by wide boreal species of red and brown algae and multizonal species of green and brown ones. Therefore the intertidal floras of the open areas of Shikotan Island' coast are characterized by the maximal species and geographic diversity. Marine floras of freshened intertidal areas (Dolphin Bay) are least diverse and и includes much more warm-water species.

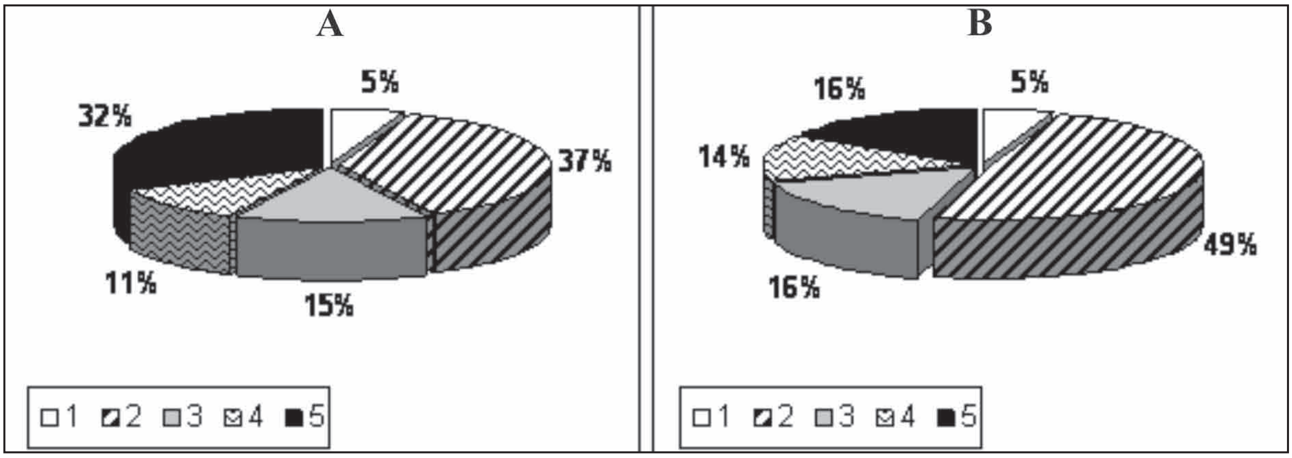

Fig. 4. Phytogeographic structure of the littoral flora of Shikotan Island: **A** – Sea of Okhotsk coast, **B** – Pacific coast. Latitude-zonal elements: 1 – wide boreal-arctic, wide boreal-arctic-notal; 2 – wide boreal, wide boreal-notal, boreal; 3 – low boreal; 4 – subtropical-boreal, boreal-tropical, wide boreal-subtropical, low boreal-tropical, low boreal-subtropical; 5 – wide boreal-arctic-subtropical, boreal-tropical-notal, boreal-subtropical-notal, boreal-arctic-tropical, multizonal.

It should be noted that inventory of the flora of study area is still not completed because of difficulties of collection of algae in this remote hard-to-reach and little-inhabited area. But its min contents are outlined and may serve as a basis for other studies on biodiversity, ecology etc.

Acknowledgements

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Meiofaunal communities on bivalve aquaculture installations in Peter the Great Bay, Sea of Japan

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Artificial substrate not only creates favorable conditions for attached forms of meiobenthos, but also promotes development of a specific associated meiofauna. For the recent time, much attention has been paid to the study of species diversity and quantitative characteristics of the meiofauna of fouling communities on anthropogenic substrates, built for settlement of bivalve mollusks, in the Sea of Japan (Galtsova, 1982; Galtsova, Pavlyuk, 1987; Belogurova, Maslennikov, 2000, 2008a, b, 2010; Maslennikov, Belogurova, 2008; Barsukova et al., 2011a, b). Studying the meiofauna is of great interest, as values of population density of its largest groups are closely related, and under changing environmental conditions ratios of their abundance can vary very fast. Some representatives of meiofauna are characterized as the most sensitive bioindicators that respond even to the smallest changes in the environment (Barsukova et al., 2011; Belogurova, 2011).

The purpose of this work is to conduct a comparative study of the taxonomic structure and quantitative characteristics of the meiofauna of fouling communities on anthropogenic substrates, built for the cultivation of bivalve mollusks in open waters of Nakhodka Bay and off Reineke Island.

Samples of meiofauna, collected from the artificial substrates of aquaculture installations in waters off Reineke Island and in Nakhodka Bay at depths of 10 to 30 m, served as a material for the analysis. Samples were processed according to the standard technique (Galtsova, 1971).

As a result, the qualitative composition and the quantitative distribution of population densities of both the entire meiofauna and its certain groups were analyzed. In the meiofauna of fouling organisms we found as many as 7 to 9 taxonomic groups. The groups registered within the eumeiofauna were foraminifers, harpacticoids, nematodes, ostracods, and halacarids. The pseudomeiofauna was represented with juvenile bivalve and gastropod mollusks, polychaetes, and amphipods (see Table).

The proportion of population density of the eumeiofauna varied from 75 to 92%, and that of pseudomeiofauna did from 8 to 25% (Fig. 1).

In Nakhodka Bay, meiofauna's mean population density on substrates, used for settlement, reached 6,528 ind./cage (see Table). Foraminifers were the predominant group in the eumeiofauna and amounted to 60% of the total meiofaunal population density. The proportion of the second densest group in the meiobenthic community – harpacticoids – was 19%. The third by density group was ostracods and the fourth nematodes, which constituted 7% and 6%, respectively. Halacarids appeared to have a low population density of 0.01%. Population density of pseudomeiofauna organisms varied

Taxonomic composition and average values of population density (individuals per cage) of organisms in fouling communities on artificial substrates

Taxon	Nakhodka Bay	Reineke Island
Foraminifera	3996	83
Harpacticoida	1307	77
Nematoda	368	106
Ostracoda	387	14
Halacarida	1	0
Eumeiofauna (total)	6029	280
Polychaeta	57	34
Bivalvia	266	52
Gastropoda	42	0
Amphipoda	134	3
Pseudomeiofauna (total)	499	89

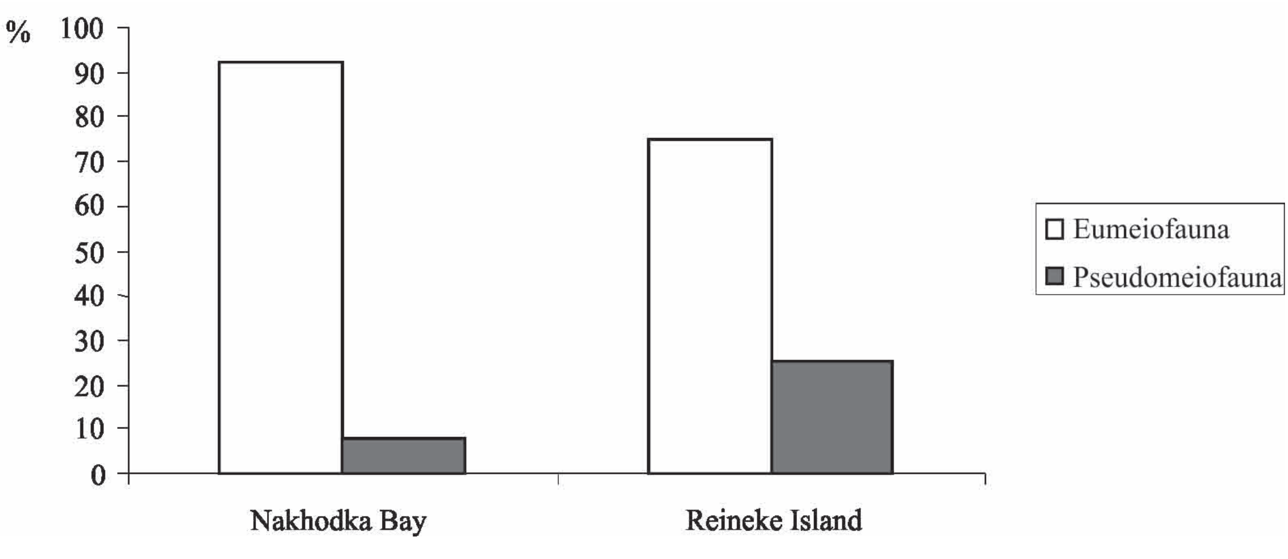

Fig. 1. Percent composition of meiofaunal groups on artificial substrates built for the cultivation of bivalve mollusks.

from 1 to 4% of the overall meiofaunal population density. This group included juvenile bivalve mollusks, amphipods, gastropods, and polychaetes, among which bivalves prevailed – 4% (Fig. 2a).

In waters off Reineke Island, the mean population density of the meiobenthos reached 369 ind./cage (see Table). Nematodes were the predominant group, which constituted 29% of meiofauna's overall population density. The population density of foraminifers was 22% and that of harpacticoids 20% of

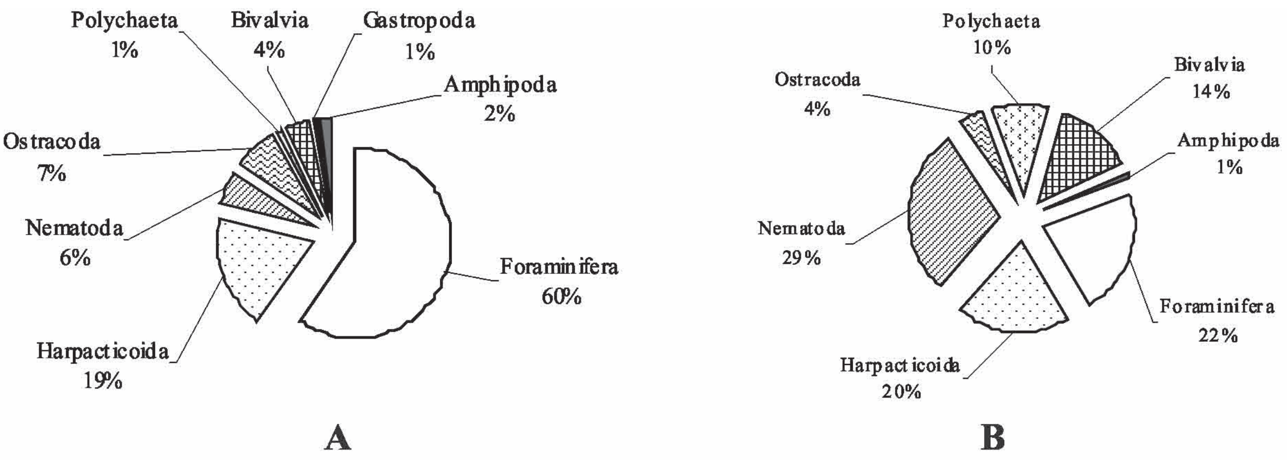

Fig. 2. Percent composition of main taxonomic groups of the meiofauna in Nakhodka Bay (**A**) and off Reineke Island (**B**).

meiofauna's overall population density. Ostracods had the proportion of 4%. In the pseudomeiofauna, bivalves, polychaetes, and amphipods varied from 1 to 14%. Juvenile bivalve mollusks prevailed, the same as those in Nakhodka Bay, although here the proportion of their population density was higher – 14%. Polychaetes were the second predominant group – 10% (Fig. 2b).

Thus, it was established that the taxonomic composition of the meiofauna in Nakhodka Bay numbered 9 groups, and that off Reineke Island 7 groups. Halacarids and gastropods did not occur on substrates off Reineke Island.

When comparing quantitative characteristics of meiofauna's fouling communities on artificial substrates of aquaculture installations, we found that the mean population density of the meiofauna in Nakhodka Bay was higher than that in waters off Reineke Island.

In fouling communities on aquaculture installations, built for the cultivation of bivalve mollusks in coastal open waters, representatives of the eumeiofauna predominated. The differences, observed in meiofauna's structure, consisted in the predominance by population density of nematodes and foraminifers off Reineke Island, and the predominance of foraminifers in Nakhodka Bay.

Large quantities of foraminifers, ostracods, and harpacticoids on artificial substrates is the evidences that these waters are unpolluted. Also the sufficient abundance of juvenile bivalves indicates that these communities are normally renewed.

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Advance of the study on the macrobenthos from the Yellow Sea and East China Sea

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The Yellow Sea is a marginal sea of the northwest Pacific located between Chinese mainland and the Korean Peninsula. Affected by the Yellow Sea coastal currents, Yellow Sea mixed water mass, the Yellow Sea cold water mass and the Yellow Sea warm current water mass, the Yellow Sea macrobenthos composition is very complex.

The East China Sea is a more open continental sea of the northwest Pacific connected with the Yellow Sea on the north, the Japan Sea on the northeast, the Pacific on the east and the South China Sea on the south. Its continental shelf is the broadest in all the China seas, slightly fan-shaped. The Changjiang River, Qiantang River and other rivers flowing into the sea in west, together with Kuroshio warm current flowing through southeast with high temperature and salinity, provide a wealth of biogenic elements for the East China Sea, making the macrobenthos with the characters of rich species, larger numbers and complex composition.

As human activities that disturb the marine habitats continue to strengthen, it is important to understand the status of Chinese coastal macrobenthic communities. Thus, based on the existing references, the ecological characteristics of the macrobenthos from the Yellow Sea and the East China Sea was summarized, in order to provide basic and background scientific information for the protection, development and sustainable use of marine living resources of Chinese coastal waters. The references based by this study are concerning the main reports, books and research papers on the macrobenthic ecology and biodiversity from the above areas since 1950. Normally, 0.1 m^2 sediment sampler (box-type grab, Dawn grab, Smith McIntyre grab or “Ocean-50” grab) has been used for the quantitative sampling with 2–5 times per station, the samples were filtered with 0.05 mm apertures sieve set. Trawl sampling mainly use 1.5–2 m wide Agassiz trawls for qualitative sampling. Both of the samples collected by quantitative and qualitative samplings were generally fixed with 75% alcohol (mostly 10% formalin before the 1990s). The sample sorting and data processing were done in laboratory. These methods of sample collecting, sorting and processing were based on the “Specification for Oceanographic Survey (GB/T 12763–2007)” and the “Specification of Ocean Monitoring (GB 17378–2007)” and their former editions.

Results and Discussion

There have been a lot of works on the ecology and biodiversity of macrobenthos from the Yellow Sea and the East China Sea since 1950's, particularly after 1980.

The Yellow Sea

In accordance with Li et al. (2010), 1124 species of macrobenthos have been found from the Yellow Sea. The dominant species were *Alpheus japonicus*, *Ampharete acutifrons*, *Antillesoma antillarum*, *Cadella narutoensis*, *Echinocardium cordatum*, *Eriopisella sechellensis*, *Euclymene lombricoides*, *Eudorella pacifica*, *Glycera onomichiensis*, *Lumbrineris cruzensis*, *L. latreilli*, *Melinna cristata*, *Nereis longior*, *Ninoe palmata*, *Notomastus latericeus*, *Ophelina acuminata*, *Ophiopholis mirabilis*, *Ophiura sarsi vadicola*, *Photis longicaudata*, *Praxillella pacifica*, *Sternaspis scutata*, *Theora lata*, *Thyasira tokunagai*, etc. The species composition and the dominant species of macrobenthos varied much since 1950's in the Yellow Sea, including the Bohai Sea and the Jiaozhou Bay.

According to Wang et al. (2010), the total macrobenthic abundances were from 5~695ind./m^2 in Bohai Bay, the mean abundance was 228.81 ind./m^2; The total biomasses were from 0.87~108.03 g/m^2, the mean biomass was 36.03 g/m^2. According to Tang (2006), the mean seasonal macrobenthic biomass was 37.17 g/m^2 in the Yellow Sea, the pattern of seasonal changes of biomass were spring (50.75 g/m^2) > Autumn (35.35 g/m^2) > summer (32.64 g/m^2) > winter (29.94 g/m^2); the mean seasonal abundance was 250 ind./m^2, the pattern seasonal changes of abundance were spring (359 ind./m^2) > winter (290 ind./m^2) > summer (290 ind./m^2) > autumn (165 ind./m^2). The macrobenthic biomass and abundance in northern Yellow Sea were higher than those in southern Yellow Sea (Li et al., 2012). The values of the biomass and abundance of the macrobenthos from different areas in the Yellow Sea were much different.

According to Li X. et al. (2009), the seasonal species richnesses (D) of the macrobenthos from the Yellow Sea during 2006–2007 were 4.95±0.28 (winter), 3.58±0.91 (spring), 3.10±0.90 (summer) and 2.90±0.74 (fall); the Shannon–Wiener diversity (H') were 2.61±0.39 (winter), 2.66±0.46 (spring), 2.44±0.45 (summer) and 2.38±0.32 (fall); the evenness (J) were 0.88±0.07 (winter), 0.88±0.11 (spring), 0.87±0.10 (summer) and 0.88±0.10 (fall).

According to Hu et al. (2000), the near-shore of the Yellow Sea macrobenthoes may be divided into 4 communities: (1), *Crangon hakodatei*–Ophiuroidea community, distributed in the northern part of the North Yellow Sea, with cold water species clearly, the dominance species are *Crangon hakodatei, Amphioplus* (*Lymanella*) *japonicus, Stegophiura sladeni, Ophiopholis mirabilis, Ophiura sarsii vadicola* etc.; (2), *Echinocardium cordatum–Nassarius* (*Zeuxis*) *oberwimen* community, distributed in the north of Shandong province and the southern part of the North Yellow Sea, the dominance species are *Echinocardium cordatum, Temnopleurus hardwickii, Distolaterias niponica, Bullacta exarata, Stegophiura sladeni,* etc.; (3), *Alpheus japonicus–Nassarius* (*Zeuxis*) *oberwimen* community, distributed in the coast of Lianyungang-Weihai area, the dominance species are *Alpheus japonicus, Odontamblyopus rubicundus, Nassarius* (*Zeuxis*) *succinctus, Nassarius variciferus* etc.; (4), *Palaemon gravieri–Collichthys* community, distributed from Sheyang (Jiangsu Province) to Changjiang River estuary coastal waters, the dominance species are *Collichthys lucidus*, *Palaemon gravieri, Sternaspis scutata, Metapenaeopsis dalei* etc, with obvious characteristic of subtropical flora.

Li et al. (2005) reported the macrobenthic secondary production in South Yellow Sea was 4.98 g m^{-2} a^{-1} in 2001–2002, the average value of P/B is 1.10 a^{-1}.

East China Sea

In accordance with Tang (2006) and Li et al. (2010), 855 and 2251 species of macrobenthos have been recorded from the East China Sea. A total of 45 dominant and common species were recorded in the East China Sea, including *Aglaophamus dibranchis*, *Alpheus distinguendus*, *Ampelisca cyclops*, *A. miharaensis*, *Amphioplus* (*Amphioplus*) *ancistrotus*, *A.* (*Lymanella*) *depressus*, *A.* (*L.*) *laevis*, *A. japonicus*, *Amphiura* (*Amphiura*) *digitula*, *A.* (*Fellaria*) *vadicola*, *Angulus lanceolatus*, *Aricidea* (*Aricidea*) *fragilis*, *Bursa rana*, *Byblis japonicus*, *Calliodentalium crocinum*, *Dentalium octangulatum*, *Episiphon kaochowwanense*, *Eriopisella sechellensis*, *Glycera chirori*, *Goniada maculata*, *Grandidierella japonica*, *Graptacme buccinulum*, *Inquisitor flavidula*, *Jassa falcata*, *Leptochela* (*Leptochela*) *aculeocaudata*, *Lophiotoma leucotropis*, *Lumbrineris latreilli, Maldane sarsi, Mitrella burchardi*, *Nassarius siquijorensis*, *Nihonotrypaea japonica*, *Ninoe palmate*, *Notomastus aberans*, *N. latericeus*, *Onuphis eremita*, *Ophiura kinbergi*, *Paralacydonia paradoxa*, *Paraprionospio pinnata*, *Prionospio malmgreni*, *P. pygmaeus*, *Sigambra hanaokai*, *Solen canaliculatus*, *Spiniplicatula muricata*, *Sternaspis scutata*, *Xenophthalmus pinnotheroides*, etc. As well as the situation in the Yellow Sea, the species composition and the dominant species of macrobenthos in the East China Sea varied much since 1950's, too.

According to Tang (2006), the seasonal mean biomass of macrobenthos from the East China Sea was 21.36 g/m^2. The seasonal dynamic of biomass were observed: spring > fall > summer > winter. The total average abundance was 283 ind./m^2. Polychaeta was the dominant group, followed by crustacean and mollusca, and the seasonal variety of abundance was fall > spring > summer > winter. The values of the biomass and abundance of the macrobenthos from different areas in the East China Sea were much different as well as in the Yellow Sea.

According to Liu L. et al. (2008), the mean indices of the Shannon–Weaver (H'), species richness (D) and evenness (J) of the macrobenthos from the East China Sea recently were 1.72 ±0.16, 1.37±0.19 and 0.64 ±0.04 (Liu L. et al., 2008).

Li (2003) treated the macrobenthos of the East China Sea as the following communities based on the Bray–Curtis similarity: (1), Coastal community in northern part of East China Sea include 2 communities: community I, *Aricidea fragilis–Calliodentalium crocinum–Raetellops pulchella–Callianassa japonica–Amphioplus* (*Lymanella*) *depressus* community; community II, *Micronephtys sphaerocirrata–Eulima bifascialis–Scapharca inaequivalvis–Ogyrides orientalis* community; (2), Coastal community in southern part of East China Sea include 2 community: community I, *Onuphis eremite–Calliodentalium crocinum–Callianassa japonica–Amphioplus* (*Amphioplus*) *ancistrotus* community; community II, *Aricidea fragilis–Calliodentalium crocinum–Grandidierella japonica–Amphioplus* (*Amphioplus*) *ancistrotus* community; (3), The open sea community in the northern part of East China Sea include 4 community: community I, *Mediomastus californiensis–Byblis japonicus–Golfingia* sp. community; community II, *Amphictene* sp.*–Calliodentalium crocinum–Leptochelia aculeocauclata–Placophiothrix striolata* community; community III, *Sigambra hanaokai–Calliodentalium crocinum–Callianassa japonica–Amphiura* (*Amphiura*) *uncinata* community; community IV, *Aricidea fragilis–Calliodentalium crocinum–Callianassa japonica–Amphioplus* sp. community; (4), The open sea community in the southern part of East China Sea include 4 communities: community I, *Aglaophamus dicirris–Turritella fascialis–Callianassa japonica–Placophiothrix striolata* community; community II, *Prionospio pinnata–Spiniplicatula muricata–Solenocera pectinulata–Ophionephthy difficilis* community; (5), The community of Taiwan Strait includes 2 communities: community

I, *Aglaophamus dicirris–Turritella fascialis–Callianassa japonica–Ophiothrix* (*Placophiothrix*) *striolata* community; community II, *Prionospio pinnata–Spiniplicatula muricata–Solenocera pectinulata–Ophionephthy difficilis* community.

Li et al. (2005) reported the macrobenthic secondary production of the East China Sea in 2000–2001 was 1.62 g (AFDW) $m^{-2}a^{-1}$, the average P/B value is 1.41 a^{-1}.

The Yellow Sea and the East China Sea are both important parts of the China seas, also the earliest areas to be developed and utilized. With the rapid economic and social developments, the environment of these areas have been suffering a growing pressure. It is an important task to research the ecology and environment so that to understand the running mechanism of the marine ecosystem of these areas for the protection and utilize of the marine ecosystem and the marine resources. The macrobenthos, as very important marine resource, should be studied deeply. The importance of the study is the innovation of the approach methods and means.

Comparison of lipid biochemical diversity of corals and hydrocorals from the Okhotsk Sea and the South China Sea

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Reef-building and soft corals are widely distributed in a tropical zone of the Pacific Ocean. Hydrocorals, such as “fire coral” *Millepora*, are also members of coral reef ecosystem. The most of reef-building coral species, as well as *Millepora*, and a lot of soft corals species contain endocellular symbiotic microalgae named zooxanthellae, which are the essential source of photosynthetic organic carbon for their host. Soft corals and hydrocorals are common species for the Bering Sea, the Okhotsk Sea, and deep water of the Sea of Japan, but these species don’t contain zooxanthellae. The absence of own phototrophic food source, quite deep habitat, and low environmental temperature are the distinctions of cold-water corals and hydrocorals.

Lipids form up to 40% of coral dry biomass. Lipids are the main energy reserve of a coral colony and the base of coral cell membranes. Lipids are involved in a majority of biochemical and physiological processes in corals; therefore, changes in the lipid composition reflect changes in the ecology, nutrition, and health of these animals (Pham, Imbs, 2012). Fatty acids (FA) are the main constituents of lipids. FA are most probably indicative of external food sources (Dalsgaard et al., 2003), the composition of symbionts and associated organisms (Bishop, Kenrick, 1980), and are applied for coral chemotaxonomy (Imbs et al., 2010a).

The overwhelming majority of coral and hydrocoral lipid investigations were performed on tropical shallow-water species but data on lipid and FA composition of cold-water corals and hydrocorals were very limited (Pham, Imbs, 2012). To determine the features of the lipid composition of coral and hydrocorals inhabited in North Pacific cold waters, eleven soft coral species and five hydrocoral species from the Okhotsk Sea were studied in comparison with tropical species from the South China Sea, which were analyzed previously (Imbs et al., 2010a).

Materials and Methods

Alcyonarian corals *Eunephthia rubiformis* (Ehrenberg, 1834) *E. fruticosa* (Sars, 1860) (Octocorallia: Nephtheidae), gorgonian corals *Paragorgia arborea* (Linnaeus, 1758) (Octocorallia: Paragorgiidae), *Amphilaphis* sp., *Plumarella* sp., *P. carinata* Kinoshita, 1908, *Primnoa pacifica* Kinoshita, 1907, *Thouarella* sp., *Calyptrophora japonica* Gray, 1866 (Octocorallia: Primnoidae), *Acanthogorgia* sp., *Calcigorgia spiculifera* Broch, 1935 (Octocorallia: Acanthogorgiidae), and hydrocorals *Allopora steinegeri* Fisher, 1938, *A. scabiosa* (Broch, 1935), *A. solida* (Broch, 1935), *Errina antarctica* (Gray, 1872) (Hexacorallia: Stylasteridae) were sampled by a dredge in the Okhotsk Sea (Kuril Islands).

Extraction of total lipids was conducted according to Folch et al. (1957). Lipid classes were analyzed by 1-dimensional silica gel thin-layer chromatography (Imbs et al., 2010a). FA methyl esters

(FAME) were obtained according to Carreau and Dubacq (1979). FAME composition was analyzed by gas chromatography, and FA structures were confirmed by gas chromatography-mass spectrometry of FAME and *N*-acylpyrrolidide derivatives (Andersson, 1978; Imbs et al., 2009).

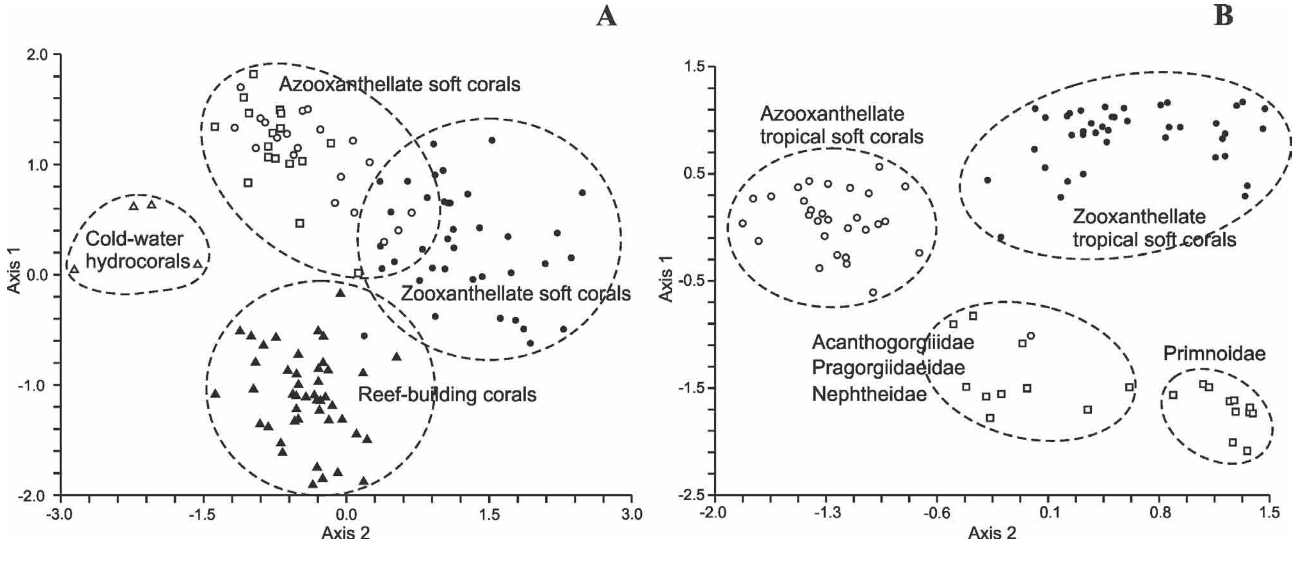
Differences in the lipid class and FA concentrations between cnidarian groups were examined with a one-way ANOVA. To represent relationships among the coral groups, all variables (square root of data) measured were included in principal components analyses (PCA).

Results

Comparison of lipid class composition of cold-water and tropical species

A variety of lipid classes (polar lipids (PL), sterols (ST), free fatty acids (FFA), triacylglycerols (TG), monoalkyldiacylglycerols (MADAG), and wax esters (WE)) were presented in total lipids of all cold-water species studied. PL was most prominent in the soft corals, whereas TG predominated in the hydrocorals. The average level of TG in the hydrocorals (32.3±3.6%) was in 2.5 times higher than that in the soft corals (12.5±3.1%), whereas total lipids of the soft coral species were rich in PL and MADAG.

The data on the lipid class composition of 15 cold-water species studied were compared with that of 106 tropical specimens published recently (Imbs et al., 2010a). These tropical specimens comprised reef-building corals, zooxanthellate and azooxanthellate soft corals, and *Millepora*. All lipid classes (except for FFA) were used as variables for PCA. As Fig., A indicates, the cold-water soft corals fall into the region of tropical azooxanthellate soft corals, but clearly divide from tropical zooxanthellate reef-building and soft corals. The boreal hydrocorals separated from all corals and tropical hydrocorals (see Fig., A). There were no significant differences ($P>0.01$) in the average contents of all lipid classes between the cold-water and tropical azooxanthellate soft corals. A comparison of cold-water and tropical hydrocorals showed significant differences in the average content of MADAG.



Principal component analysis (PCA) plots: (**A**) lipid class content of 15 cold-water and 95 tropical cnidarian species; (**B**) fatty acid content of 11 cold-water and 58 tropical soft coral species. (●) Tropical zooxanthellate soft corals, (○) tropical azooxanthellate soft corals, (▲) tropical reef-building corals, (□) cold-water soft corals, (Δ) cold-water hydrocorals.

Comparison of fatty acid composition of cold-water and tropical species

Polyunsaturated FA (PUFA) was a major part of total lipid FA from the cold-water corals. 20:5n-3 constituted 12–23% of total FA of Nephtheidae, Paragorgiidae, and Acanthogorgiidae, but over 30% of total FA of Primnoidae. Concomitantly, Primnoidae contained only a small amount of 20:4n-6 whereas this acid was a major component of lipids of other coral families. Very-long-chain tetracosapolyenoic acids (24:5n-6 and 24:6n-3) were present in all samples. Except of Primnoidae, the level of 24:5n-6 in a total FA of the corals investigated was higher than that of 24:6n-3. The major tetracosapolyenoic acid in Primnoidae was 24:6n-3, and 24:5n-6 was a minor component. The major components of cold-water hydrocoral PUFA were 20:4n-6, 20:5n-3, and 22:6n-3. In average, the hydrocorals contained more saturated FA than the corals, whereas the reverse was true for PUFA. No tetracosapolyenoic acids were found in total lipids of the hydrocorals. PCA analysis of FA composition indicated that all cold-water hydrocorals clearly divided from all cold-water soft corals (data are not shown).

The FA of 11 cold-water soft coral species studied and 73 tropical soft coral specimens (Imbs et al., 2010a) were used as variables for PCA. The tropical specimens comprised zooxanthellate and azooxanthellate soft corals. The outcome of the analysis is shown in Fig., B. The boreal soft corals fall out the regions of tropical both zooxanthellate and azooxanthellate soft corals. The family Primnoidae divided from other boreal coral families. Azooxanthellate tropical and cold-water soft coral species didn’t form a joint region according to their FA composition whereas they jointed according to their lipid class composition (see Fig., A).

Discussion

Lipid composition of corals and hydrocorals depend on their taxonomic position, nutrition, environment, and presence of symbionts. Zooxanthellae strongly influence on a proportion between reserved and structural lipid classes in tropical coral tissues (Imbs et al., 2010a). In nature, zooxanthellae can’t live at water temperature lower than 12ºC, and corals lose their zooxanthellae at 33ºC. The loss of zooxanthellae (coral bleaching) lead to considerable decreasing of reserved lipid classes, such as TG and WE (Yamashiro et al., 2005; Rodrigues et al., 2008; Imbs et al., 2012). Our study showed that lipid class composition of cold-water corals strongly differ from that of tropical zooxanthellate corals because of the absence of the symbionts, but there are no significant differences in lipid class composition of all azooxanthellate soft corals irrespective of environment conditions.

On the contrary, FA composition of the cold-water coral species strongly differed from that of tropical azooxanthellate soft corals. The most contribution to this difference were made by PUFA of n-6 and n-3 series, which were biomarkers of phyto- and zooplankton – important food source of corals. Probably, differences of plankton species composition from cold and warm waters with different FA compositions lead to the changes of FA profiles of azooxanthellate soft corals. The low content of 20:4n-6 in Primnoidae in comparison with other cold-water coral families indicate the increasing of phytoplankton portion in Primnoidae nutrition. High level of PUFA in both cold-water and tropical soft corals does not come to an agreement with the hypothesis about increasing of PUFA content in cold-water animals.

20:4n-6 and 20:5n-3 are biosynthetic precursors of 24:5n-6 and 24:6n-3, respectively. The different levels of 20:4n-6 and 20:5n-3 in Primnoidae and other cold-water coral families lead to the different levels of 24:5n-6 and 24:6n-3. Hydrocorals lack for tetracosapolyenoic acids, which can not to be synthesized in tissues of Hexacorallia (Imbs et al., 2010b).

Thus, lipid and their FA are useful indicators of the influence of environment conditions on biochemical diversity of North Pacific cnidarians.

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Phylogeography and population genetic structure in four Far Eastern redfin species of the genus *Tribolodon* (Pisces, Cyprinidae) in the northwestern Pacific

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Understanding of the relative importance of historical and contemporary ecological factors that determine the intraspecies subdivision, appearance of population genetic structure, and formation of new species is a topic central to evolutionary biology. The separation of one species into genetically isolated populations leads over time to the formation of two or even more species. In the Northwestern Pacific, climate oscillation during some last million years has had a great impact on the divergence of species and genetic structuring of freshwater and marine fishes (Lindberg*,* 1972). During glacial periods, most species were forced into southern refuges from which they expanded northwards when the climate warmed up and glaciers retreated. In case of a few refuges for a species there is the possibility for evolution and formation of two or more species.

Historical factors (e.g. fragmentation, recolonization, expansion, hybridization) influence patterns of the distribution of genetic variation of species similar to effects of ongoing gene flow (Avise, 2000). Comparative analysis of genetic variability in closely related species makes it possible to reveal the most essential factors, which influence the formation of their population genetic structure.

Phylogeographical approach, based on the analysis of mtDNA variation is very useful for understanding the ways and mechanisms of formation of species and intraspecies units. The break of the genetic exchange between the populations creates difference in mtDNA as a result of mutations and stochastic processes. The divergence of populations isolated in space or time leads to the formation of different mtDNA phylogroups (Avise, 2000). Moreover, the analysis of the molecular variability of mtDNA makes it possible to retrospectively reconstruct the events of divergent evolution inside species and groups of species, and to place the events of divergence in one or other historical periods (Avise, 2000).

The genus *Tribolodon* endemic for the Northwest Pacific presents a unique possibility for the comparative study, since its species differ by history of their origin and special traits of biology. The genus includes five species: *T. sachalinensis* and *T. nakamurai* are freshwater; *T. hakonensis* is capable of inhabiting both fresh water and in the estuary zones, therefore is amphidromous; *T. brandtii* and the recently discovered species *Tribolodon* sp. have anadromous lifestyle and migrate to fresh water to spawn and winter (Semina et al., 2006). Natural habitats of all the species are restricted to the coastal waters of Primorye, Sakhalin, Shantarsky and Kuril Islands, Japanese Archipelago, Korean Peninsula; and the habitats overlap for some species.

The goal of the present work was to comparatively study mtDNA variation in four species of Far Eastern redfins of the genus *Tribolodon* over a substantial part of their habitats to better understand how special features of their divergence correlate with the history of origin and biological traits of the species.

Methods

The locations of sampling are presented in Table and Fig. 1. From each DNA sample, three mtDNA segments coding for A6/A8/COIII, ND3/ND4L/ND4, and ND5/ND6 genes were amplified via PCR. RFLP analysis was performed using restriction endonucleases that previously had been chosen to reveal intra-specific variability. Demographic patterns were assessed using mismatch distributions comparing observed distributions of divergences among haplotypes to distributions predicted under a model of sudden expansion in Arlequin 3.5.1.2. (Schneider et al., 2000).

Samples of *T. sachalinensis*, *T. brandtii, T. hakonensis* and *Tribolodon* sp. examined in the present study

Species	N	Locality	Number of specimens	Number of haplotypes	Haplotype diversity	Nucleotide diversity
T. sachalinensis	1	Sakhalin Island, Aniva Bay, Susuya River	33	9	0.7424±0.07142	0.0149559
	2	Sakhalin Island, Aniva Bay, Lutoga River	25	7	0.8500±0.03300	0.0101767
	3	Sakhalin Island, Bolshoe Chibisanskoe Lake	25	4	0.6400±0.06879	0.016795
	4	Sakhalin Island, Protochnaya Creek	29	6	0.7438±0.06034	0.018908
	5	Western coast of the Tatar Strait, Tumnin River	24	1	0.0000±0.00000	0.000000
T. brandtii	6	Sea of Japan, Primorye, Razdolnaya River	35	8	0.6471±0.08029	0.009337
	7	Sea of Japan, Primorye, Kiparisovka River	27	3	0.5527±0.08440	0.009308
	8	Sea of Japan, Primorye, Vostok Bay	40	7	0.6640±0.06740	0.010195
	9	Sea of Japan, Primorye, Artemovka River	25	5	0.6167±0.09686	0.009741
	10	Sea of Japan, Primorye, Lebedinka River	32	4	0.6391±0.05561	0.010762
	11	Sea of Japan, Primorye, Cape Krasnyi	31	6	0.6409±0.07316	0.010313
	12	Sea of Japan, Primorye, Cape Falshivyi	36	7	0.7238±0.06033	0.010426
	13	Sakhalin Island, Aniva Bay	32	3	0.4617±0.07446	0.005638

TABLE (*Continued*)

Species	N	Locality	Number of specimens	Number of haplotypes	Haplotype diversity	Nucleotide diversity
T. hakonensis	14	Okhotsk Sea, East Sakhalin, Bakhura River	35	15	0.8723±0.04231	0.013570
	15	Okhotsk Sea, East Sakhalin, Firsovka River	42	12	0.8049±0.04160	0.012566
	16	Tatar Strait, West Sakhalin, Il'inskoe	39	10	0.8016±0.04398	0.012067
	17	Sakhalin Island, Aniva Bay, Korsakovka River	31	9	0.7869±0.04293	0.012527
	18	Western coast of the Tatar Strait, Tumnin River	27	11	0.8775±0.04233	0.009795
	19	Okhotsk Sea, Northeast Sakhalin, Nabilsky Bay	12	8	0.8939±0.07773	0.012448
***Tribolodon* sp.**	20	Sea of Japan, Primorye, Razdolnaya River	32	6	0.6855±0.04807	0.002008
	21	Sea of Japan, Primorye, Vostok Bay	37	4	0.6291±0.05805	0.001967
	22	Sea of Japan, Primorye, Ternei, Russkaya Bay	22	5	0.6234±0.09943	0.002038
	23	Sea of Japan, Primorye, Ternei, Blagodatnaya Bay	19	4	0.5752±0.10133	0.001395

Results

T. sachalinensis* and *T. brandtii. PCR-RFLP-analysis of the three mtDNA segments 6935 bp total long revealed 13 composite haplotypes in *T. sachalinensis* and 14 composite haplotypes in *T. brandtii*. *T. sachalinensis* haplotype network revealed two phylogroups (*a*, *b*) each having more or less pronounced star-like structure, and divided about 2.9% of nucleotide divergence between them (Fig. 2: 1A). *T. brandtii* haplotype network revealed three closely related phylogroups (*a*, *b*, *c*) divided by mutational distances of 3 and 4 substitutions (Fig. 2: 2A), the divergence level between phylogroups *a* and *b* was estimated to be about 1.1%, between *a* and *c* – 1.5%, and between *b* and *c* – 2.3% of nucleotide substitutions. Mismatch distribution of both species haplotypes was clearly bimodal (Fig. 2: 1B, 2B) and each phylogroup fitted the predicted distribution under the model of sudden expansion. At the same time, the first two phylogroups of each species co-occurred in all samples and were not geographically confined. The representatives of the third phylogroup of *T. brandtii* occurred only in Sakhalin sample.

***T. hakonensis* and *Tribolodon* sp.** Earlier we showed (Semina et al., 2006; Gudkov et al., 2010) that species described as *T. hakonensis* was, in fact, presented by two closely related species. The one that dwells in the coastal waters of Sakhalin Island, continental part to the north from Primorye, and Japanese Islands, appears to be "true" *T. hakonensis* since this species was first described in

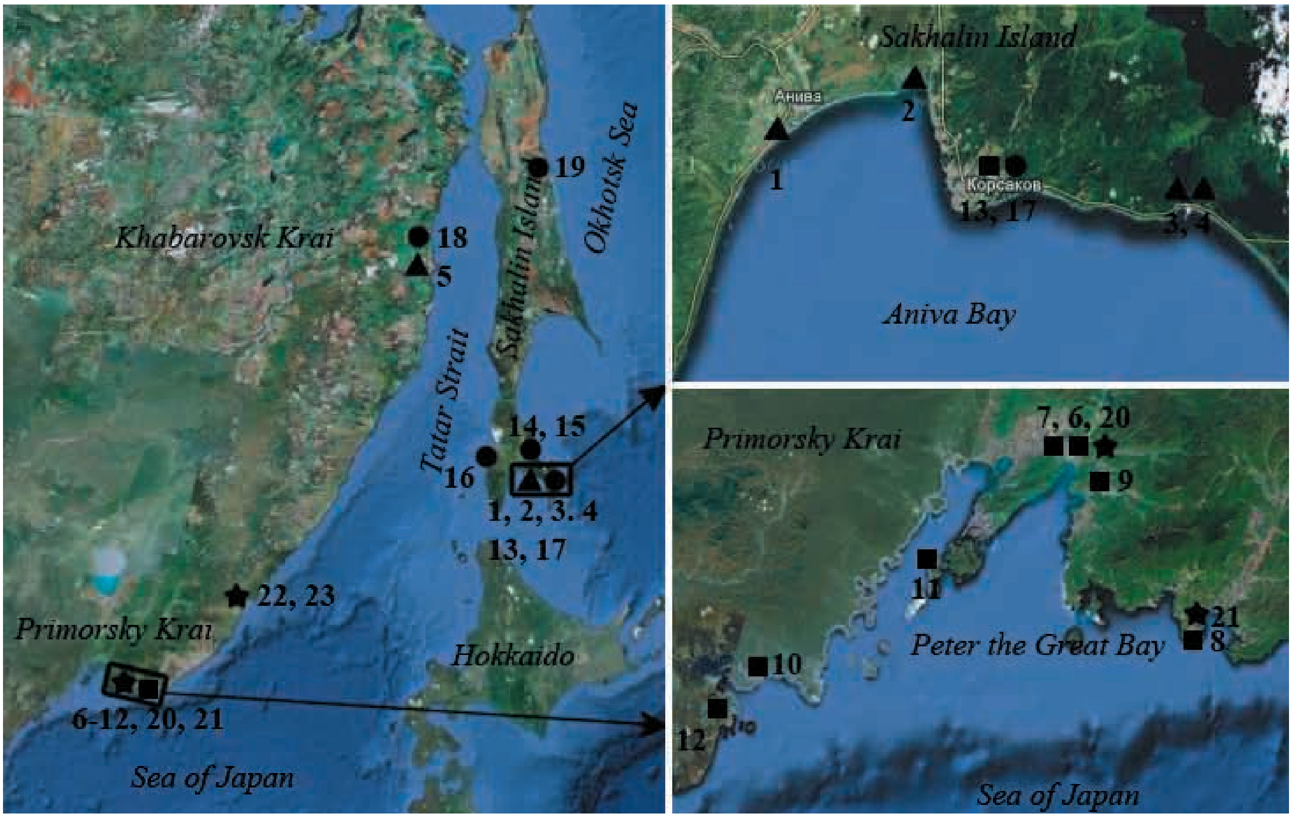

Fig. 1. Sampling sites of the species of *Tribolodon* genus studied. Numbers of the sites correspond to the numbers of localities in Table. ▲ – *T. sachalinensis*, ■ – *T. brandtii*, ● – *T. hakonensis*, ★ – *Tribolodon* sp.

Japan. The southern species (*Tribolodon* sp.) inhabits Primorye and, apparently, Korean Peninsula waters. In this work we examined these species separately. Our analysis revealed 35 composite haplotypes among 186 specimens of *T. hakonensis*. *T. hakonensis* minimal spanning haplotype network is presented in Fig. 2: 3A. This species has two distinctly pronounced phylogroups of closely related haplotypes (*a*, *b*) divided by 16 nucleotide substitutions; the value of divergence between them is 2.1%. Similarly to the previously mentioned two species, mismatch distribution of all *T. hakonensis* haplotypes was bimodal (Fig. 2: 3B), the representatives of the haplotypes from both phylogroups were distributed continuously on the area studied and occurred in all the samples with approximately equal frequencies. *T. species* revealed only 8 haplotypes among the investigated 114 individuals in four samples. The haplotype network of this species is represented as one phylogroup (Fig. 2: 4A), and mismatch distribution between the haplotypes does not differ from the expected distribution under the model with the recent increase in number (Fig. 2: 4B).

Discussion

Population genetic structure in the Far Eastern redfin species

As evident from our data, population genetic subdivision depends on the degree of confinement of their lifestyle to the freshwater reservoirs. Population genetic structure of the freshwater *T. sachalinensis* is apparent, the interpopulation component of its mtDNA variation comprising almost

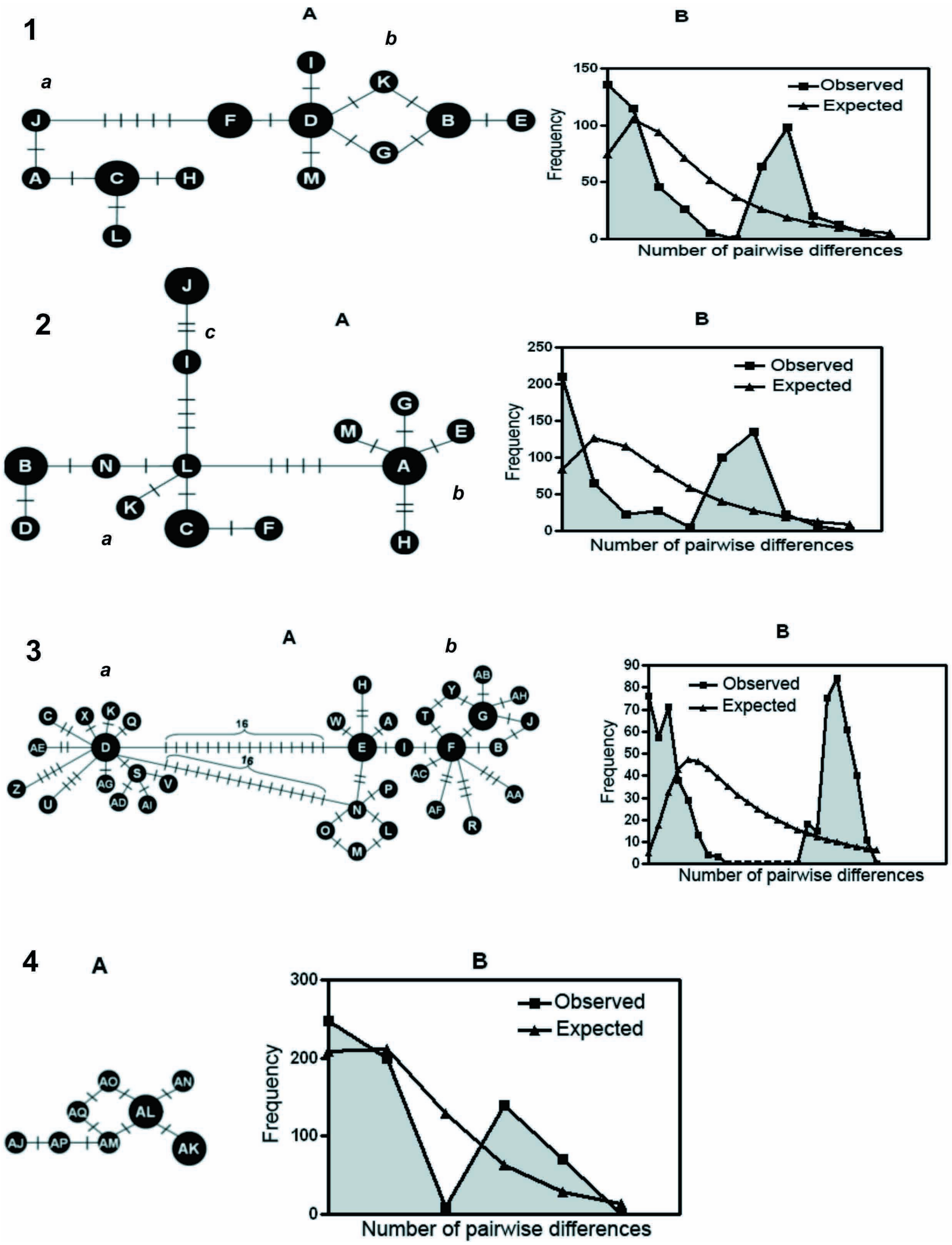

Fig. 2. Minimum spanning haplotype network showing maternal mtDNA genealogies of *T. sachalinensis* (**1A**), *T. brandtii* (**2A**), *T. hakonensis* (**3A**), *Tribolodon* sp. (**4A**). Mismatch distributions comparing observed distributions of divergences among haplotypes of *T. sachalinensis* (**1B**), *T. brandtii* (**2B**), *T. hakonensis* (**3B**), *Tribolodon* sp. (**4B**) to distributions predicted under a model of sudden expansion.

a third of the total variation. This agrees with the existing data, according to which, freshwater fishes are characterized by more pronounced population genetic structure as the consequence of geographical subdivision and the presence of physical barriers for the gene flow between populations. Population genetic structure of *T. brandtii* was not as clearly pronounced. Despite the fact that about 20% of genetic variation was attributable to the variation among populations, most of it was determined by the differences between the Sakhalin and mainland coastal (Primorye) samples, suggesting that the genetic exchange is high between the mainland samples and low between the latter and Sakhalin Island populations. This data support the idea of existence of the population genetic structure of this species and this structure determined by restriction of gene flow by the distance or by the fragmentation of its habitat in the past of this species. The results of allozyme analysis (Sakai et al., 2002) support population genetic subdivision of these species.

The differences between samples of *T. hakonensis* were low. Only 1.39% of total variation accounted for the inter-population component while more than 98% was distributed within populations. The significant differences were revealed only between the geographically remote populations. Our data also agree with the fact that *T. hakonensis* has an amphidromous lifestyle, and that it freely migrates from fresh water to estuary zones.

In *Tribolodon* sp. samples studied there was no heterogeneity revealed by both pairwise nucleotide variation comparison and frequencies of the haplotypes. These data confirm observations about the high capability for the extensive migrations of this species.

Influence of origin on genetic variation in the species of the genus *Tribolodon*

The phylogenetic relationships between the *Tribolodon* species have been studied and published in the series of papers (Sakai et al., 2002; Sasaki et al., 2007; Semina et al., 2007). The majority of *Tribolodon* species is characterized by the values of mtDNA divergence ranging from 8 to 11% of nucleotide replacements. The calculated basic interval of divergence in this group of species is placed to the boundary between Miocene and Pliocene, i.e., 4.5–6 million years ago (mya) (Brykov et al., 2010). The diversifying factors were, apparently, great geological and paleoclimatic changes, among which the formation of the Western-Antarctic glacial shield was the most significant (Lindberg, 1972). In the northwestern Pacific, this period was characterized by a significant regression of the ocean level and, as a result, by complete or partial isolation of the Sea of Japan and, potentially, the Bering and Okhotsk Seas from the Pacific Ocean.

The species of the genus *Tribolodon* have freshwater, amphydromous, and anadromous life style. Thus, differentially directed influence of factors, such as salinity and temperature, might have underlain the radiation of the species. The last period of divergence (the divergence of *Tribolodon* sp.) is evaluated from 1 to 2 mya (Brykov et al., 2010). This period (1.25–2.5 mya, late Pliocene – early Pleistocene) is also thought to undergo a change of the paleoclimate in the northwestern Pacific resulting in periodic climatic fluctuations and formation and retreats of the glaciers (Lindberg, 1972; Jakobs et al., 2004). The Sea of Japan and the Okhotsk Sea might have served as refugium, where different redfin species were formed.

In theory, the oldest species within a genus should possess a relatively higher level of genetic variation in comparison to a younger one due to its more prolonged existence and, consequently, the accumulation of a larger number of neutral mutations. Freshwater *T. sachalinensis* is considered to be the first species separated from its freshwater ancestor in the group under this study. *T. brandii* is believed to be the next one diverged from the *Tribolodon* ancestor. However, the level of variation in *T. sachalinensis* and *T. brandtii* did not prove to be the highest among the *Tribolodon* species while the number of haplotypes revealed were relatively small, only 13 and 14 correspondingly. The variation of mtDNA in *T. hakonensis*, in particular, the largest number of haplotypes, the presence of the well-defined phylogroups, gives grounds to consider this form as the most ancient, which preserved greatest level of variation in its mtDNA (see Table). It is possible to assume that this species kept a large part of the ancestral form's variability, while consecutively diverging from the ancestor resulting in the formation of separate species that lost a substantial portion of its changeability due to a bottleneck and/or founder effects.

The youngest species in the genus *Tribolodon* is *Tribolodon* sp. (Semina et al., 2007). This finding is confirmed by the comparative analysis of the nucleotide variability of mtDNA, which proved to be practically by an order of magnitude lower than in the other species (see Table). It is plausible that this is caused by the founder effect during stages of the origin of this species.

In case of all *Tribolodon* species, it is obvious that mechanisms of biological isolation of the populations within the period of isolation were not completely formed. When the barriers disappeared, most probably, due to the transgression of the ocean level and the connection of the marginal seas to the Pacific Ocean, the integrity of nuclear genomes of the early independent units was reestablished. MtDNA, however, owing to the absence of recombination still preserved the "tracks" of its history in the form of different mtDNA phylogroups. The basins of the Sea of Japan, Okhotsk Sea and the Pacific Ocean could have been the refugium, where those different mtDNA phylogroups of all the Far Eastern redfin species were formed.

Acknowledgments

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Filamentous fungi associated with the giant oyster *Crassostrea gigas* (Bivalvia) from Peter the Great Bay of the Sea of Japan

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In this study, mycological investigation of the Pacific (giant) oyster *Crassostrea gigas* (Thunberg, 1793) (Bivalvia) from Peter the Great Bay of the Sea of Japan was carried out. The taxonomic composition of filamentous fungi associated with *C. gigas* was studied. The taxonomic composition of the fungi associated with the giant oyster included 22 species of filamentous fungi of which 17 species were identified. The latter belonged to six genera: *Alternaria*, *Aspergillus*, *Botrytis*, *Fusarium*, *Penicillium*, and *Trichoderma.* The distribution of filamentous fungi in the internal organs of the bivalve mollusk was studied.

Materials and Methods

The giant oyster *C. gigas*, a bivalve mollusk, was the material for mycological investigations. The material was collected in the Peter the Great Bay near the Rikorda Island at a depth of 3–4 m in April 2010.

The dissected internal organs of the mollusks – the gills, the mantle, the kidneys, the digestive gland (DG), the muscle, and the male gonads – were soaked in an antibiotic solution (500000 U of penicillin and 0.5 g of streptomycin per 1l of sterile seawater) for 2 h to inhibit the growth of bacteria. The material was then washed in sterile seawater and plated on the wort agar medium and Czapek medium with peptone.

The strains of filamentous fungi are stored in the Collection of Cultures of Marine Fungi of the A.V. Zhirmunsky Institute of Marine Biology, Far Eastern Branch, Russian Academy of Sciences.

Results and Discussion

The mycological study of the giant oyster revealed 22 species of filamentous fungi; 17 species were identified. These species were anamorphic micromycetes (Anamorphic fungi). The species identified belonged to six genera: *Alternaria* (Fig. 1), *Aspergillus* (Fig. 2, 3, 4), *Botrytis* (Fig. 5), *Fusarium*, *Penicillium*, and *Trichoderma* (Fig. 6) (see Table). The genus *Aspergillus* was the most common (five species). The fungi of the genera *Penicillium* and *Alternaria* were also comparatively widely represented (four and three species, respectively). The genus *Botrytis* represented by one species, *Botrytis cinerea*, was the least numerous (see Table). The species designated by us as Mycelia Sterilia (five species) formed mycelium without sporulation and differed from each other in colony morphology.

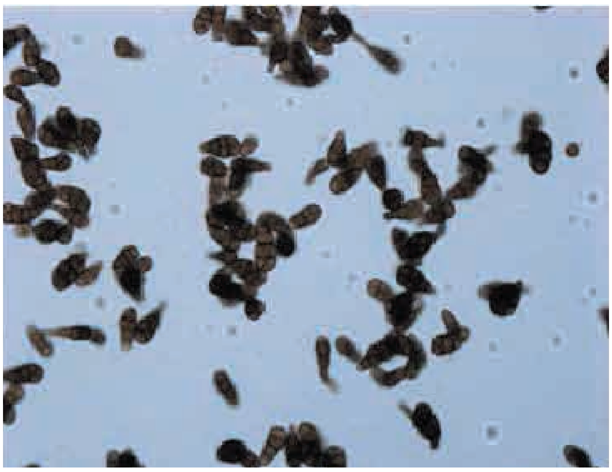

Fig. 1. *Alternaria alternata* (Fr.) Keissl.: conidia (Leica DM 4500).

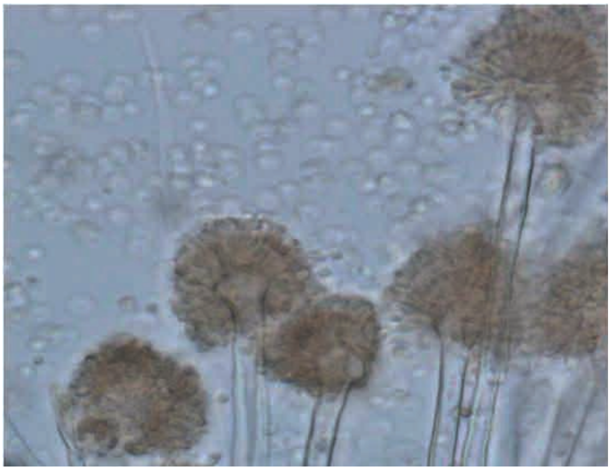

Fig. 2. *Aspergillus phoenicis* (Corda) Thom: conidial apparatus (Leica DM 4500).

Fig. 3. *Aspergillus candidus* Link: strain on the agar medium.

Fig. 4. *Aspergillus candidus* Link: conidial apparatus (Leica DM 4500).

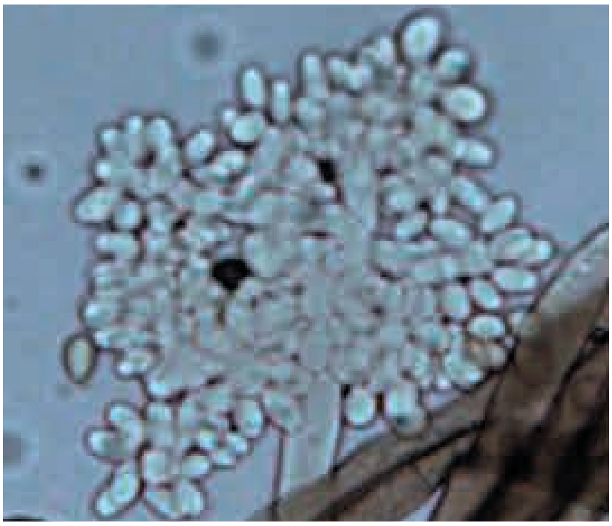

Fig. 5. *Botrytis cinerea* Pers.: conidial apparatus (Leica DM 4500).

Fig. 6. *Trichoderma aureovirive* Rifai: conidial apparatus (Leica DM 4500).

Distribution of filamentous fungi on the shell surface and in the internal organs of the giant oyster *Crassostrea gigas* from Peter the Great Bay of the Sea of Japan

Fungal taxon	Valve	Mantle	Muscle	DG	Gonads	Gills	Kidney
Alternaria alternata (Fr.) Keissl.	+		+				
A. litorea (Pivkin et Zvereva) Ging.	+						
A. tenuissima (Fr.) Wiltshire	+						
Aspergillus anthodesmus Bartoll. et Maggi		+					
A. candidus Link		+					
A. carbonarius (Bain.) Thom	+			+			
A. ochraceus K. Wilh.		+					
A. phoenicis (Corda)Thom		+	+	+	+	+	+
Botrytis cinerea Pers.					+		+
Fusarium oxysporum var. *orthoceras* (App. Et Wr.) Bilai	+						
F. lateritium Nees	+						
Penicillium brevicompactum Dierckx.		+					
P. digitatum Sacc. var. *californicum* Thom	+						
P. lanosum Westling	+			+			
P. simplicissimum (Oud.) Thom		+					
Trichoderma aureoviride Rifai	+		+				
T. viride Pers.		+			+		+
Mycelia Sterilia 1	+			+		+	
Mycelia Sterilia 2						+	
Mycelia Sterilia 3						+	
Mycelia Sterilia 4	+						
Mycelia Sterilia 5					+		
Total: 22	11	7	3	4	4	4	3

Note: DG – digestive gland.

The character of the distribution of filamentous fungi in the mollusk internal organs was studied: the mantle contained seven species; the muscle, three species; the digestive gland, fours species; the gonads, four species; the gills, four species; the kidneys, three species (see Table). Eleven species of filamentous fungi were revealed on the shell valves.

The species *Alternaria litorea*, *A. tenuissima*, *Fusarium oxysporum* var. *orthoceras*, *F. lateritium*, *Penicillium digitatum* var. *californicum*, and Mycelia Sterilia 4 were detected only on the valves of the giant oyster (Zvereva, Borzykh, 2010; Borzykh, Zvereva, 2012). The anamorphic fungus *Aspergillus phoenicis* was isolalated from all the internal organs studied, but not from the mollusk valves.

The species *Aspergillus antodesmus*, *A. candidus*, *A. ochraceus*, *Penicillium brevicompactum*, and *P. simplicissimum* were revealed only in the mantle. The Mycelia Sterilia 5 isolate was revealed in the gonads; Mycelia Sterilia 2 and Mycelia Sterilia 3 were isolated exclusively from the gills. The kidneys, the muscle, and the digestive gland were found to contain the species of filamentous fungi also detected in the other organs.

The fungi of the genus *Aspergillus* revealed in the internal organs of the oyster are assigned to the group of opportunistic and toxicogenic fungi (Bilai, Koval, 1988; Zvereva et al., 2009).

Acknowledgements

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The changes of macrobenthic community structure in the Jiaozhou Bay (Yellow Sea) in the past 30 years

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The Jiaozhou Bay located on the northwest of the Yellow Sea within the area of 35.38–36.18° N, 120.04–120.23° E. The Jiaozhou Bay is about 446 km^2, the average depth is 7 meters, and the maximum depth is 64 meters near the mouth part of the bay. It belongs to the warm temperate monsoon climate with four distinct seasons. The Jiaozhou Bay is impacted by human activities and natural changes.

Macrobenthos is an important component in marine ecosystems. The changes of macrobenthic community can reflect the impacts on marine ecosystem by natural changes and human activities. In the recent 30 years, especially the Jiaozhou Bay Ecology Station became the member of Chinese Ecosystem Research Network (CERN). In total, four cruises were carried out in the four seasons every year, a large amount of data of macrobenthos were accumulated. Based on the survey data, long-term changes of the macrobenthic community were analyzed in this study.

In recent 30 years, average 161.15 species of macrobenthos was found in Jiaozhou Bay for one year; of them, Polychaeta was the best represented taxon with 63.42 species (accounting for 38.48%), followed by Crustacea with 40.08 species (26.59%), Mollusca with 32.15 species (20.30%), Echinodernata with 8.54 (5.49%), and the other group with 13.96 (9.14%). The species number of macrobenthos yearly fluctuated during the period, and recently the species number showed an increasing trend. In the period, the species number of Polychaeta showed an increasing trend from 1983; the species number of Crustacea yearly fluctuated 12–64, and Mollusca 19–62, Echinodernata 5–14. The species number in each station showed an increasing trend from 1980 to 1999, but then showed a decreasing trend.

The species were identified as dominant species in this study in accordance with their values ($Y = (n_i/N) \times f_i$). The top 5 dominant species were counted in the period, Polychaeta appeared 82 times, Crustacea 16, *Branchiostoma belcheri tsingtauense* 12, Mollusca 9, and Echinodernata 6. The dominant species also changed in the past 30 years. In 1980–1990s, *Branchiostoma belcheri tsingtauense* was one of the most important dominant species, but it rarely appears as a dominant species in recent years, and *Raphidopus ciliates* also lost its dominance after 1990's.

The average abundance in the period was 239.11 ind/m^2, and the highest abundance was 389.42 ind/m^2 in 2006. The abundance was gradually increasing in 1980–1985, and lower in 1989–1991, then increasing, from 1999 the average abundance was high and relatively stable. The average biomass of the research region was 49.03 g/m^2, the highest biomass appeared in 1998, and the lowest biomass was 22.73 g/m^2 in 2003. From 1980's, the biomass showed a decreasing trend. The Shannon–Wiener index was high in 1980–1981, which descended suddenly in 1982, increased gradually from that time, and in 1998–2000 reached a high level, then decreasing. The distribution patterns of the Shannon–Wiener index were different, usually higher values appeared in the northern and central parts of the bay. The secondary production fluctuated from 4.52–17.98 g (AFDW)/(m^2·a), the highest value appeared in 1998. The general trend of the secondary production was gradually decreasing from 1980 to 2010. The secondary production in winter was lower than that in the other seasons, and the highest value appeared in spring.

The macrobenthic community in Jiaozhou Bay were divided into six communities based on the data of 1980–1981 (Liu et al., 1992): (1) *Branchiostoma belcheri tsingtauense* community, (2) *Ophelina aulogaster–Ampharete* sp. community, (3) *Temnopleurus toreumaticus–Amphioplus japonicas* community, (4) *Ruditapes philippinarum–Cirolana japonica* community, (5) *Protankyra bidentata–Leionucula* sp. community, (6) *Raeta pulchella–R. philippinarum* community. The community structure had changed in the past 30 years. In recent survey cruises, *Branchiostoma belcheri tsingtauense* disappeared in the station near the mouth part of the bay. *Ophelina aulogaster–Ampharete* sp. community was distributed in the outer part of the bay, but recently they are not the dominant species in that region, and the species belong to Polychaeta (*Paralacydonia paradoxa*, *Nephtys oligobranchia*, *Sternaspis scutata*) were the main dominant species. *Temnopleurus toreumaticus–Amphioplus japonica* community distributed in the central part of the bay, but they disappeared in recent cruises. *R. philippinarum–Cirolana japonica* community was distributed in the eastern part of the bay, but *R. philippinarum* was not collected collected in 2010. *Protankyra bidentata–Leionucula* sp. community was distributed in the northern part of the bay, and *Protankyra bidentata* and *Leionucula* sp. appeared in that region, but there are some cultured species like *R. philippinarum*. *R. pulchella–R. philippinarum* community was distributed in the northeastern part of the bay, but it also disappeared in recent survey cruises.

Biology and taxonomy of Spionidae, one of the largest groups of the polychaetous annelids (Annelida) in Peter the Great Bay of the Sea of Japan (East Sea)

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Spionidae is one of the most numerous and abundant groups of the polychaetous annelids, comprising more than 500 recent species. Adult spionids occur in a wide variety of habitats in benthic communities from the intertidal to the deep sea, while their larvae are common world-wide in the plankton. Although the Spionidae is not defined by synapomorphy but, as some other annelid families, by a combination of homoplasious characteristics, adult spionids and their developing larvae are readily recognized by their general body morphology and the pair of long prehensile palps extending from the head. From other palpate polychaetes they can be distinguished by the shape of body, parapodia and chaetae even when palps are lost due to damage or fixation. Most spionids live on soft bottoms (usually sandy, silty, rarely muddy), either moving freely in sediment near the surface or dwelling in more or less temporary or permanent tubes. The population density of such tube-dwellers may reach hundreds of thousands of individuals per square meter. Some polydorin spionids burrow into sponges, mollusc and barnacle shells, corals, lime-, mud- and sandstones. Most of the borers are associated with various host species; these usually use the host shell only as a suitable substratum and, as with tube-dwellers, collect food from the surrounding medium by long palps. The borers may form dense colonies with hundreds of individuals in one shell or sponge that greatly affect the host. Infestation by boring polydorins is a serious concern for mollusc fisheries and aquaculture. The larvae of intertidal and shallow subtidal spionids, especially those occurring in estuaries (often used by man as port areas) easily survive in ballast waters and are transported worldwide. There have been numerous unintentional transportations through aquaculture, especially of oysters, some of which have had dramatic consequences.

Polychaetous annelids have been subject of studies since early biological investigations on the Russian Far East and 259 species were reported from Peter the Great Bay situated in the northwestern part of the Sea of Japan (East Sea) (Adrianov & Kussakin, 1998). About 50 species of 17 genera of Spionidae were described from this area by Zachs (1933), Annenkova (1937, 1938), Uschakov (1955), Buzhinskaja (1967, 1971), Koblikov (1977), Bagaveeva (1981, 1986, 1988, 1992), Radashevsky (1983, 1985, 1986, 1988, 1989, 1993), Britayev & Rzhavsky (1985), Ozolinsh (1990a, b, 2002), Belan (1992, 2003), Buzhinskaja & Britayev (1992), Manchenko & Radashevsky (1998), Ozolinsh & Bagaveeva (2004), and Radashevsky & Pankova (2006). Most of these spionids live on soft bottom, whereas adults of 13 species bore into mollusc shells, and one species bores into sponges. Adults of eight polydorin spionids bore into shells of commercial molluscs, such as scallop *Mizuhopecten yessoensis* (Jay, 1857), giant oyster *Crassostrea gigas* (Thunberg, 1793), and mussel

Crenomytilus grayanus (Dunker, 1853). Two spionids, *Polydora cornuta* Bosc, 1802 and *Polydora limicola* Annenkova, 1934, were introduced to Peter the Great Bay with ship fouling and ballast water, while *Pseudopolydora achaeta* Radashevsky & Hsieh, 2000 enters the bay with warm water currents from the south (Zvyagintsev et al., 2011).

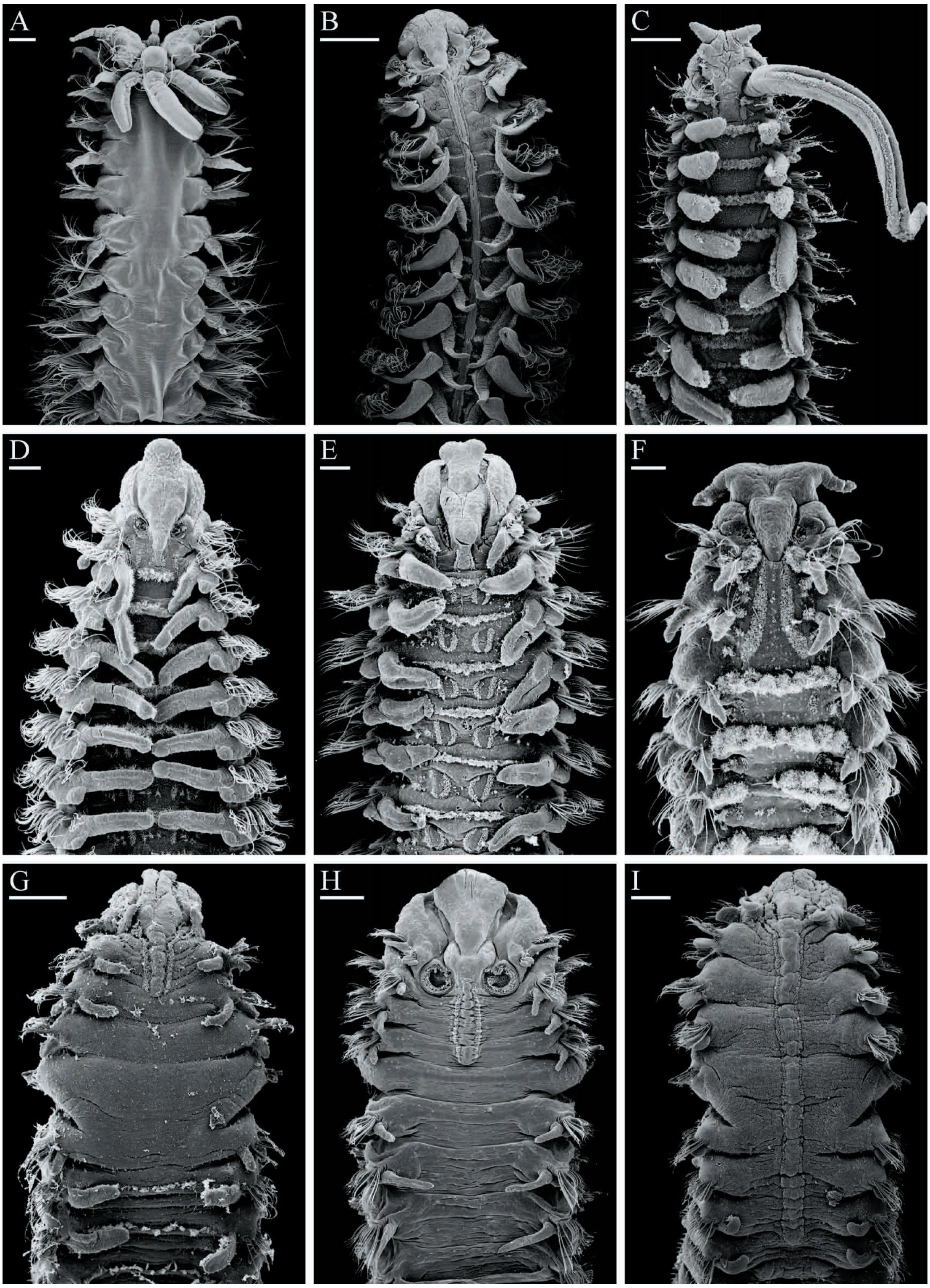
The morphology, ecology, reproductive biology and phylogeny of Spionidae are the objectives of the author's studies over the world during more than 30 years (see Radashevsky, 2012). The identification key to spionid genera occurring in Peter the Great Bay is given below and some representatives of the family are shown on figure.

List of genera of Spionidae reported from or likely to be found in Peter the Great Bay

1. *Aonides* Claparède, 1864
2. *Apoprionospio* Foster, 1969
3. *Boccardia* Carazzi, 1893
4. *Boccardiella* Blake & Kudenov, 1978
5. *Carazziella* Blake & Kudenov, 1978
6. *Dipolydora* Verrill, 1881
7. *Laonice* Malmgren, 1867
8. *Microspio* Mesnil, 1896
9. *Poecilochaetus* Claparède in Ehlers, 1875
10. *Polydora* Bosc, 1802
11. *Prionospio* Malmgren, 1867
12. *Pseudopolydora* Czerniavsky, 1881
13. *Pygospio* Claparède, 1863
14. *Rhynchospio* Hartman, 1936
15. *Scolelepis* Blainville, 1828
16. *Spio* Fabricius, 1785
17. *Spiophanes* Grube, 1860

Key to genera of Spionidae reported from or likely to be found in Peter the Great Bay

1. Dorsal branchiae absent 2
– Dorsal branchiae present 3
2(1). Dorsal lip of peristomium forming elongated facial tubercle. Neuropodia of chaetiger 1 with only capillaries. Postchaetal lamellae ampulaceous from chaetiger 7. Plumose chaetae present in noto- and neuropodia .... *Poecilochaetus*
– Dorsal lip of peristomium convex, not elongated. Neuropodia of chaetiger 1 with one or two large crook-like spines in addition to capillaries. Postchaetal lamellae short, subtriangular to cirriform and subulate, not ampulaceous. Plumose chaetae absent .... *Spiophanes*
3(1). Chaetiger 5 with only capillaries 4
– Chaetiger 5 with heavy spines in addition to capillaries in notopodia 11
4(3). Branchiae beginning after chaetiger 10, limited to middle and posterior chaetigers. One pair of dorsal appendages (horns) present on chaetiger 2 in males. Pygidium with two pairs of cirri .... *Pygospio*
– Branchiae from chaetigers 1–3 for a variable number of chaetigers. Horns absent on chaetiger 2 in males. Pygidium with cirri or otherwise 5
5(4). Branchiae throughout most of body length 6
– Branchiae limited to anterior half of body 8



Spionid polychaetes from Peter the Great Bay of the Sea of Japan (East Sea), anterior ends of adult worms, all in dorsal view with palps missing (except the right palp is present in C). **A**, *Poecilochaetus trilobatus*. **B**, *Laonice cirrata*. **C**, *Rhynchospio arenincola*. **D**, *Microspio kussakini*. **E**, *Spio* cf. *filicornis*. **F**, *Spiophanes uschakowi*. **G**, *Boccardiella hamata*. **H**, *Boccardia perata*. **I**, *Dipolydora carunculata*. Scale bars: A, C, G–I – 200 µm. B – 500 µm. D–F – 100 µm.

6(5). Head anteriorly conical and distally pointed. Nuchal organs entire U-shaped ciliary bands over 1–2 anterior chaetigers. Branchiae from chaetiger 2. Posterior notopodia with hooks in addition to capillaries .... *Scolelepis*

– Head anteriorly wide, rounded to truncate, not pointed. Nuchal organs metameric ciliated bands over a number of anterior chaetigers. Pygidium with two pairs of cirri 7

7(5). Branchiae from chaetiger 1. Posterior nuchal metamers double ciliary bands .... S*pio*

– Branchiae from chaetiger 2. Posterior nuchal metamers single ciliary bands .... *Microspio*

8(5). Occipital antenna present. Nuchal organs extending beyond chaetiger 3. Branchiae present over most of anterior body. Posterior notopodia with only capillaries. Lateral pouches present between middle neuropodia .... *Laonice*

– Occipital antenna present or absent. Nuchal organs extending over 1–2 anterior chaetigers or absent. Branchiae limited to first 22 chaetigers. Posterior notopodia with hooks in addition to capillaries. Lateral pouches absent between middle neuropodia 9

9(8). Prostomium conical with narrow and rounded tip anteriorly. Occipital antenna present on prostomium. Nuchal organs absent. Branchiae up to 21 pairs .... *Aonides*

– Prostomium broadly rounded to truncate anteriorly. Occipital antenna absent on prostomium. Nuchal organs U-shaped ciliary bands over 1–2 anterior chaetigers. Branchiae up to 10 pairs 10

10(9). Branchiae all smooth or some with digitiform pinnules .... *Prionospio*

– Branchiae on chaetigers 2–4 apinnate, on chaetiger 5 with plate-like appendages ... *Apoprionospio*

11(3). Hooks in neuropodia from chaetiger 7. Branchiae from chaetigers 2–10 12

– Hooks in neuropodia from chaetiger 8. Branchiae from chaetiger 7 15

12(11). Branchiae from chaetiger 2 (from chaetiger 7 in early juveniles) 13

– Branchiae from chaetigers 7–10 in all-size individuals 14

13(12). Notopodia of chaetiger 5 with heavy falcate spines alternating with bilimbate-tipped companion chaetae .... *Boccardiella*

– Notopodia of chaetiger 5 with heavy falcate spines and bristle-topped spines .... *Boccardia*

14(12). Chaetiger 1 with notochaetae. Branchiae from chaetigers 7–10. Hooks accompanied by inferior capillaries at least in anterior neuropodia; upper part of hook shaft without constriction .... *Dipolydora*

– Chaetiger 1 without notochaetae. Branchiae from chaetiger 7. Hooks not accompanied by inferior capillaries; upper part of hook shaft with constriction .... *Polydora*

15(11). Notopodia of chaetiger 5 with heavy falcate spines and enlarged capillaries with wide distal limbation Upper part of hook shaft with constriction; lower part of hook shaft bent at about right angle .... *Pseudopolydora*

– Notopodia of chaetiger 5 with heavy falcate spines and bristle-topped spines. Upper part of hook shaft without constriction; lower part of hook shaft slightly curved .... *Carazziella*

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A preliminary analysis on the polychaete fauna of the deep Sea of Japan

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Investigations of the deep-sea fauna of the Sea of Japan started with the expedition of 1932 conducted by K.M. Derjugin when qualitative samples were taken at depths to 3535 m. In 1950–1976, deep-sea investigations of the Sea of Japan were continued on board RV "Vityaz". Quantitative biological sampling sites covered the lower continental shelf and slope down to the abyss. Results of these expeditions showed that the number of bottom living fauna of the Sea of Japan decreases markedly with depth (Derjugin, 1939; Zenkevich, 1963; Levenstein and Pasternak, 1976). The deep-sea fauna of the Sea of Japan is poor and composed of eurybathic species found in adjacent high-boreal regions. About 24 polychaete species were reported from depths >1000 m and only one of them, *Harmothoe derjugini* (Annenkova, 1937) was considered to be a truly endemic deep-sea species (Mokievsky, 1954). According to Annenkova (1937, 1938) only 8 polychaete species were found in depths of 1000–3900 m. Levenstein (1969) recorded about 30 species of Polychaeta inhabiting depths greater than 2000 m in the Sea of Japan. To date, little is known about the Polychaeta from the slope and the deep-sea basin of the Sea of Japan, although they belong to one of the most abundant and most diverse invertebrate groups in benthic communities.

The main purpose of this study is to give a preliminary estimation of the composition and distribution of the polychaetes sampled during the *SoJaBio* (*Sea of Japan Biodiversity Studies*) expedition from the deep-water areas in the north-western part of the Sea of Japan.

Material and Methods

During the joint Russian-German expedition *SoJaBio* on board of RV "Akademik Lavrentjev" (51st cruise) in August–September 2010 samples with the camera-epibenthic sledge (C-EBS) were taken mainly in the northwestern sector of the Sea of Japan. A total of 13 EBS-stations along 4 transects down the continental slope to abyssal depths (from 455 to 3666 m) have been sampled. On deck the samples were washed with ice-cold water and sieved through 300-µm mesh size. The samples were fixed with 4% formaldehyde and later transferred to 96% ethanol for preservation and morphological studies. Epi- and supranet samples were sorted separately, but for the current analysis supra- and epi-net data were pooled. In the laboratory the sampled polychaete specimens were sorted to species or genus level. For analyses of a-diversity the Shannon–Wiener Index (H' based on $\log_e$), the Pielou's evenness (J') and species richness after Margalef (d) were calculated (Primer 5).

Results and Discussion

To date, 92 polychaete taxa belonging to 70 genera and 28 families and 3 indeterminate species have been identified. The most abundant families are the Spionidae, Opheliidae, Polynoidae, Ampharetidae and Cirratulidae. The most speciose family is the Sphaerodoridae with 10 species followed by the Maldanidae and Paraonidae with 8 and 7 species, respectively, Syllidae and Cirratulidae with 6 species each, Ampharetidae, Phyllodocidae, Polynoidae, Spionidae and Terebellidae with 5 species each. The remaining families are represented by 1–3 species. A total of 37 polychaete taxa were identified to species level, the other species taxa were identified to genus or family level. It is likely that some of the undetermined polychaete species belonging to the genera *Sosane*, *Aphelochaeta*, *Chaetozone, Lacydonia*, *Sphaerodoridium*, *Sphaerodoropsis* while others are new to science. Among the identified species, 12 are new for the polychaete fauna of the Sea of Japan: *Chaetozone* cf. *commonalis* Blake, 1996, *Gyptis hians* Fauchald and Hancock, 1981, *Macellicephala violacea* (Levinsen, 1887), *Anguillosyllis* cf. *capensis* Day, 1963, *Eusyllis tubicola* Uschakov, 1950, *Lanassa* cf. *nordenskjoldi* Malmgren, 1866, *Sagitella kowalewskii* Wagner, 1872, *Travisiopsis lanceolata* Southern, 1910, *Travisiopsis lobifera* Levinsen, 1885, *Sosane* sp., *Apistobranchus* sp. and *Lacydonia* sp. Eight genera (*Sosane*, *Gyptis*, *Anguillosyllis*, *Apistobranchus*, *Macellicephala*, *Lacydonia*, *Sagitella*, *Travisiopsis*) have not been reported from the Sea of Japan before but were registered from other basins including deep-sea areas (Uschakov, 1972, 1982; Hilbig, 1994; Jirkov, 1994; Blake, 1996; Böggemann, 2009).

The present results show that the composition of Polychaeta in the deep sea of the Sea of Japan does not seem to differ at higher taxonomic level from that of other deep-sea regions world-wide (Schüller et al., 2009). Noteworthy is the spionid polychaete prevalence in abundance in our samples. Recent studies have shown that the dominance of the Spionidae in number of individuals and sometimes species is also a typical pattern observed in the Southern Ocean deep sea (Hilbig, 2001; Wilmsen and Schüller, 2011) and Pacific Ocean (Hilbig and Blake, 2006).

The highest number of species (84 species) was found at the shallow station B7-7 positioned on the continental slope in the central part of the investigated area. This station also yielded the highest number of specimens (5406). The lowest abundance (25 specimens) and number of species (3) was found at the abyssal plain station D1-4 (Fig. 1).

Species richness and diversity of Polychaeta strongly decreased with depth: between ~450–550 m 84 species were found, between ~1000–1500 m – 36 species, between ~2500–2700 m – 14 species and at ≥3300 m – 8 species (Figs. 1, 2). At abyssal depths greater than 3300 m the number of species per station ranged from 3 to 6. In comparison a total of 45 species were found at bathyal depths between 1000–3431 m. This result is similar to what Levenstein (1969) reported from the deep sea of the Sea of Japan.

The diversity indices indicate that the Sea of Japan continental slope (st. B7-7 and B6-7) is the area of highest polychaete diversity among all stations analyzed (Fig. 2). Shannon diversities (H') were between 0.3 and 2.8 and Margalef diversities (d) varied from 0.46 to 9.66 between stations. The lowest diversity and evenness (J') were found for stations B4-8 and C3-4 where the polychaete community was clearly dominated by the spionid *Laonice* sp. In contrast, station D1-4 with the lowest number of species (3) showed the highest evenness with J'=0.8 due to the presence of only few individuals of each species at this station.

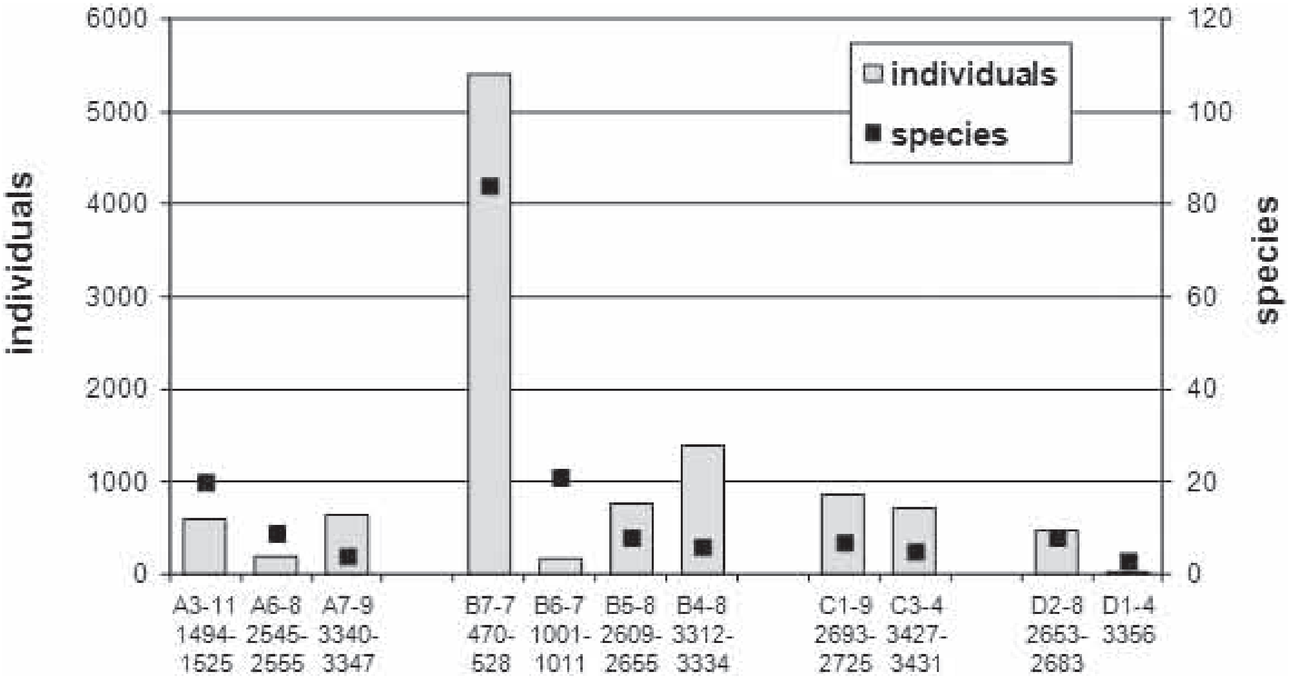

Fig. 1. Numbers of polychaete individuals and species at C-EBS stations ordered by transects and depth.

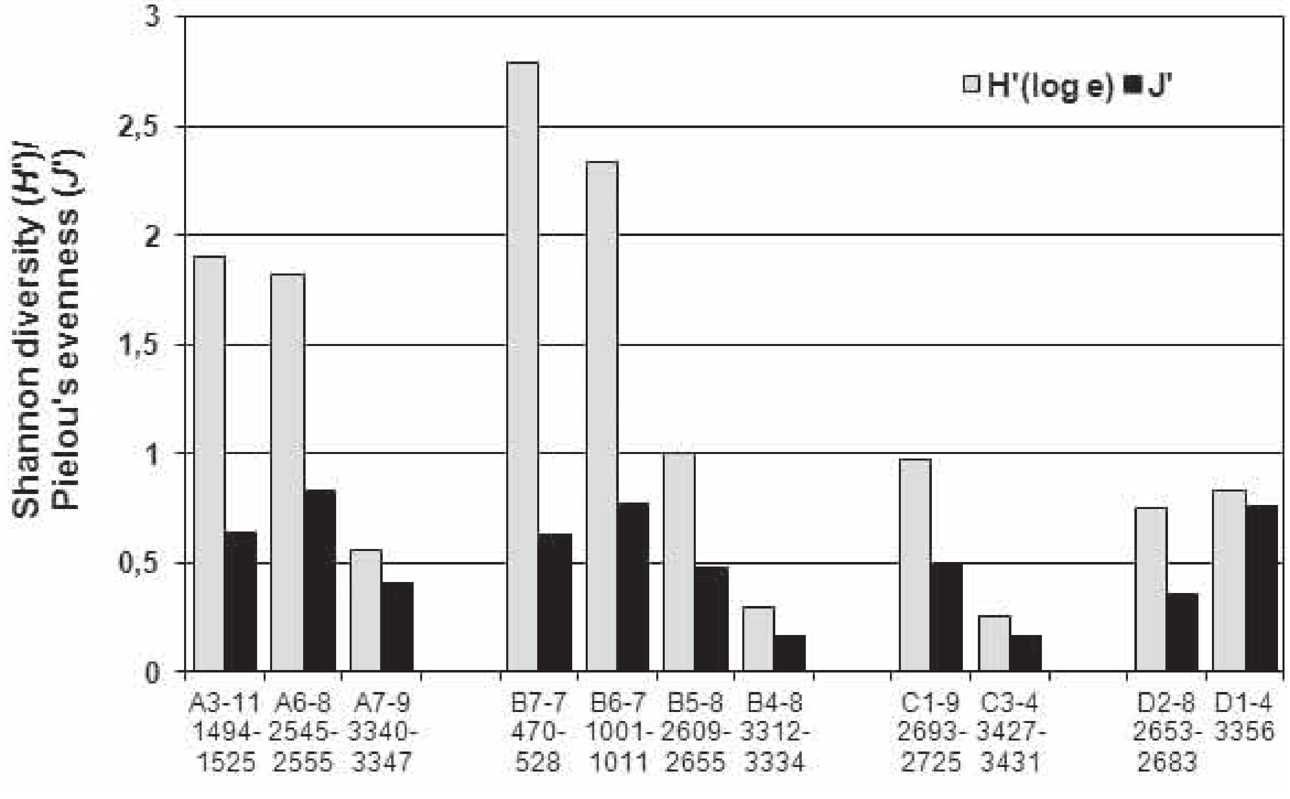

Fig. 2. Shannon diversity (based on $\log_e$) and Pielou's evenness for Polychaeta sampled in the north-western part of the Sea of Japan.

For polychaetes Shannon diversities (based on $\log_e$) are commonly reported between 1.9 and 3.5 with evenness values above J'=0.7 due to the findings of many rare species (e.g., Hilbig and Blake, 2006; Hilbig et al., 2006; Schüller et al., 2009). These values are higher than we have observed. However, all authors described a decrease in polychaete diversity with depth. High diversity of the slope stations might therefore be evidence for the presence of faunal elements from both shelf and deep-sea communities (Brandt et al., 2007).

In this study the most widely distributed species were *Terebellides* sp., *Ampharete* sp., *Laonice* sp., *Chaetozone* sp. 1, *Aglaophamus malmgreni* (Theel, 1879), *Flabelligera affinis* Sars, 1829. They were found at 6–9 stations. More than half of all species (50 species or 53%) were sampled at one station only (B7-7), positioned on the continental slope at depths around 500 m. These results are similar to data reported by Wilmsen and Schüller (2011) from the Antarctic and Subantarctic waters where 52% species were limited to one station. Recent works (Hessler and Jumars, 1974; Glover et al., 2001, 2002) show that the patchiness is a common feature for deep-sea polychaetes worldwide in which species are often only represented in far-flung accumulations. Therefore, the lack of a species in samples does not necessarily rule out its occurrence in the respective area, but might result from undersampling or too small sample sizes (Wilmsen and Schüller, 2011).

In general, analyses of geographical distribution patterns indicate that many deep-sea polychaetes have wide distributional ranges at depths below 2000 m (Hilbig, 2004; Hilbig et al., 2006; Méndez, 2007; Wilmsen and Schüller, 2011). According to our data, among 17 species of Polychaeta found in depths below 2000 m 6 species (*Chaetozone* cf. *setosa* Malmgren, 1867, *Harmothoe impar impar* (Johnston, 1839), *Polydora cornuta* Bosc, 1802, *Scalibregma inflatum* Rathke, 1843, *Flabelligera affinis*, *Aglaophamus malmgreni*) are widely distributed worldwide, 10 species identified to genus level and only *Harmothoe derjugini* is a truly endemic deep-sea species of the Sea of Japan.

Preliminary analyses of the polychaete fauna of the Sea of Japan show that the upper bathyal is an area of higher species richness than the abyssal plain. The increased richness and diversity here could be explained by the overlapping with the shallower (and richer) species complex of the shelf. More than half of all species were sampled at one shallowest slope station only in this study. About 30% polychaete species found in the Sea of Japan at depths below 2000 m are characterized by wide-spread species.

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Techniques and applications for the investigation of marine microbenthos and Foraminifera in marine environmental monitoring and global change studies

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Marine microbenthos comprise unicellular prokaryotes and eukaryotes including benthic bacteria, microalgae, and Protozoa, which contained foraminifers, ciliates, dinoflagellates, amoeba. etc. These organisms constitute the basic and key components in marine microbial food webs and may play important roles in carbon and energy flows as well as in the marine biogeochemical cycle. Nevertheless, methodological shortcomings have long been the main problems in estimating the ecological roles of these microorganisms. The current Chinese regulations of “Specifications for Oceanographic Survey – Part 6: Marine Biological Survey” of the National Standard of China involved only marine meio- and macrobenthos, but marine microbenthos were absolutely neglected. This situation impeded the understandings of the carbon and energy flow and ecological interactions among micro-, meio- and macrobenthos within the entire marine food webs. The present study provides a series of standard methods and protocols for the surveys of marine microbenthos and foraminifera. The methods and protocols have been adopted by the Oceanographic Professional Standard as the “Specifications for the Survey of Marine Microbenthos”. These techniques could be applied in diverse marine sediments, such as intertidal flat, shallow sea, and deep sea to the investigation of microbenthos, as well as their applications in marine environmental monitoring and global changing studies. This study was supported by the Knowledge Innovation Program of Chinese Academy of Sciences (41176132, No. KSCX2-YW-Z-0914, 10-3-3-38jh, Y22330102H).

Zonal-biogeographical analysis of bivalve molluscan faunas in three major areas of the western Sea of Japan

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In the recently published catalogue of bivalve mollusks of the continental coast of the Sea of Japan, we divided the continental coast of the sea from Busan to Tatarsky Strait into four main areas: **SK** – South Korea; **NK** – North Korea; **SP** – South Primorye (Peter the Great Bay), Russia; and **MNP** – Middle and North Primorye, Russia (Fig. 1; Lutaenko, Noseworthy, 2012). When analyzing the biogeographical structure of the bivalve molluscan faunas of the western Sea of Japan, we follow the zonal-geographical (zonal-biogeographical) approach widely used in the Russian biogeographical literature. Although such an analysis has been undertaken several times in the past for larger areas of the sea (Scarlato, 1981; Romeyko, 1985) and for embayments (Possjet, Amursky, Ussuriysky, Yeongil bays – see bibliography in the catalogue), sufficiently new data, especially for South Korea, were obtained since that time. Temperate waters and fauna of the northern hemisphere are called boreal (Kussakin, 1990) and the term "boreal" corresponds to the American term (e.g., Hall, 1964; Briggs, 1974, 1995) "cold-temperate" or "cool-temperate". The term "warm-temperate" of the American workers should correspond to "subtropical". For more information about this approach and a clarification of terms see: Scarlato (1981); Kussakin (1990); Lutaenko (1993). This method allows to characterize faunas as more "warm-water" or less "warm-water" based on a proportion of species of different biogeographic nature.

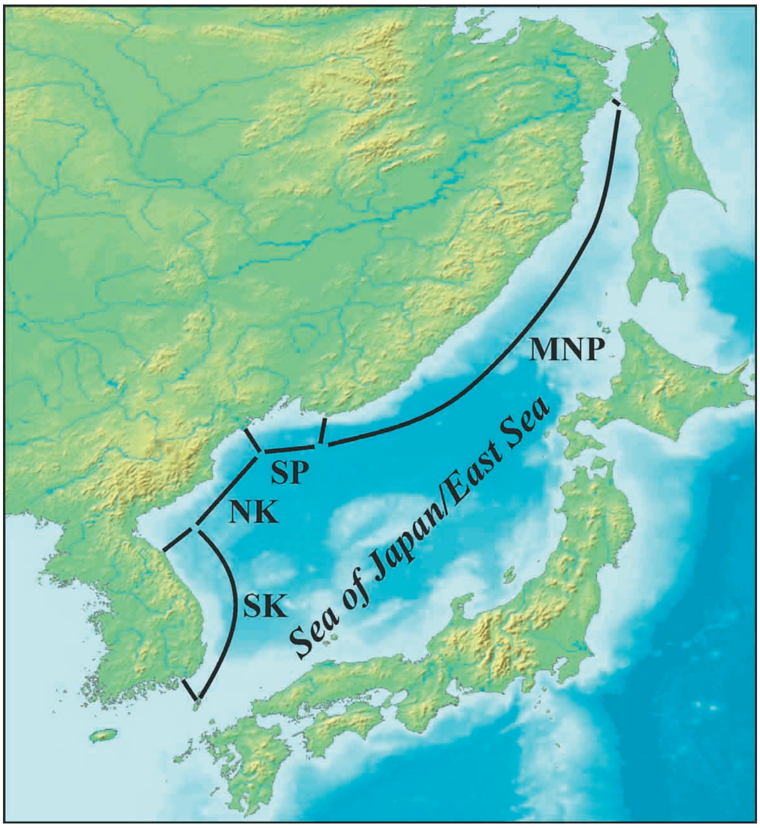

Fig. 1. A map of the Sea of Japan with indication of the four main regions of the continental coast: **SK** – South Korea, **NK** – North Korea, **SP** – South Primorye (corresponds to Peter the Great Bay), and **MNP** – Middle and North Primorye.

We recognize six major zonal-geographical groups: **1.** *tropical-subtropical* (distributed southward to the Philippines, Vietnam and Indonesia);

2. *subtropical* (distributed southward to Taiwan and the northern part of the South China Sea); **3.** *subtropical-lowboreal* (limited both to subtropical seas and the Sea of Japan, southeastern Sakhalin, and the southern Kuril Islands); **4.** *lowboreal* (limited to the Sea of Japan from Peter the Great Bay, northern Korea, and northern Honshu to southwestern Sakhalin, Aniva and Terpenya bays, and the southern Kurile Islands); **5.** *widely distributed boreal* (limited to the Sea of Japan and Hokkaido to the Bering Strait, along the Asian coast, and along the northern American coast southward to California), and *circumboreal* (limited mainly to temperate latitudes, both in the Atlantic and Pacific Oceans, but also partly to subtropical and arctic zones); **6.** *boreal-arctic* (limited to both the temperate zone of the Pacific Ocean and the Arctic, and partly to the temperate Atlantic). We checked the geographical distributions of many species using numerous literature sources not cited herein, and unified zonal-geographical characteristics are given for each species in our catalogue (Lutaenko, Noseworthy, 2012).

Tropical-subtropical, subtropical and subtropical-lowboreal species are warm-water groups. Presence of tropical-subtropical species in boreal waters may cause some misunderstanding, however, some of them have high tolerance range to temperature and can only slightly penetrate to relatively cold waters. To avoid confusion, we would like to emphasize that findings of tropical-subtropical and subtropical species in boreal waters merely mean that the **main** part of their distributional ranges lies in the respective climatic zones, but they can survive in winter and can reproduce in certain, warmed-up in summer, areas of the boreal Pacific; in Russian literature, these areas are termed inter-zonal regions (Scarlato, 1981). Winter cooling in itself does not prevent warm-water fauna from living in boreal waters as in some low-boreal areas an intense summer warming takes place which is necessary for successful reproduction (Scarlato, 1981). Climatic optimum of the Holocene played an important role in migration of warm-water mollusks to the north in the Sea of Japan and was an additional reason for penetration of those species to boreal areas (Lutaenko, 1991a, b; 1993; Taira, Lutaenko, 1993). Briggs (1995) calls these species as “eurythermic tropicals” – this group, although it ranges broadly in the tropics, also habitually occupies the warm-temperate zones that lie next to the tropics. It seems that part of the tropical-subtropical bivalve mollusks can live in the low-boreal waters too, in semi-enclosed bays and other protected shallow-water localities, as we can see in Peter the Great Bay. However, taxonomy of some species is still unclear and they may appear “mixed” species containing, in fact, several sibling species.

Kafanov (1991) cautions of the mixing of biogeographic proper and ecological terminology and proposes a “faunal”, neutral equivalent for zonal-geographical characteristics “tropical”, “subtropical” and “low-boreal” – thermotropic (in another paper, tropical, tropical-subtropical, low-boreal, boreal-subtropical and boreal-tropical are included into thermotropic – Kafanov and Volvenko, 1997), whereas “arctic”, “boreal-arctic”, “high-boreal” and “widely distributed boreal” (and amphiboreal= circumboreal, see Kafanov and Volvenko (1997)) are combined under term “psychrotropic”. Necessity of this terminological innovation is not obvious.

Zonal-biogeographical analysis

Based on updated species lists and verified zonal-geographical characteristics of species living in the western part of the Sea of Japan (MNP, SP and SK), we can undertake a biogeographic analysis of these regional faunas. North Korean waters are poorly studied, and only 50 species of bivalves are known there (Fig. 2), thus, we excluded this area from the analysis. Unfortunately, there are no exact bathymetric data for all three regions, but it is obvioius that we analyze the shelf fauna as an overhelming majority of records refer to depth down to 100–200 m deep. Although Scarlato (1981) and Lutaenko (2003, 2006) have shown clear bathymetric patterns in the distribution of species of different biogeographic nature on the example of embaymental faunas, the shelf fauna can be regarded as a whole. Moreover, deep-water molluscan fauna of the Sea of Japan is impoverished due to geological isolation of the basin and not well-studied at present.

Middle and northern Primorye bivalve fauna is represented by 124 species (Fig. 2) but one species (*Macoma* sp.) is out of the analysis. Among them, boreal species (low-boreal, widely distributed boreal and circumboreal) are predominant (56%) constituting together with boreal-arctic species a proportion of 74%. Warm-water subtropical and subtropical-low-boreal species constitute only 25%, and only one tropical-subtropical species (1%) – *Theora lubrica* – is known from MNP.

In the **southern Primorye** (Peter the Great Bay), among 156 species (Fig. 2; in total, 157 species; one species is not identified to species level), boreal species have share of 50%, constituting together with boreal-arctic ones 65% whereas a proportion of warm-water mollusks increases to 35%. There are nine tropical-subtropical bivalves in SP (*Trapezium liratum*, *Pillucina pisidium*, *Mactra quadrangularis*, *Raeta pulchella*, *Th. lubrica*, *Dosinia japonica*, *Dosinia penicillata*, *Barnea manilensis*, and *Laternula marilina*), and more subtropical proper species (23, or 15%). This clearly shows a high role of semi-enclosed inlets and inner parts of large ria-type bays with an intense summer-warming as well as the Holocene climatic warmings in the formation of the fauna of Peter the Great Bay. These interzonal species make the character of the southern Primorye fauna mixed: boreal in general but with an important role of boreal-arctic mollusks in the lower shelf, and warm-water species – in the upper subtidal zone. Comparing to data of Scarlato (1981) who found 135 species of bivalves in SP, the proportions of zonal-geographical gropus are similar to ours (in Scarlato's calculations, 69.5% – boral and boreal-arctic species, and 30.5% – warm-water species).

In coastal waters of **South Korea**, 313 species are found (Fig. 2) but five forms were not identified to species level and we analyzed 308 species. Among them, warm-water mollusks exceedingly predominate constituting 78% with prevalence in this biogeographical group of subtropical (38% of the total fauna), and many tropical-subtropical (95 species, or 31%). The role of boreal (low-boreal and widely distributed boreal) species is much lower – only 18% but boreal-arctic mollusks are also present (14 species, or 4%). These proportions evidence that the bivalve molluscan fauna of the eastern coast of Korea is subtropical and related more to the fauna of East China Sea than to northern Sea of Japan. An abrupt increase of number of species of warm-water mollusks in southern Sea of Japan (Fig. 3) and decrease of boreal and boreal arctic species although not significant (Fig. 4) confirm this opinion. The species richnes of subtropical

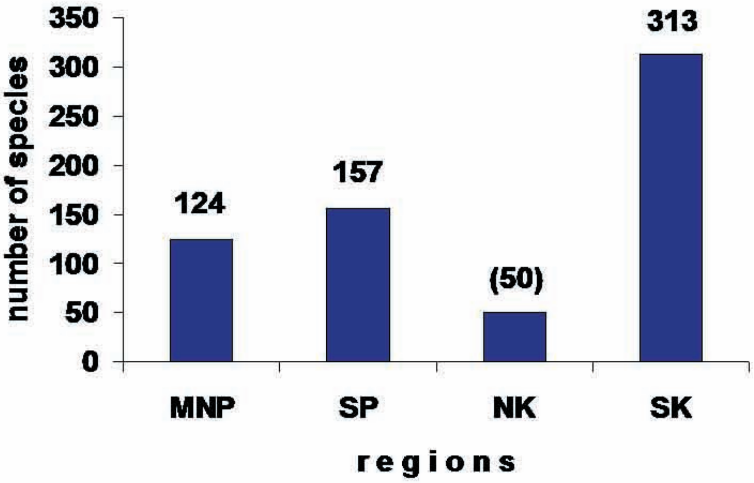

Fig. 2. Species richness of bivalve mollusks in four areas of the continental coast of the Sea of Japan. For other abbreviations, see Fig. 1.

and tropical-subtropical bivalves shows clear gradient from north to south along the continental coast of the Sea of Japan: for subtropical, **9** (MNP) → **23** (SP) → **117** (SK), and for tropical-subtropical: **1** (MNP) → **9** (SP) → **95** (SK), whereas low-boreal (**29** (MNP) → **30** (SP) → **23** (SK)) and even boreal-arctic (**22** (MNP) → **24** (SP) → **14** (SK)) do not exhibit well this tendency. Thus, in South Korea coast, the number of subtropical species as compared to southern Primorye increases by 5 times, and tropical-subtropical – more than 10 times. It is obvious that the transitional zone between two faunas lie in North Korea although prelinimary data on bivalves of East Korean Bay (area of Wonsan) document rather cold-water character of North Korean fauna: molluscan assemblages of East Korean Bay (31 species) bear close resemblance in their composition and biogeographical aspects to those of Peter the Great Bay; all of them are known from southern Primorye, and the predominant role belongs to boreal and cold-water species (Evseev, 1996). In other words, the main trend in zonal-biogeographical structure change from north to south can be described as significant enrichment of the southern part of the sea by subtropical and tropical-subtropical faunal elements whereas boreal species occupy in both faunas rather similar position. However, due to biogeographical submergence (a phenomenon when relatively cold-water animals occur in deeper areas at the edge of their ranges), the cold-water mollusks proportion and role may be different at various bathymetric ranges. Moreover, warm-water mollusks also submerge into deeper areas in southern part of the sea as was demostrated by Lutaenko (1999).

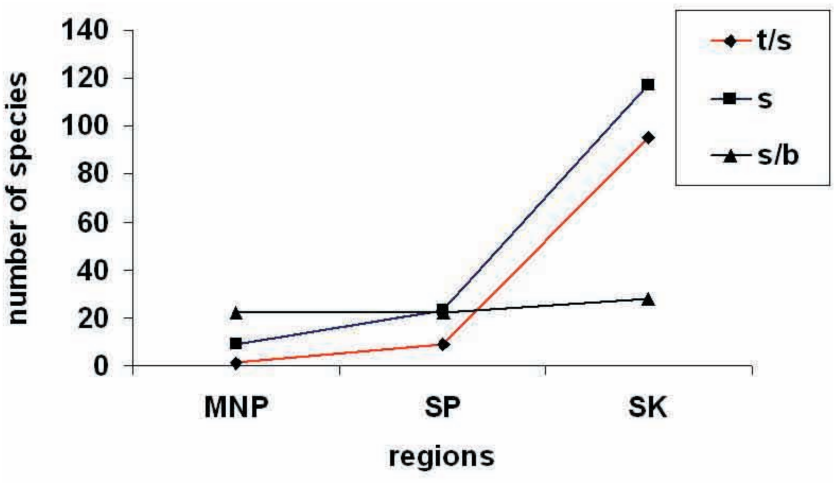

Fig. 3. Changes of number of species of warm-water bivalve mollusks along the continental coast of the Sea of Japan (**t/s** – tropical-subtropical species, **s** – subtropical species, **s/b** – subtropical-boreal (mostly subtropical-lowboreal) species). For other abbreviations, see Fig. 1.

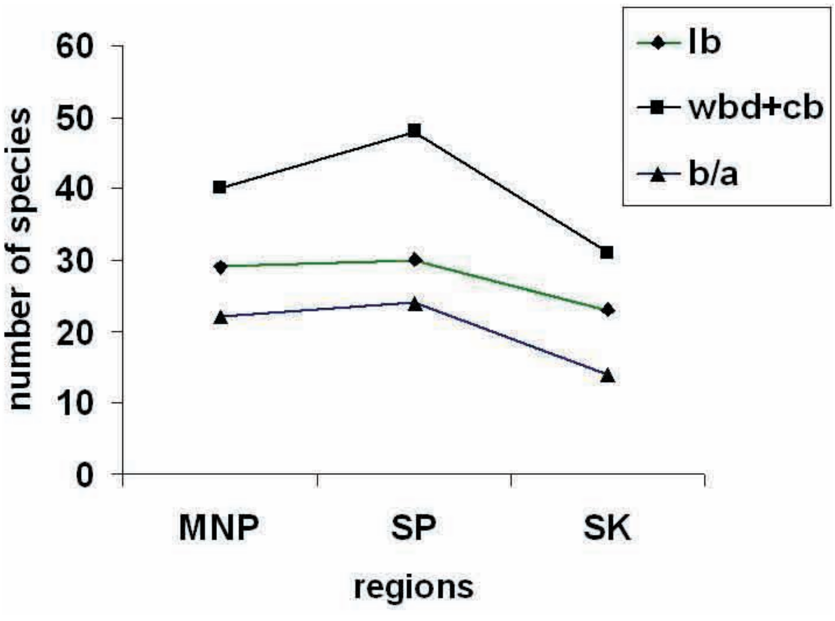

Fig. 4. Changes of number of species of boreal (**lb** – low-boreal, **wdb+cb** – widely distributed boreal and circumboreal species) and boreal-arctic (**b/a**) bivalve mollusks along the continental coast of the Sea of Japan. For other abbreviations, see Fig. 1.

Chinese malacologists (Tchang et al., 1963) regarded Yellow Sea, Korean and Russian coasts of the Sea of Japan as belonging to the Far Eastern Subregion of the temperate North Pacific Region. According to Briggs and Bowen (2011), the Sea of Japan belongs to three provinces: cold-temperate (or boreal) Kurile (most northern part of the sea) and Oriental (middle to southern part of the sea including south-western coast), and warm-temperate Sino-Japanese.

A detailed review of the demarcation lines in the sea based on studis if various animal groups can be found in Nishimura (1965, fig. 1) who identified at least four cross-boundary lines (between east and west coasts) proposed by previous authors (Olga Bay – Soya Strait; Wonsan (East Korean Bay) – Soya Strait; Yeongil Bay – Tsugaru Strait; Busan – north of Wakasa Bay) and 8 boundary lines along the Honshu and Hokkaido. Along the eastern and southern Korean coasts, Nishimura (l.c. and references therein) mentioned demarcation around Chinhae Bay (fishes), Busan (crabs), zone Yeongil Bay – Korea Strait (decapod crustaceans), Yeongil Bay (cirrripeds). The boundary between boreal and subtropical parts of the sea was roughly set by Scarlato (1981) between East Korean Bay and around Sado Island based on the fact that in these points subtropical species of bivalve mollusks begin to prevail over boreal ones. Kafanov (1991) considered the Korean coast south to Busan as belonging to the North Pacific (boreal) Region. Ophiuran biogeography suggests that the zoogeographic boundary line exists on the Korean coast to the northeast of Yeongil Bay (Shin, Koh, 1993).

Biogeographical bondaries are not frontier lines but transitional zones between different faunas and it is rather difficult to determine them precisely. We propose that there is a transitional zone marking significant increase of warm-water species between Yeongil Bay and Busan on the continental coast of the Sea of Japan, and, similarly, corresponding zone between Noto Peninsula and Tsugaru Strait in eastern part of the sea which can be compared with earlier "discontinuity belt" of abrupt change of number of southern elements of Nishimura (1965). This belt marks boundary between subtropical and boreal zones in the Sea of Japan, or Warm-Temperate and Cold-Temperate zones in the sense of Briggs (2007). This viewpoint is confirmed by our zonal-geographical analyses. Horikoshi (1962) noted that on the Pacific side of Japan, the subtropical region directly adjoins the temperate region without a warm-temperate region between them whereas no such sharp boundary between the subtropical and temperate regions is found on the Sea of Japan side. Usage of term "subtropical zone" seems to be more desirable as subtropical species in their origin are related to tropical fauna, but not the boreal one ("the warm-temperate regions border the tropics and have evident tropical relationship" and "the cold-temperate regions have biotas that are markedly different than those of the warm-temperate regions" – Briggs (1995, p. 284)). In this sense, South Korean bivalve molluscan fauna on the south-eastern coast of the peninsula is subtropical.

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Amphipod distribution off the oil-and-gas platform Moliqpak on the shelf of northeastern Sakhalin (Okhotsk Sea)

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Amphipods are among the dominant macrobenthic taxa on the shelf of the eastern coast of the Sakhalin Island (Nadtochy et al., 2004; Moshchenko et al., 2005; Fadeev, 2011). Amphipod species composition and their distribution on the shelf of eastern Sakhalin were previously studied (Budnikova, Bezrukov, 2003; Demchenko, Fadeev, 2011). It is well known that amphipods are very sensitive to oil pollution and any environmental disturbances (Bellan-Santini, 1980; Gómez-Gesteira, Dauvin, 2000; Nikitik, Robinson, 2003; Guerra-Garcia, Garcia-Gomez, 2004; De-la-Ossa-Carretero et al., 2012). Amphipod quantitative characteristics and species diversity can be used to indicate water quality. Over the last decade, oil and gas have been extracted on the shelf of the north-eastern part of the Sakhalin Island. Thus, monitoring biota in this region is very urgent now (Labay et al., 2008; Fadeeva, Maslennikov, 2009). The aim of the present work is to study species composition and quantitative distribution of amphipods in the region of the oil-and-gas platform Moliqpak (the Piltun-Astohskoye oil-and-gas field). The macrobenthic samples were taken by collaborators of the A.V. Zhirmunsky Institute of Marine Biology in October 2004 in the region of the oil-and-gas platform Moliqpak. The sampling was carried out on four transects (Fig. 1). The sites were located 125, 250, 500, 1000, 3000 m to the north, south, west and east from the platform in depths of 23–33 m. A total of 25 sites were observed. Four replicates were taken at each site by means of an 0.2 m^2 Van Veen grab. Amphipods were preserved in 4% buffered formaldehyde solution and then transferred to 75% ethanol solution after a week fixation. Amphipod biomass per site was expressed in g/m^2 and transformed by the fourth root and the similarity of species composition among sites was compared by the Bray–Curtis coefficient (Clarke, 1993). The ordination of sites was performed by means of nonparametric multidimensional scaling MDS (Clarke, Warwick, 2001). Twenty-six species of amphipods belonging to 16 genera and 9 families were found in the region of the oil-and-gas platform Moliqpak. The most representative families as to the number of species were Oedicerotidae (7 species), Lysianassidae (5), Uristidae (3) and Corophiidae (3). Only 8 of 26 species had frequencies of occurrence over 50%: *Anonyx nugax* (88%), *Anonyx lilljeborgi* (84%), *Pleusymtes* sp. (64%), *Psammonyx kurilicus* (56%), *Protomedeia fasciata* (52%), *Monoculodes zernovi* (52%), *Orchomenella minuta* (52%) and *Bathymedon* sp. (52%).

Average amphipod biomass in the region of the oil-and-gas platform Moliqpak (Fig. 1) in October 2004 varied from1.9 to 81.2 g/m^2 with an overall mean of 23.9±5.0 g/m^2. The maximum amphipod biomass was at 125 m from the platform on the northern and eastern transects. Three species had the greatest biomass: *Anonyx nugax* (45% from total amphipod biomass), *Anisogammarus pugettensis* (32%) and *Anonyx lilljeborgi* (9%). The maximum amphipod biomass was in the gravel sediments, while minimum biomass – was in the sandy sediments. The distributions of some amphipod species

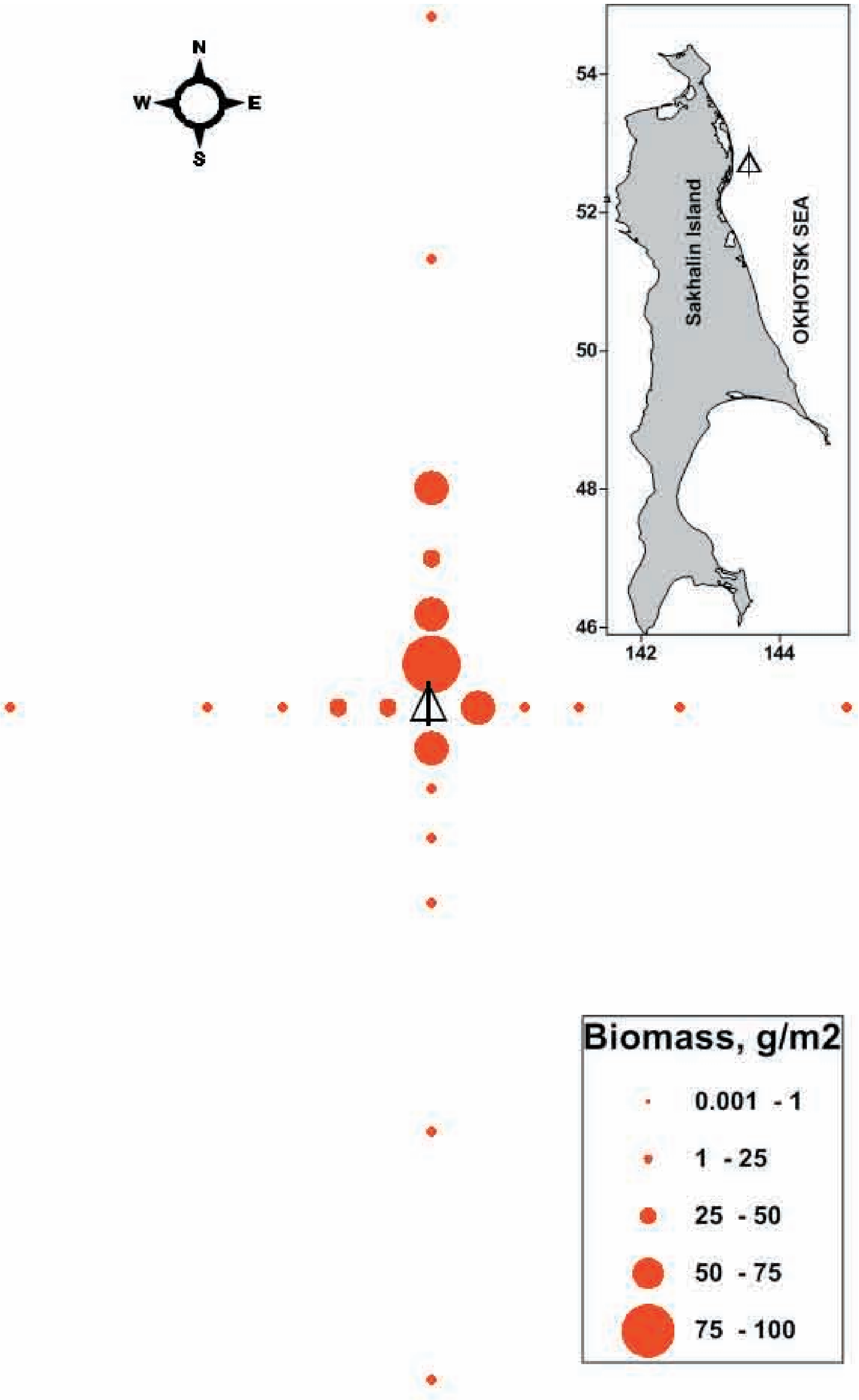

Fig. 1. The spatial and quantitative distribution of amphipods in the region of the oil-and-gas platform Moliqpak.

varied greatly among sediment types. For instance, *Psammonyx kurilicus*, *Eogammarus schmidti* and species of genera *Eohaustorius*, *Grandifoxus* occurred in well sorted sandy sediments. In contrast, *Protomedeia fasciata*, *Pleusymtes* sp., *Ischyrocerus* sp. were most common in the gravel. The 50% of the amphipod species were not associated with particular sediment types. Three species: *Anisogammarus pugettensis*, *Anonyx nugax* and *Anonyx lilljeborgi* were found on all types of sediment. The observed sites were fell into three groups (clusters) as to similarity of amphipod species composition (Fig. 2).

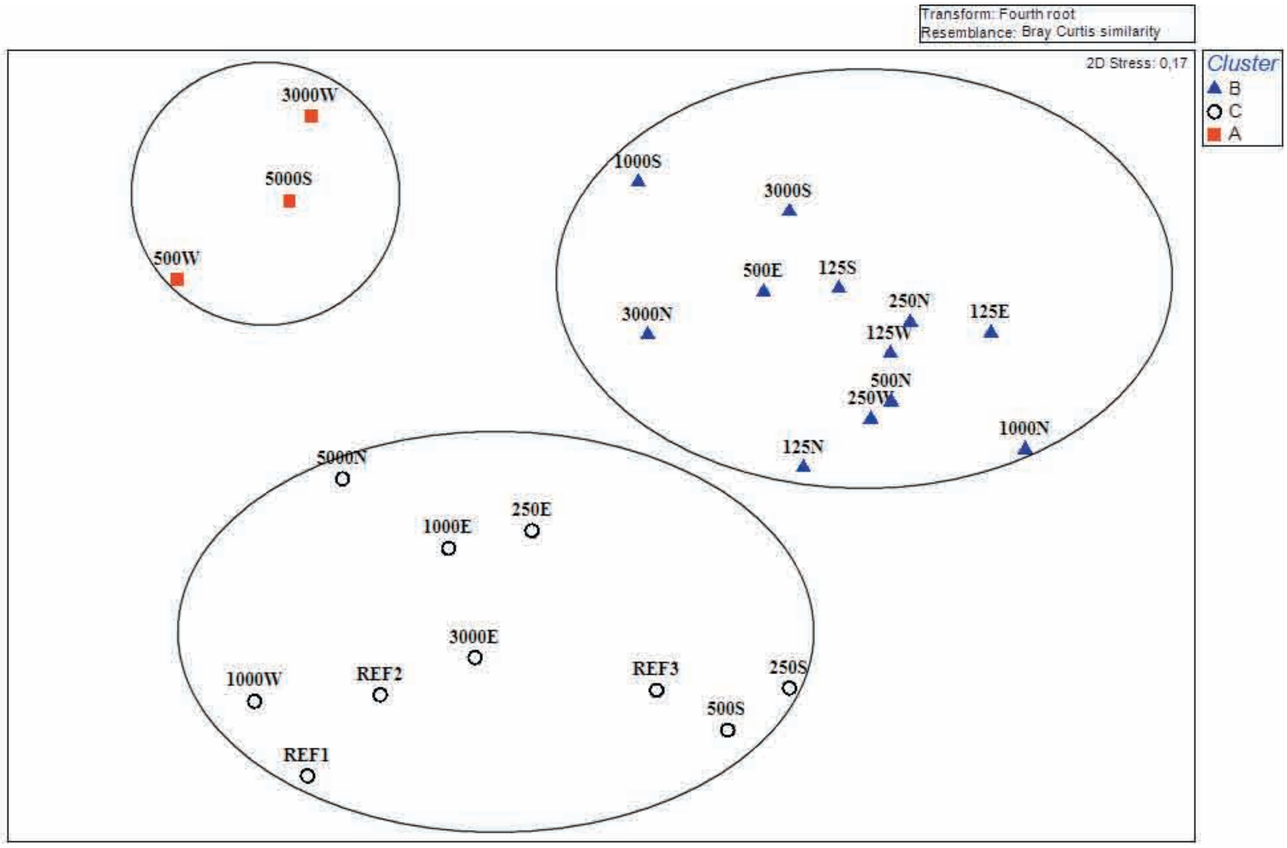

Fig. 2. The MDS ordination of sites as to similarity of amphipod species composition.

Group A (sites on sandy sediments) – *Psammonyx kurilicus+Anonyx lilljeborgi+Eogammarus schmidti*; Group B (sites on gravel and gravel-sand sediments) – *Anonyx nugax+Anisogammarus pugettensis+Protomedeia fasciata*; Group C (sites on sand and sandy-gravel sediments) – *Anonyx nugax+Psammonyx kurilicus*.

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A preliminary analysis of the Cumacea fauna (Crustacea, Peracarida) from deep water basin of the Sea of Japan based on the material of the Russian-German SoJaBio 2010 Expedition

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In total, 1634 species and 140 genera of Cumacea (Crustacea, Peracarida) are known worldwide. About 350 deep-sea species including 48 eurybathic species are presently known; 190 species live exclusively deeper than 2000 meters; 42 genera have a wide distribution throughout several oceans and 26 of them can be found below >2000 meters (Brandt et al., 2012).

Recently, only 13 cumacean species living deeper 500 meters were known in the Sea of Japan (Lomakina, 1958; Gamo, 1999; Vassilenko, Tzareva, 2004). There is a suggestion than the deep-sea fauna in the Sea of Japan is mostly composed of cold-adapted eurybathic species rather than true deep-sea ocean species due to its isolation from the abyssal of the Pacific and physical peculiarities of the waters (Derjugin, 1939; Zenkevich, 1963; Nishimura, 1983; Tyler, 2002; Malyutina, Brandt, in press). Thus, the question whether any true deep water species inhabit the deep-sea basin of the Sea of Japan is still opened.

Material and Methods

The material for this study was collected in August–September 2010 on the continental slope and in the deep-sea basin of the Sea of Japan during the Russian-German expedition SoJaBio (Sea of Japan Biodiversity studies) at 51st cruise of R/V *Akademik M.A. Lavrentyev* (Fig. 1). Macrofauna was sampled using a newly modified camera epibenthic sledge (C-EBS). During the expedition 13 stations using C-EBS were accomplished in the northwestern part of the Sea of Japan at depths ranging from 455 to 3666 meters.

All samples were sieved through of 300 µm mesh size sieve onboard immediately after hauling. Material from the first deployment at each station was fixed in pre-cooled 96% ethanol for genetic studies. The sample from the second deployment (if it was accomplished) was primarily preserved in 4% formaldehyde for morphological studies, and after 48 hours washed with fresh water and re-fixated in 96% ethanol.

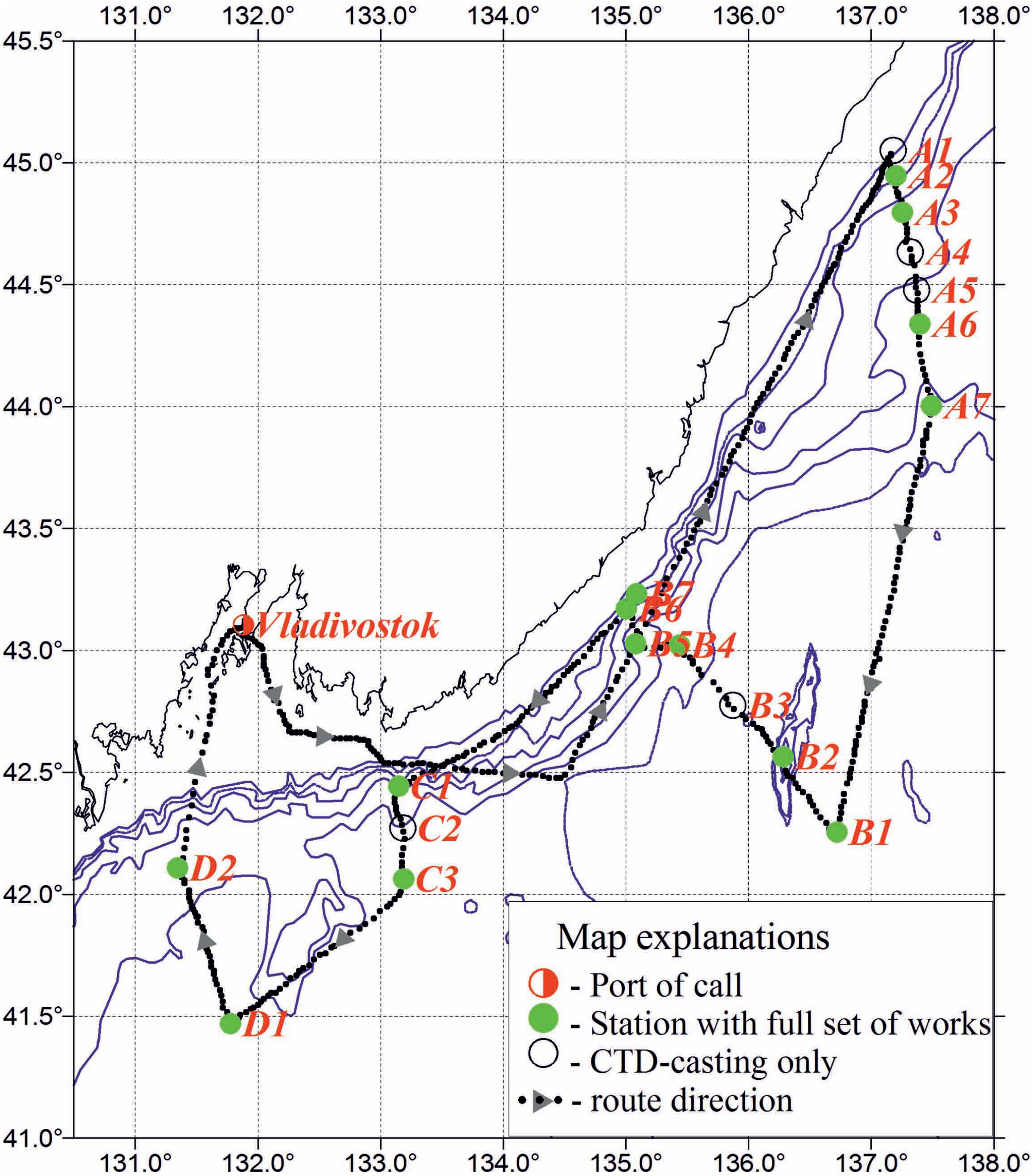

Fig. 1. Map of the stations of the SoJaBio 2010 expedition.

Results and Discussion

As the result, 3567 specimens belonging to 36 taxa of Cumacea (Crustacea, Peracarida) from 5 families and 14 genera were found in the material. In the studied material 22 species were identified to species level, the other species taxa were identified to genus or family level. The predominant families are Leuconidae (13 species) with the richest genera *Leucon* (6 species) and *Eudorella* (4 species, probably in complexes). They are followed by Diastylidae (10) with the genus *Diastylis* (4) and

Nannastacidae (9) with the genus *Campylaspis* (6). Family Lampropidae is presented by 2 species belonging to *Lamprops* and *Hemilamprops*, and familiy Pseudocumidae – by a single species *Petalosarsia declivis* (Sars, 1865). Some of undetermined cumacean species possibly are new to science.

The following taxa were recorded for the first time for the depths more than 500 meters at the Sea of Japan: the genera *Lamprops* (at the depth ~500 meters), *Pavlovskeola* (down to 1,000 meters depth), *Cumella* (at the depth ~500 meters) and the family Pseudocumidae with the genus *Petalosarsia* (down to 1356 meters depth in SoJaBio 2010 material).

Among the identified cumacean taxa the widespread boreal species prevail (12 species from Leuconidae, Diastylidae and Lampropidae); most of them (9 species) show the western Pacific distribution. This group is followed by the boreal-arctic species (6 species from Diastylidae, Nannastacidae and Pseudocumidae) and low-boreal species (3 species from Nannastacidae).

The highest numbers of species (25 species) and specimens (1766 specimens) is found at the relatively shallow-water continental slope station B7-7 (470–528 meters) (Fig. 2). The second station by the species and specimens richness (23 species, 1392 specimens) is station A2-10 (455–465 meters). As we found, the species richness (diversity) of Cumacea decrease with the depth: 31 cumacean species is found at the depths between ~450–550 meters, 25 species – at the depth ~900–1600 meters, 5 species – ~2500–2700 meters and 2 species at the depths ≥3300 meters. Totally, 22 species are recorded from bathyal depths between 1000–3357 meters.

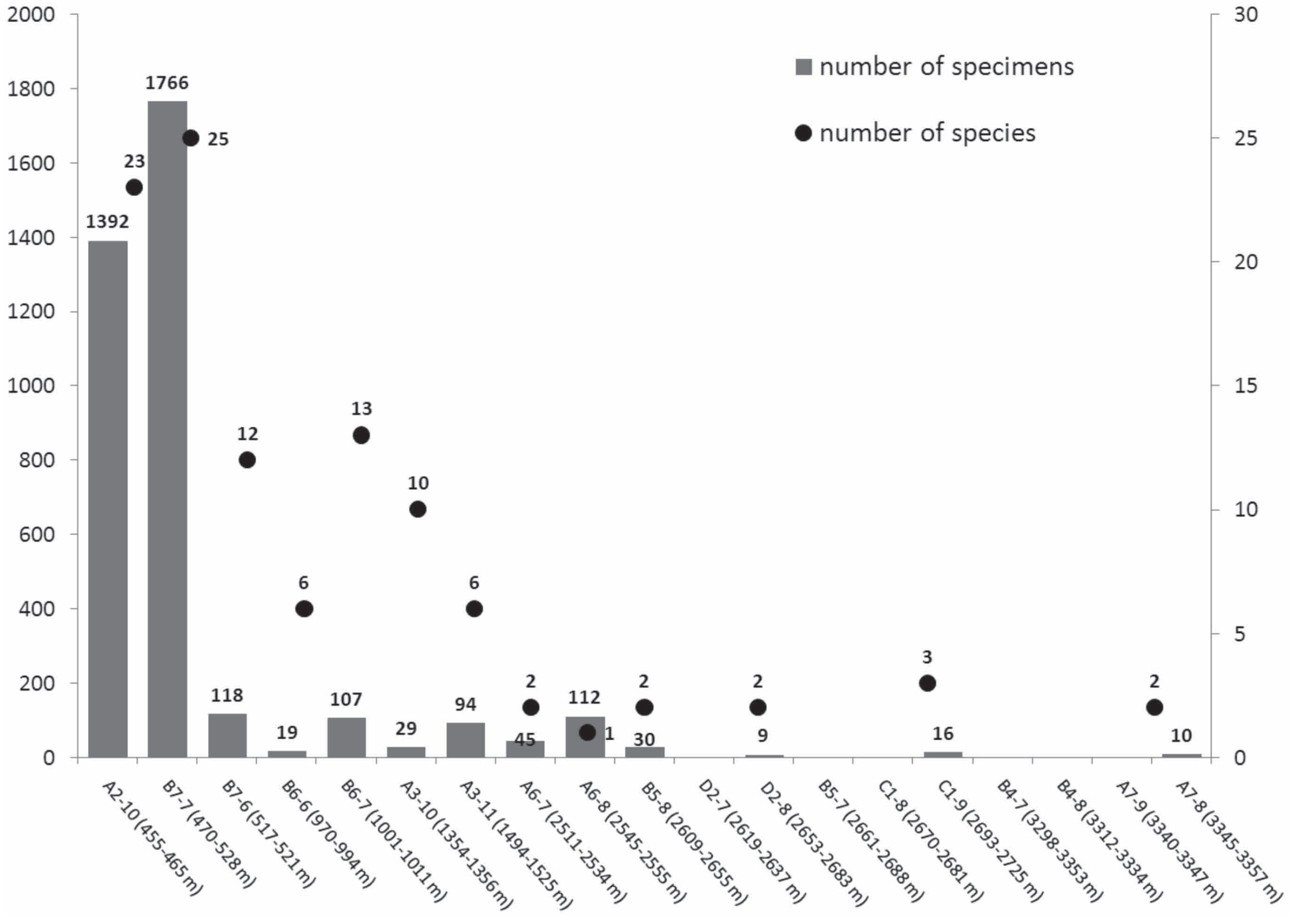

Fig. 2. The species diversity and abundance per stations of the SoJaBio 2010 expedition.

In our material *Leucon* cf. *fulvus* (Sars, 1865) (Leuconidae) is most widely distributed species found at 13 stations with the depths 450–3357 meters (totally 664 specimens). Four species are widely distributed among relatively shallow water stations, but rare deeper than 500 meters: 1) *Cumella* sp. 2 (Nannastacidae) – 354 specimens found at station A2-10 (455–465 meters) and 1 specimen collected at the depth 1350 meters; 2) *Petalosarsia declivis* (Sars, 1865) (Pseudocumidae) – 347 specimens collected at the depths shallower than 550 meters, and the species is rare (only 2 specimens found) at the depths down to 1350 meters; 3) *Campylaspis crispa* (Lomakina, 1955) (Nannastacidae) – 260 specimens collected at the two relatively shallow water stations with depths less than 550 meters, and several specimens were found down to 1000 meters; 4) *Leptostylis* sp. 1 (Diastylidae) – 267 specimens collected at the station A2-10 (455–465 meters) and 2 specimens collected at the depths down to 1350 meters.

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Biodiversity of anomuran crabs in North China and the Pacific seas of Russia

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Anomuran crabs are a group of crustacean species within the infraorder Anomura MacLeay, 1838, which are morphologically like brachyuran crabs. They consist of two superfamilies in the North Pacific: Hippoidea Latreille, 1825, Lithodoidea Samouelle, 1819, and one family Porcellanidae Haworth, 1825 within superfamily Galatheoidea Samouelle, 1819. Hippoidea consists of three families in the world: Albuneidae Stimpson, 1858, Blepharipodidae Boyko, 2002 and Hippidae Latreille, 1825. Lithodoidea includes two families: Lithodidae Samouelle, 1819 and Hapalogastridae Brandt, 1850. Anomuran crabs are widely distributed in marine environment, with complex, diversiform habitats. As important benthic crustaceans, anomuran crabs play a significant role in the benthic ecosystem, with great importance for assessment of ecology, biodiversity and marine biological conservation.

Hippoid crabs prefer sandy and muddy substrates, dredging holes in the bottom for refuges in intertidal and subtidal zones, and some species with depth records of 200 meters. Most Albuneid and Hippid species inhabit tropical and subtropical areas in the world, while Blepharipodid crabs are usually found in temperate seas of North Pacific.

Lithodoid crabs are global distributed, including tropical seas and Polar Regions. Some Lithodoid crabs, like Hapalogastrid species, are small-shaped and living in intertidal and subtidal zones of North Pacific. But the most well-known Lithodoid crabs, which are called king crabs, are of huge creatures in cold and deep waters, and some of them, as *Paralithodes camtschaticus* (Tilesius, 1815), possess great values for fishery.

Porcellanid crabs (or porcelain crabs) are a kind of fragile crustaceans worldwide distributed except Polar regions. They are best known for their high biodiversity in tropical coral reefs, as well as interesting commensalism of some species with sea anemone, sponge, polychaeta and so on. Porcellanid crabs are common in temperate seas of northeast Pacific, living on rocky or muddy bottom of shallow waters, sometimes on blades of kelps.

There are abundant anomuran crabs living in adjacent waters of North China and Pacific seas of Russia, including the Yellow Sea, Sea of Japan, Sea of Okhotsk and Bering Sea. North China and Pacific Russia, each of which represents temperate and cold sea areas respectively, exhibit distinct biodiversity and zoogeographic characters. Porcellanid and hippoid fauna, which prefer warm and shallow waters, are especially common in the Yellow Sea. In contrast, most lithodoid species inhabit deep and cold waters, with high occurrence in Pacific seas of Russia. According to present data, there are 10 species of anomuran crabs in the Yellow Sea, including 3 species of Hippoidea, 2 species of Lithodoidea and 5 species of Porcellanidae; there are 14 species inhabit Pacific Russia, including 1 species of Hippoidea, 12 species of Lithodoidea and 1 species of Porcellanidae. Among them, 4 species are distributed on both waters of the two countries. Human activities, as well as geographic and hydrographic environments, impact mostly on the biodiversity of anomuran crabs in North Pacific.

The species of anomuran crabs distributed in North China and Pacific Russian are list as follows:

Anomuran crabs	North China	Pacific Russia
Hippoid crabs	*Albunea symnista* (Linnaeus, 1766) *Blepharipoda liberata* Shen, 1949 *Lophomastix japonica* (Durufle, 1889)	*Blepharipoda liberata* Shen, 1949
Lithodoid crabs	*Hapalogaster dentata* (De Haan, 1849) *Oedignathus inermis* (Stimpson, 1860)	*Dermaturus mandtii* Brandt, 1850 *Hapalogaster dentata* (De Haan, 1849) *Oedignathus inermis* (Stimpson, 1860) *Hapalogaster grebnitzkii* Schalfeew, 1892 *Lithodes aequispinus* Benedict, 1895 *Lithodes couesi* Benedict, 1895 *Paralithodes brevipes* (H. Milne-Edwards & Lucas, 1841) *Paralithodes camtschatic*a (Tilesius, 1815) *Paralithodes platypus* (Brandt, 1850) *Paralomis multispina* (Benedict, 1895) *Paralomis verrilli* (Benedict, 1895) *Sculptolithodes derjugini* Makarov, 1934
Porcellanid crabs	*Pachycheles stevensii* Stimpson, 1858 *Pisidia serratifrons* (Stimpson, 1858) *Polyonyx sinensis* Stimpson, 1858 *Porcellana pulchra* Stimpson, 1858 *Raphidopus ciliatus* Stimpson, 1858	*Pachycheles stevensii* Stimpson, 1858

Modern benthic Foraminifera in the area around Zhitkova Peninsula (Peter the Great Bay, Sea of Japan)

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Benthic Foraminifera are single-celled animals similar to amoeboid organisms in cell structure. Benthic foraminiferal morphologies are very diverse, from simple single-chambered to multi-chambered, complex forms are composed of calcite or mineral grains. They occupy a wide range of marine environments, from brackish estuaries to ocean basins and occur at all latitudes and depths from intertidal to ultra-abyssal. In many marine areas foraminifers are a dominant meiofaunal group in terms of both numerical abundance and biomass (Murray, 1979; Gupta, 2002).

Benthic Foraminifera are used as environmental indicators. Changes in benthic foraminiferal assemblages and test morphologies are becoming increasingly useful for assessing environmental quality (changes in water salinity, temperature, dissolved oxygen, nutrient input, heavy metals and other toxic materials) in coastal regions (Scott et al., 1980; Bresler, Yanko-Hombach, 2000).

Since 2008 until present, the water area around the Zhitkova Peninsula (Peter the Great Bay, Russky Island) is under increasing anthropogenic pressure caused by construction of the Research and Educational Centre "Primorsky Aquarium" and the new campus of Far Eastern Federal University. Study of foraminiferal assemblages in the water area around the peninsula before construction activity is the beginning of the research of foraminiferal communities' long term changes under the human impact.

The purpose of this research is to investigate the species composition and distribution of benthic foraminifera in the area around Zhitkova Peninsula for further determination of temporal changes in their communities.

Material and Methods

Sediment samples were collected at 25 stations in the water area around Zhitkova Peninsula: Zhitkova Bay (8), Paris Bay (9), water area out of the bays (8) (Fig. 1). Two samples were taken at each station from a depth range of 1 to 20 m during November 2008 with SCUBA, using a 5-cm core sampler (area 20 cm^2). The samples were preserved with a 70% ethanol solution and Rose Bengal (Walton, 1952). After 24 hours, the samples were washed through a 63 µm sieve and dried at 80°C. Foraminifers were separated from residue by floatation method using carbon tetrachloride (CCl_4) (Saidova, 1975). Foraminifera were selected using a binocular microscope. Population density was calculated per square meter.

Species composition, total number, proportion of calcareous and agglutinated tests, percentage of live individuals were investigated to analyze foraminiferal assemblages (Fursenko, Fursenko, 1973).

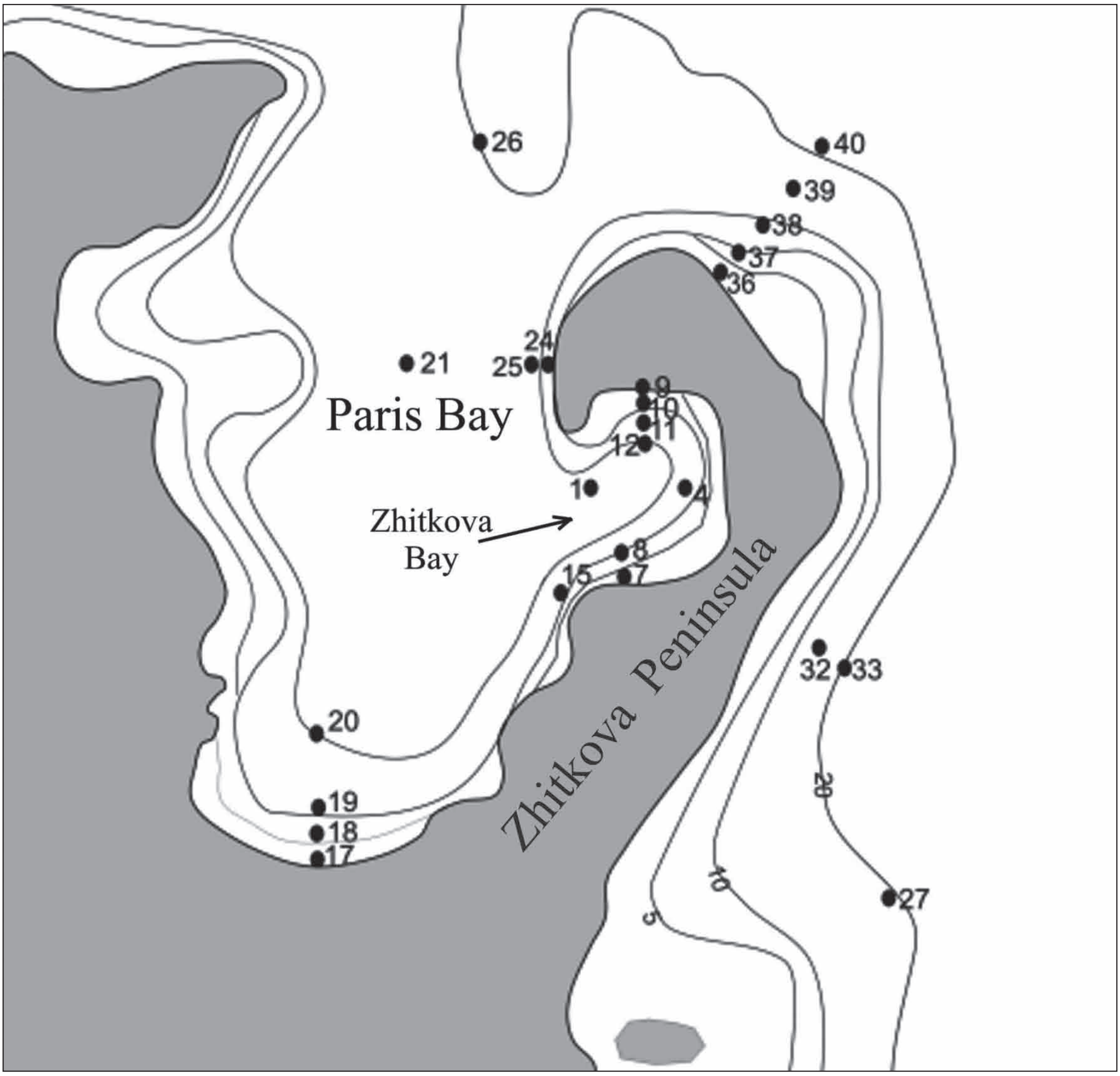

Fig. 1. Schematic map of the study area and the locations of sampling stantions.

Results

120 species of benthic foraminifers belonging to 48 genera and 20 families were identified in bottom sediments in the area around Zhitkova Peninsula. 97 species were calcareous. Among calcareous taxa the most prevalent were Elphidiidae (*Canalifera fax*, *Cribroelphidium frigidum*, *Cribroelphidium goesi cognatum*, *Cribroelphidium kusiroense*, *Elphidium advenum depressulum*, *Protelphidium asterotuberculatum*, *Retroelphidium subgranulosum*), Discorbidae (*Buccella frigida*, *Discorbis bradyi*, *Discorbis subaraucana*, *Rosalina globularis*, *Glabratella opercularis*), Rotaliidae

(*Pararotalia nipponica*, *Ammonia beccarii)*, Buliminidae (*Buliminella elegantissima*), Miliolidae (*Quinqueloculina cf. quinquecarinata*, *Quinqueloculina laevigata*, *Quinqueloculina stalkeri*). Among agglutinated foraminifera (23 species) the most common were Trochamminidae (*Trochammina inflata*), Ataxophragmiidae (*Eggerella advena*), Lituolidae (*Labrospira jeffreysi*). These species were the major component of foraminiferal assemblages, contained more than 90% of total number of foraminifers in the study area.

Distribution of foraminiferal assemblages was patchy. The number of species at each station varied from 18 to 80, population density varied from 14.8 to 5411 thousand ind/m^2.

Foraminiferal quantitative indices was the lowest in Zhitkova Bay (stations 1, 4, 7, 8, 9, 10, 11, 12). The number of species comprised a total of 52 species, agglutinated taxa attributed to 14 species (Fig. 2). The number of species at each station varied from 18 to 33. Minimal population density (14.8 thousand ind/m^2) was observed in the northern part of Zhitkova Bay (station 11) at a depth of 5 m, maximal (451.08 thousand ind/m^2) – at the bay entrance (station 1) at a depth of 10 m. The most common benthic foraminiferal species were *Cribroelphidium frigidum*, *Buccella frigida*, *Eggerella advena*, *Protelphidium asterotuberculatum*, *Trochammina inflate*.

94 species of benthic foraminifera were identified in Paris Bay (stations 15, 17, 18, 19, 20, 21, 24, 25, 26), 23 species were agglutinated (Fig. 3). The number of species at each station varied from 22 to 62, population density varied from 88.36 to 3013.36 thousand ind/m^2. The lowest population density (88.36 to 311.20 thousand ind/m^2) was observed in the southern apical part of the bay at depths to 5 m (stations 17, 18, 19). Here, the strong dominance of agglutinated forms was identified that contained at the average 80% of total number of foraminifera. The dominant and subdominant species were *Trochammina inflata* and *Eggerella advena* respectively. As the depth increased from 10 to 20 m (stations 20, 21, 26) population density increased from 372.64 to 2189.68 thousand ind/m^2. Also, abundance of foraminifers was extremely high in the eastern part of Paris Bay (stations 15, 24, 25) amounting from 1162.64 to 3013.36 thousand ind/m^2. *Protelphidium asterotuberculatum*, *Cribroelphidium frigidum* и *Eggerella advena* were the most common benthic foraminifers of these stations.

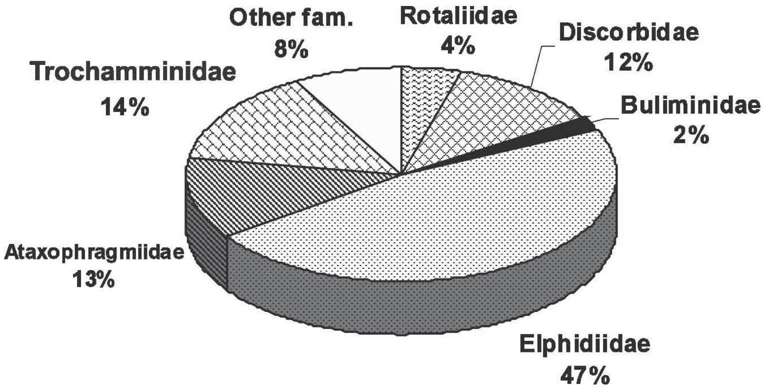

Fig. 2. Percent ratio of the most common families of Foraminifera in Zhitkova Bay.

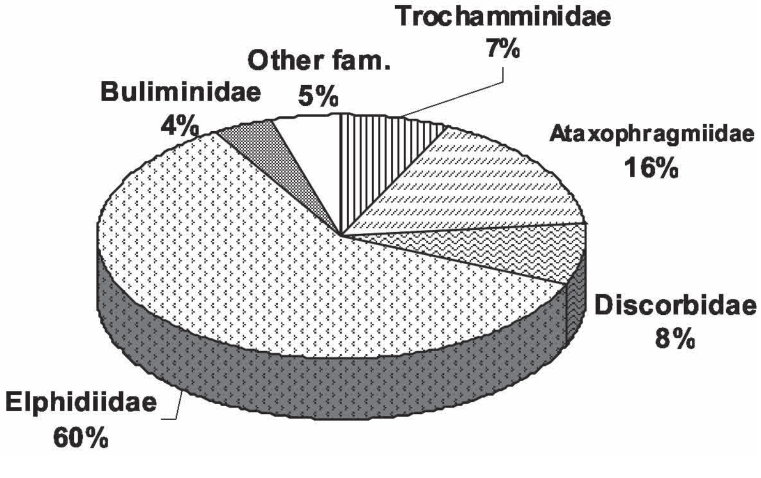

Fig. 3. Percent ratio of the most common families of Foraminifera in Paris Bay.

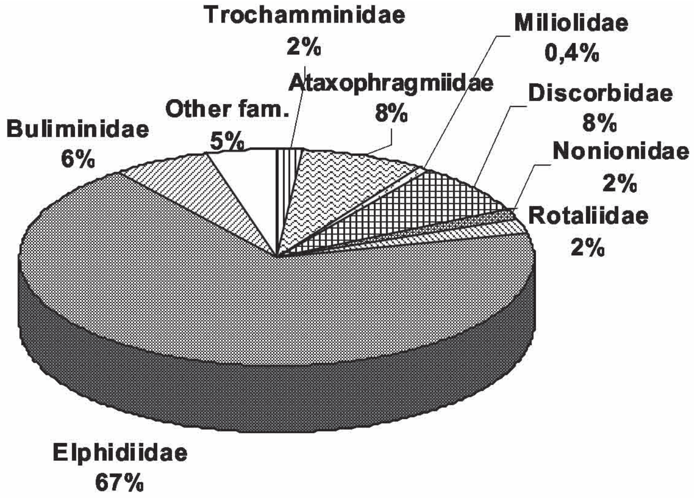

Fig. 4. Percent ratio of the most common families of Foraminifera in the outer water area off the Zhitkova Peninsula.

The percentage of living individuals in the total foraminiferal complexes varied from 9% to 51% and averaged 29% in Zhitkova Bay and Paris Bay.

The richest and most abundant fauna of benthic foraminifera was observed in the water area beyond the peninsula (stations 27, 32, 33, 36 37, 38, 39, 40). The number of species comprised a total of 114 species, agglutinated taxa attributed to 22 species. Specimen of Miliolidae (11 species) and minor species of Bolivinitidae, Nodosariidae, Glandulinidae, Reophacidae, Ammodiscidae were found only in this area (Fig. 4). The number of species at each station varied from 32 to 80. Population density was maximal and varied from 1208.16 to 5638.24 thousand ind/m^2. An exception to this was a shallow-water site by the northern extremity of the peninsula (station 36); here, population density was 269.68 thousand ind/m^2 at a depth of 2 m. *Cribroelphidium frigidum* and *Protelphidium asterotuberculatum* were dominant species in the water area beyond the peninsula. The percentage of living individuals in the foraminiferal assemblages varied from 15% to 29% and averaged 22%.

Discussion

The study of benthic foraminiferal assemblages in the area around Zhitkova Peninsula revealed rich and diverse fauna with patchy distribution. Taxonomic composition and fauna abundance differed in Zhitkova Bay, Paris Bay and the water area lying out of the peninsula.

Benthic foraminiferal assemblages in Zhitkova Bay contained poor species diversity and low population density comparing with Paris Bay and the water area beyond the peninsula. Probable cause is scallop *Mizuhopecten yessoensis* fostering in marine aquaculture farms in Zhitkova Bay. Mariculture operations in the bay lasted for 5 years and ceased in 2008 by reason of forthcoming construction of the “Primorsky Aquarium” and the campus of Far Eastern Federal University. It is known that mariculture affects environmental conditions. Environmental impacts from marine farms can be high siltation of the bottom, oxygen level reduction, huge accumulations of organic compounds and toxicants, including heavy metals (Kucheryavenko, 2002). As a consequence, mariculture exerted negative and suppressing impact on marine benthic ecosystems, including foraminiferal density and species composition (Galtsova, Pavluk, 1993; Schafer et al., 1995; Scott et al., 1995; Tarasova, Preobrazhenskaya, 2000; Tarasova, 2006). However, maricultural environmental impact is usually highly local. It is observed that the foraminiferal population density and species richness always decreased in immediate proximity to marine farm sites, but at a distance of several hundred meters a phenomenal increase in species number and density was identified. The abundance is related to close source of organic matter, which impacted as stimulant (Scott, Medioli, 1980; Schafer, 1991; Tarasova, 2004). This difference in conditions influenced the foraminiferal assemblages and explained the faunal differences of Zhitkova Bay comparing with Paris Bay and the outer water area of the peninsula.

Benthic foraminiferal assemblages data indicate favorable ecological conditions in coastal ecosystem of the area around Zhitkova Peninsula. A recurring study is planned at the moment. Identification and analysis of foraminiferal complexes would enable to assess the influence of large-scale construction activity on Russky Island.

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Intraspecific male gamete diversity in the mussels *Crenomytilus grayanus* and *Mytilus trossulus*; phenomenon of sperm pattern overlap

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Sperm structure have been described for bivalve molluscs belonging to the Mytilidae in a number of reports (see Hodgson, Bernard, 1986a, b; Reunov, Drozdov, 1986, 1987; Reunov, Hodgson, 1994; Drozdov, Reunov, 1997). The results showed that the Mytilidae possess variations of "primitive" spermatozoa which are typically found in invertebrates with external fertilization (see for review: Reunov, 2005). Only a single pattern of sperm cells has been presumably found for mytilids. However, in our study, we have revealed some unusual cases of heteromorphism in sperm populations of species *Crenomytilus grayanus* and *Mytilus trossulus*. The aim of this paper is to investigate this unusual finding in more details and try to analyse its reason.

Materials and Methods

Mussels, *C. grayanus* and *M. trossulus*, were collected from the intertidal zone of "Vostok" Biological Station (Japan Sea, Russia) during July–August, 2011.

Scanning electron microscopy. Gonads were removed, cut into small pieces and fixed for 2–3 h (in 2.5% glutaraldehyde in 0.1M cacodylate buffer, pH 7.4). Primary fixed materials were washed gradually in the same buffer. Washed samples were rinsed in buffer and distilled water, dehydrated in a graded series of ethanol solutions. Sperm suspension was prepared by crushing pieces of fixed materials. The suspension was pipetted onto a Thermanox coverslip (Cat. # 72280) and allowed to settle for 1 h. Coverslips with attached sperm cells were transferred to acetone and critical-point dried in CO_2. Dried materials were mounted on to aluminum stubs, coated with gold, and examined with a scanning electron microscope LEO-430.

Quantitative analysis. The 250 cells of each species were considered, sperm patterns were identified and frequency of each pattern was calculated. The results were analysed by the Microsoft XL program using Student's *t*-test.

Results

***Mytilus trossulus*.** Five morphological patterns of spermatozoa were found. All types have trapeziform or ovoid nucleus attached by five mitochondria in their bottom and are peculiar in having anteriorly situated acrosomes. Flagellum is protruded from the mitochondrial area (Fig. 1A–E).

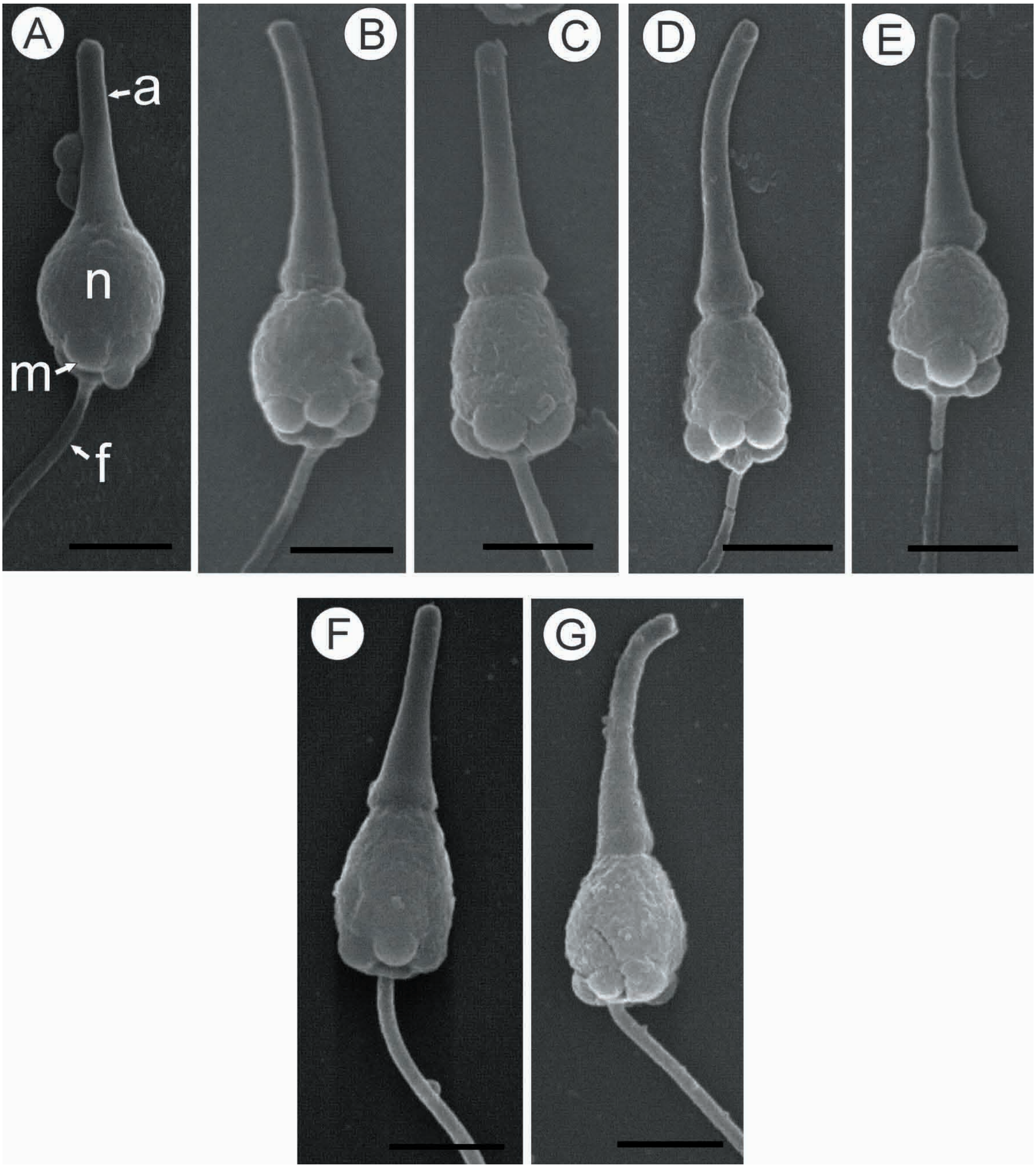

Fig. 1. Various patterns of spermatozoa in the mussels *Mytilus trossulus* (**A–E**) and *Crenomytilus grayanus* (**F–G**). Note morphologically close sperm pattern that takes place both in *M. trossulus* (Fig. 1C) and *C. grayanus* (Fig. 1F); ***a***, acrosome; ***n***, nucleus; ***m***, mitochondria; ***f***, flagellum.

The differences between sperm patterns concerned shape of both the acrosomes and nucleus. There is one dominant sperm pattern (Fig. 1A) that comprise about 36% of sperm population (Fig. 2A). The next four patterns (Fig. 1B–E) were calculated as 29, 13, 11 and 9% respectively (Fig. 2A).

***Crenomytilus grayanus*.** Two morphological types of sperm cell were found. Both types were peculiar in having trapeziform nucleus attached by five mitochondria in their bottom. Flagellum is protruded from the mitochondrial area (Fig. 1F, G). The first type, which was more common and comprised about 67% of sperm population (Fig. 2B), was characterized by anteriorly directed acrosome which is straight (Fig. 1F). The second type was presented by lowest amount evaluated as 33% of sperm population (Fig. 2B). This sperm pattern is characteristic in having longer acrosome which is curved (Fig. 1G).

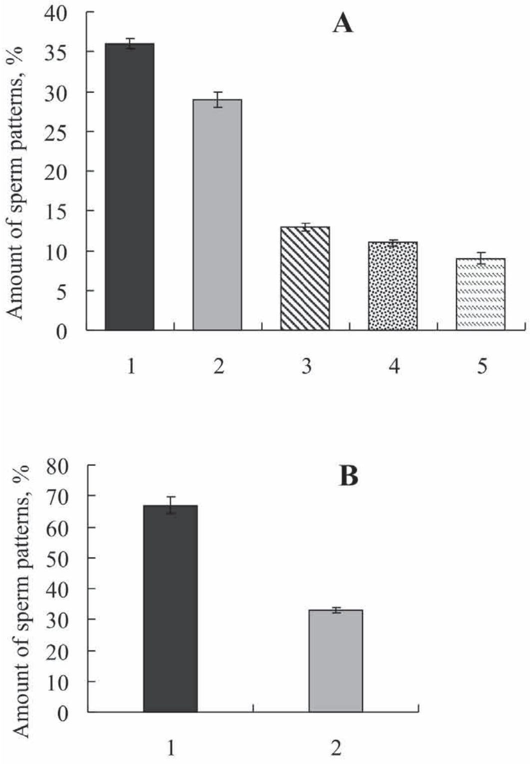

Fig. 2. Diagrams of the distribution of sperm patterns in the mussels. (**A**), *Mytilus trossulus*, 1, 2, 3, 4, 5 – the graphs respectively corresponding to sperm patterns showed in Fig. 1A–E. (**B**), *Crenomytilus grayanus*, 1, 2 – the graphs respectively corresponding to sperm patterns showed in Fig. 1F–G.

Discussion

Results of the present study demonstrated that in sperm populations of mussels *M. trossulus* and *C. grayanus* male gametes differentiate as heteromorphic cells. This finding is novel because previous reports recorded mytilid species as having only one species specific sperm pattern (see for review: Reunov et al., 1999). Thus, *M. trossulus* and *C. grayanus* belongs to the group of multicellular animals that are known to have polymorphic gametes. Indeed, the phenomenon of polymorphism in sperm cells was revealed in the nineteenth century by Siebold in the example of a gastropod *Paludina* (Siebold, 1836, cited from Roosen-Runge, 1980). Aftewards, dimorphism and polymorphism of male gametes were described in various taxa of multicellular animals, like mollusks, echinoderms, insects, fish, and mammals (Beatty, Fechheimer, 1972; Healy, Jamieson, 1981; Eckelbarger et al., 1989; Feito, 1990; Hodgson, 1997; Au et al., 1998; Jespersen et al., 2002). The phenomenon of additional sperm types in different animals is sometimes linked to transport and nutrient functions performed by these gametes (Roosen-Runge, 1980; Hodgson, 1997). However, in some reports the presence of heteromorphic gametes is characterized as an "intriguing" or "mysterious" (Jespersen et al., 2001; Lutzen et al., 2001).

It seems obvious that heteromorphic sperms of *C. grayanus* and *M. trossulus*, appear normal since there is no any structural signs of abnormality in these cells. These sperms have some common features that are characteristic for "primitive spermatozoa" of marine invertebrates (see for review: Reunov, 2005). However, these spermatozoa are differing from each other in the shape of the nucleus and acrosomes. It is interesting that an amount of sperm morphs found in two mussels is species specific. Indeed, two sperm patterns were recorded for *C. grayanus* and five patterns were found in sperm population of *M. trossulus.* Besides, an amount of sperm patterns was intraspecifically varied. Each species is peculiar in having one dominant pattern although there are another patterns existing in smaller amount. It could be suggested that several sperm types is aimed to increase adaptive possibities of molluscs in terms of their reproduction. Probably, an adaptive ability of *M. trossulus* is higher since this species has so many variants of reproductive cells.

Surprisingly, one of five sperm patterns that is typical for *M. trossulus* (see Fig. 1C) was morphologically similar to one of two sperm variants characteristic for *C. grayanus* (Fig. 1F). The reason for this similarity still could not be explained. Presumably, it might be assumed that sperm populations of both species have this common sperm pattern due to close phylogenetic affinity of *M. trossulus* and *C. grayanus.*

Acknowledgments

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Introduction to the China Jellyfish Project – the key processes, mechanism and ecological consequences of jellyfish bloom in China coastal waters

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As a National Basic Research Program (973 Program) of China, *the Key Processes, Mechanism and Ecological Consequences of Jellyfish Bloom in China Coastal Waters* (2011–2015) led by Prof. Sun Song from the Institute of Oceanology, Chinese Academy of Sciences (IOCAS) was endorsed by the Ministry of Science and Technology.

As well known, jellyfish plays an important role in marine ecosystems. It grows very fast and feeds mainly on zooplankton, fish eggs and fish larvae; however, it is preyed by few marine creatures. Jellyfish population blooms damage the structure and function of the marine ecosystem, causing many economic and social problems to the tourism because of jellyfish sting, to the coastal industries by blocking the pipeline of its cooling system and to the fishery resources and fishery activities. Jellyfish blooms occurred all over the world in recent years, especially the giant jellyfish blooms in the Far East seas including China coastal waters. Frequent jellyfish blooms would depress fish and other species population, making jellyfish dominant in the ecosystem, which may last for many years. This situation has been observed in several seas in the world, mainly in the fishing ground; however, the causes, consequences of jellyfish bloom and whether it will cause regime shift of the ecosystem remain largely unexplored.

This project will focus on the giant jellyfish bloom in the East China Sea and the Yellow Sea, aiming to understand the causes, consequences and the role of jellyfish bloom in marine ecosystem, to evaluate the marine ecosystem status, and to avoid the ecosystem incidents caused by jellyfish outbreak. It is our wish that by conducting this comprehensive research program, we will be able to provide scientific evidence in the mechanism of jellyfish bloom, to understand the ecosystem dynamics in the East China Sea and Yellow Sea under human impacts, and to improve the ecosystem management in these areas.

The key scientific issues of this project are: 1) understanding the main controlling factors, key processes and driving mechanisms of jellyfish blooms in China coastal waters; 2) revealing how jellyfish blooms influence the marine ecosystem and the disaster-causing mechanism; 3) evaluating ecological disasters and establishing the counter-measures.

The main research contents are: 1) the life history of jellyfish and the regulatory mechanisms of environmental factors in the different developmental stages; 2) the control of marine environmental and food web changes to jellyfish population growth; 3) the ecological consequences and disaster-causing mechanisms of jellyfish blooms; 4) the influence of jellyfish bloom on ecosystems succession in China coastal waters and the counter-measures.

Eight institutions are involved in this project, including Institute of Oceanology, Chinese Academy of Sciences, Ocean University of China, Xiamen University, Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Tianjin University of Science and Technology, National Marine Environmental Forecasting Center, Jinan University and Liaoning Ocean & Fisheries Science Research Institute. Scientists will focus on six topics: 1) jellyfish life history & regulation of population growth; 2) relationship between marine environmental changes and bloom; 3) relationship between phytoplankton succession and jellyfish bloom; 4) interaction between zooplankton change and jellyfish bloom; 5) interaction between fish, benthos and jellyfish bloom; 6) ecological model on blooming mechanism and ecological consequences. Based on the synthesis and integration of the historical data, scientists will combine mesocosm experiments of different scales, field survey, and ecological model to reveal the underlying mechanism of jellyfish bloom in China coastal waters and the ecological consequences.

Bivalve pinnid populations in the western South China Sea

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All species of the family Pinnidae Leach, 1819 (Bivalvia) are sessile semi-infaunal suspension feeders and live vertically embedded in the bottom sediments, usually in mud or muddy sand, anchoring by net of byssus threads (Yonge, 1953; Purchon, Purchon, 1981). They are commercial species. The large posterior adductor muscle is used as dainty food, and the shell is utilized to carve decorative ornaments. Beautiful black pearls are sometimes produced by the pinnids. Because it attains a large size, pinnids are probably one of the most economically important members of the family in the Indo-West Pacific (Poutiers, 1998).

However, little is known about ecology of pinnids. Only *Pinna nobilis* and *Pinna bicolor* from the Midetteranean Sea are well studied. For other pinnids, usually only synonymy was referred (e.g., Hedley, 1924; Yonge, 1953; Rosewater, 1961; Purchon, Purchon, 1981; Abbott, Dance, 1982; Bernard et al., 1993; Huber, 2010; etc.). The decline in pinnids' populations is observed for the last decades due to various pollutants, sea resorts, anchoring of yachts, as well as unauthorized fishing. Despite the need for conservation, knowledge of the ecology and monitoring of the structure and condition of the main populations of pinnids inhabiting the western part of the South China Sea is especially limited.

The family Pinnidae consists of the single genus *Pinna* Linne, 1758, which comprises three subgenera *Atrina* Gray, 1842; *Pinna* Linnaeus, 1758 and *Streptopinna* von Martens, 1880 (OBIS Indo-Pacific Molluscan Database 2006). At the coasts of southern Vietnam, two Pinnidae species are found. They are *Atrina vexillum* (Born, 1778) (=*Pinna nigra Dillwyn, 1817*; *Pinna nigra* Chemnitz, 1785; *Pinna vexillum* Born, 1778; *Atrina nigra* Okan and Hosgor, 2009) and *Pinna trigonium* Dunker, 1852 (=*Pinna fumata* Reeve, 1858; *Pinna philippinensis Reeve, 1858*) (Silina, 2011a). The shells of the Pinnidae bivalves are very large and always triangular in outline, tapering to a point anterior (Yonge, 1953) (Figs. 1, 2).

It is poorly known in world literature about *P. trigonium* and there is a knowledge gap about their distribution near Vietnam. Studies about biology and ecology of this species are few (Yonge, 1953; Malakhov et al., 1985). As a rule, pinnids live vertically embedded in the ground, however, *P. trigonium* was found not only in the ground, but also among the biofouling of the sunken vessel (Silina, 2010).

P. trigonium was found in Nha Trang Bay (6–10 m of depth) and in the eastern Gulf of Thailand (3–12 m). Near the coasts of Nha Trang, *P. trigonium* was collected and compared in 1980, 1984 and 1986. *P. trigonium* population was not numerous in 1980–1986 in Nha Trang Bay, which is under anthropogenic pressure. Now this pinnid is not found near the coasts of Nha Trang. Here, the mean value of shell length of this bivalve was 315±22 mm in 1980–1984, but in 1986 it was only 207±20 mm

(Silina, 2010). In the Gulf of Thailand in 1986, specimens of *P. trigonium* were sampled near the coasts of An Thoi Archipelago, mainly near Hon Vang Island (Echo Island) (6–7 m depth), Hon Roi (Lamp Island), and Hon May Rut Island (Cold Cloud Island) (3–12 m). In the Gulf of Thailand, mean and maximum parameters of *P. trigonium* individuals were higher then those of specimens from Nha Trang Bay. At the coasts of An Thoi Archipelago, the total wet weight of *P. trigonium* varied from 262 to 1590 g (559±118 g on the average), wet weight of the posterior adductor muscle was 7–25 g (15±2 g) and shell length varied from 249 to 377 mm (303±15 mm) (Silina, 2010).

A. vexillum is a cosmopolitan tropical species distributed in Indo-Pacific region from East Africa to eastern Polynesia; Western Pacific, South-Western Pacific, Central Pacific; north to Japan and Hawaii, and south to Australia (Hedley, 1924; Habe, 1968; Morris, Purchon, 1981; Abbott, Dance, 1982; Bernard et al., 1993; Huber, 2010). Common name of *A. vexillum* is Indo-Pacific pen shell. A shell of *A. vexillum* reaches large size and usually attains 30 cm (maximum to 48 cm) (Poutiers, 1998).

Specimens of *A. vexillum* were found in 1986 in the eastern Gulf of Thailand, near the coasts of Phu Quoc Island and An Thoi Archipelago located in the inner part of the Gulf, and near Tho Tu Island situated in the outer part of the Gulf. In 2010, *A. vexillum* was not registered near both Phu Quoc Island and An Thoi Archipelago (Latypov, Selin, 2011). Population structure varied with areas and habitat types. In the outer area of the Gulf, shell length (mean±S.E.) was the highest, 272.7±13.2 mm. In the inner area of the Gulf, shell length did not exceed 182 mm (166.7±9.5 mm). Shell length frequency of *A. vexillum* populations was wide-ranging; nevertheless, the specimens with a shell length of 170–190 and 290–310 mm were the most frequently found in the inner and outer areas, respectively. In the population lived at the coasts of An Thoi Archipelago, the total wet weight of *A. vexillum* varied from 82 to 500 g (236±18 g) and the wet weight of the posterior adductor muscle was 5–30 g (13±1 g) (Silina, 2011b).

A. vexillum individuals inhabited the inner part of the gulf had slower growth rates than the specimens from the outer area of the gulf. The local differences in growth rates

Fig. 1. Flag pen shell *Atrina vexillum* with epibionts on its shell.

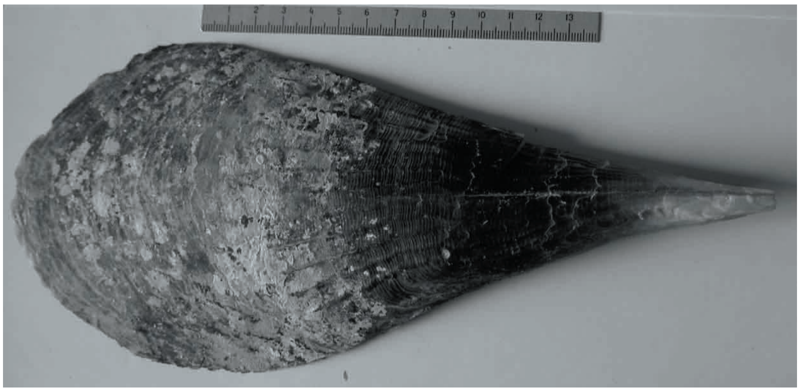

Fig. 2. Pen shell *Pinna trigonium*.

of *A. vexillum* indicate that the molluscs are under different levels of environment pressure in the areas studied. The water temperature, concentration of suspended organic carbon, and the concentration of chlorophyll “a” increase from the outer to inner locations, but oxygen concentration decreases in this direction due to the high level of organic matter decomposition. So, in the inner area, *A. vexillum* is subject for the whole set of unfavourable environmental factors.

During the last decades, studied pinnid populations have declined drastically due to increasing anthropogenic pressure. In the turbid waters of Nha Trang Bay, *P. trigonium* was practically petered out. Due to rise in tourist industry in the studied part of the Gulf of Thailand, especially on the Phu Quoc Island, pinnid populations that lived near the coasts of Phu Quoc Island and adjacent An Thoi Archipelago are the most vulnerable. These 15 islands and islets can be visited by boats, and it’s a fine area for sightseeing, fishing, swimming and snorkelling. In addition, pinnids are important component of the soft sediment communities of sublittoral zone. They are large bivalves that, alive and died, provide shelter for motile species and hard substrate for settling larval invertebrates and egg-laying (Fig. 1). Sometimes, for instance, in St. Joseph Bay, Florida, pen shells *Atrina rigida* are the most abundant source of hard substrate. Therefore, pinnids are need in their conservation and implementation of sustainable resource management in the studied areas.

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Blooms of *Pseudo-nitzschia* and domoic acid producers from Amursky Bay, the northwestern East/Japan Sea, 2005–2011

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Since the 1987 Prince Edward Island incident, increased attention has been paid to species of the diatom genus *Pseudo-nitzschia* that are presently known as potential producers of domoic acid (DA), the neurotoxin causing Amnesic Shellfish Poisoning (Bates, Trainer, 2006; Trainer et al., 2008).

Beginning in 1992, the events of blooms of *Pseudo-nitzschia* species has been observed in the Amursky Bay of the East/Japan Sea: in the vicinity of Vladivostok, in areas of mariculture farms, and in recreational zones (Orlova et al., 1996; Stonik et al., 2001, 2008). The Vladivostok city region's large human population makes it potentially vulnerable to ASP. However, limited information is available on population dynamics of bloom-forming *Pseudo-nitzschia* species or their domoic acid content in this area during last decade (Orlova et al., 2008; Stonik et al., 2011, 2012).

The present research is focused on the study of the species composition and dynamics of *Pseudo-nitzschia* species and their domoic acid content during 2005–2011 in Amursky Bay, East/Japan Sea.

Materials and Methods

Phytoplankton samples were collected from January 2005 to December 2011 for *Pseudo-nitzschia* enumeration, species identification and toxin analysis. One-liter samples were taken from depths of 0–0.5, 2.5, and 5 m from station situated in the northeastern part of Amursky Bay (43°12′ N, 131°54′ E). Samples were fixed with Utermoehl's solution, postfixed with 4% formalin, and concentrated by the precipitation method. Species identification was carried out using an Olympus BX 40 (Tokyo, Japan) light microscope (LM) and a JEOL JEM-100S (Tokyo, Japan) transmission electron microscope (TEM). For TEM examination, a drop of the washed sample was placed on formvar-coated grids and dried in the air. Concentrations of domoic acid in clones of *Pseudo-nitzschia* species were measured by Enzyme Linked Immunosorbent Assay using "ASP direct cELISA" kit (Biosence Laboratories AS, Norway, AOAC official method 2006.02). For DA analysis, unialgal cultures were established for *P. americana* (one isolate) and *P. multistriata* (one isolate).

Results and Discussion

We identified 9 species of *Pseudo-nitzschia* via TEM in field samples collected from Amursky Bay from 2005 to 2011: *P. americana*, *P.* cf. *caciantha*, *P.calliantha*, *P. delicatissima*, *P. fraudulenta*, *P. multiseries*, *P. multistriata*, *P. pungens*, *P. seriata.* Morphological analysis revealed *P. multistriata*, *P. calliantha*, and *P. delicatissima* as the dominant species. These species have been associated with domoic acid production in other regions of the world (Bates, Trainer, 2006; Trainer et al., 2008).

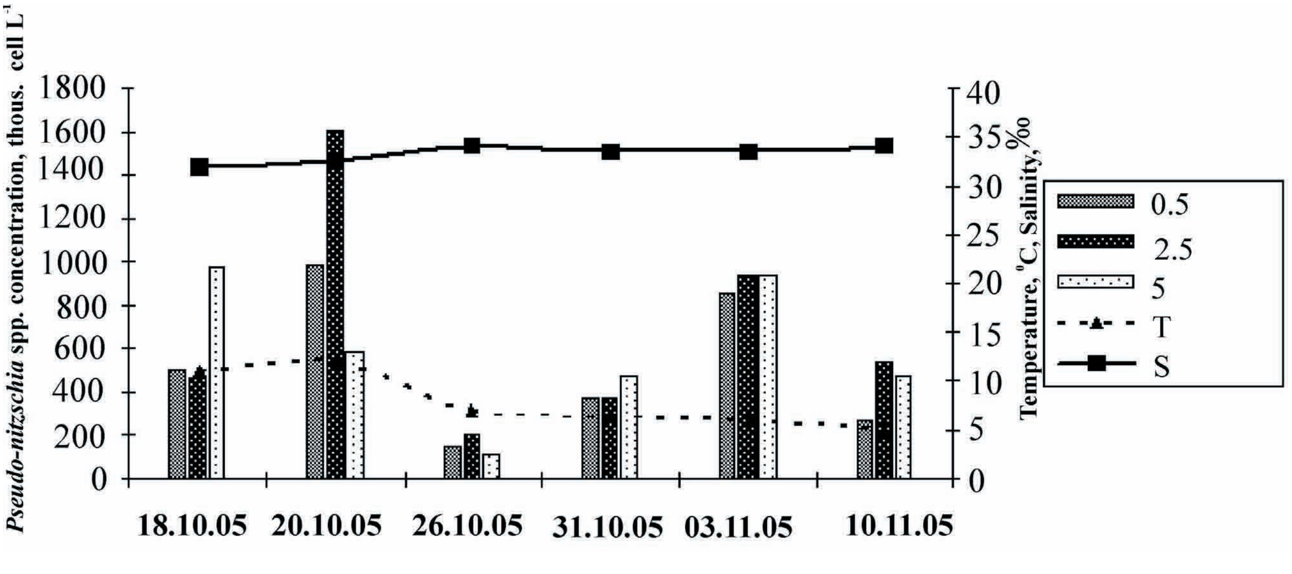
An analysis of the samples collected between January 2005 and December 2011, revealed the occurrence of multispecific blooms of *Pseudo-nitzschia*. The peaks of the *Pseudo-nitzschia* spp. density were recorded in summer and in autumn. Abundances of *Pseudo-nitzschia* were highest in autumn 2005 with up to $1.6 \cdot 10^6$ cell L^{-1} (see figure).

The first most intense bloom of *Pseudo-nitzschia* species was observed in October–November 2005 at a salinity of 31–33.5‰ and a water temperature of 6–12°C. The first peak of *Pseudo-nitzschia* spp. cell density (with a mean value of $1.4 \cdot 10^6$ cell L^{-1}), which was recorded in the second half of October after heavy rains, was mainly caused by the massive development of *P. multistriata* (67% of the total phytoplankton density) and *P. calliantha* (9%). The second relatively small peak of cell density of *Pseudo-nitzschia* species (water column average $0.9 \cdot 10^5$ cell L^{-1}) was recorded at the beginning of November when water temperature decreased to 6°C and salinity slightly increased to 33.5‰. The second cell density peak was also connected with the intense development of *P. multistriata* (an average of 60% of total density) and P. *calliantha* (10%). The field samples collected during this bloom event did not contain detectable and quantifiable domoic acid (DA).

The second bloom occurred in September 2007 at a salinity of 29–30‰ and a water temperature of 18–20°C. It reached cell concentrations of $2 \cdot 10^5$ cell L^{-1} consisting of *P. calliantha* (up to $1.7 \cdot 10^5$ cell L^{-1} or 29%) and *P. delicatissima* (up to $2.6 \cdot 10^4$ cell L^{-1} or 4%). The field samples collected in September 2007 were not tested for DA.

The small peaks of cell density of *Pseudo-nitzschia* species were recorded in October 2009 (up to $2.1 \cdot 10^4$ cell L^{-1}), in August 2011 (up to $2.4 \cdot 10^4$ cell L^{-1}) and in September–October 2011 (up to $2.5 \cdot 10^4$ cell L^{-1}). These events were dominated by *P. calliantha* with an abundance of $1.9 \cdot 10^4$ cell L^{-1}, and *P. delicatissima* with an abundance of $1.2–1.8 \cdot 10^4$ cell L^{-1}.

Our observations indicate that the peaks of the *Pseudo-nitzschia* spp. density were recorded in summer and in autumn. High concentrations of *P.calliantha*, *P. multistriata,* and *P. delicatissima* have



Dynamics of the *Pseudo-nitzschia* spp. densities in relation to salinity and temperature variations during October–November 2005.

been observed in summer and autumn, with abundances exceeding $1.6 \cdot 10^6$ cell L^{-1} and constituting 29–67% of the total density of the phytoplankton assemblage.

DA analysis of unialgal cultures of *Pseudo-nitzschia* (two isolates) collected from Amursky Bay in October 2011 has been performed. DA was found in stationary-phase *P. multistriata* culture (days 30–39) at concentrations varying between 334.4 and 1081,8 pg DA ml^{-1} and DA cell quota varying between 0.017 and 0.024 pg DA per cell, which fall within the lower range reported for other isolates of *Pseudo-nitzschia* (Thessen, Stoecker, 2008; Alvarez et al., 2009). No DA was detected (<10 pg ml^{-1}) in culture of *P. americana* from the same locality. Toxicity of a culture of *P. multiseries* isolated from Amursky Bay has previously been documented (Orlova et al., 2008). Domoic acid was found in stationary-phase (days 20–35) cultures of *P. multiseries* isolated from Peter the Great Bay at concentrations varying between 180 and 5390 ng ml^{-1} or 2 to 21 pg $cell^{-1}$, which is in the range reported for other isolates of *P. multiseries*. No domoic acid has been detected in cultures of *P. calliantha* and *P. multistriata* from the same locality (Orlova et al., 2008).

The relatively low toxicity level of *Pseudo-nitzschia* cultures isolated from Amursky Bay during 2005–2011 argues against making *Pseudo-nitzschia* a monitoring priority; however, having historical data on abundance and toxicity could prove valuable in the future. Current conditions in Amursky Bay appear to be unfavorable to highly toxic *Pseudo-nitzschia* blooms; however, cultural eutrophication and climate change may alter environmental conditions, making them more conductive to DA production. The potential toxicity of *Pseudo-nitzschia* clones from Amursky Bay suggests that phytoplankton and shellfish toxicity monitoring is warranted in Amursky Bay, where bivalves are commercially harvested for human consumption.

Acknowledgements

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Gamete morphology and evolution of reproductive biology in Spionidae, one of the most diverse groups of the polychaetous annelids (Annelida)

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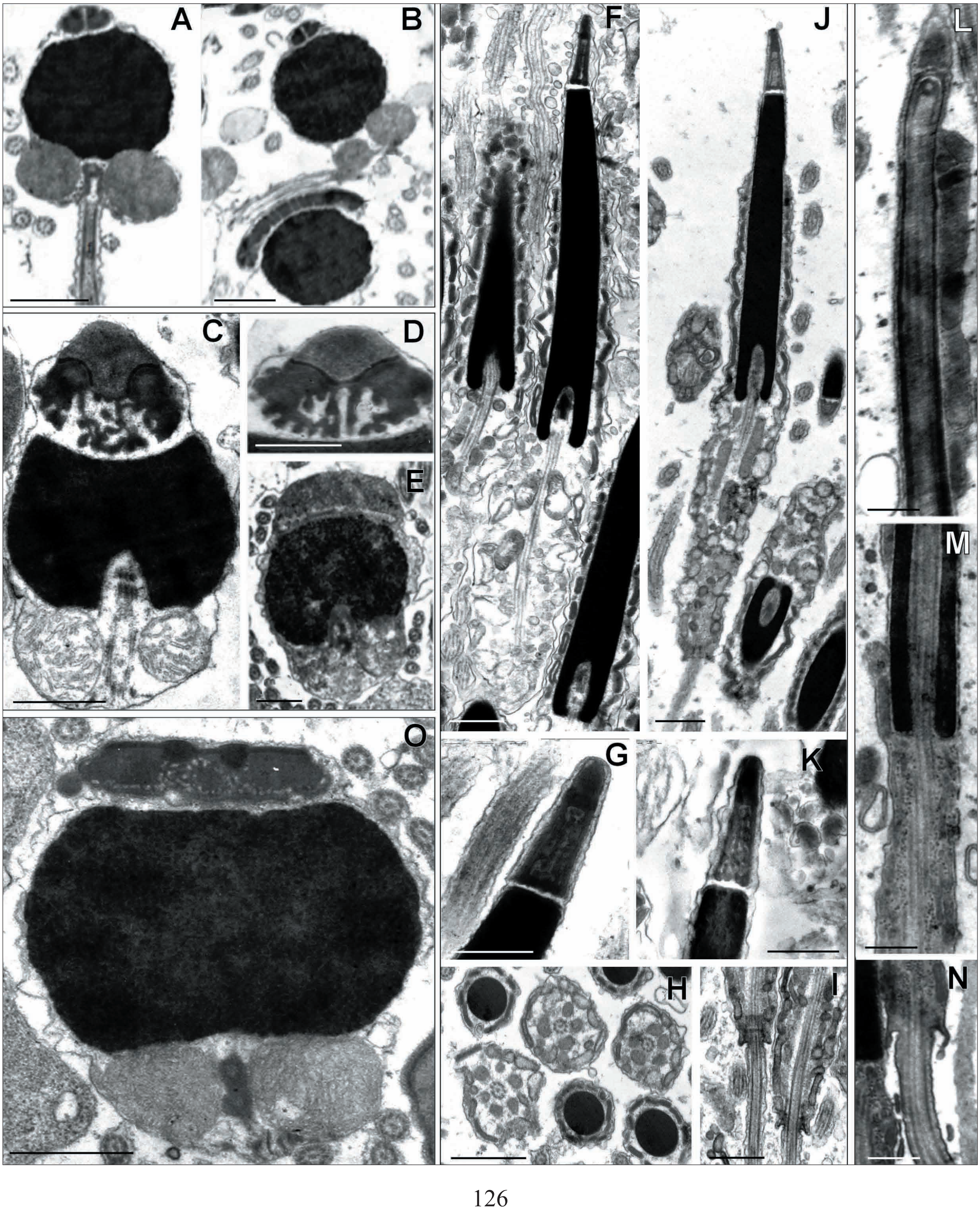
Spionidae is one of the most diverse groups of the polychaetous annelids, comprising more than 500 recent species. Adult spionids occur in a wide variety of habitats in benthic communities from the intertidal to the deep sea, while their larvae are common world-wide in the plankton. The spionids demonstrate great diversity of kinds of the reproduction and modes of larval development. Some species are known to include in their life history an asexual reproductive phase by architomy or paratomy. Asexual reproduction occurs more frequently among species associated with a particular rare host or substratum (some sponge and shell borers) but some opportunistic tube-dwelling species exploit this reproductive strategy as well (e.g., *Pygospio* spp.). The majority of spionids are gonochorists with genotypic sex determination but hermaphroditism and environmental sex determination occur in some commensal species. Sexual dimorphism is rare and females and males usually can be distinguished only by their gametes during the gametogenic period. Many spionids discharge their gametes directly into water where fertilization and planktotrophic larval development occur while various kinds of brooding are common in polydorins, *Microspio*, *Rhynchospio*, *Spio*, *Streblospio* and at least some *Malacoceros* and *Scolelepis* species. Brooding is usually associated with sperm transfer via spermatophores, sperm storage in various seminal receptacles, and mixed development, including lecithotrophy at early stages and planktotrophy at late stages of larval development. Parental investment into offspring varies a great deal in terms of quantity of the yolk allocated per offspring by females, the site of yolk storage, and also in terms of the kind and duration of brooding care. Different kinds of brooding have probably evolved independently in various groups of Spionidae and include development on the parent's dorsum in epithelial pouches, between long notochaetae, in various capsules inside the parent's tube or burrows, and in outside jelly or mucous masses. Consequently, larval development of spionids is extremely diverse, from entirely pelagic and planktotrophic to almost entirely brooded and lecithotrophic, i.e., demersal. Free spawners usually have oocytes with a thick sculptured envelope and short-headed spermatozoa with spherical to egg-shaped nuclei and spherical mitochondria, while brooders have oocytes with a thin, smooth envelope and spermatozoa with elongated nuclei and mitochondria (see Hannerz, 1956; Blake, 2006; Radashevsky, 2012).

Two opposed hypotheses have been provided on the evolution of reproduction in Spionidae. One of them considers oocytes with thin and smooth envelope as a plesiomorphic character shared by Spioninae members and many other polychaetes, and the oocytes with thick sculptured envelope as a derived character evolved within Spionidae (Söderström, 1920). Another hypothesis suggests that thick-envelope oocytes evolved in a primary spionid and thin-envelope oocytes evolved in Spioninae due to the reduction of the original thick envelope in connection with the evolution of brooding (Hannerz, 1956).

The morphology, ecology, reproductive biology and phylogeny of Spionidae are the objectives of the authors' studies over the years (see Radashevsky, 2012). We use the following techniques and the methods to describe the ultrastructure of spermatozoa in spionid polychaetes (see figure):



Light microscopy. Gamete-bearing segments of mature males are cut off, placed onto a slide with filtered sea water and examined alive using a differential interference contrast light microscope Leica DM4500 B equipped with a digital camera Leica DFC300 FX.

Transmission electron microscopy (TEM). The gamete-bearing segments of mature males are cut-off and fixed for 2 h at 4°C. The fixative is 2.5% glutaraldehyde in 0.1 M cacodylate buffer with 21 mg/ml NaCl to make the solution isotonic to sea water. Specimens are washed in several changes of buffered sodium cacodylate with added NaCl and then post-fixed in 2% buffered OsO_4 for 2 h. After dehydration in a graded ethanol series and acetone, they are embedded in Spurr resin (Spurr, EMS). Semi- and ultra-thin sections are made using a Leica UC6 ultramicrotome. Semi-thin sections are stained with methylene-blue and examined with a light microscope Leica DM4500 B. Ultra-thin sections are stained with 2% alcoholic uranyl acetate and aqueous lead citrate, and then viewed with transmission electron microscopes JEOL JEM 100S and Zeiss Libra 120 at 80–120 kV.

Scanning electron microscopy (SEM). The gamete-bearing segments of mature males are dissected in filtered sea water on a slide, and the coelomic fluid with sperm is placed with pipette onto nucleapore filters coated with poly-L-lysine hydrobromide (MP Biomedicals Inc). The filters are fixed and dehydrated as described above in TEM procedure, and then critical point dried in carbon dioxide, coated with platinum-iridium, and observed with a Zeiss EVO 40 scanning electron microscope at 10–15 kV.

Immunohistochemistry and confocal laser scanning microscopy (CLSM). The gamete-bearing segments of mature males are cut-off and fixed in 4% paraformaldehyde in phosphate buffered saline (PBS, 100 mM sodium phosphate and 140 mM sodium chloride; pH 7.4) for 1 h at room temperature, washed in PBS three times for 15 min each and blocked overnight in PBS with 10% normal goat serum, 0.1% Triton X-100 (TX), and 0.03% sodium azide. The specimens are labeled by incubating in monoclonal anti-α-tubulin antibody (Sigma-Aldrich, Cat. No. T-6793, developed in mouse) diluted 1:1000 in same solution used for blocking for a day at 4°C. Then they are washed three times in PBS for 1 hour each and incubated overnight in secondary antibody Alexa Fluor 488 goat anti-mouse IgG (MP Biomedicals Inc) diluted 1:600 in PBS-sodium azide. After incubation in secondary antibodies, the specimens are washed in PBS, treated for 5 min with 0.05% bisbenzimide (Hoechst 33258) in PBS to stain DNA material, and washed in PBS. Then the specimens are dissected in 80% glycerol in PBS on a slide, and the coelomic fluid with sperm is examined using Leica SPE confocal laser scanning microscope with appropriate wavelength-filter configuration settings. Photographs are taken with a digital camera.

Sperm ultrastructure of various spionid polychaetes (Annelida: Spionidae). **A**, **B** – spermatozoa of *Prionospio japonica*: **A**, longitudinal section; **B**, longitudinal sections through two spermatozoa, the upper one with an acrosome sectioned through the short axis, and the lower one with an acrosome sectioned through the long axis. **C**–**E** – *Aonides oxycephala*: **C**, longitudinal section through a spermatozoon; **D**, longitudinal section showing five layers in the complex acrosome of a spermatozoon; **E**, longitudinal section through a middle-stage spermatid with the three-layered trapezoid acrosomal vesicle in the distal part of the cell; nucleoplasm reduced to small areas scattered within the condensed chromatin. **F**–**I** – spermatozoa of *Dipolydora bidentata*: **F**, longitudinal sections; **G**, longitudinal section through an acrosome; **H**, transverse sections through nuclei and midpieces; **I**, longitudinal sections through midpieces and flagella. **J**, **K** – spermatozoa of *Dipolydora caruncu-lata*: **J**, longitudinal sections; **K**, longitudinal section through an acrosome. **L**–**N** – spermatozoon of *Dipolydora commensalis*: **L**, longitudinal section through an acrosome and anterior part of nucleus; **M**, longitudinal section through posterior part of nucleus and anterior part of midpiece; **N**, longitudinal section through posterior part of midpiece and flagellum. **O** – spermatozoon of *Spiophanes uschakowi*, longitudinal section. Scale bars: A–C, E, F, H–J, O – 1 µm. D, G, K–N – 0.5 µm.

We examined sperm ultrastructure in various spionid species (Radashevsky et al., 2010, 2011, in preparation; Reunov et al., 2010) and included a series of gamete characters into phylogenetic analysis of Spionidae (see figure). Preliminary results of this analysis suggest that the first spionid was a free-living in sediment and gonochoristic polychaete that had branchiae on most segments, cirri-form pygidium and uniform metanephridial segmental organs distributed throughout body, except the first three segments. These worms produced oocytes with a thick sculptured envelope and ect-aquasperm with subspherical acrosome, spherical nucleus, and four external spherical mitochondria. They released gametes into water where fertilization and entirely planktotrophic larval development occurred. The oocytes with thin and smooth envelope and brooding evolved not in the nearest ancestor of Spioninae but in an ancestor of a clade that includes *Rhynchospio* and Spioninae. Similar mode of reproduction evolved independently in an ancestor of *Streblospio*, thus thin-envelope oocytes, long-head spermatozoa and brooding are homoplasious characteristics resulted in a parallel evolution in two different clades of Spionidae.

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The distribution and biodiversity of benthic Harpacticoida (Copepoda) in Jiaozhou Bay, Yellow Sea

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Benthic copepods are mainly harpacticoid copepods. Harpacticoid copepods are small, predominantly less than 1 mm long, and inhabit all kinds of marine sediments. They are usually the second most abundant metazoan taxon after free-living nematods in marine sediment. The observation of shapes of habitus and appendages is necessary for the identification of benthic copepods, however, the tiny body size of these animals and the disputed systematics of Harpacticoida make the identification of harpactiocoid copepods very difficult. The Jiaozhou Bay is a semi-enclosed bay on the southern coast of the Shandong Peninsula, Yellow Sea, at Qingdao City. The benthic copepods of Jiaozhou Bay have been poorly investigated. Based on the samples collected during the Ecological Monitor Research Cruises (May 2008 and August 2011) from different depth and sediment, a compendium of the Harpacticoida of the Jiaozhou Bay, Shandong peninsula is presented, and the distributional range of species is discussed. Up to now, a total of 22 harpacticoid species belonging to 21 genera and 13 families have been found in this region for the first time. The most speciose families are the Miraciidae (7 species). Most of the genus just had one species to be found in this region which was attributed to less survey. The majority of the identified Harpacticoid species are new records for the Jiaozhou Bay and others are likely to be new species. For the difficult identification of harpacticoids, most of the species were just identified to genus level. List of the known harpacticoid copepods is as follows:

Family Longipediidae Boeck, 1865
Longipedia sp.
Family Canuellidae Lang, 1944
Scottolana bulbifera (Chislenko, 1971)
Family Ameiridae Boeck, 1865
Ameira sp.
Nitocra sp.
Family Ectinosomatidae Sars, 1903
Halectinosoma sp.
Bradya sp.
Family Miraciidae Dana, 1846
Amphiascus sp.
Bulbamphiascus plumosus Mu & Gee, 2000
Delavalia qingdaoensis Ma & Li,2011
Delavalia sp.
Onychostenhelia bispinosa Huys & Mu, 2008
Sinamphiascus dominatus Mu & Gee, 2000
Typhlamphiascus sp.
Family Pseudotachidiidae Lang, 1936
Danielssenia typica Boeck, 1873
Family Tachidiidae Boeck, 1865
Tachidiidae sp.
Family Tisbidae Stebbing, 1910
Tisbe sp.
Family Zosimidae Seifried, 2003
Zosime sp.
Family Canthocamptidae Brady, 1880
Canthocamptidae sp.
Family Aegisthidae Giesbrecht, 1892
Cervinia sp.
Family Cletodidae T. Scott, 1905
Enhydrosoma sp.
Limnocletodes sp.
Family Idyanthidae Lang, 1944
Idyanthidae sp.

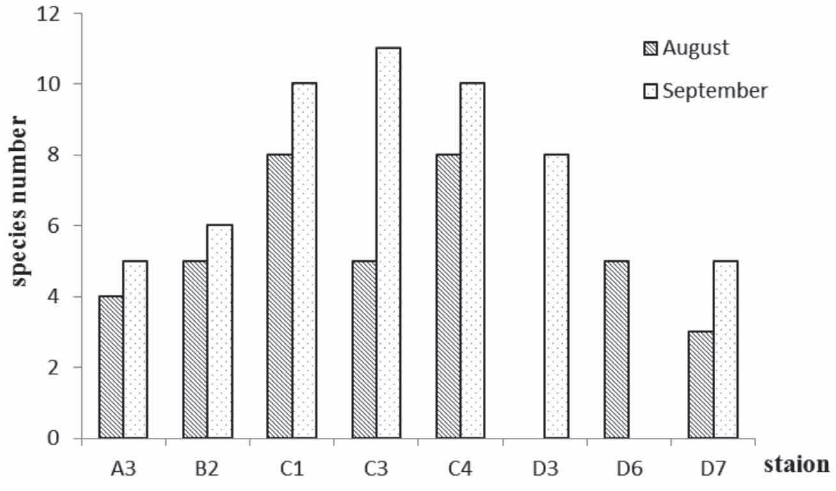

Fig. 1. The number of benthic Harpacticoida in all stations of the Jiaozhou Bay (August and September, 2011).

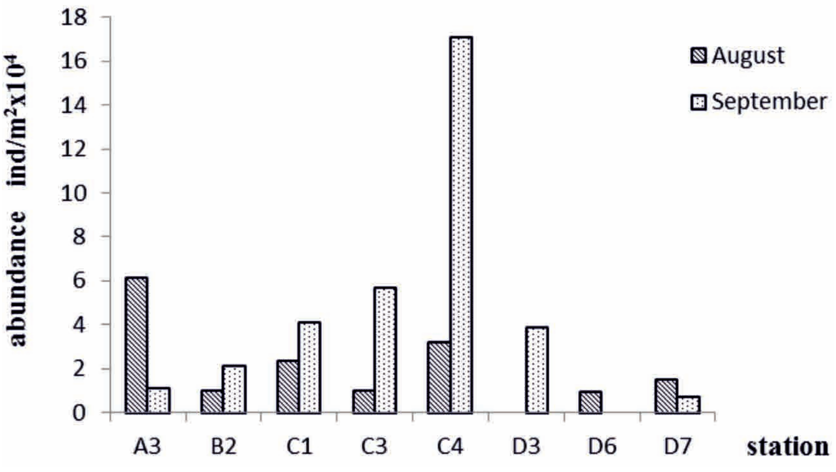

Fig. 2. The abundance of benthic Harpacticoida in all stations of the Jiaozhou Bay (August and September, 2011).

Sediments of all stations range from muddy sand to mud and sediment samples were collected at depth range 3–25 m. The number of benthic harpacticoida of all stations in Jiaozhou Bay can be seen in Fig. 1. The biggest number of benthic harpacticoida was in station C3 in September, but in August which was the station C1 and C4 had the biggest harpcticoid species number. The distribution of harpacticoida were unbalance, which was attributed to the depths of sample stations, seasonal variation and types of sediment. All stations were found more species number of harpcticoida in September than in August. For the abundance of harpacticoida, the station C4 had the highest abundance in September (Fig. 2). Except the station A3 and D7, the other stations all had higher harpacticoida abundance in September than that in August. Maybe the environmental factors and types of species contributed to this result. Our study just gave a basic data of benthic harpacticoids in Jiaozhou Bay. To clarify the biodiversity of benthic harpacticoids in Jiaozhou Bay, it needs more investigation.

Spawning desynchronization in populations of the sea urchin *Strongylocentrotus intermedius* inhabiting coastal waters of the northwestern Sea of Japan: is it an effect of pollution?

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The coincidence of the periods when a large number of planktotrophic larvae of marine bottom invertebrates appear in plankton with the periods of phytoplankton blooming is considered to be the evidence of the synchronization of reproductive cycles (in particular, the spawning period of invertebrates) with natural processes (Pearse, Cameron, 1991). Spawning synchronization appears to be crucial for reproduction. In addition to the fact that it facilitates efficient fertilization, the appearance of the larvae during the period of phytoplankton blooming provides them with the food required for their growth and development (Levitan, 2004). The available data of field and laboratory studies revealed also that temperature (Himmelman et al., 2008), lunar phases (Reuter, Levitan, 2010), and water salinity (Wilson, 1969) might also serve as the stimuli that synchronize or trigger spawning of bottom invertebrates. In marine coastal zone, these environmental variables may be extremely changeable. Besides, coastal waters are subject to pollution which would be associated with eutrophication and, therefore, would contribute to regulation of spawning schedule of marine invertebrates.

The sea urchin *Strongylocentrotus intermedius* (A. Agassiz, 1863) is a commercially important species inhabiting hard substrates in Northern Asian Pacific coastal waters, from the Japanese Islands to the southern Sea of Okhotsk (Bazhin, 1998; Agatsuma, 2007). The first studies of the reproductive cycle of *S. intermedius* in Russian waters of the Sea of Japan were performed in Peter the Great Bay in 1970–1980s. Proceeding from the results summarized by Khotimchenko et al. (1993), a conclusion has been made that the spawning period of *S. intermedius* in Peter the Great Bay usually falls on late summer–early autumn. In the mid-1980s the studies were initiated on the effects of marine environment pollution on reproductive functions of the sea urchin *S. intermedius* inhabiting Peter the Great Bay. An analysis of results of long-term investigations showed that in late 1990s – early 2000s the degree of gonad maturation in female sea urchins collected in the closest to Vladivostok part of Amursky Bay was significantly lower than that observed in the 1980s (Vaschenko et al., 2001). It was suggested that pollution could disturb the reproduction cycle in *S. intermedius,* which was manifested in the changed of spawning schedule in these animals. This suggestion was corroborated in 2003, during studies of seasonal dynamics of *S. intermedius* gonad condition in several areas of Amursky Bay with different pollution level. One early-summer spawning peak was observed in the sea urchin populations inhabiting the closest to Vladivostok polluted area of the bay, whereas in seaward insular area the sea urchins demonstrated two spawning peaks, in early summer and in autumn.

The purpose of the present study was to reveal the spawning schedule in the populations of the sea urchin *S. intermedius* inhabiting Peter the Great Bay and northward off Cape Povorotnyi (Sea of Japan).

Materials and Methods

Sea urchins *S. intermedius* were sampled using scuba in 2003, 2005–2010, at 21 stations located in the northwestern Sea of Japan (see Table), once a month: throughout the year, at stations Alekseeva Bay, Kievka Bay 2nd Island, Sportivnaya Gavan' and Gornostai; from May to November, at stations Rudnaya Bay, Kievka Bay 1st Island, Patrokl Bay, Vostok Bay, Russky Island, Cape Tokarevskogo, Verkhovskogo Islands, Starka Strait and Reineke Island; from May to July, at stations Abrek Bay, Pod'yapol'skogo Bay, Andreeva Bay, Manchzhur Bay, Narva Bay and Cape Perevoznyi; from May to September, at station Novitskogo Bay; from May to October, at station Vityaz' Bay.

Reproductive characteristics of the populations of the sea urchin *Strongylocentrotus intermedius* in the northwestern Sea of Japan

Station (N, E)	Year	GI±SD*	Period of spawning		Proportion of spawned males/females, %	
			early	late	in earlier period	in later period
Rudnaya Bay (44º20′, 145º49′)	2009	24.0±4.9	29.05–07.07	07.08–16.09	3/6	100/100
Kievka Bay						
1st Island (42°50′50″, 133°40′15″)	2010	10.6±3.1	absent	06.08–8.09	0	100/100
2nd Island (42º50′, 133º41′)	2007	18.6±4.5	absent	28.08–10.10	0	98/100
	2008	20.0±5.4	absent	21.08–25.09	0	100/100
	2009	19.5±6.7	02.06–26.06	21.08–15.09	8/67	100/100
	2010	22.0±4.1	absent	06.08–8.09	0	100/100
Nakhodka Bay						
Novitskogo Bay (42°47′7″, 132°54′10″)	2010	24.1±6.8	absent	19.07–27.09	0	100/100
Vostok Bay (42º53′41″, 132º43′59″)	2009	21.9±5.7	19.06–27.07	12.08–21.09	18/29	95/95
	2010	17.8±5.4	absent	02.08-22.10	0	90/100

TABLE (*Continued*)

Station (N, E)	Year	GI±SD*	Period of spawning		Proportion of spawned males/females, %	
			early	late	in earlier period	in later period
Strelok Bay						
Abrek Bay (42°52′36″, 132°21′58″)	2010	14.1±6.5	04.06–15.07	N.d.	74/100	N.d.
Ussuriysky Bay						
Pod′yapol′skogo Bay (43°0′6″, 132°17′56″)	2010	14.9±6.1	04.06–18.07	N.d.	25/66	N.d.
Andreeva Bay (43°6′24″, 132°19′20″)	2010	10.4±4.1	04.06–18.07	N.d.	43/62	N.d.
Manchzhur Bay (43°14′3″, 132°13′51″)	2009	20.7±4.7	11.06–29.07	N.d.	64/87	N.d.
Gornostai (43°7′50″, 132°2′36″)	2009	19.4±7.8	11.06–27.07	08.09–13.10	71/88	26/80
	2010	15.7±5.0	15.06–22.07	21.09–20.10	72/73	22/75
Patrokl Bay (43°4′26″, 131°57′1″)	2009	16.5±6.4	02.06–27.07	09.09–29.09	53/61	52/90
Russky Island (43°1′25″, 131°56′8″)	2009	7.8±3.6	13.06	14.09–26.10	77/59	18/56
Verkhovskogo Islands (42°54′22″, 131°50′22″)	2009	14.9±5.6	08.06–19.07	10.09–23.10	39/60	61/85
Amursky Bay						
Sportivnaya Gavan′ (43°7′14″, 131°52′27″)	2003	23.3±5.2	24.05–25.06	06.08–06.10	78/82	10/68
	2005	22.5±4.8	31.03–04.06	02.08–06.09	82/80	10/62
	2006	27.0±4.6	15.05–11.07	25.08–17.10	92/91	17/80
	2009	21.0±4.8	28.05–07.07	06.09–22.10	92/98	43/95
	2010	23.8±5.2	24.06–17.17	26.08–30.09	100/100	8/80
Cape Tokarevskogo (43°5′5.10″, 131°50′45″)	2003	14.3±3.2	24.05–25.06	06.08–06.10	54/68	52/79
Alekseeva Bay (42°59′10″, 131°42′58″)	2003	7.2±4.1	10.06–2.07	22.08–27.09	15/30	70/63
	2005	12.5±4.7	17.04–10.06	01.09–12.10	44/60	51/45
	2006	13.6±6.5	16.05–02.07	26.08–06.10	50/38	44/60
	2009	9.6±3.9	27.05–16.06	11.09–13.10	38/47	54/44
	2010	11.1±3.7	05.05–16.06	30.08–10.09	39/54	43/52

TABLE (*Continued*)

Station (N, E)	Year	GI±SD*	Period of spawning		Proportion of spawned males/females, %	
			early	late	in earlier period	in later period
Starka Strait (42°58′4″, 131°45′8″)	2009	14.4±5.5	07.06–15.07	11.09–13.10	81/83	17/77
	2010	16.1±6.4	05.05–18.06	10.09–22.10	96/75	8/73
Reineke Island (42°54′53″, 131°44′48″)	2003	12.5±4.3	24.05–11.06	21.08–27.09	44/74	55/58
	2009	9.1±3.8	08.06–19.07	12.08–23.10	38/56	50/70
Cape Perevoznyi (43°3′25″, 131°35′51″)	2010	27.4±5.0	31.05–15.07	N.d.	55/97	N.d.
Narva Bay (42°57′49″, 131°30′19″)	2010	11.9±5.3	31.05–15.07	N.d.	32/32	N.d.
Possjet Bay						
Vityaz′ Bay (42°35′5″, 131°9′55″)	2007	15.3±5.6	15.05–4.06	05.08–03.10	35/88	56/61
	2009	13.7±6.3	25.05–6.07	N.d.	28/56	N.d.

* Provided are the maximum values of gonadal index (GI)±the standard error.

Note. "N.d." means the absence of respective data.

The sea urchins were dissected with a circumferential incision at a distance of 5–8 mm from the peristomial membrane. Gonads of 30 animals were weighed in order to determine the gonad index (GI), which was calculated as gonad weight to total body weight percentage ratio. After weighing, the pieces taken from the medial part of the gonad were fixed in Bouin's fluid for histological examination. For all animals from the sample, small pieces of gonads were used to prepare smears for further determination of the sex and stage of gonad development. The occurrence of either early (spring–summer) or late (autumn) spawning was identified by decreasing GI and the relative proportions of animals in prespawning and postspawning stages of the reproductive cycle in two consecutive samples.

Results and Discussion

An analysis of seasonal dynamics of gonadal condition showed the spawning schedules to be different in different *S. intermedius* populations of the northwestern Sea of Japan. Three types of populations of the sea urchin have been found that differ from each other in the relative proportions of individuals having different spawning schedules. Among 21 populations of *S. intermedius* examined within this study, populations of the first type (spawning in September–early October) were found in Vostok, Novitskogo, Kievka and Rudnaya bays; in these areas the proportions of females spawned in late August–early October were 90–100% (see Table). Populations of the second type (spawning in late May–June) were located in the adjacent to Vladivostok zone (Sportivnaya Gavan' in Amursky

Bay and Gornostai Stn. in Ussuriysky Bay, close to the city dumping ground) as well as in Starka Strait, in the dumping area near Russky Island and in Abrek Bay. In different years, the proportions of females completed their spawning in late May–early June were 71–100%. The populations of the third type (almost equal proportions of individuals with early and late spawning schedules) were located in the insular area of Peter the Great Bay (the Verkhovskogo and Reineke Islands and Alekseeva Bay) as well as in Vityaz' Bay, Patrokl Bay and at the stations close to the west coast of Amursky Bay (Narva Bay and Cape Perevoznyi) and the east coast of Ussuriysky Bay (Andreeva and Pod'yapol'skogo bays).

The spawning schedule of *S. intermedius* did not depend on the relative weight of gonads. The greatest GI values (>20%) were registered in sea urchins from Rudnaya Bay and Kievka Bay 2nd Island, Vostok Bay and Sportivnaya Gavan' (see Table); however, late spawning was characteristic of the first three populations, whereas on the fourth station early spawning was observed. The smallest GI values (approximately 10%) were found in sea urchins from the stations Kievka Bay 1st Island and Alekseeva Bay; however, late spawning was revealed in the first population, while both early and late spawning peaks were observed in the second one. The numbers of males and females that took part in spawning events during the year were different (see Table). The total proportions of females and males that took part in early and late spawning equaled 99±11% and 138±28%, respectively (the differences were significant at $p<0.0001$). This is evidence that females participated in the spawning only ones, whereas significant proportion of males took part in both spawning periods. An exception was a sample of sea urchins from Sportivnaya Gavan' Stn. collected in 2009 and comprised 92% females that had spawned in early summer and 43% females that had spawned in autumn period.

The sea urchins inhabiting temperate climatic zone usually have annual reproductive cycle and one spawning period per year (Pearse, Cameron, 1991). However, some sea urchin species, in some areas of their geographic range, can spawn more than once a year. In the most detail the reproductive biology has been studied in the sea urchin *Paracentrotus lividus* distributed along the western coast of the Atlantic, from Ireland to the southern extremity of Morocco and in the Mediterranean. The data from 52 publications were involved into a statistical analysis to reveal spatial and temporal regularities in the reproductive processes of this species over the entire geographical range (Ouréns et al., 2011). It is shown, that in Atlantic populations *P. lividus* spawns once a year, in spring–early summer. In Mediterranean populations this species, according to most authors, spawns twice (in spring and autumn), although some authors reported either a single spring spawning or multiple summer spawning peaks.

The reasons of the regional differences in the reproductive cycle of the same sea urchin species are still unknown. According to one of the hypotheses, the reproductive cycles of sea urchins and other echinoderms change in accordance with latitudinal gradient; in temperate waters the individuals spawn during a short period of time, while in the conditions of tropical zone the spawning period extends and some species can even spawning throughout the year (Mercier, Hamel, 2009). However, this hypothesis cannot explain, why *P. lividus* populations located along the western coast of the Atlantic, for approximately 2000 km from south to north, are characterized by a single spawning, whereas the populations of the same species inhabiting the Mediterranean and located at the same latitude have several spawning peaks that mostly fall on spring and autumn (Ouréns et al., 2011). This hypothesis also cannot explain different spawning schedules of the sea urchin *S. intermedius* in Peter the Great

Bay of the Sea of Japan; all the examined populations of this species were located at approximately the same latitude (see Table). The populations of *S. intermedius* around Hokkaido that is washed by waters of the Sea of Okhotsk, Sea of Japan and Pacific are also located at the same latitude, however, in this region the sea urchin spawn in different time; in the Sea of Japan they spawn in autumn, in the Sea of Okhotsk and eastern Pacific – from June to October, while the sea urchins inhabiting the southern coast of the island (Tsugaru Strait and Funka Bay) spawn twice a year, in spring and in autumn (Agatsuma, 2007). The experiments on transplantation of the offspring, obtained from *S. intermedius* from the Sea of Japan, into the region of the eastern Pacific coast and vice versa, the offspring of sea urchins from the oceanic region into the Sea of Japan showed that in both the cases the sea urchins retained the parental pattern of spawning cycle. It was speculated that these two populations of *S. intermedius* are genetically isolated from each other (Agatsuma, 2007), however this idea has not been corroborated yet.

One more fact merits attention that also was obtained during the studies of reproductive cycle of *S. intermedius* in the Sea of Japan, namely the changes in spawning schedules of some populations happened during the recent 50–60 years. For example, in the 1950s the spawning in sea urchin populations of eastern Tsugaru Strait and Funka Bay occurred from September to November (the Sea of Japan spawning pattern) (Fuji, 1960). The disagreement between these data and the results of studies of 1980–1990s that revealed double spawning in *S. intermedius* inhabiting this area were explained by changes in the warm Tsushima Current and Oyashio Current, which provided prerequisites for transfer of sea urchin larvae from other regions of the Pacific (Agatsuma, 2007). Late (autumn) spawning was also registered in *S. intermedius* from Ussuriysky Bay in the late 1960s and early 1980s (Khotimchenko et al., 1993), whereas in the 2000s we revealed double spawning in this species; moreover, in the population at Gornostai Stn. most animals (71.8±6.3% females) spawned during early summer (see Table). If to follow the Japanese scientists and suppose that the spawning schedule in the sea urchin *S. intermedius* is strictly determined by genetic mechanisms, so it can be concluded that genetically isolated populations of this species exist in Peter the Great Bay at distances of a few tens kilometers from each other. However, taking into account active hydrodynamics of this area owing to monsoon climate, such a conclusion appears unlikely. Summer winds of mostly southern directions and northerly autumn winds initiate pronounced wind currents mixing the water and transferring planktonic invertebrate larvae from the open part of Peter the Great Bay to its inner areas and vice versa. Also, no information is available about any changes in hydrological regime of the bay for the recent 50 years, which could benefit the transfer of *S. intermedius* larvae from remote areas, like, for example, the Sea of Okhotsk.

Our observations provided evidence that early summer spawning is the most characteristic of *S. intermedius* populations located close to sources of pollution; thus, we supposed that the shifts of spawning period could be due to a phenotypic response of the sea urchin to changes in environmental conditions caused by chronic anthropogenic pollution. This conclusion is corroborated by a positive correlation between the relative proportions of females of the sea urchin *S. intermedius* from Amursky and Ussuriysky bays spawning in early summer and the potential toxicity of bottom sediments at these stations (Zhadan et al., 2011). It is also pertinent to note that marine environment pollution is associated with eutrophication of waters; thus, the effects of anthropogenic pollution on the reproductive cycle of the sea urchin could be indirect, conditioned by great concentrations of phytoplankton and its metabolites in seawater. It has been shown previously that the increase of phytoplankton concentration in seawater is the primary and necessary prerequisite of spawning triggering in the populations of *S. intermedius* (Zhadan et al., 2010).

The results of the present study give evidence that in the northwestern Sea of Japan, in the area from Rudnaya Bay to Vostok Bay, spawning of the sea urchin *S. intermedius* takes place in late summer-autumn period, which is characteristic for this species. At the same time, in the area adjacent to Vladivostok City (Amursky and Ussuriysky bays), *S. intermedius* spawning time has been shifted to the early (spring-summer) season. We suggest that this shift in spawning period of *S. intermedius* can be explained by a phenotypic response of this species to changing environmental conditions due to chronic anthropogenic pollution of the bay.

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Marine Ecosystems under the Global Change
in the Northwestern Pacific

Distribution and ecological morphs of northwestern Pacific gastropod *Batillaria attramentaria* (G.B. Sowerby II, 1855) (Cenogastropoda: Batillariidae)

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Intertidal gastropods of the genus *Batillaria* Thiele, 1931 are widely distributed along the east coast of Asia. They inhabit intertidal marine to brackish water, ranging up to about mid-tide level and form a dominate group in the muddy tidal flats, bottom of estuaries and coastal lakes where they may occur in aggregations. Snails of *Batillaria* prefer the zone above mid-tide level to search for their food of organic detritus.

In northwestern Pacific, the most common *Batillaria* species is *B. attramentaria* (G.B. Sowerby II, 1855). Its junior synomyn *B. cumingi* (Crosse, 1862), or *B. cumingii* (erroneous spelling used after Sowerby (1866)) is more frequently used in Chinese, Japanese and Russian literature (Golikov, Scarlato, 1967; Habe, 1970; Volova et al., 1979; Higo et al., 1999; Hasegawa, 2000; Seashells of China, 2004; Kantor, Sysoev, 2006; and many others) and sometimes even with synonym of *B. attramentaria* (Miura et al., 2005; Torchin et al., 2005) in contradiction with International Code of Zoological Nomenclature (ICZN). *B. attramentaria* is distributed mainly in Sea of Japan, Yellow and East China seas. This subtropical-lowboreal species occurs in littoral zone to a depth of 3 m (Adrianov, Kussakin, 1998; Gulbin, 2004).

Batillaria attramentaria extends its native area from Taiwan (Golikov, Scarlato, 1967) and Hong Kong (Golikov, Kussakin, 1978) in the south to south Primorye, southern Sakhalin and South Kuriles in the north (Golikov, Scarlato, 1967, 1985; Golikov, Gulbin, 1978; Golikov et al., 2001; Gulbin, 2006; and others). More southern records of the species are misidentifications. For example, "*B. cumingii*" from north Vietnam (picture N 125 in Thach, 2005) is considered to be not *B. attramentaria* but instead belong to *B. sordida* (Gmelin, 1791). Additionally, *B. attramentaria* was introduced to the west coast of North America with shipment of Pacific oysters, imported from Japan for aquaculture production in the early part of the last century (Bonnot, 1935). First, it was recorded under name *B. multiformis* (Lischke, 1869). Now *B. attramentaria* has become naturalized from Boundary Bay, British Columbia to Elkhorn Slough, Monterey, California (Taylor, 1981; Byers, 1999; Torchin et al., 2005; Ozawa et al., 2009; and others). Latitudinal range of the species is from 50° N (British Columbia) to 20° N (Hong Kong).

Having a rather wide range and shell variable in color (Miura et al., 2007) and form, the species is sometimes misidentified with closely related *B. zonalis* (Brouguerre, 1972) and *B. flectosiphonata* Ozawa, 1996, especially in sympatric populations (Kojima et al., 2001). For example, species identified as *B. flectosiphonata*, endemic for Ryukyu Islands (Kojima et al., 2003), from Jeju Island in a Korean color atlas (Min et al., 2004) is considered to be *B. attramentaria* (T. Ozawa, personal communication).

Taxonomic confusions put obstacles in ecological, zoogeographical and phylogenetic studies. So, here we consider distribution and shell morphology of the *B. attramentaria* from different sites of northwestern Pacific based on published illustrations, specimens stored in Zoological Museum, Far East Federal University (ZMFU), Vladivostok and original material stored in Institute of Biology and Soil Science (IBSS), Far Eastern Branch, Russian Academy of Sciences.

In Russian mainland, distribution of *B. attramentaria* extends from Sivuchja Inlet (Fig. 1D) and Possjet Bays (collection of ZMFU) (Fig. 1C) through the Peter the Great Bay to Vostok Bay (Fig. 1I). Snails are often found in high densities (more than 300 individuals per square metre) grazing on surface microalgae and detritus. Their populations are known in inner parts of Amursky Bay (Fig. 1E–H) in bays of Russky Island, in Popov Island and some other localities (ZMFU and IBSS collections). Continental coastal distribution of *B. attramentaria* is not restricted by the Peter the Great Bay in southern Primorye and it is recorded northward in Kievka Bay (Fig. 1G) and Olga Bay (Fig. 1K) in central Primorye.

Southern Kuril Islands, Kunashir (Fig. 1A) and Shikotan (Fig. 1B) are inhabited by *B. attramentaria* as well (Prozorova et al., 2010; original data from the collection of IBSS). In southern Sakhalin, *B. attramentaria* is recorded in Bousse Lagoon (Kantor, Sysoev, 2006; original data from the collection of IBSS). The latter site is the northernmost edge of native distributional area of the species.

Shells of *B. attramentaria* from both continental and insular Russian sites have a tall, 30 mm and more, spire composed more than 10 slightly-rounded whorls and an acute apex. Russian specimens of *B. attramentaria* quite resemble those from northeastern coast of the Korean Peninsula, where Liman Current flows down (North Korea and Gangwon Province in South Korea), as well as snails of Hokkaido, northwestern Tohoku Region of the Sea of Japan (T. Ozawa, personal communication). This ecological form of the *B. attramentaria* is named *attramentaria*-like morph because of similarity with types of *Lampania attramentaria* Sowerby G.B. II, 1855 (Fig. 1L) from unknown locality (Sowerby G.B., 1855, 1866), stored in the Natural History Museum, London (BMNH). Originally they are syntypes as G.B. Sowerby (l.c.) did not designate any types of *L. attramentaria* in his publications.

The rest of more southern distributional area of the *B. attramentaria* is inhabited by a less tall and elongate (nearly 20 mm in high and 8–10 whorls) *cumingi*-like ecological form, described as *Lampania cumingi* Crosse, 1862. The types are not located, but specimens from the type locality are available from the ZMFU collection (Fig. 2). Type specimens were collected in "sinu Talienwhanensi" (Crosse, 1862; Lischke, 1869), that is, nowadays, Dalian Bay, Liaodong Peninsula, northeastern China. Other records of *Batillaria cumingi* (Grosse) are Tschi-fu (now Cape Chantong), northern China; Korean coast of Yellow Sea; Pehio River, Bohai Bay, Yellow Sea, northern China (Lischke, 1869), South Korea with Jeju Island excluding Gangwon Province (Min at al., 2004; Min-Ho Son, Sung-Yun Hong, 2005; T. Ozawa, personal communication; original data), Taiwan (The Taiwan Malacofauna Database, 2012), central and southern Japan (Habe, 1968; Ozawa, 1996; Adachi, Wada, 1998; Higo et al., 1999; Hasegawa, 2000; and many others), China from Liaoning to Fujian (Seashells of China, 2004; Checklist of Marine Biota of China Seas, 2008; Zhang Suping, 2008).

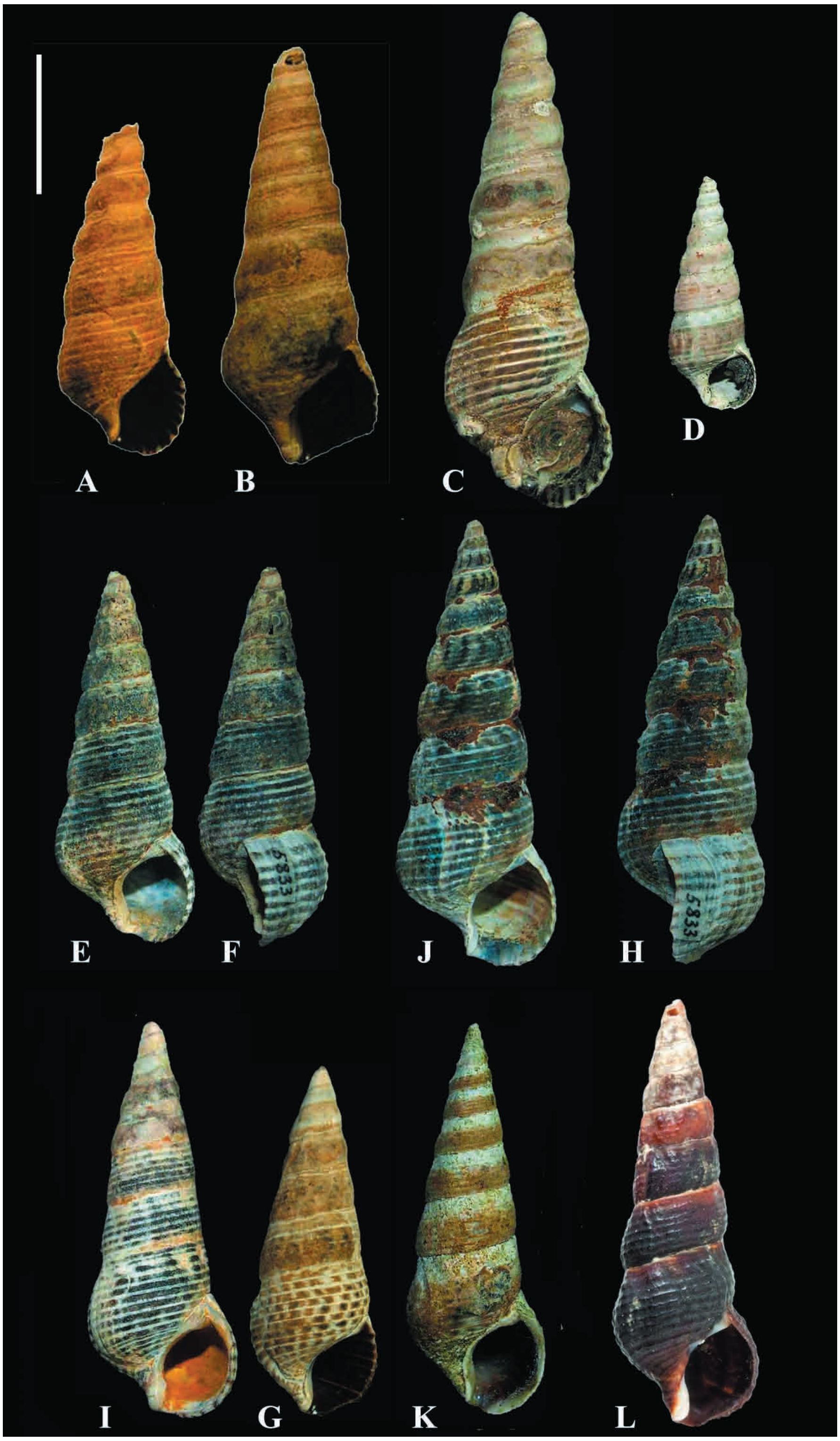

Fig. 1. *Batillaria attramentaria* (G.B. Sowerby, 1855), shells of *attramentaria*-like morph: **A** – Kunashir Island, Southern Kuriles; **B** – Shikotan, Southern Kuriles; **C** – Possjet Bay, Peter the Great Bay; **D** – Sivuchja Inlet, Peter the Great Bay; **E–H** – Amursky Bay, Peter the Great Bay; **I** – Vostok Bay, Peter the Great Bay; **G** – Kievka Bay, central Primorye; **K** – Olga Bay, central Primorye; **L** – syntype of *Lampania attramentaria* (= *Cerithium attramentarium*) from the BMNH no.1991021. Scale bar: 1 cm.

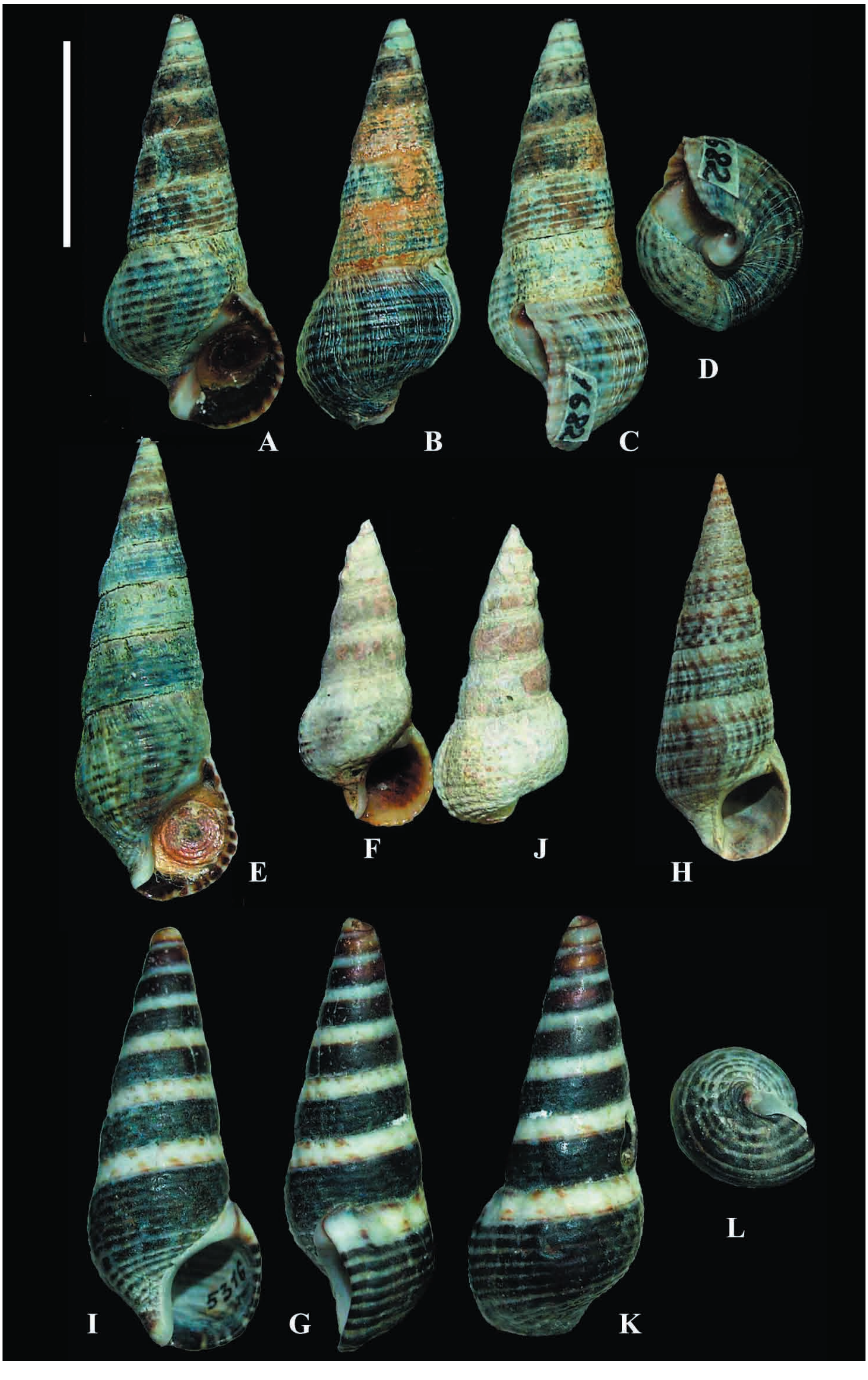

Fig. 2. *Batillaria attramentaria* (G.B. Sowerby, 1855), shells of *cumingi*-like morph: **A–E** Dalian Bay, north China (type locality); **F**, **J** – Qingdao, China; **H–L** – Jeju Island, South Korea. Scale bar: 1 cm.

Batillaria attramentaria with *cumingi*-like shells is similar to *B. flectosiphonata,* originally described from Ryukyu (Ozawa, 1996) and endemic for this island group (Kojima et al., 2003). Specimens with *cumingi*-like shells occur in area with warm temperate water in Korea, China, central and southern Japan. Morphological form with *attramentaria*-like shells extends its distribution northwards in rather cold water areas of Western Pacific – Primorye, southern Sakhalin, and Southern Kuril Islands in Russia, Hokkaido and northwestern Honshu in Japan, and also eastern coast of the Korean Peninsula where Liman Current comes down (T. Ozawa, personal communication and original data). Mitochondrial DNA analyses revealed that these different morphs distributed from Hokkaido to Kyushu are conspecific (Kojima et al., 2004). The oldest valid name for this species is *B. attramentaria* (G.B. Sowerby II, 1855) (syn. *B. cumingi* (Crosse, 1862)).

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Study of population genetic diversity and structure of cultivated and wild *Saccharina japonica* (Laminariales, Phaeophyta) with SSR markers system

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Saccharina japonica (Areschoug) Lane, Mayes, Druehl, and Saunders, is one of the common kelps along northwest coasts of Pacific Ocean. Attaching to sublittoral solid substrates, this kelp grows in temperate cold seawater along the coastline of Russia Far East, northern Japan and northeast Korea. As it is unable to complete its life cycle in higher temperature seawater in more southern latitude, this kelp occurs as south as 36°N latitude Although the oceanographic conditions along the northern coast of the Yellow Sea are similar to those of its “home” near western Hokkaido of Japan in some respects, *S. japonica* does not spread to the China coast only by itself, because the wide warmwater region lying at both sides of Korean Peninsula prevents its southward extension. *S. japonica* is not indigenous to China but was accidently introduced from Hokkaido to Dalian on the northern coast of the Yellow Sea in Liaoning Province, China. The transplanted *S. japonica* settled down in the vicinity of Dalian coast, and benefiting from the suitable habitat conditions, it propagated and developed into wild populations. In China, the range of *S. japonica* wild population is also restricted to north of 36°N latitude, however, the range of cultivated population has been successfully extended southward to Fujian Province (about 26°N latitude) by artificial seedling-rearing techniques.

In the present study, we selected the sporophytes of eight representative cultivars and four wild populations (Fig. 1; Table 1) as specimens to assess their genetic diversity level and investigate their population genetic structure with simple sequence repeat (SSR) markers (Table 2). Eight cultivars such as “Zhong ke No. 1” (ZK1), “Zhongke No. 2” (ZK2), “Ben niu” (BN), “901” (901), “Dong fang No. 2” (DF2), “Dong fang No. 3” (DF3), “Lian za” (LZ) and “Guan wu” (GW) were widely cultivated nowadays (cultivars of LZ and GW are cultivated in southern China such as Fujian Province, and the others are cultivated in northern China, such as Shandong and Liaoning provinces). Two wild populations RU1 and RU2 from Sakhalin island of Russia Far East, and two wild populations JA1 and JA2 from Hokkaido of Japan were sampled, respectively (Fig. 1). The genetic diversity analysis showed that the average genetic diversity of the wild populations from Russian Far East was higher than that of the wild populations from northern Japan, which was in turn higher than that of the cultivars from China. The wild populations harboring higher genetic diversity level than the cultivars implied that *S. japonica* domestication might be accompanied by genetic diversity decrease. The four wild populations and the eight cultivars were taken as two groups respectively (Fig. 2). Analysis of molecular variance (AMOVA) results indicated that there was significant

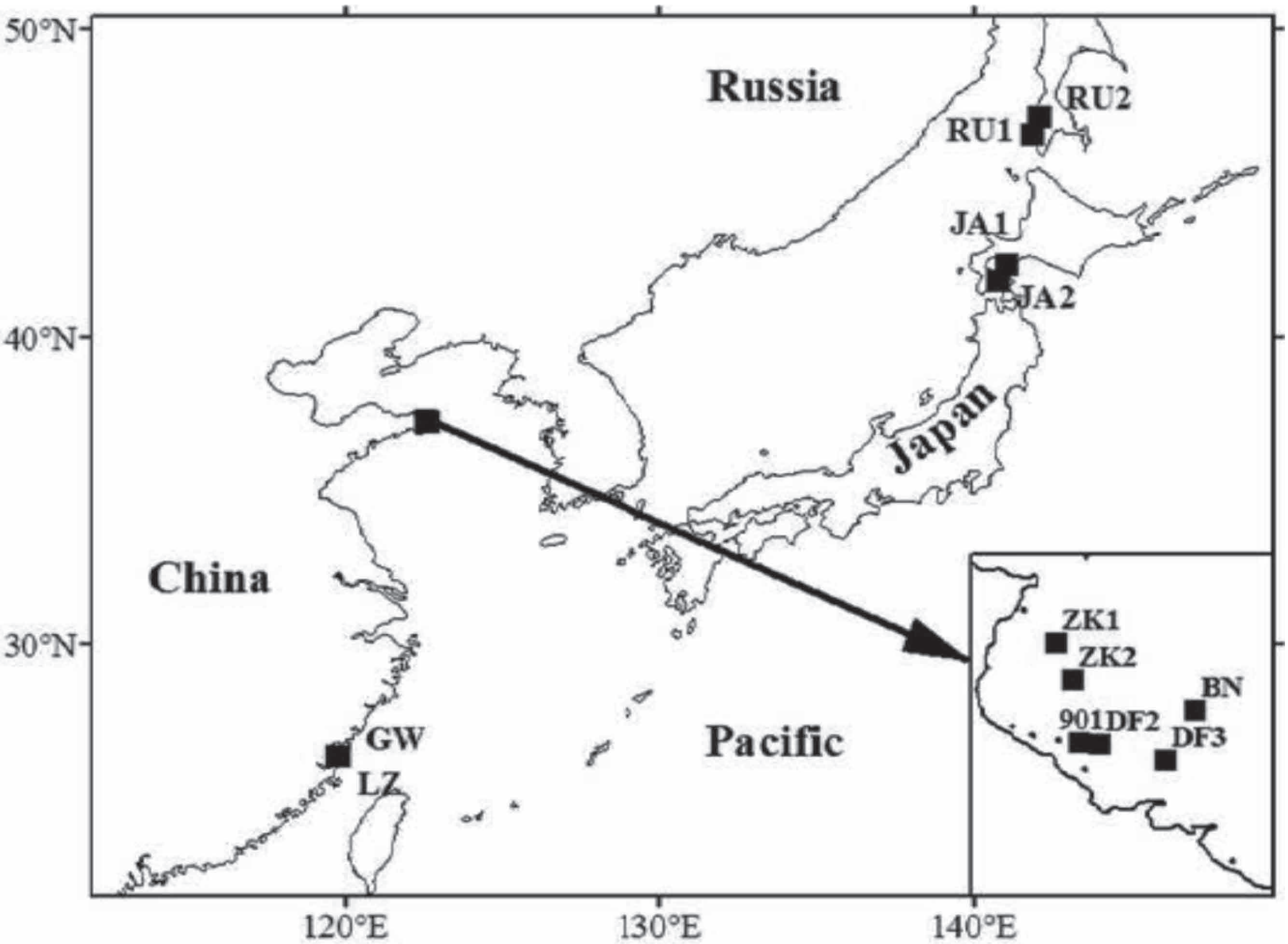

Fig. 1. Geographic loci of four wild populations (RU1 and RU2 along coast of Sakhalin Island; JA1 and JA2 along coast of Hokkaido) and eight cultivars (ZK1, ZK2, BN, 901,DF2, DF3, LZ and GW cultivated in China).

TABLE 1
Parentage information and phenotypic characteristics of wild populations and cultivars

Population	Parentage information	Phenotypic characteristics
ZK1	Descendants of *S. japonica* intraspecific hybridization	Much longer and thicker frond; shorter stipe; much stronger rhizoid; high resistance; and high yield
ZK2	No parentage information available, artificial selected from *S. japonica* aquaculture farm	Much wider and thicker frond; narrower frond edge shorter stipe; much stronger rhizoid; high resistance; and high yield
BN	Landrace, widely cultivated in northern China, presumably the descendants of the transplanting *S. japonica*	Much longer, wider and thicker frond; much stronger rhizoid; strong high temperature resistance; and high yield

TABLE 1 (*Continued*)

Population	Parentage information	Phenotypic characteristics
901	Hybrid of *Laminaria longissima* (maternal parent) and *S. japonica* (paternal parent)	Much longer, wider and thicker frond; clear channel in the middle of blade; thinner and longer of stipe; much stronger rhizoid; strong high temperature resistance; and high yield
DF2	Hybrid of *Laminaria longissima* (paternal parent) and *S. japonica* (maternal parent)	Wide, long and deep-brown uniform blade; distinct middle groove; robust holdfast; obvious heterozygous vigor; appropriate vegetative maturation time; and high adaptive ability
DF3	Hybrid of *Laminaria longissima* (paternal parent) and *S. japonica* (maternal parent)	Wide and dark brown frond; deep vertical groove; short stipe; very robust holdfast; strong irradiance and high temperature resistance; and later mature period
LZ	Landrace, widely cultivated in southern of China, artificial selected from descendants of transplanting S. japonica from northern China	Much wider and thickness frond; shorter stipe; much stronger rhizoid; much stronger irradiance and high temperature resistance; and earlier mature period
GW	The same with “LZ”	Similar to “LZ”
RU1	Wild population	Narrower but relative longer frond; not obvious vertical groove; thinner and longer stipe; medial strength rhizoid; lower high temperature resistance; and lower yield
RU2	Wild population	Similar to “RU1”
JA1	Wild population	Similar to “RU1”
JA2	Wild population	Similar to “RU1”

TABLE 2

Information of the 13 SSR markers used in the analysis

Primer code	Accession no.	Repeat motif	Primer sequence (5–3)	Ta (°C)
SJ1	CX943061	$(GCCT)_{18}$	CGGCTTCATCTCCCACAG TGACGGAATAGACCCAAA	55
SJ2	CX942983	$(AC)_5$	GCAGGCTCGTGTCGTGTA CGCAGCATCAAGAAGGTA	55
SJ3	DQ978335	$(AC)_7$	AGAATCGGCACGAACACT CAAACACGAACGACGAAG	53

TABLE 2 (*Continued*)

Primer code	Accession no.	Repeat motif	Primer sequence (5–3)	Ta (°C)
SJ4	DQ978342	$(ACAAC)_5(ACAAA)_6$	TCTTACCCGACTGACCGTGAC CTCATCGGTTTTCCCCAAGT	59
SJ5	DQ978337	$A_7(CA)_5CG(CA)_3$	CCGTCTACCGCTGTATTGTGA CGAAAGCATAAGGACGGT	57
SJ6	DQ978343	$(AC)_{39}$	TGGGTATGATGGATGTCGC CAATAATAGCATGGCCGTAA	59
LD1	CN467174	$(GGAA)_9$	AACAAAGGACGGTTGGACG TAGCCTGGTATTTACGGGTG	55
LD2	CN467886	$(AGC)_6$	GGGAGGGGACATCCTGAGTA AGAAAGAGACCAAGCACGGC	55
LD3	CN468120	$(TGC)_7$	TTCGCAAGAAGGGACACC AAAGCAGGAAAAGACGCA	53
LD4	AW400562	$(AT)_{10}$	TGCGAAGATAATAAATGGGA CGTTGTAGCATAGGTTGGAG	52
LD5	CN468232	$(GGAA)_{10}$	CGAGGGGTTTGAGAAGCA TACCGAAAGTTCTGCCGC	55
LD6	Y17347	$(ACC)_7$	GGCGATGAGTGAATGTGAG GTCTTCAAAACCCGAGAAAGAG	60
LD7	Y17349	$(CA)A(CA)_9\ C(CA)$	TCTAGGATGAAGAATCAGCACG TTTCTTGCGATGGATACACCAG	60

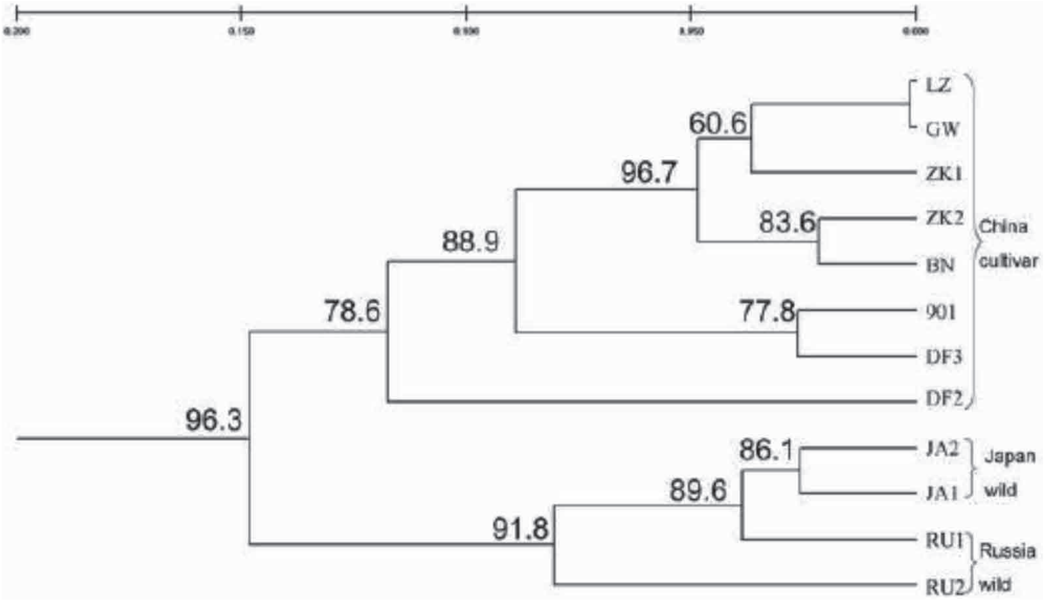

Fig. 2. UPGMA dendrogram of four wild populations (RU1, RU2 from Russia; JA1 and JA2 from Japan) and eight cultivars (ZK1, ZK2, BN, 901, DF2, DF3, LZ and GW were cultivated in China) based on Nei’s unbiased genetic distances.

genetic differentiation between the two groups (Φ_{CT}=0.212, P<.01), and the differentiations among populations or cultivars within group were also significant (Φ_{SC}=0.115, P<0.01) (Table 3). Intensive artificial selection, cultivation method and dispersal model together affected the population genetic structure, and the artificial selection was the main factor caused the significant genetic differentiation between wild and cultivated *S. japonica*. In addition, the four wild populations and

TABLE 3

AMOVA for eight cultivars and four wild populations of *S. japonica*

Source of variation	Variance component	Percentage of variation	Φ-Statistic	P
Among groups	0.157	21.16	Φ_{CT}=0.212	<0.01
Among populations within group	0.067	9.03	Φ_{SC}=0.115	<0.01
Within populations	0.518	69.81	Φ_{CT}=0.302	<0.01

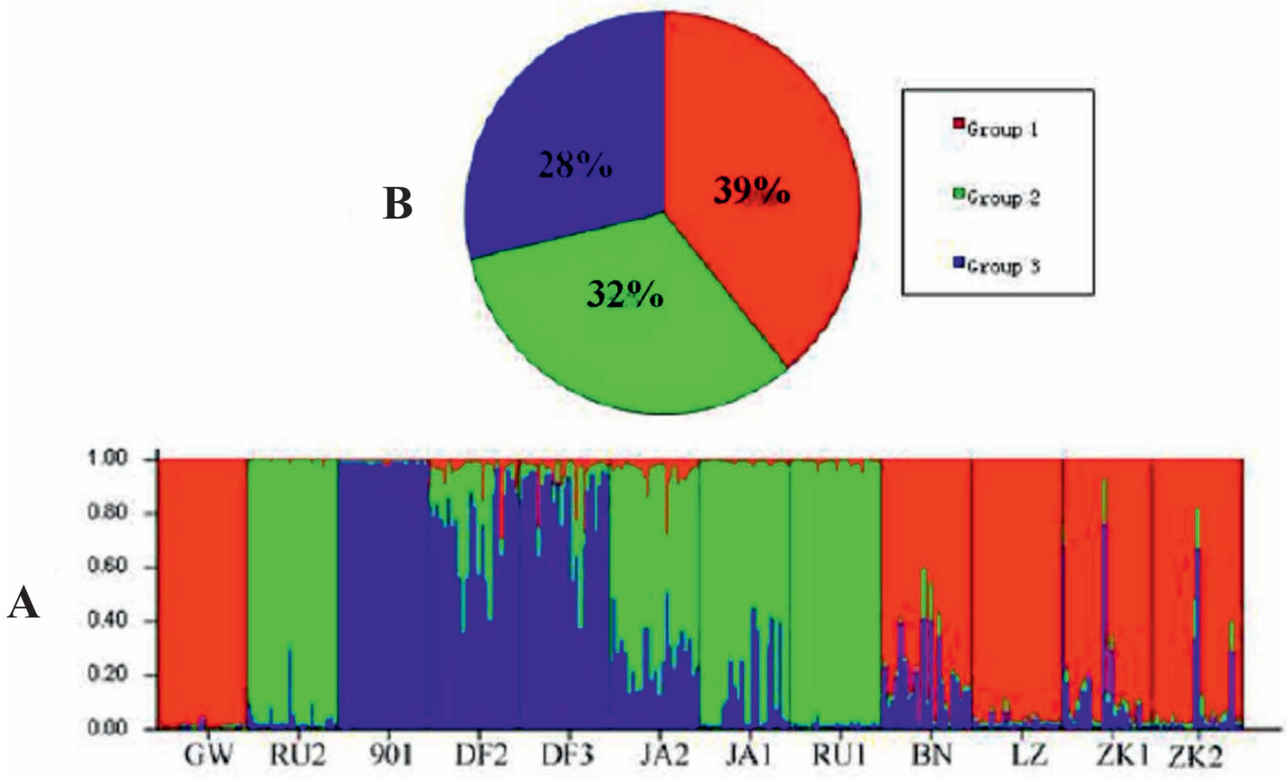

Fig. 3. Population genetic structure analysis with software Structure 2.2. **A**: Genetic clusters obtained with three groups. Each individual is represented by one vertical line with 3 segments colored proportionally according to their belonging to a genetic group. Black lines separate individuals from different populations or cultivars. **B**: The percentage of genetic variations contained by each genetic group.

eight cultivars were divided into three genetic groups by Bayesian model-based cluster analysis. The proportion of the genetic variations contained by each genetic group was 39.12%, 32.18% and 28.70% respectively (Fig. 3). These discoveries will shed light on the effects of historical and ongoing domestication and cultivation on population genetic structure and diversity of *S. japonica*, and also provide the foundation for high-efficiency utilization of *S. japonica* germplasm resources in *S. japonica* breeding practice.

Genetic and ecomorphological aspects of determining morphologically distinct forms of *Undaria pinnatifida* in Peter the Great Bay (Sea of Japan)

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Undaria pinnatifida (Harvey) Suringar is endemic to Japan, Korea, and China. Lately, *Undaria* is introduced to the France, New Zealand, Australia, and Argentina. In Russia, *Undaria pinnatifida* is distributed only in Peter the Great Bay (Skriptsova et al., 2004).

Polymorphism of *U. pinnatifida* was recorded in many works (Castric-Fey et al., 1999; Cecere et al., 2000; Skriptsova, 2008). Previously, two forms within this species were detected as well recognizable on the basis of morphological differences: f. *typica* Yendo and f. *distans* (Miyabe, Okamura) Yendo (Yendo, 1911; Okamura, 1915). U. *pinnatifida* f. *typica* (Yendo, 1911) has a short stipe, shallow pinnate division of the blade and sporophyll development is often confluent with the base of the blade. *U. pinnatifida* f. *distans* is characterized by an elongated stipe and deeply-divided blade, with large sporophyll limited at the basal portion of the stipe, the main distinctive feature is a wide transitional zone between sporophyll and lamina (Okamura, 1915). Third form, f. *narutensis*, was described by Yendo (1911) as containing a short stipe, with less folded sporophylls which became confluent with the lamina and grew sterile ligules from the sporophyll margin. This form was subsequently recognized as an extreme expression of f. *typica* (Okamura, 1915).

Unfortunately, until the present time researchers have not reached common understanding about taxonomical status of these morphs. It was shown that morphological variability of *U. pinnatifida* depends on local conditions of microhabitat only (Stuart et al., 1999), but other researchers suggested that specific morphology of two forms is genetically determined (Lee, Sohn, 1993; Dan, Kato, 2008). According to our previous studies (Skriptsova, 2008; Shibneva, Skriptsova, 2010), polymorphism of *U. pinnatifida* in Peter the Great Bay (Sea of Japan) is rather high. Three morphologically distinct forms were detected in this region. Samples of *U. pinnatifida* collected on the Cape Butakova (Form B) are characterized by high frond length (119±4.1 cm) and elongated stipe (up to 30–50 cm length) with marked transitional zone between sporophyll and lamina. Lamina is deeply divided. This form is close to f. *distance*, described by Okamura (1915) from the Honshu Island in the North of Kurosaki Cape. Samples of *U. pinnatifida* found on the shallow of Daragan (Form D) in Peter the Great Bay have a short (40–60 cm) and wide (50–80 cm) thalli with broad undivided parts of the lamina (12–20 cm). Frond width usually exceeds its’ length. Sporophylls are confluent with lamina. Besides these two forms which are characterized by extremely different morphology there is a transitional form (Form C) in Peter the Great Bay. This form is the most distributed in this region. Individuals of this form are characterized by interjacent values of morphological parameters between B and D forms. Frond varied in a range of 60–80 cm, lamina width is in average 35–60 cm. Stipe usually has a short transitional zone between lamina and sporophyll (up to 5 cm). Samples with this morphology are close to *U. pinnatifida* f. *typica*, described by Yendo (1911). The detected forms, presented by one or several populations in Peter the Great Bay, may be conditioned by external and internal factors in unequal degree.

The objective of this study was to reveal whether detected morphs of *U. pinnatifida* in Peter the Great Bay are determined genetically. According to this objective we exposed genetic inter-population heterogeneity of *U. pinnatifida* using AFLP-analysis, also we have grown three morphs in the similar conditions at the seaweed farm.

Materials and Methods

Samples for genetic analysis were collected during June 2010 at 5 sites in Peter the Great Bay from 1–2 meters depth by SCUBA. Form B was collected in Cape Butakova, form D was collected from Shallow of Daragan, and form C was collected from three sites: Engelm Island, Lazurnaya Bay and Cape Mikhelsona (Fig. 1).

Six to 13 samples were selected in 5 populations for genetic analysis. Fragments of lamina tissue from the middle part of lamina up to 150 mg were sampled from fresh material and then were frozen at −20°C for following DNA isolation. Genomic DNA was extracted from each of 49 samples with

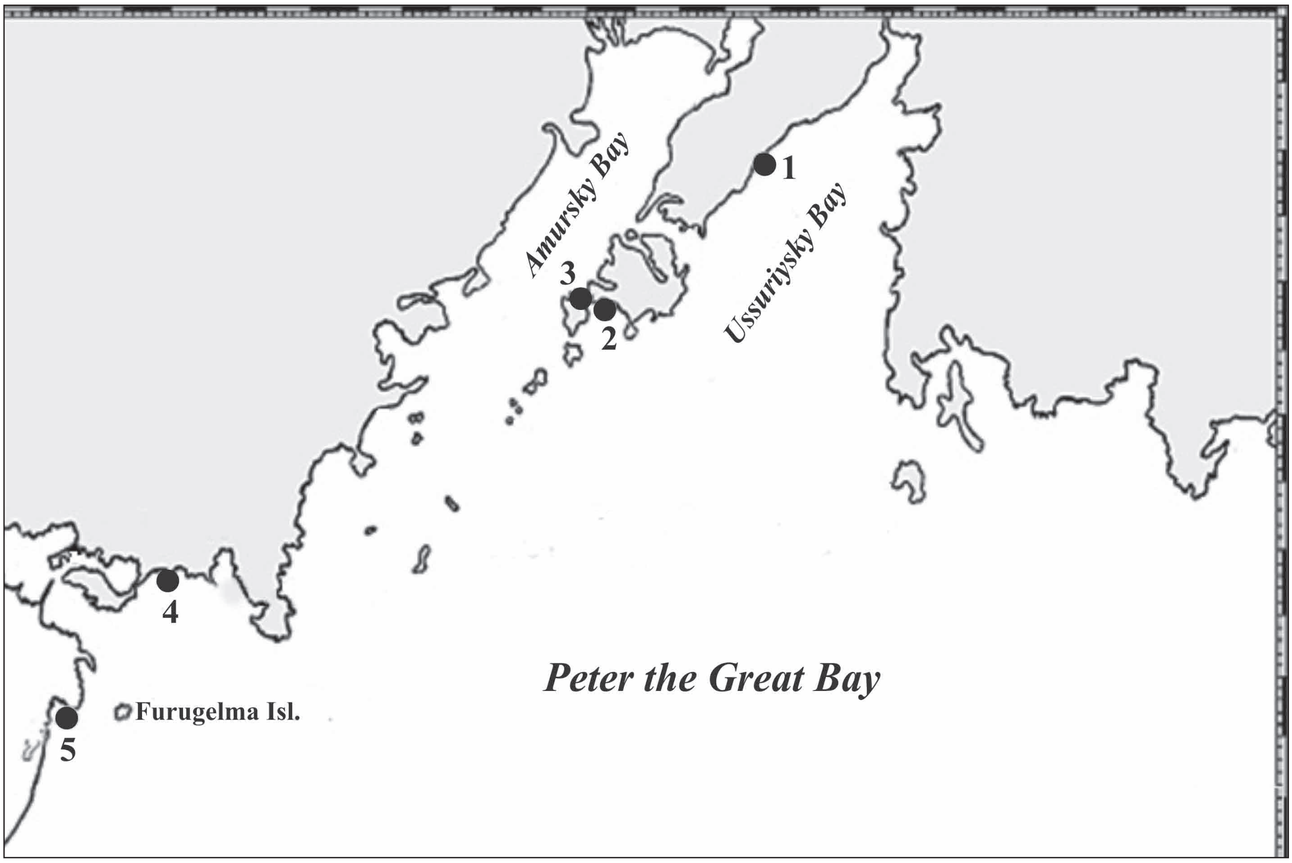

Fig. 1. Collecting sites for *Undaria pinnatifida* in Peter the Great Bay: **1** – Lazurnaya Bay, **2** – Engelm Island, **3** – Shallow of Daragan, **4** – Cape Mikhelsona, **5** – Cape Butakova.

CTAB-buffer. Analysis of fragment length polymorphism (AFLP) was performed according to Vos et al. (1995), adapted for kelps by Shan et al. (2011).

A data binary matrices based on gel-electrophoresis were prepared for genetic analysis. Individual-wise genetic similarity were calculated with Dice coefficient (Dice, 1945). Similarity matrices were subjected to cluster analysis using the unweighted pair group method with arithmetic averages (UPGMA) clustering approach and a dendrogram was constructed using a tree clustering module in Statistica 7.0. Bootstrap analysis by 1000 permutations was performed to evaluate the robustness of the groupings using WINBOOT (Yap, Nelson, 1996). Nei's genetic identities and distances were calculated using PopGene version 1.31 (Yeh et al., 1999).

Three different morphs of *U. pinnatifida* were grown in the same conditions to reveal whether morphological differences among morphs are genetically determined. Spores were collected in June 2011 from plants of three morphs: Form D (Shallow of Daragan), Form B (Cape Butakova) and Form C (Cape Andreeva) (Shibneva, Skriptsova, 2010, 2012). Algae were obtained from spores and were grown on the seaweed farm from October 2011 to April 2012. Main morphological parameters were measured after collecting sample: thallus length (TL), stipe length (StL), blade length (BL), sporophyll length (SpL), whole blade width (BW), width of undivided blade of lamina (WW) (Shibneva, Skriptsova, 2012). Ratio thallus length to stipe length (TL/StL), blade length to blade width (BL/BW) and blade width to undivided part of blade (BW/WW) were calculated based on measured morphological parameters.

Morphological data were analyzed statistically using Mann-Whitney test. Differences, where $p<0.05$, were taken as significant. Computations were done with Statistica 7.0 (StatSoft).

Results and Discussion

The studied populations of *U. pinnatifida* were genetically close. Genetic identity coefficient between populations was 0.907 on the average and varied from 0.831 to 0.978 (see Table). Minimal genetic identity was between Cape Butakova population and other populations, the former is far from other populations geographically. The largest genetic identity was detected between populations from Cape Mikhelsona and Lazurnaya Bay.

Nei's genetic identity index (1978) and genetic distance coefficients among five populations of U. pinnatifida. Genetic identity coefficients are given above diagonal, genetic distance coefficients are given below diagonal

	E	D	B	L	M
E		0.9254	0.8786	0.9152	0.924
D	0.0775		0.8319	0.9598	0.9502
B	0.1294	0.1843		0.8448	0.866
L	0.0886	0.041	0.1687		0.9778
M	0.079	0.0511	0.1452	0.0224	

Genetic polymorphism of *U. pinnatifida* in Peter the Great Bay is low enough, genetic distance between populations do not exceed 0.1 according to AFLP results. Such values are typical for inter-population intraspecific differences of macrophytes along geographic gradient (Waycott et al., 1997; Wang et al., 2006). Intraspecific inter-population heterogeneity of *U. pinnatifida*, most probably is not charged with morphological differences and is relating to geographical extent of species areal. Thus, high level of genetic identity and low heterogeneity allows concluding that morphological differences among three morphs are not genetically fixed, that is suggested by results of experiment.

The result of experimental growing of three *U. pinnatifida* forms have shown that two most morphologically distant forms B and D after growing at the same conditions lost their specific morphological differences and became like form B (Fig. 2). Notably, samples, presented form D, lost their specific morphology traits such as short and wide lamina and lack of transitional zone. Samples of form B also lost one of distinctive traits – deeply divided lamina. Similarity between form B and D also observed in relative morphological parameters. Thus, the results of growing different forms in the same conditions showed that all of these parameters have only phenotypical basis. However, such results are not charactericed for morphologically distinct forms of *U. pinnatifida* from other locations. For example, transplantation experiment performed by Dan and Kato (2008) for two forms of *U. pinnatifida* revealed fixedness of morphological differences, despite change of conditions.

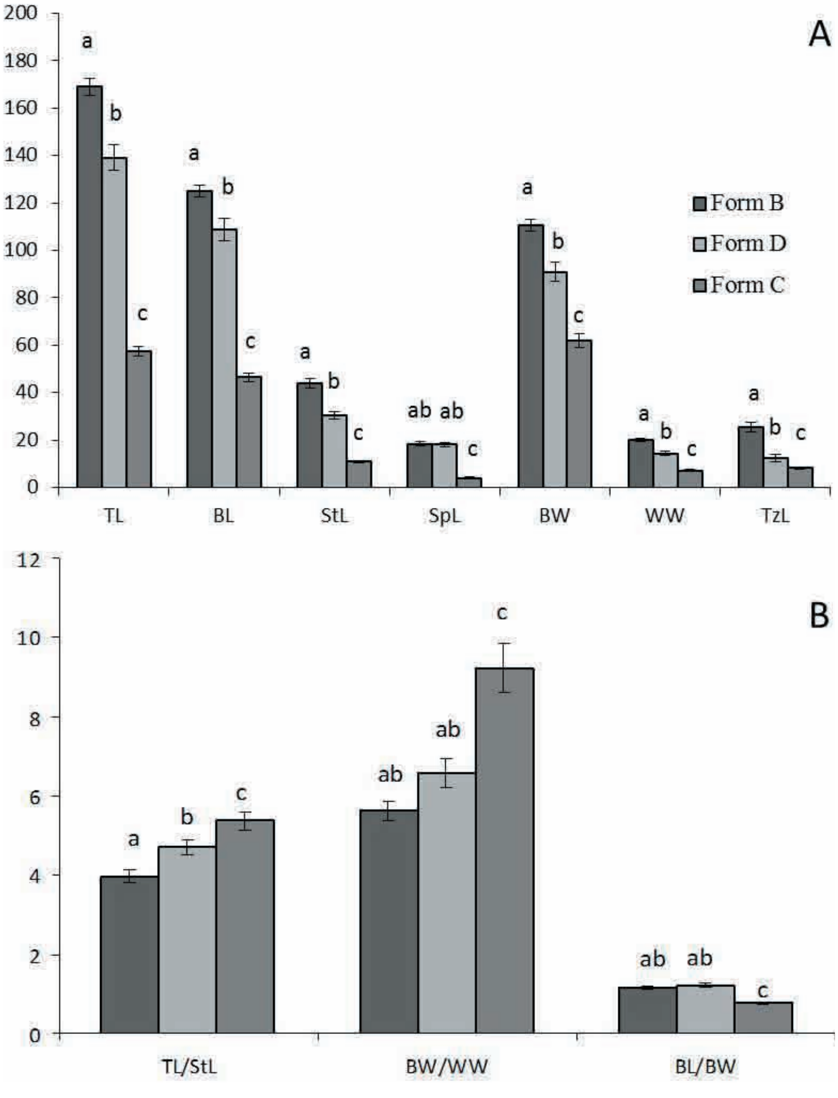

Fig. 2. Comparison of measured (**A**) and relative (**B**) morphological parameters between forms grown at seaweed farm. Bars show standard errors (SE). Different small letters indicate significant differences between forms by Mann–Whitney test ($p<0.05$) within each group (Wild, Cultivated).

Individuals of form C (Cape Andreeva) after growing in the same conditions differed from forms B and D in relations BW/WW, BL/BW, and in smaller size. These samples differ most probably as a result of more late transplantation to the seaweed farm (approximately 2 months) of Cape Andreeva population samples (form C). We may suppose that this form is not genetically separated from other two forms since population from Cape Andreeva is not isolated geographically and form C presented in this population is common in Peter the Great Bay.

Results of complex studies including genetic analysis and growing three morphologically different forms of *U. pinnatifida* in the same conditions suggested that morphological differences which previously revealed and conditioned differentiation of populations in Peter the Great Bay into three forms, are not fixed on the genetic level and result from ontogenetic adaptation to influence of environmental factors. The present study confirms previous data that *U. pinnatifida* has rather high adaptation strategy which determines form of thallus depending on complex of environmental factors and respectively phenotypical polymorphism of *U. pinnatifida*.

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Porphyra aquaculture technology in China: a critical review of its history and current status

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China has a long history of algal aquaculture and is the largest aquaculture-based algae producer, accounting for 70% of world production, annually. Among the dominant seaweed species in China, *Porphyra* was the earliest one selected for aquaculture. *Porphyra* is now the most widely distributed, occupies the largest aquaculture area, and has the most output value. The *Porphyra* aquaculture is a long and complicated technological process. The Chinese government and aquaculture enterprises have been paying more attention to improving the technology for *Porphyra* aquaculture. Researchers and cultivators have made a great progress in recent years in developing aquacultural practices. To provide a comprehensive understanding of *Porphyra* aquaculture in China, this review summarizes the history of *Porphyra* aquaculture and its current status from three angles: the life history of *Porphyra*, the methods for *Porphyra* mass cultivation, and the current status of *Porphyra* aquaculture in China. First, we describe the various life-history styles among different species of *Porphyra* and the production of three types of spores (carpospores, conchospores, and monospores). Conchospores are widely used as seeds for *Porphyra* aquaculture. Second, we summarize the methods of *Porphyra* mass cultivation, including the methods based on freezing nets, artificial seeding, and floating raft structures. In recent years, artificial seeding is gradually replacing the practice of semiartificial seeding in China. For sea farming, the technique based on semi-floating raft structures is still widely used along coastal areas in China. Finally, we describe the current status of *Porphyra* aquaculture in China, reviewing the two principal cultivated species (*P. yezoensis* and *P. haitanensis*), the distribution of aquaculture areas, the yield and output value, processing, and marketing. We point out current problems in the laver industry and predict future development of the industry.