

Fauna of microcrustaceans (Cladocera: Copepoda) of shallow freshwater ecosystems of Wrangel Island (Russian Far East)

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Information on the freshwater microcrustaceans of the remote arctic islands is very limited. The species composition of freshwater zooplankton and meiobenthos of Wrangel Island (Russia) is reported here for the first time. We identified five species of Cladocera, and 20 species of Copepoda. Twenty-three taxa are new records for the island. Additionally, we discuss the taxonomy of some species with doubtful taxonomical status. We also analysed data from other arctic and subarctic territories to compare with that of Wrangel Island and evaluate the latitudinal trends of species richness and distribution of Cladocera and Copepoda and their relation to environmental factors. The effects of different environmental factors were statistically examined to establish which are important in controlling distributional patterns and diversity of species. On Wrangel Island the fauna of microcrustaceans is primary controlled by three factors: low temperatures, the remoteness from the nearest mainland and geological history of the island – its relation to ancient Beringia and lack of Pleistocene glaciation.

Keywords: Cladocera; Copepoda; freshwater; Wrangel Island; Arctic

Introduction

Arctic freshwater ecosystems are often considered to be species poor. Information on the microcrustaceans of high latitude archipelagos and remote islands of the Arctic and Subarctic is limited due to their geographic isolation and very limited access to them. The existing species inventories also suffer from taxonomic limitations, in particular relating to unidentified synonymies and misidentifications (Coulson 2007; Ávila-Jiménez et al. 2011); and detailed knowledge of the distributions and biogeography of the majority of invertebrate species remains limited (Coulson et al. 2014). However, such ecosystems are particularly valuable for studies addressing insular biogeography, species colonization, impact of glaciation and others.

Wrangel Island is located in the Arctic Ocean on the border of the East-Siberian and Chukchi seas, belonging to the Nature Reserve, which also includes Herald Island and the surrounding 12 miles of water. Wrangel Island is about 125 km wide and 7600 km² in area. Its relief consists of a southern coastal plain, a central belt of low-relief mountains, and a northern coastal plain. With the 180th meridian crossing its territory, Wrangel Island falls into both western and eastern hemispheres. The terrain is mostly mountainous, strongly partitioned, with seacoast lowlands in the

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south and the north. There are 1400 rivers and streams, about 900 small lakes and several coastal lagoons on the island (Stishov 2004). The island lies in the arctic tundra sub-zone with spotted and polygonal tundra prevailing. In the south-west and the centre of Wrangel Island, there are relict steppe and tundra-steppe vegetation communities with a variety of flowering plants. There are also sedge hypnum bogs with sphagnum, areas of sedge cotton grass bogs, and willow scrubs. Wrangel Island has a severe polar climate. Average summer temperature is 3.4°C, and average year temperature is -10.3°C. The region is blanketed by dry and cold Arctic air masses for most of the year.

The invertebrate fauna of Wrangel Island has been studied only fragmentarily. If entomofauna is considered it is now one of the best-studied areas in the Arctic (Stishov 2004); however, researchers have largely focused their attention on the terrestrial groups of arthropods, while the freshwater inhabitants have remained almost unstudied. A few publications on separate groups of benthic organisms exist (Makarchenko and Makarchenko 1981, 2001, 2013a, 2013b, 2014) but only one complex study on the benthic community of Wrangel Island (Makarchenko et al. 1980). The species composition of freshwater zooplankton and meiobenthos of Wrangel Island remains almost unknown, and publications concerning both the description of species richness and the community structure of this ecological group of invertebrates are absent. There is only a single study of the brackish fauna of Wrangel Island (Yashnov 1935), in which 12 taxa of various ecological groups have been reported. All the species encountered can be considered as typical inhabitants of brackish waters.

This paper presents the results of an investigation of the cladocerans and copepods from the shallow freshwater bodies of Wrangel Island and an analysis of biogeographical and environmental features of the fauna. These biodiversity data augment existing inventories of taxa, which are then compared with inventories from other insular and some continental northern territories to evaluate latitudinal trends of species dispersal, possible biogeographic relationships and influence of environmental factors.

Materials and methods

Sampling

The samples were collected during a hydrobiological survey from 18 June to 5 August 2013 in 74 water bodies (Figure 1). At the time of sampling, the sites varied from 0.15 to several metres depth (1.2 m in the place of sampling), and 4–18°C. Studied sites included water bodies of various origin, hydrological mode and size; most of them were small thermokarst ponds. Environmental variables such as bottom sediment type (silt, sand, or rock), depth, estimated surface area, water temperature and the presence (or absence) and species composition of macrophytes were noted for each site. At each site three replicate samples were collected by hauling a plankton net (diameter 0.1 m, 50 µm mesh) through the water column, engaging the upper layer of the bottom with the detached sediment filtered through the net up to the surface. The sampling was performed from the shore (in puddles and small lakes) augmented with artificial constructions that allowed the net to reach the centre of a lake (in larger lakes) such that each sample was reflective of the species composition of the whole

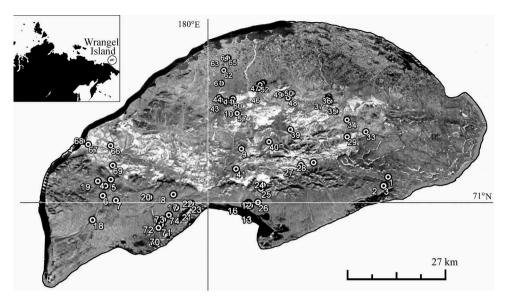


Figure 1. Location of the sampled stations in inner water bodies on the map of Wrangel Island.

water body, including the pelagial, littoral and near-bottom zones. The abundance of species at each site was calculated as the mean value of the three replicate hauls. Upon collection, all samples were preserved in ethanol (96%). Species identification and enumeration was carried out primarily in Bogorov counting chambers; the total numbers of Cladocera and Copepoda were recorded.

Identification of crustaceans and literature review

Crustacean identification followed standard taxonomic treatises and recent taxonomic revisions: Cladocera: Lieder (1996), Kotov et al. (2009), Sinev (1999, 2002, 2009), Smirnov (1971); Copepoda: Alekseev and Tsalolikhin (2010), Brtek and Mura (2000), Borutsky (1952), Borutsky et al. (1991), Dahms et al. (2006), Rylov (1948).

The existing literature for freshwater Crustacea (Cladocera, Copepoda) from major Arctic and Subarctic territories, such as Iceland, Faroe Islands, Shetland Islands, Svalbard, Greenland, Franz Josef Land, Novaya Zemlya, Novosibirskie islands, Bering Island, Ellesmere Island, Hebrides, Chukotka Peninsula, Bolshezemelskaya tundra, Lena Delta, Western Siberia, Northern Alaska, Norway and some regions of arctic-subarctic Canada (Alberta, British Columbia, Northwest territories, Yukon, Nunavut, Ontario) were compiled into regional inventories of taxa (see Supplement).

Statistical analysis

The data were subjected to statistical analysis to establish which environmental factors and characteristics of area affect distributional patterns and species richness of crustaceans. Analysis of the data was performed by using regression models in SYSTAT (SYSTAT Software, Inc. 2004) to identify significant trends. Factors expected to influence species richness, including average summer temperature, latitude, presence of glaciation, ice-free area size and distance from the mainland were chosen. Most of the variables were log-transformed (base 10) to homogenize residual variation.

To determine the degree of overlap in faunal composition between regions we determined the number of species that were in common between regions (percentage overlap) and proportions of endemic and cosmopolitan species. We compiled species lists using data from the current study and from previously published literature. Faunal similarity between regions was estimated using Bray-Curtis similarities (S_{B-C}):

$$S_{B-C} = \sum_{R} min(p_{1i}; p_{2i}),$$
 (1)

where p_{1i} is the abundance of *i*-species in sample 1, and p_{2i} the abundance of *i*-species in sample 2.

The resemblance matrix was built using hierarchical agglomerative clustering with a group average linkage mode. The clustering was performed using PRIMER (Clarke & Gorley 2001; http://www.primer-e.com). Also, Margalef index of species richness (*D*) was calculated:

$$D = (S-1)/log(N); (2)$$

where S is total species and N is total number of individuals.

Results

Fauna of Wrangel Island

We identified 25 crustacean species, comprising five species in five genera of Cladocera, and 20 species in 16 genera of Copepoda (Table 1). Almost all of these taxa (23 species) have not been previously documented on Wrangel Island. Only two of the species, *Eurytemora affinis raboti* Richard and *Tachidius discipes* Giesbrecht, had been already reported (Yashnov 1935). Microcrustaceans were found only in 65% of the studied sites. The number of species encountered in each water body varied from one to seven.

Crustacean fauna of Wrangel Island is poor even in comparison with other arctic territories ($D_{\text{Wrangel}} = 8.53$; mean value among other territories D = 16.69) (Figure 2). The most common and abundant species were widespread eurythermal species (Daphnia cf. pulex Leydig, Chydorus cf. sphaericus (Muller), Eudiaptomus gracilis Sars and Megacyclops gigas (Claus)). They were dominant in most of the lakes and ponds and occurred frequently and in large numbers (Table 1). Other species of Crustacea were found rarely and seldom reached considerable number (0.7–22.6% of the total abundance). There were no taxocenes or species assemblages determined.

The most widely present group of Crustacea on Wrangel Island was Copepoda (80%). Along with freshwater species, there were four brackish species all found in a lake located near the lagoon on the southern edge of island (site 21 Figure 1, salinity >1000 ppm NaCl). There were only brackish species in this lake. The

Table 1. List of species Cladocera and Copepoda (including brackish species) recorded on Wrangel Island in the current study and in literature data (Yashnov 1935). Numbers in column indicate the number of water bodies in which the species was found.

	Species	Present in original data	Literature sources
Cladocera			
Bosminidae	Bosmina cf. longispina (Muller)	2	_
Chydoridae	Alona werestschagini Sinev	2	_
	Chydorus cf. sphaericus (Muller)	35	_
Daphnidae	Daphnia cf. pulex Leydig	21	_
Eurycercidae Copepoda	Eurycercus (Eurycercus) longirostris Hann	5	_
Calanoida	Acartia bifilosa (Giesbrecht)*	_	+
	A. longiremis (Lilljeborg)*	_	+
	Diaptomus glacialis Lilljeborg	8	_
	Eudiaptomus gracilis Sars	12	_
	Eurytemora affinis raboti Richard	2	+
	E. canadensis Marsh*	-	+
	Heterocope borealis (Fischer)	4	_
Cyclopoida	Diacyclops abyssicola (Lilljeborg)	2	_
	D. bisetosus (Rehberg)	9	_
	Cyclops canadensis Einsle	11	_
	C. kolensis Lilljeborg	5	_
	C. scutifer Sars	1	_
	C. sternuus Fischer	6	_
	Macrocyclops albidus (Jurine)	1	_
	Megacyclops gigas (Claus)	12	_
	Mesocyclops leuckarti (Claus)	1	_
	Microcyclops varicans (Sars)	2	_
Harpacticoida	Archisenia sibirica (Sars)*	_	+
•	Canthocamptus glacialis (Lilljeborg)	4	_
	Microarthridion littorale (Poppe)*	1	_
	Nannopus palustris Brady*	1	_
	Neomrazekiella nordenskjöldi nordenskjöldi (Lilljeborg)	4	_
	Scutellidium arthuri Poppe*	1	_
	Tachidius discipes Giesbrecht*	1	+

Note: *Marine and brackish species.

depth of the lake does not exceed one metre. Less than half a metre apart in two neighbouring smaller ponds the salinity was slightly lower (518 ppm and 329 ppm NaCl) and the species composition was different. The freshwater species D. cf. pulex was dominant in these lakes, but there was also E. affinis raboti - a stenothermal cold-water inhabitant of arctic water bodies, which dwell both in brackish and fresh water, and also in marine waters according to some reports (Borutsky et al. 1991). All the other water bodies were inhabited by typically freshwater crustaceans.

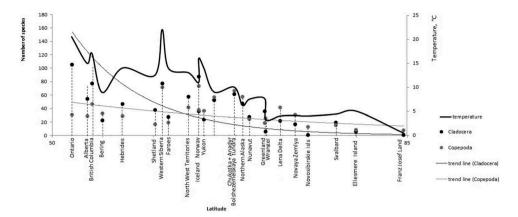


Figure 2. Species number of Cladocera and Copepoda in different areas along a latitude gradient following average summer temperatures, based on literature sources and original data.

Comparative analysis of Wrangel Island with other Arctic territories

On the basis of our data and literature sources we compared faunal composition of major northern islands and some continental territories (Figure 3). The species richness index D varied among all the territories from 3.08 (Franz Josef Land) to 31.33 (Norway). The number of freshwater species documented on islands ranged from seven (Franz Josef Land) to 74 (Hebrides) and was generally lower than for the continental mainland. The similarity in faunal composition between Wrangel Island and other territories was rather low (not more than 34.8%). The most similar in faunal composition to Wrangel Island were Novosibirskie islands and Ellesmere Island (Figure 4), where the faunas are also very poor and reflect severe arctic conditions. Placed separately from them on the dendrogram was Franz Josef Land – the northernmost and the poorest arctic island. Another cluster grouped territories with more temperate climatic conditions and more diverse species composition. Among this broad group there were four clusters with high similarity: group of North Atlantic islands (Iceland, Greenland, Hebrides, the Faroes and Shetland islands); group of Nearctic territories - Canada and Alaska; group of Palaearctic mainlands (Chukotka, Western Siberia, Norway, Bolshezemelskaya tundra) and one more group of islands with severe climate conditions and scarce fauna (Svalbard, Novaya Zemlya, Bering Island) and Lena Delta.

The analysis of the proportion of Cladocera and Copepoda in different arctic regions shows that the number of species of both crustacean groups declines along latitude gradient northward in accordance with temperature (Figure 2). At the same time, the number of cladoceran species drops strongly in correlation with temperature, while copepod species number declined more gradually. Wrangel Island, which had one of the lowest average summer temperatures among all the observed areas, was strongly dominated by copepods (Figure 5). Thus, there is good reason to believe that temperature factor is a major force affecting the number of crustacean species and the community structure in arctic regions.

Environmental factors (average summer temperature, latitude, presence of glaciation, ice-free area size and distance from the mainland) known to influence species richness were examined in zooplankton communities of different Nordic territories. According to the regression analysis, species richness in both Cladocera and Copepoda, as well as the



Figure 3. Simplified map of the compared regions.

proportion of these groups, appears to be primarily controlled by temperature (Table 2). The results revealed that more than 70% of variation in number of species is due to the factors related to temperature: the average summer temperature itself and the latitudinal gradient, which also reflects temperature variation. The distance of the island from the mainland also influenced fauna of microcrustaceans but to a lesser degree. Correlations between number of species and other factors were not significant.

Discussion

A major feature of Arctic landscapes is their large number of lakes and ponds, which in some regions can cover up to 90% of the total surface area (Raatikainen and Kuusisto 1990; Pienitz et al. 2008). Many Arctic freshwater ecosystems were formed following the last glacial period when depressions were left behind by the retreating ice masses (Pienitz et al. 2008). Other ponds have been formed by the thermal erosion of

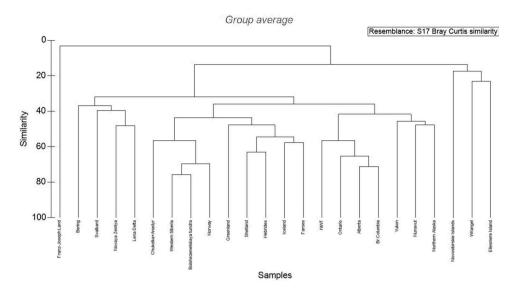


Figure 4. Dendrogram for hierarchical clustering (group average) of faunas of different arctic regions.

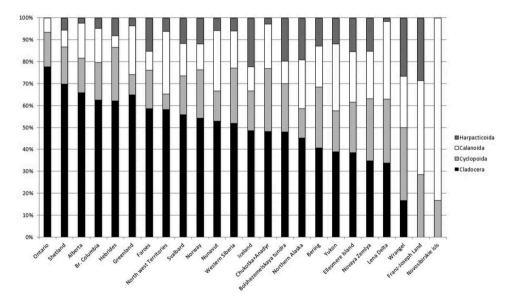


Figure 5. The percentage ratio of the species number of different crustaceans (Cladocera, Copepoda: Cyclopoida, Calanoida, Harpacticoida) from different arctic regions.

permafrost soils, and are abundant throughout the circumpolar Arctic (Rautio et al. 2011). These so-called thaw (or thermokarst) lakes and ponds are dominant inner water bodies of Wrangel Island. Almost all studied lakes are shallow. In such types of water bodies, the separation of fauna into planktonic and benthic communities is provisional. Even moderate stirring of the water column leads to mixing of the planktonic and

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Dependent variable*	Multiple R	Squared multiple R	Standard error of estimate	Effect	Coefficient	Standard error
LG_Total	0.866	0.750	0.188	Constant	1.375	0.153
				Temperature	0.085	0.026
				Distance	0.119	0.034
LG_Cladocera	0.855	0.731	0.303	Constant	0.529	0.245
				Temperature	0.165	0.042
				Distance	0.120	0.055
LG_Copepoda	0.739	0.546	0.192	Constant	1.653	0.055
				Distance	0.155	0.030
LG_CCR	0.771	0.594	0.519	Constant	5.440	0.838
				Latitude	0.072	0.013

Table 2. The results of regression analysis for identifying significant environmental factors.

Note: *LG Total = log-transformed (base 10) total number of species in the area; LG Cladocera = log-transformed (base 10) number of cladoceran species; LG_Copepoda = log-transformed (base 10) number of copepods; LG_CCR = log-transformed (base 10) Cladocera/Copepoda ratio.

benthic cladocerans and copepods. According to recent data (Kotov 2006), a substantial portion of Cyclopoida and Cladocera traditionally considered to be planktonic are, in fact, closely related to the benthic community and should be referred to meiobenthos. The majority of species retained in nets from these shallow waters are unlikely to be truly planktonic, given both water depth and the connection between individual species life histories and water body marginal or bottom habitats. Some taxa may also move between environments in active and passive ways. Many species that are retained in nets in such shallow habitats could be meiobenthic, living on or in bottom sediments; some may even be phytophilous, associated with certain types of plants or any thickets; and some may even be species of the open littoral area.

Taxonomic commentary

Several taxa found during our survey could not be reliably identified to species. To prevent confusion, any departure from taxonomic convention is discussed here. The taxonomic status of many taxa in the genus Bosmina is still unclear. B. (Eubosmina) cf. longispina is a very variable species complex, with numerous local races that have no systematic meaning (Kotov et al. 2009). This is the subgenus with the most confusion in the Bosminidae. Now it includes three tentative 'species': Palaearctic B. cf. coregoni, Nearctic 'B. cf. longispina' and Japanese B. tanakai. Nearctic populations differ from European eubosminids, but these traits are known only for males or can be detected by genetic analysis. We are not sure if our species belong to the European or Beringian 'species'.

The accurate classification of *Chydorus sphaericus* (Muller) requires inspection of both male and female samples. Unfortunately, we did not recover any male specimens. However, because the female specimens exhibit traits consistent with the descriptions by Smirnov (1971) we classify our specimens as C. cf. sphaericus. Currently, it is recognized as a complex of very similar species, determination of which is possible based on male characters (Belyaeva and Taylor 2009).

One of the most significant discoveries of microcrustaceans inhabiting Wrangel Island is *Eurycercus* (*Eurycercus*) longirostris Hann. This 'Nearctic' species is widespread in different types of biotopes through the whole USA and Canada up to Yukon in the north (Bekker et al. 2012). In the Palaearctic region this species has been reported only once – on Bering Island (Commander Islands, Russian Federation) (Novichkova 2013). The presence of some populations of *E. longirostris* on Wrangel Island and their absence on the Eurasian mainland could be explained as a recent colonization through some birds, whose migratory routes pass through the Aleutian–Commander Arc and the shore of Chukchi Sea (Madge and Burn 1988; Alerstam et al. 2007). In contrast, the possibility that *E. longirostris* appeared on Wrangel Island due to an anthropogenic invasion is extremely low. During the whole Holocene, Beringia was a main route for human migrations, but mainly from Siberia to the north (Kitchen et al. 2008). During the twentieth century, when transportation activity was greatly increased, Wrangel Island was strongly isolated from Alaska, a part of USA, due to well-known political reasons (Bekker et al. 2014).

One more notable species of crustaceans registered on the island is *Alona werestschagini* Sinev, 1999, a species with a specific disjunct area. Presently it is known from the mountains of Central Asia – Pamir, Tien Shan, Altai (Sinev 1999; Belyaeva 2003), Tibet (Chiang and Du 1979), Kyrgyzstan (Sinev 1999) and Western Mongolia (Sarmaja-Korjonen and Sinev 2008; M. Alonso, pers. comm.) as well as northernmost European Russia (Komi Republic and Kola Peninsula) (Sinev 1999), the Russian Far East (Commander Islands; Novichkova 2013), Iceland (Novichkova et al. 2014) and northern Norway (Walseng et al. 2006). Subfossil remains of *A. werestschagini* have also been found in lake sediments above the treeline in northernmost Finnish Lapland (Sarmaja-Korjonen and Sinev 2008) and in the Lake Piramide Inferiore in the Nepalese Himalayas (Nevalainen et al. 2014). Such a patchy distribution in cold climates suggests this species is a cold-adapted postglacial relict (Sinev 2002). Its occurrence on Wrangel Island supports this assumption and, probably, is a result of lack of Pleistocene glaciation on the island.

Characteristics of the fauna

Arctic ponds in general are species-poor when it comes to zooplankton and zoobenthos. The cold northern climate and shallowness exert overriding influences on their ecosystem structure. For example, most have 'much lower hydrological connectivity and freeze solid during winter, which prevents the occurrence of fish but allows extensive crustacean communities to inhabit the systems during the open water period' (Rautio et al. 2011, p. 205). The lack of efficient predators is an important factor that contributes to the success of polar aquatic invertebrates (Rautio et al. 2008).

Cladoceran fauna of Wrangel Island is extremely poor even in comparison with other arctic territories (Figure 2). One of the most likely reasons is lack of macrophytes, which are necessary for Chydoridae species, the most abundant inhabitants of arctic shallow lakes. Changes in the chydorid assemblage have been even used to identify major changes in abundance of plants (Einarsson 1982). In the observed water bodies the vegetation was mostly represented with submerged terrestrial plants, while true water macrophytes were rare. Otherwise, the number of Copepoda species on the island is relatively high. Differences in life cycles among the various

microcrustacean groups may be involved in their differential colonization success in the arctic. The most abundant group presented on Wrangel Island is Cyclopoida. They provide considerable flexibility and adaptability for highly variable environmental conditions, frequently producing eggs throughout all the growing season. Moreover, various copepodite stages of cyclopoids are able to encyst (Tash and Armitage 1967). This ability could account for abundance of cyclopoids on Wrangel Island and other high arctic areas (Figure 5).

The invertebrate fauna of freshwaters of Wrangel Island is unique. The island is located in a so-called Beringian sector of Holarctic. This region covers territories which once belonged to Beringia – an ancient biogeographical area connecting together the north-east of Asia and the north-west of North America – and functioned as a united natural complex. During the periods of regression the shelves of Chukchi and Bering Seas cropped out and the continents were connected via the Bering land bridge. During the maximum regressions its border on the west extended to Kolyma, in the south – practically to the Commander–Aleutian island arc, and in the North occupied an extensive shelf, including Wrangel Island and Chukchi Sea. This ancient area was not only the migratory bridge between Nearctic and Palaearctic, but also was the centre of speciation of many organisms. The possibility that the diversity patterns of zooplankton communities reflect the effects of survival in glacial refugia, especially Beringia, merits consideration. The presence of American species of invertebrates, such as *E. longirostris* and variety of benthic chironomids, on Wrangel Island may be evidence of its ancient connection with the Nearctic.

The island is isolated from the mainland by the De Long Strait. This, coupled with severe climate, serves as migration filter. Thus, the insular fauna is poorer than the continental fauna ($D_{\text{Chukotka}} = 8.95$). The severity of the physical environment (low temperature, low primary production) is clearly a major factor limiting the size and composition of the microcrustacean community in arctic habitats. But also Subarctic and Arctic freshwater zooplankton communities have been profoundly impacted by Pleistocene glaciations (Weider and Hobaek 2000). The current-day distribution of invertebrate fauna may reflect the species composition present during the preglacial period, but it may also represent recent secondary colonization. The Arctic fauna appears to have been the result of postglacial recolonization rather than local survival (Pugh and McInnes 1998). The series of Pleistocene glaciations have clearly had a major influence on the distribution of the lake biota. 'Any present-day inhabitants of lakes in previously ice-covered areas would have to have migrated from non-glaciated areas that provided habitats (refugia) in which the animals survived' (Rautio et al. 2008, p. 232). The distance of an island from the mainland determines the degree of isolation and, therefore, the success of colonization and migration of species. This effect has much stronger influence on the environments, which has experienced climatic shifts during the glaciations leading to the extinction of fauna. Thus, the relative species richness (per square km of ice-free area) of microcrustaceans on Wrangel Island is high. It may be attributable to the fact that much of this area escaped glaciation during the Pleistocene. The presence of relict species on the island supports this hypothesis. The persistence of tundra habitats through this period has had important biological effects as evidenced by the large number of plant species which are either endemic to the area or of Beringian distribution. According to statistical analysis, the general species diversity and amount of cladoceran species vary depending on the distance from the mainland

and strongly correlate with average summer temperatures (Table 2). While the diversity of copepods depends significantly only on the degree of isolation. It is likely that low species diversity on Wrangel Island is due to these two major factors operating independently and in combination: (1) the climate, which regulates the possibility of colonization and occurrence of species; and (2) the remoteness from the nearest mainland, which is also determines the success of colonization and migration of species.

Another factor that did not have a significant effect on the diversity of microcrustaceans, but plays a role in the diversity of the whole fauna of the island, is (3) the age of the ecosystem, which is mainly characterized by the presence (or absence) of the latest Pleistocene glaciation. Subsequent to the results of the current study and literature data, it is seems that the relative importance of these factors can differ. The impact of each factor needs additional confirmation and further investigations, but, undoubtedly, the temperature factor appears to be the most significant in species composition and distribution on Arctic territories.

Thus, the fauna of microcrustaceans of freshwater ecosystems of Wrangel Island is extremely poor. It is characterized by lack of cladocerans and dominance of copepods, which are more tolerant to low temperatures and can successfully penetrate into high latitudes. It also shows traces of the geological history of the island: the presence of relict species and Nearctic species is likely the result of lack of Pleistocene glaciation on the island and its relation to ancient Beringia.

Disclosure statement

No potential conflict of interest was reported by the authors.

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Supplemental material

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