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SHORT COMMUNICATIONS

Peripheral Blood Cell Composition of Baikal Seal *Phoca sibirica*

I. I. Gordeev[a, b], E. A. Boltnev[a], T. A. Suvorova[c, *], D. V. Mikryakov[c], and L. V. Balabanova[c]

[a] *Russian Federal Research Institute of Fisheries and Oceanography, Moscow, Russia*
[b] *Lomonosov Moscow State University, Moscow, Russia*
[c] *Papanin Institute for Biology of Inland Waters, Russian Academy of Sciences, Borok, Nekouzskii raion, Yaroslavl oblast, Russia*
**e-mail: tanya@ibiw.ru*

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Abstract—The cellular composition of the peripheral blood of the Baikal seal *Phoca sibirica* (Gmelin, 1788) has been studied. Various cells have been found in the blood smears: large and small lymphocytes; monocytes; and neutro-, eosino-, and basophils. Analysis has shown a high percentage of band neutrophils, eosinophils, and lymphocytes in the leukogram of young seal individuals when compared to adult individuals. Similar forms of leukocytes have been recorded during the study of some marine representatives of true seals (Phocidae). However, the proportion of band neutrophils is significantly lower in the leukograms of adult gray, harp, and ringed seals. The differences may be determined by physiological features and living conditions.

Keywords: Baikal seal (*Phoca sibirica*), leukogram, leukocytes
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The Baikal seal *Phoca sibirica* (Gmelin, 1788) is an endemic species, a representatives of true seals of the family Phocidae, which has fully adapted to life in inland freshwater bodies. The body of the Baikal seal is fusiform and has a thick subcutaneous layer of fat up to 12–14 cm. The body length of adult seals from the tip of the nose to the tip of the tail varies from 110 to 142 cm. The body weight of adult individuals ranges from 45 to 55 kg; however, some individuals can reach ≥100 kg (*Mlekopitayushchie* ..., 1976). Svatosh (1926) indicated the length of an adult female up to 130 cm and length of an adult male up to 180 cm at a body weight of 56–160 kg. Sexual dimorphism can be traced in most of the skull dimensions: almost all the average values for the absolute skull features are higher in males than in females (Pastukhov, 1969). Sexual dimorphism is particularly pronounced in adult individuals: males and females differ in the muzzle and body shape due to different levels of fatness, particularly in some seasons of the year; however, these differences can only be noticed by an experienced observer (Pastukhov, 1993).

The Baikal seal is endemic to Lake Baikal and does not spread beyond the lake. In the winter months, seals generally stay afloat under continuous ice cover using holes in hummocky ice for breathing. They form ice rookeries during ice destruction and coastal rookeries in summer and autumn in places difficult to reach for humans, off the northeastern coast above the Svyatoi Nos and on the Ushkany Islands (Pastukhov, 1993; Petrov, 2009). According to recent estimates, the population size of Baikal seal reaches dozens of thousands of individuals (Il'ina et al., 2022; Peterfel'd et al., 2022) and its population structure is possibly divided into separate spatial groups outside the mating season (Meshcherskii et al., 2022). Females reach maturity in the 4th–6th years of life and males in the 5th–7th years of life. The Baikal seal feeds on different fish species (17 species were found in its food bolus) and invertebrates. More than 90% of food is represented by two Baikal oilfish species, whose biomass is 69% of the biomass of all fishes in Lake Baikal. Sand, pebbles, and mica are often found in the food tract of the Baikal seal (Ivanov, 1936; Starikov, 1977; Pastukhov, 1993; Petrov, 2009; Tkachev et al., 2016; Watanabe et al., 2020).

The composition of leukocytes is known in sea-dwelling representatives of true seals of the family Phocidae. The proportion of different forms of white blood cells depends on the species and environmental features, functional state of the body, age, and type of nutrition (Kavtsevich, 2011; Kavtsevich and Minzyuk, 2017; Gulland et al., 2018; Goertz et al., 2019; Erokhina et al.., 2020; Kavtsevich et al., 2020a, 2020b). Thus, the number of lymphocytes was higher than that of neutrophils in gray, harp, and hooded seals in some periods of their early postembryonic development. Significant differences were recorded in the leukocyte formula of the blood of gray seal puppies of different age groups. The blood of newborn gray seal individuals

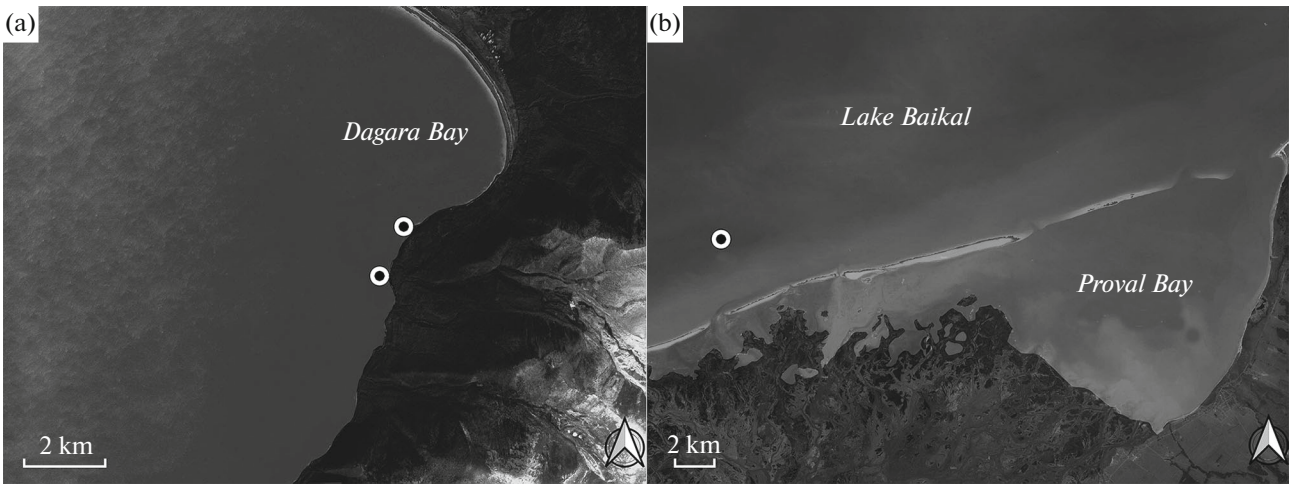

Fig. 1. Schematic location of the coordinates for net setting on Lake Baikal (marked with circles): (a) northern basin, near the Dagara Bay; (b) southern basin, near the Proval Bay.

contains immature neutrophils and metamyelocytes. These cells were rarer in puppies fed with milk and puppies that completed milk feeding and they were not recorded in individuals feeding on fish. A high proportion of eosinophils was recorded in adult harp seal individuals compared to the literature data for pinnipeds (Kavtsevich and Minzyuk, 2017). In seals living under natural conditions and seals adapting to living under captive conditions, the numbers of lymphocytes and neutrophils become equal at certain stages of individual development ("physiological decussation" of the leukocyte blood formula as a result of the formation of a specific immune system). However, the available literature provides no data on the composition of white blood cells of the freshwater species of this family.

Therefore, the study of the composition of leukocytes in the peripheral blood of the Baikal seal and description of their morphology are an extremely important task, which should become a subject for a full article. This paper presents the first data on the composition, size, and proportion of different forms of leukocytes in the peripheral blood of the Baikal seal.

The Baikal seal was caught from September 29 to October 23, 2018, using stake nets with a mesh size of 120–150 mm in accordance with permits from the Angara–Baikal Territorial Administration of the Federal Fisheries Agency.[1] The biological material that was retained after the dissection of Baikal seal carcasses was disposed according to the order of the Russian Federal Fisheries Agency.[2] Territorially, the seal was captured in the Dagara Bay, Severo-Baikalsky District (Fig. 1), and in the Proval Bay, located in the central part of the southern basin of the lake. Places for setting net lines were selected taking into account the behavioral features of the Baikal seal. Fishing depths varied from 1.5 to 4.5 m in the Dagara Bay (55°38′2″ N, 109°54′42″ E and 55°38′39″ N, 109°55′18″ E) and from 3 to 5 m near the Proval Bay (52°24′28″ N, 106°25′51″ E).

A total of 14 individuals (seven males and seven females) were caught. Nine of them are young immature individuals in the first and second years of life with a body length of 87–98 cm (mean 92.11 ± 1.30) and a body weight of 20.57–34.16 kg (mean 27.80 ± 1.36), and the other five are adult mature individuals aged 7–23 years with a body length of 115–132 cm (mean 122.60 ± 3.50) and a body weight of 57–83 kg (mean 71.40 ± 4.22). Blood was taken from seals in vivo using a needle of a 10-mL syringe or a G20/G22 catheter from the vein located in the region of the caudal third of the hind flipper. Blood smears were made onto a defatted glass slide, dried, and fixed in 96% ethanol for 30 min. Under laboratory conditions, smears were stained according to Romanovsky–Giemsa. Stained preparations were studied using oil immersion (×100 lens and ×10 eyepiece) under a Biomed-6PR1-FK light microscope. Two hundred leukocytes were analyzed in each preparation. Cells were photographed and measured using a KEYENCE VHX-1000 digital microscope (Keyence, Japan). The research results were statistically processed using standard algorithms implemented in Statistica v. 12.0. The peripheral blood smears of the test individuals contained leukocyte types characteristic of marine mammals (Kavtsevich, 2011) (Table 1).

[1] Permits nos. 032018030195 and 032018030101 from the Angara–Baikal Territorial Administration of the Federal Fisheries Agency (valid from March 29 to January 12, 2018) throughout the Republic of Buryatia.

[2] Order no. 34 of the Federal Fisheries Agency dated January 25, 2010 "On Approval of Forms of Acts Provided for by Decree no. 921 of the Government of the Russian Federation as of November 13, 2009 "On Approval of Fishing Regulations for Research and Monitoring Purposes.'"

Table 1. Composition and size of leukocytes in the peripheral blood of seals

Cell type	Proportion of cells, %	Large and small diameter, μm
Lymphocytes		
large	0.5 ± 0.01 / 0.5 ± 0.01	10.46 ± 0.49 × 10.06 ± 0.28
small	39.46 ± 5.85 / 28.30 ± 11.57	6.77 ± 0.17 × 6.04 ± 0.18
Monocytes	4.44 ± 0.79 / 5.30 ± 1.65	14.87 ± 0.48 × 11.62 ± 0.98
Neutrophils		
band	22.22 ± 3.04 / 36.30 ± 6.79*	12.22 ± 0.56 × 10.95 ± 0.57
segmented	28.94 ± 4.10 / 27.70 ± 6.30	13.82 ± 0.46 × 11.04 ± 0.49
Eosinophils	4.44 ± 1.02 / 1.10 ± 0.78*	14.10 ± 0.32 × 11.90 ± 0.37
Basophils	0.0 / 0.80 ± 0.33	13.74 ± 0.45 × 10.77 ± 0.59

Indicators for young seals are above the line; indicators for adult seals are below the line.

* Significant differences between different age groups of seals at $p \leq 0.05$.

Neutrophils prevail in the leukogram of the Baikal seal (about 50% in juvenile individuals and over 60% in adults). Significant differences between different-aged individuals were recorded in the percentage of band forms; the differences in the number of segmented forms were insignificant between them. Lymphocytes were the next most numerous form: about 40% in young individuals and 30% in adult individuals. The leukogram showed a low content of other cell pools: monocytes (4.44% in young individuals and 5.30% in adult individuals), basophils (0 and 0.8%), and significantly different eosinophils (4.44 and 1.10%, respectively). Similar cell types were previously found in ringed (*Pusa hispida* (Schreber, 1775)); bearded (*Erignathus barbatus* (Erxleben, 1777)); harp (*Phoca* (*Pagophilus*) *groenlandica* (Erxleben, 1777)); gray *Halichoerus grypus grypus* (Fabricius, 1791)); and hooded (*Cystophora cristata* (Erxleben, 1777)) seals living in the White, Barents, and Kara seas (Kavtsevich and Minzyuk, 2017; Erokhina et al., 2020; Kavtsevich et al., 2020a, 2020b) and in bearded (*Erignathus barbatus* (Erxleben, 1777)), ringed (*Pusa hispida*), spotted (*Phoca largha* (Pallas, 1811)), and striped (*Histriophoca fasciata* (Zimmermann, 1776)) seals living in seasonal sea ice in Arctic and Subarctic regions (Goertz et al., 2019). The proportion of agranulocytes is lower than that of granulocytes in the blood of seals. As in other vertebrates, most of the lymphocytes are small; very few of them are large. The greater part is occupied by a nucleus surrounded by a narrow cytoplasm rim (Figs. 2a, 2b). They differ from each other in the large and small diameter (Table 1); however, these cells are smaller in size than other types of white blood cells. Monocytes are quite large cells, with a bean-shaped or horseshoe-shaped nucleus; vacuoles are encountered in the cytoplasm (Fig. 2c). Granulocytes are large cells; most of them are represented by neutrophils. The nucleus is horseshoe-shaped in smaller band neutrophils and consists of several lobes in segmented neutrophils; small granules are present in the cytoplasm (Figs. 2d, 2e). Eosinophils were identified by orange-stained cytoplasmic granules (Fig. 2f) and basophils by blue granules and a horseshoe-shaped nucleus (Fig. 2g). Our study showed a similarity in the composition of white blood cells and proportion of most of the leukocyte forms in the peripheral blood of Baikal seal and other pinnipeds (Kavtsevich, 2011; Erokhina et al., 2020; Kavtsevich et al., 2020a, 2020b). It should be noted that the proportion of band neutrophils is lower in the leukograms of adult gray, harp, and ringed seals (≤5%). Also, the proportion of lymphocytes in ringed seal does not exceed 15%. The detected differences are presumably determined by the species and age-related features and habitat conditions. Another feature is the high content of eosinophils in ringed and harp seals (13 and 19%, respectively). The most likely reasons for eosinophilia are parasitic invasions or allergic responses of various origins. Also, the phenomenon of "physiological decussation" (i.e., the equalization of the number of neutrophils and lymphocytes) was recorded in bearded, gray, harp, and hooded seals (Kavtsevich and Minzyuk,

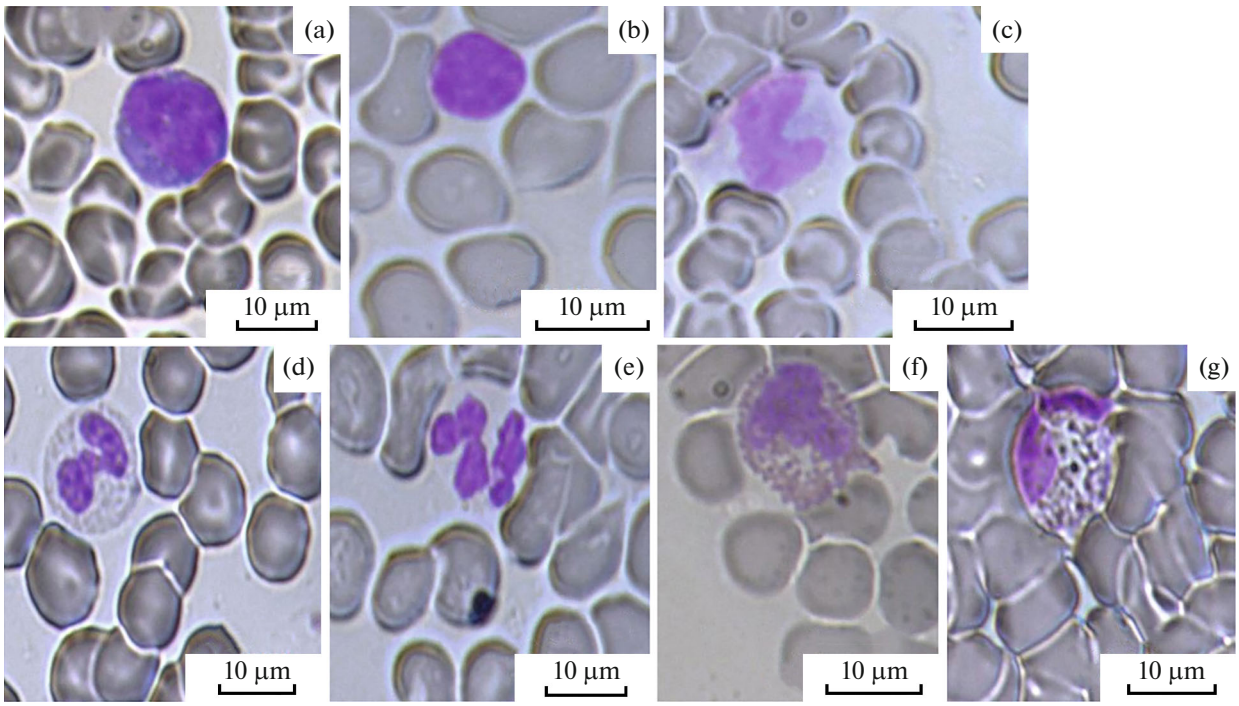

Fig. 2. Blood cells of the Baikal seal: (a) large lymphocyte, (b) small lymphocyte, (c) monocyte, (d) band neutrophil, (e) segmented neutrophil, (f) eosinophil, and (g) basophil. Magnification (×2000).

2017; Kavtsevich et al., 2020a, 2020b) in different ontogenetic periods.

CONCLUSIONS

Data on the composition, size, and proportion of the content of different forms of leukocytes in the peripheral blood of Baikal seal were obtained for the first time. The predominance of neutrophils and lymphocytes and an insignificant content of other forms of leukocytes were established. Young individuals had a significantly low percentage of band neutrophils and a significantly high percentage of eosinophils when compared to adult individuals. Similarities in the composition of most of the leukocyte forms and differences in the proportion of band neutrophils were recorded between the Baikal seal and sea pinnipeds.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest. The authors declare that they have no conflicts of interest.

Statement on the welfare of animals. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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