PERIPHERAL BLOOD LEUKOCYTE COMPOSITION OF THE ATKA MACKEREL *PLEUROGRAMMUS MONOPTERYGIUS* (HEXAGRAMMIDAE) AND CHUM SALMON *ONCORHYNCHUS KETA* (SALMONIDAE) FROM THE BERING SEA

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In the Atka mackerel *Pleurogrammus monopterygius*and chum salmon *Oncorhynchus keta* from the Bering Sea, lymphocytes predominate among leukocytes in peripheral blood. Atka mackerel lacks segmented neutrophils and basophils, while chum salmon lack eosinophils.. The studied species differ in the size of leukocytes and the values of the leukocyte abundance index.

Keywords: Atka mackerel *Pleurogrammus monopterygius*, chum salmon *Oncorhynchus keta*, leukogram, leukocytes, Bering Sea.

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INTRODUCTION

Northern Greenling *Pleurogrammus monopterygius* (Pallas, 1810) (Perciformes: Hexagrammidae), also known as sea lenok, is distributed in the northern part of the Pacific Ocean, along the Asian coast - from the middle part of the Kuril ridge to the Gulf of Anadyr, along the American coast - to the south to California, as well as in the waters near the Commander and Aleutian Islands (Commercial fish ..., 2006).Larvae and juveniles lead a pelagic lifestyle and can be carried by ocean currents over considerable distances. At the age of two, they come closer to the shores.Greenlings are schooling fish, predators leading a coastal bottom lifestyle. They feed on zooplankton, benthos, fish eggs and juveniles. The northern greenling is one of the main commercial demersal fish species in the Russian Far East. From 2015 to 2020, its catch was 19.5–35.0 thousand tons per year (Golovatyuk et al., 2023).

Chum salmon *Oncorhynchus keta* (Walbaum, 1792) (Salmoniformes: Salmonidae) is widely distributed along both shores of the Pacific Ocean - from San Francisco to the Bering Strait along the American coast and from Providence Bay to Peter the Great Bay and the Tumen-Ula River along the Asian coast. It is also found in the Arctic basin (Commercial fish..., 2006). Chum salmon is an object of ranching aquaculture in Russia, USA, Canada, Japan, and the Republic of Korea, so its actual habitat range may vary following the distribution and success of aquaculture. The life cycle of chum salmon, like other anadromous fish, involves changing habitats. Shortly after hatching in streams, rivers, and lakes, juveniles migrate to the sea and feed in the oceanic waters of the northern Pacific. The duration of the life cycle and time spent in the ocean varies from two to five years. After feeding, chum salmon undertakes a pre-spawning migration to river mouths (Commercial fish..., 2006; Gordeev, Klovach, 2019).

Hematological studies are used to assess both the physiological condition and immunological status of fish. One of the most important blood indicators is the leukocyte formula. In a normal physiological state, deviations in the percentage composition of leukocytes from the norm are insignificant. Shifts in the leukocyte formula indicate a disruption of homeostasis in the fish organism and the beginning of pathological processes long before clinical signs appear. Such data can be used to monitor physiological condition, poisoning, and helminth infections, and to determine the influence of biotic and abiotic factors on the organism (Ivanova, 1983; Zhiteneva et al., 1997; Yakhnenko, Klimenkov, 2009; Izergina et al., 2014; Mineev, 2015; Koroleva, 2016; Basova, 2017; Gordeev et al., 2017 ; Golovina, 2018).

Leukocyte composition of fish from the greenling family (Hexagrammidae) has been poorly studied. Comparative studies between wild and cultivated individuals of the Japanese greenling *Hexagrammos otakii* showed differences in some hematological parameters, including lower total white blood cell content in individuals from the natural habitat (Gao et al., 2022). Studies of cultivated individuals of this species did not reveal significant differences in the total number of leukocytes at different levels of water salinity (Zhou et al., 2021). In salmonid fish, the blood leukogram is studied in juveniles during early periods of ontogenesis and in broodstock at fish hatcheries and in net pens located in the sea (Sandnes et al., 1988; Ciereszko et al., 2007; Izergina et al., 2014; Lulijwa et al., 2019; Dessen et al., 2020).

Previously, we (Gordeev et al., 2022) investigated the cellular composition of peripheral blood leukocytes in pink salmon *O. gorbuscha* and chum salmon caught in the open waters of the northwestern Pacific Ocean (east of the Kuril Ridge). Among leukocytes, lymphocytes predominated, eosinophils were absent, and in pink salmon, basophils were also absent. Compared to pink salmon, chum salmon had a lower proportion of segmented neutrophils and smaller sizes of some cell types. However, there is little data in the available literature on the leukocyte composition of fish blood inhabiting the northern Pacific Ocean. In our work, we studied the blood leukocyte composition of the northern Atka mackerel and chum salmon caught in the Bering Sea.

MATERIAL AND METHODS

Immature chum salmon (11 specimens with gonads at stages I-II of maturity) and young greenling (10 specimens) with an average absolute length of 338.7 ± 11.37 and 209.0 ± 2.99 mm and an average weight of 528.1 ± 40.13 and 78.9 ± 4.55 g, respectively, were caught 29.09 - 05.10.2019 using an epipelagic trawl during a trawl survey from the research vessel "Professor Kaganovsky" in the southwestern part of the Bering Sea (Fig. 1).

Blood was collected from the caudal vein 90 min after capture and keeping the fish in flowing water. Blood smears were applied to a degreased slide, fixed for 30 min in 96% ethanol, stained according to Romanovsky-Giemsa and examined under a Biomed-6PR1-FK light microscope ("Biomed," Russia) using an immersion objective (×1000 magnification). In each preparation, 200 leukocytes were counted and identified according to Ivanova (1983). Photographs of cells and their measurements were performed using an EVENCE VHX-1000 digital microscope ("Keyence," Japan). To determine the leukocyte abundance index , or the frequency of occurrence of white blood cells, in the peripheral blood smear, 100 fields of view were examined in different areas of the preparation at ×400 magnification. In each field of view, the number of leukocytes was counted, the obtained data were summed and divided by 100, resulting in the average number in one field of view (Mikryakov, Lapirova, 1997) . To determine deviations in hematological parameters, the leukocyte shift index (LSI) was calculated as the ratio of the number of granulocytes to agranulocytes:

LSI = granulocytes/agranulocytes (Zhiteneva et al., 1997).

Statistical processing of the results was carried out according to standard algorithms implemented in the Statistica v. 6.0 software package, using *t* -test. Differences were considered significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

In peripheral blood smears of Atka mackerel and chum salmon, the types of leukocytes characteristic of most fish species were found (table). Lymphocytes predominated in both species, their proportion in Atka mackerel was 91.80%, in chum salmon - 90.57%. The content of other cell types was significantly lower: after lymphocytes, in descending order, there were blast cell forms, neutrophils, basophils, eosinophils, and monocytes. Unlike chum salmon, the leukogram of Atka mackerel lacked segmented neutrophils and basophils but contained eosinophils. Comparison of the ratio of different forms of chum salmon leukocytes with data from this species caught earlier (Gordeev et al., 2022) showed similarity with minor deviations. Previously, a low number of eosinophils and basophils were noted in peripheral blood smears of Far Eastern salmonid fish (Izergina et al., 2014).

Composition and size of peripheral blood leukocytes of Atka mackerel *Pleurogrammus monopterygius* and chum salmon *Oncorhynchus keta*

Cell type	Cell proportion, %		Size, µm	
	Atka mackerel	Chum salmon	Atka mackerel	Chum salmon
Lymphocytes	91.80 ± 0.86	90.56 ± 1.61	6.16 ± 0.19 / 5.28 ± 0.19	7.08 ± 0.12* / 6.09 ± 0.10*
Monocytes	0.80 ± 0.20	0.84 ± 0.40	11.65 ± 0.15 / 10.50 ± 1.30	14.72 ± 0.47* / 14.02 ± 0.38*
Neutrophils:				
– band	1.40 ± 0.24	1.85 ± 0.55	10.53 ± 1.36 / 8.90 ± 0.73	11.61 ± 0.32 / 10.36 ± 0.22*
– segmented	0	3.14 ± 0.55		13.47 ± 0.21 / 12.02 ± 0.34
Eosinophils	3.60 ± 0.81	0	10.38 ± 0.18 / 9.60 ± 0.17	
Basophils	.0	0.33 ± 0.21		15.30 ± 0.30 / 11.52 ± 0.90
Blast forms	2.40 ± 0.40	3.28 ± 0.35	11.06 ± 0.26 / 9.60 ± 0.40	13.72 ± 0.21* / 12.68 ± 0.37*

Note. Mean values and their errors are shown, above the line - large diameter, below the line - small diameter; *differences between species are significant at $p \leq 0.05$.

In chum salmon, compared to greenling, the same-named leukocytes are generally larger (table; Fig. 2, 3). The average size of chum salmon lymphocytes is significantly larger than that of greenling. These agranular cells are small in size with a rounded structure; the nucleus occupies most of the cell and is surrounded by a thin ring of cytoplasm (Fig. 2a, 3a). Lymphocytes are immune system cells that perform functions of recognizing foreign bodies, destroying antigens, synthesizing antibodies, forming specific immunity and memory cells (Mikryakov, 1991; Roitt et al., 2000; Galaktionov, 2005; Van Muiswinkel, Vervoorn-Van Der Wal, 2006; Uribe et al., 2011; Scapigliati, 2013). Like lymphocytes, monocytes are agranular but larger in size, with an eccentrically located kidney-shaped or oval nucleus. They have phagocytic activity against cell and tissue breakdown products, so vacuoles and parts of other cells are often found in their cytoplasm. The studied fish species differ significantly in size and shape of these agranulocytes: in greenling they are oval, in chum salmon they are round (Fig. 2b, 3b). Granulocytes are cells with an eccentrically located nucleus; granules contained in the cytoplasm of neutrophils are small (Fig. 2c, 3c, 3d), while in eosinophils and basophils they are large (Fig. 2d, 3e). In greenling, eosinophils stain orange. Granulocytes participate in phagocytosis of microorganisms, synthesis of immune response mediators and non-specific immunity factors (Ellis, 1977; Mikryakov, 1991; Zapata et al., 1996; Van Muiswinkel, Vervoorn-Van Der Wal, 2006; Havixbeck, Barreda, 2015; Hodgkinson et al., 2015). In chum salmon, band neutrophils are significantly smaller than segmented neutrophils. However, granulocytes and blast cells of chum salmon exceeded those of greenling in size. In blast cells, the nucleus occupies most of the cell and is surrounded by a narrow layer of cytoplasm (Fig. 2e, 3f). The proportion of these cells in the leukogram can be up to 10% and depends on the species and ecological characteristics of fish (Ivanova, 1983). The sizes of chum salmon leukocytes in our study correspond to published data (Izergina et al., 2014; Gordeev et al., 2022).

The leukocyte abundance index characterizes the intensity of leukopoiesis and the level of leukocyte content in a unit volume of blood (Mikryakov, Lapirova, 1997; Yakhnenko, Klimenkov, 2009) . This index is significantly higher in chum salmon (28.07 ± 5.83) compared to greenling (15.42 ± 2.02). The obtained data exceeded the values of this indicator in chum salmon (10.0 ± 1.28) and pink salmon (8.96 ± 1.61) caught in the open waters of the northwestern part of the

Pacific Ocean (east of the Kuril Ridge) (Gordeev et al., 2022). Probably, this difference is due to the fact that the chum salmon and greenling specimens caught in the Bering Sea are younger and therefore have higher intensity of leukopoiesis. Also, the fishing took place in the autumn period and geographically much further north, and therefore, at lower water temperatures, which could affect the intensity of the body's life processes.

The Lymphocyte Shift Index (LSI) calculated from the leukocyte formula data is a criterion for assessing the condition of an individual specimen, which reflects deviations from the conditional norm. A shift in the LSI value in either direction is considered a sign of disease or increased negative pressure from the environment. A high frequency of occurrence of individuals with such disorders is a sign of trouble in the population as a whole (Zhiteneva et al., 1997; Mineev, 2015). The acceptable LSI value may differ for different fish. For example, in most species of the Cyprinidae family, the normal LSI value is 0.30, and in sturgeons, it is 0.25–0.40 (Zhiteneva et al., 1997). In the fish we studied, this indicator is significantly lower – 0.07 ± 0.01 in greenling and 0.09 ± 0.01 in chum salmon. There is no literature data on the conditional norm of LSI for these species. No differences were found when comparing the LSI of chum salmon with this indicator calculated based on previously obtained (Gordeev et al., 2022) leukograms of chum salmon (0.10 ± 0.01) and pink salmon (0.11 ± 0.01).

Thus, lymphocytes predominate in the peripheral blood of greenling and chum salmon from the Bering Sea. In the greenling leukogram, segmented neutrophils and basophils are absent, while in chum salmon, eosinophils are absent. Compared to chum salmon, greenling has smaller sizes of all cell types and a lower leukocyte abundance index in peripheral blood .

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COMPLIANCE WITH ETHICAL STANDARDS

The research was conducted in accordance with the legislation of the Russian Federation and the provisions of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes." The animal study protocols were approved by the Bioethics Commission of the Institute of Biology of Inland Waters, Russian Academy of Sciences (Protocol No. 16 dated April 12, 2024).

CONFLICT OF INTEREST

The authors of this paper declare that they have no conflict of interest.

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FIGURE CAPTIONS

Fig. 1. Map of the study area and capture sites of Atka mackerel *Pleurogrammus monopterygius* (■) and chum salmon *Oncorhynchus keta* (●); the number of analyzed specimens is indicated inside the symbols. Scale: 200 km.

Fig. 2. Blood cells of Atka mackerel *Pleurogrammus monopterygius* : a – lymphocyte (*L*), b – monocyte (*M*), c – band neutrophil (*BN*), d – eosinophil (*E*), e – blast cell (*Bl*). Scale here and in Fig. 3: 10 µm.

Fig. 3. Blood cells of chum salmon *Oncorhynchus keta* : a – lymphocyte (*L*), b – monocyte (*M*), c – band neutrophil (*BN*), d – segmented neutrophil (*SN*), e – basophil (*B*), f – blast cell (*Bl*).