
SHORT
COMMUNICATIONS

Composition of Peripheral Blood Leukocytes of Pink Salmon *Oncorhynchus gorbuscha* and Chum Salmon *O. keta* (Salmonidae) during the Marine Life Period

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Received March 9, 2021; revised April 8, 2021; accepted April 9, 2021

Abstract—In pink salmon *Oncorhynchus gorbuscha* and chum salmon *O. keta* lymphocytes predominate among leukocytes in the peripheral blood during the marine period of life. In both species eosinophils are absent, while in pink salmon basophils are also absent. The proportion of segmented neutrophils and the size of lymphocytes, monocytes, and stab neutrophils are lower in chum salmon than in pink salmon.

Keywords: pink salmon *Oncorhynchus gorbuscha*, chum salmon *Oncorhynchus keta*, blood, leukogram, leukocyte size, Pacific Ocean

DOI: 10.1134/S0032945222020060

INTRODUCTION

The life cycle of pink salmon *Oncorhynchus gorbuscha* and chum salmon salmon *O. keta*, like those of other anadromous fish, is associated with the change of the habitat. After spawning in streams, rivers and lakes, they migrate into the sea and feed in the oceanic waters of the northern part of the Pacific. At the same time, pink salmon and chum salmon differ significantly from each other in terms of the duration of their life cycle and the time spent in the ocean. Pink salmon is a short-cycle species and performs pre-spawning migration to river mouths the next year after the migration of juveniles, while chum salmon spends in the sea from two to five years (*Promyslovyye ryby ...*, 2006; Gordeev and Klovach, 2019).

The study of fish leukocytes allows one to judge about the physiological and immunological state of the organism and the influence of the habitat on it (Serpunin, 2002; Kuzina, 2011; Koroleva, 2016). The leukocyte formula is an important informative indicator for assessing the physiological state of the body in complex studies of fish populations in the nature and in aquaculture (Ivanova, 1983; Golovina and Trombitsky, 1989; Yakhnenko and Klimenkov, 2009; Izergina et al., 2014; Basova, 2017; Gordeev et al., 2017; Golovina, 2018; Suvorova and German, 2021). Changes in the ratio of leukocytes are detected long before the appearance of clinical signs of disease and pathologies. Shifts in the leukocyte formula of fish in a normal physiological state are insignificant (Zhite-

neva et al., 2004), which allows one to use them as markers of various physiological and pathological processes occurring in the body. In salmonids (Salmonidae), hematological parameters are mainly studied at hatcheries in spawners and juveniles in the early periods of ontogeny, before the migration to the sea (Ciereszko et al., 2007; Izergina et al., 2014; Lulijwa et al., 2019). There are data on haematological parameters of salmon reared in net sea pens under marine conditions (Sandnes et al., 1988; Dessen et al., 2020), but no data on the ratio of leukocytes during the marine feeding period of life. Such studies are necessary to understand the adaptive capabilities of salmonids, monitor their physiological state and assess survival, which is important for calculating the forecast for the return of salmon fish.

The purpose of this work is to study the leukocyte composition of the blood of pink salmon and chum salmon during the marine period of life.

MATERIALS AND METHODS

Sexually mature individuals of pink salmon and chum salmon (12 specimens each) with an average weight of 844.91 ± 27.60 and 1592.00 ± 105.50 g, respectively, and a total length (*TL*) of 441.25 ± 5.44 and 547.66 ± 9.46 mm, respectively, were caught on 06.06–01.07.2018 in the open waters of the northwestern part of the Pacific Ocean (to the east of the Kuril chain) using an epipelagic trawl from the R/V *Profes-*

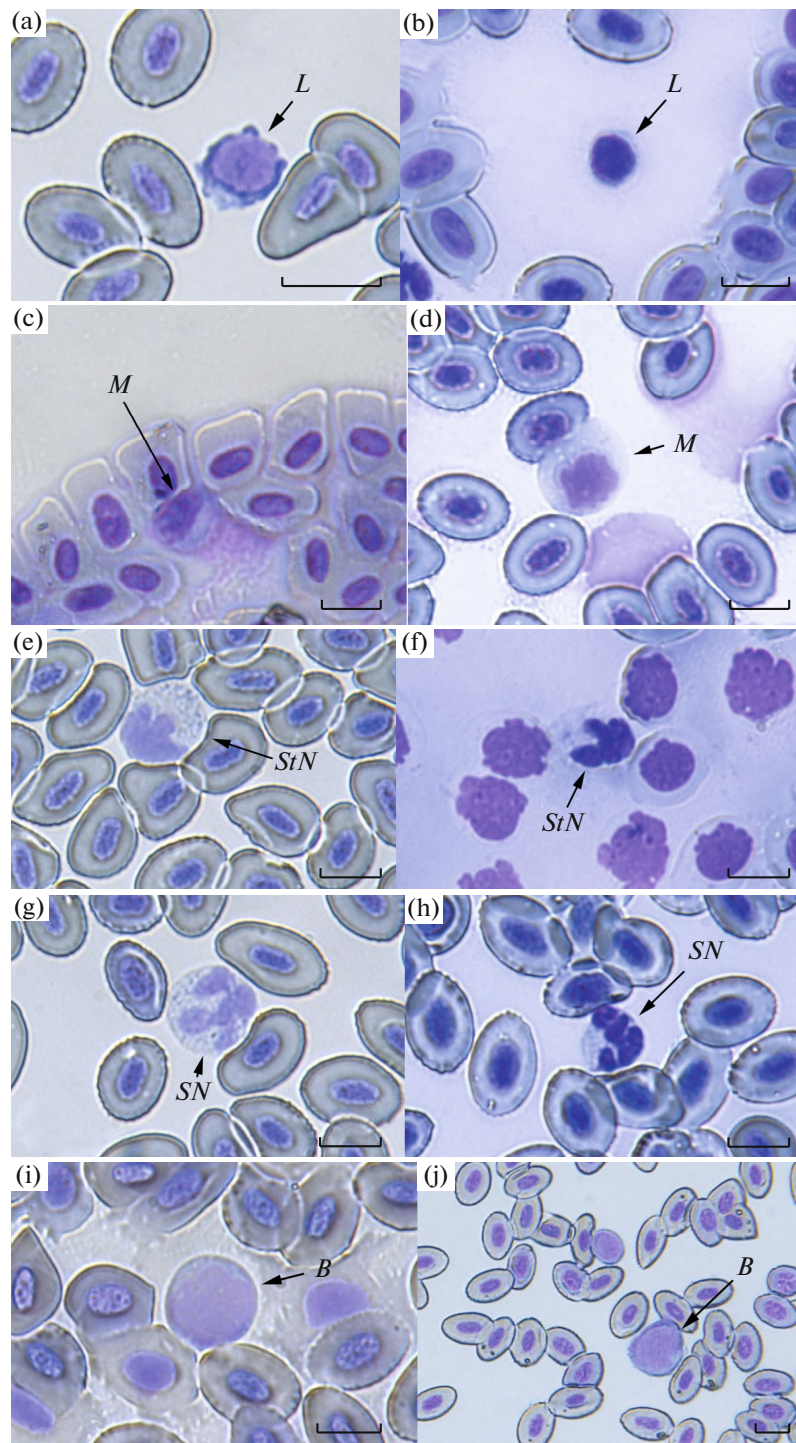


Fig. 1. Blood cells of pink salmon *Onchorhynchus gorbuscha* (a, c, e, h, j) and chum salmon *O. keta* (b, d, f, i, k): a, b—lymphocytes (*L*); (c, d)—monocytes (*M*); e, f—stab neutrophils (*StN*); h, i—segmented neutrophils (*SN*); j, k—blast cells (*B*). Scale: 10 μm .

sor Kaganovsky during a trawl survey of Pacific salmon in the marine life period.

Blood was taken from the tail vein 90 min after catching and keeping the fish in running water. Blood smears were made on degreased glass slides, fixed in 96% ethanol for 30 min, stained according to Roma-

novsky–Giemsa, and examined under a Biomed-6PR1-FK light microscope (magnification $\times 360$). In each preparation, 200 leukocytes were identified according to Ivanova (1983). Cell photographs and measurements were taken on a Digital Microscope EVENCE VHX-1000. To determine the index of

Table 1. Composition and size of peripheral blood leukocytes of pink salmon *Onchorhynchus gorbuscha* and chum salmon *O. keta*

Cell type	Proportion of cells, %		Diameter, μm	
	Pink salmon	Chum salmon		Chum salmon
Lymphocytes	88.40 ± 0.97	89.71 ± 1.01	8.02 ± 0.19 7.03 ± 0.23	$7.08 \pm 0.12^*$ $6.09 \pm 0.10^*$
Monocytes	0.90 ± 0.23	0.42 ± 0.20	17.70 ± 0.95 12.76 ± 0.63	$14.72 \pm 0.47^*$ 14.02 ± 0.38
Neutrophils:				
— stab	1.80 ± 0.24	1.45 ± 0.22	12.18 ± 0.24 11.27 ± 0.38	11.61 ± 0.32 $10.36 \pm 0.22^*$
— segmented	5.00 ± 0.86	$2.57 \pm 0.29^*$	13.92 ± 0.17 12.37 ± 0.36	13.47 ± 0.21 12.02 ± 0.34
Basophils	0	1.00 ± 0.30	0	15.00 ± 0.40 11.90 ± 0.40
Blast forms	3.90 ± 0.27	4.85 ± 0.40	14.80 ± 0.63 12.30 ± 0.43	13.72 ± 0.21 12.68 ± 0.37

A large diameter is above the line, a small diameter is below the line; * Differences between chum salmon and pink salmon are significant at $p \leq 0.05$.

abundance of leukocytes, or the frequency of occurrence of white blood cells, 100 fields of view were examined in a peripheral blood smear in different parts of the preparation at a magnification of $\times 400$. In each field of view, the number of leukocytes was counted, the obtained data were summed up and divided by 100, obtaining an average number in one field of view (Mikryakov and Lapirova, 1997).

The results were statistically processed 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

The types of leukocytes characteristic of most fish species were found in peripheral blood smears of the studied individuals (table). Lymphocytes predominate in the leukogram of pink salmon and chum salmon (88.40 and 89.71%, respectively), while content of other cell types is insignificant: monocytes (0.90 and 0.42), band- (1.80 and 1.45) and segmented neutrophils (5.00 and 2.57), basophils (0 and 1.00), blast forms (3.90 and 4.85); eosinophils are absent. Leukograms of pink salmon and chum salmon significantly differ in the content of segmented neutrophils and the absence of basophils in pink salmon. Many researchers have also noted that basophils and eosinophils are very rare in the blood of Far Eastern salmon (Khovansky and Khovanskaya, 1994; Kalinina, 1997; Pustovit and Pustovit, 2005; Izergina et al., 2014).

When comparing the ratio of different forms of pink salmon and chum salmon leukocytes with the lit-

erature data (Sergeenko, 2007; Izergina et al., 2014), similarities and differences were found. Leukograms of mature individuals during the sea feeding period differ from juveniles switching to exogenous feeding by a low proportion of agranulocytes and a high content of granulocytes, while the level of lymphocytes, monocytes, and neutrophils is similar to those of young individuals that have completed smoltification in sea water. At the same time, young forms of neutrophils predominate in juvenile pink salmon. The observed increase in the proportion of neutrophils and a decrease in the number of lymphocytes in juvenile salmon during smoltification (Kalinina, 1997; Izergina et al., 2006) are associated with a change in the direction of leukopoiesis (Konratieva and Kitashova, 2002).

Leukocytes are, as a rule, larger in pink salmon, compared to chum salmon (Table 1). The average size of pink salmon lymphocytes is significantly greater than that of chum salmon (Fig. 1). These cells are small, usually form pseudopodia and have a typical rounded structure: a large nucleus is surrounded by a thin ring of cytoplasm (Figs. 1a, 1b). Lymphocytes are the key cells of the immune system, which are divided into two main subpopulations: T- and B-lymphocytes. T-lymphocytes perform the functions of recognizing foreign bodies, destroying an antigen, forming specific immunity, and adapting fish to parasites and toxic factors, while B-lymphocytes perform the functions of antibody synthesis, the formation of precursors of antibody-forming cells, and the formation of memory cells (Mikryakov, 1991). Unlike lymphocytes, monocytes are large cells with an eccentrically located bean-

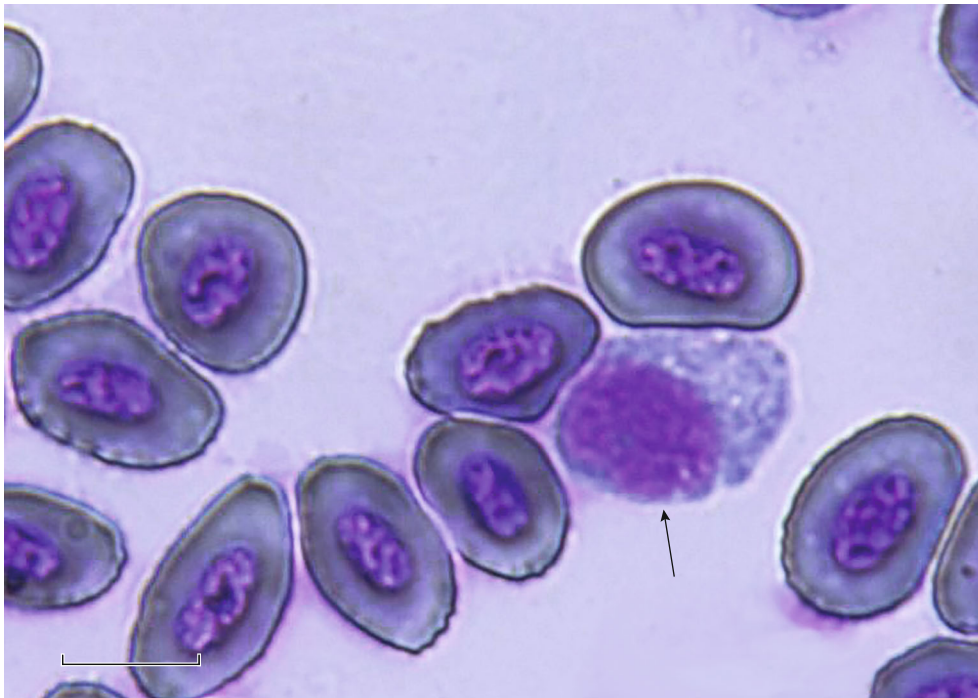


Fig. 2. Basophil (→) chum salmon *Onchorhynchus keta*. Scale: 10 μ m.

shaped or oval-shaped nucleus; their cytoplasm often contains vacuoles and parts of other cells. The studied species significantly differ in size and shape of these agranulocytes: in pink salmon they are oval, while in chum salmon they are round (Figs. 1c, 1d). Granulocytes are cells with an eccentrically located nucleus; the granules contained in the cytoplasm are small in neutrophils (Figs. 1e–1h) and large in basophils (Fig. 2). In pink salmon, segmented neutrophils are significantly larger than in chum salmon. The basophils found in the blood of chum salmon differ in shape and size from neutrophils. Blast cells have a large nucleus surrounded by a narrow layer of cytoplasm (Figs. 1i, 1j). The sizes of lymphocytes, monocytes, and neutrophils generally correspond to the published data (Izergina et al., 2014).

The leukocyte abundance index characterizes the intensity of leukopoiesis and the level of leukocytes per unit volume of blood (Mikryakov and Lapirova, 1997; Yakhnenko and Klimenkov, 2009). No significant differences in this index between pink salmon (8.96 ± 1.61) and chum salmon (10.0 ± 1.28) were not found.

Thus, lymphocytes predominate in the peripheral blood of pink salmon and chum salmon during the marine period of life. In both these species eosinophils are absent, while in pink salmon basophils are also absent. The proportion of segmented neutrophils and the size of lymphocytes, monocytes, and stab neutrophils are lower in chum salmon than in pink salmon.

FUNDING

The work was carried out within the framework of the state order no. AAAA-A18121050500046-8.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests. 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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Translated by P. Kuchina