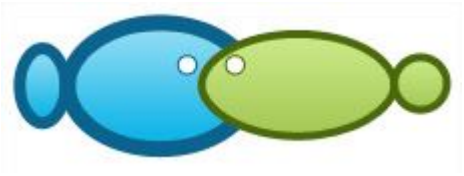

Influence of light conditions on the hematological parameters of fish and amphibians

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Abstract. Light has different effects on animals. However, its role in many cases is still poorly studied. This study is aimed at studying the effect of light on the hematological parameters of 4 fish species (*Carassius gibelio*, *Carassius auratus*, *Cyprinus carpio*, and *Glossolepis incisa*) and one amphibian species (*Xenopus laevis*). For experiments, fish were placed in flowing aquariums with a volume of 30-40 liters with a controlled water temperature. The lighting was created by fluorescent lamps of white light. The photoperiod was adjusted using automatic photo timers. Standard filters were used to study the effect of lighting color on fish. If the light conditions are optimal for the growth of animals, there is a tendency to improve blood characteristics (lymphophilia, an increase in the amount of hemoglobin), which indicates their satisfactory condition. If there is the deterioration of growth under certain conditions of photoperiod, illumination and monochromatic illumination, these indicators change to the opposite (lymphopenia, monocytophilia, neutrophilia and basophilia, a decrease in the content of hemoglobin and the number of red blood cells). From a practical point of view, the light conditions in aquaculture should be carefully selected for each stage of the life cycle and in accordance with the species preferences of the animals.
Key Words: erythrocytes, hemoglobin, light color, light intensity, lymphocytes.

Introduction. As a primary abiotic factor, light plays a major role in the life of most animal species throughout their ontogenesis. There is an exception: species from the caves, muddy waters, and at great depths (Boeuf & Le Bail 1999; Kuznetsov & Ruchin 2001; Ruchin 2020). Scientists (Boeuf & Le Bail 1999; Gaston et al 2013; Hänel et al 2018; Grubisic et al 2019; Ruchin 2020) indicate such important components of light as photoperiod (duration of illumination), illumination (light intensity), wavelength (color of illumination) and polarization. The intensity of light penetration into the water environment, together with the wavelength, depends on such factors as the reflection and refraction of sunlight by the water surface, their absorption and scattering in the water column. The amount of reflected radiation depends on the position of the sun above the horizon and increases with a decrease in the angle of incidence of the sun's rays (Lindström 2000; Rich & Longcore 2006; Ruchin 2021). Light penetrates the water best when the sun is at its zenith (Pegau et al 1997; Lindström 2000; Kuznetsov & Ruchin 2001; Rich & Longcore 2006; Kyba et al 2017). Therefore, light conditions are among the most fundamental for the biotechnics of growing aquatic organisms (Konstantinov et al 2000; Kim et al 2018; Ruchin 2020, 2021).

Lighting conditions affect not only the growth and development of fish and amphibians, but also the physiological and biochemical parameters of the body, the quality of aquaculture products and, ultimately, the performance of aquaculture systems. Therefore, it is important to understand the requirements for creating certain lighting conditions in aquaculture systems where the breeding conditions of organisms interfere with natural light (Delabbio 2015; Nguyen & Winger 2019; Lopez-Betancur et al 2020; Ruchin 2019, 2021).

Blood is involved in metabolic processes and shows changes in the animal's body (Conte 2004; De Souza Neves et al 2014; Ahmed et al 2020). The blood transports a variety of substances (gases, water, minerals, nutrients, hormones, immune effectors,

toxins, waste products). Hematological indicators are most often used to monitor the physiological state of animals during rearing (Ruchin 2007; Jeffrey et al 2015; Sopinka et al 2016; Romanova et al 2018; Abdel-Tawwab et al 2019; Seibel et al 2021). For example, increased blood cortisol levels were recorded in *Solea senegalesensis* exposed to 200 lx (Figueiredo et al 2020). However, there was no dependence of cortisol and blood glucose levels on light in experiments on *Thunnus orientalis* larvae (Honryo et al 2013). Light intensity affected catalase and total glutathione peroxidase during the development of *S. senegalensis* larvae (Cañavate et al 2007). The hepatopancreas oxidative stress of juvenile gibel carp, *Carassius gibelio* were induced in a dark environment. When the light intensity increased to 670 lx, cortisol, glucose, and lactic acid increased significantly (Wei et al 2019). The light intensity changed the amount of trypsin and chymotrypsin in *Dicentrarchus labrax* larvae during the first 7 days of the experiment. But this phenomenon was not observed later. Low light intensity induced significantly lower specific activity of both enzymes than at other intensities (Cuvier-Peres et al 2001). Detailed studies were conducted on *Megalobrama amblycephala* fingerlings (Tian et al 2015). The juvenile *M. amblycephala* had plasma levels of glucose and lactate that increased with light intensity rising from 100 to 1600 lx while the lowest plasma levels of cortisol was observed at 400 lx group. The application of light intensity at 1600 lx significantly lowered liver glutathione activity (Tian et al 2015). An increase in hemoglobin, hematocrit, and individual hemoglobin isomorphs in *Salmo gairdneri* was associated with hypoxia, shortened day length, and increased temperature (Tun & Houston 1986). *Seriola lalandi* fish exposed to green light had levels of antioxidant enzyme significantly lower than in the control, while levels of immune-related parameters were significantly higher compared to other groups (Choi et al 2016). However, there is not much information about the effect of light on blood counts. The aim of our work is to study the effect of light on the hematological parameters of fish and amphibians.

Material and Method. The studies were conducted in 2009 (from March to October), 2011 (from September to November) and 2014 (from September to December). Several species of fish and amphibians were used for experiments. Gibel carp *Carassius gibelio* lives in low-flow reservoirs. According to our observations, fry usually stay at a shallow depth near macrophyte thickets. It is omnivorous, it feeds on algae, rotifers, detritus, seeds and other parts of higher plants, planktonic crustaceans. Goldfish *Carassius auratus* is an object of aquarium breeding, a traditional object of ichthyological research. Carp *Cyprinus carpio* is one of the main objects of both pond and industrial fish farming (Ruchin 2019). In the latter case, plants with a closed water supply cycle are usually used for its cultivation. Red rainbowfish *Glossolepis incisa* is an aquarium fish that lives in the near-surface layers of water (Ohee 2013). Clawed frog *Xenopus laevis* is a species with a long period of larval development, a permanent inhabitant of the aquatic environment. In nature, tadpoles live in muddy water with an abundance of various microalgae and suspended particles. They are typical filtrators and filter water with an abundance of microalgae through the gill arches, capturing it with their mouth (Rothman et al 2016).

Fingerlings of *C. gibelio, C. auratus*, and *C. carpio* were captured in natural reservoirs. The weight of the fingerlings of these species varied from 8 to 19 g (body length 54-79 mm). Eggs and fry of *G. incisa*, as well as eggs and fingerlings of *X. laevis* were obtained in laboratory conditions (constant temperature 21±1°C and constant lighting 500 lx). At the same time, *G. incisa* fry and *X. laevis* fingerlings were kept in large aquaria at the usual daily natural photoperiod and illumination. In order to acclimate the animals, they were kept for 15 days in a common aquarium at a constant temperature and round-the-clock lighting before the start of the experiments. Then, for experiments, individuals were randomly caught and placed in flow aquaria with a volume of 30-40 liters with a controlled water temperature of 21±1°C and forced aeration. Each series of experiments was carried out with one type of animal. A total of 24 series of experiments were performed. A series refers to one experiment that was conducted with one type of animal. Thus, each series included at least three experiments. In each experiment, the number of animals in one aquarium ranged from 12 to 30 specimens, depending on the animals' size and species. In total, 300 individuals of *X. laevis*, 750

individuals of *G. incisa*, 560 individuals of *C. auratus*, 588 individuals of *C. gibelio* and 270 individuals of *C. carpio* were involved in the research. The duration of the experiments was 30 days in the experiments of *C. gibelio* and *C. carpio*. The number of fish in aquariums depended on the type of fish and its size. The number of large fish (for example, carp) in one aquarium was less than the number of small fish (for example, G. incisa). In experiments with other animals, the duration was 40 days. The duration of the experiments depends on the type of fish. Since some species grow faster, the effect of light on their body becomes clearly visible after 30 days. But those fish species that grow slower were kept in experiments for 40 days.

The lighting was created by white-light fluorescent lamps. To create complete darkness (0 lux), a light-tight hood was used. To increase or decrease the illumination, we changed the number of fluorescent lamps. The photoperiod was adjusted using Aquael automatic photo timers. The following photoperiod variants (L/D, h) were used: 0L/24D, 12L/12D, and 24L/0D. The illumination was created by changing the number of lamps. To study the effect of light color on *C. gibelio,* we used standard filters with transmission in a certain range of the spectrum: red – 680-760 nm, green – 510-540 nm, blue – 440-500 nm, dark blue – 430-470 nm, violet – 420-440 nm. The control aquarium was illuminated by a white fluorescent lamp. These studies were performed only with young *C. gibelio*.

In all series of experiments, all aquaria were separated from each other and from any other external influences by impenetrable partitions. The light intensity (from 0 to 10 000 lx) was measured on the water surface with a Yu–116 luxmeter.

Blood was taken from the caudal vein. The concentration of hemoglobin was determined in the Sali hemometer, the number of red blood cells and white blood cells - in the Goryaev chamber (Ivanova 1983). At the same time, two smears were taken from one individual to study the leukocyte formula. The count of the number of white blood cells was carried out according to the method of Ivanova (1983). At the same time, at least 600 cells were counted on each smear. The total number of neutrophils, eosinophils, monocytes and lymphocytes was taken into account. Blast forms were not taken into account in the analysis. Data between treatments and sampling times were compared by analysis of variance (ANOVA). The data were statistically processed using a standard method with Student's Test.

Results. In the blood of *X. laevis*, the number of neutrophils, eosinophils and monocytes in different variants of the photoperiod differed unreliably and did not exceed 2-3% of the total number of white blood cells. The number of other forms of white blood cells varied. Thus, the level of basophils in the blood of frog fingerlings was almost the same in conditions of round-the-clock darkness or round-the-clock lighting (6.4-6.6%). At the same time, when alternating light and dark, an increase in their number was recorded by 60% compared to other modes. This also affected the ratio of lymphocytes, which decreased in the 12L/12D photoperiod, and increased in other variants (Figure 1).

Slightly different results were obtained in experiments on *C. auratus* fry (Table 1). The number of neutrophils and basophils in the blood of this species did not depend much on the illumination and did not exceed 0.7%. The number of eosinophils and monocytes was significantly higher by 5-15% in conditions of constant darkness. The number of lymphocytes decreased in the dark. Thus, in the dark, lymphopenia, eosinophilia and monocytosis were recorded. When comparing these blood elements in fish contained in any of the illumination variants, no significant differences were revealed.

G. incisa had a significantly higher number of neutrophils and basophils in the blood when grown for a month in the dark and at a significant light intensity (2400 lx). At the same time, the number of lymphocytes and monocytes decreased in these modes. At the same time, well-expressed lymphophilia was observed at 600 lx illumination (Table 1).

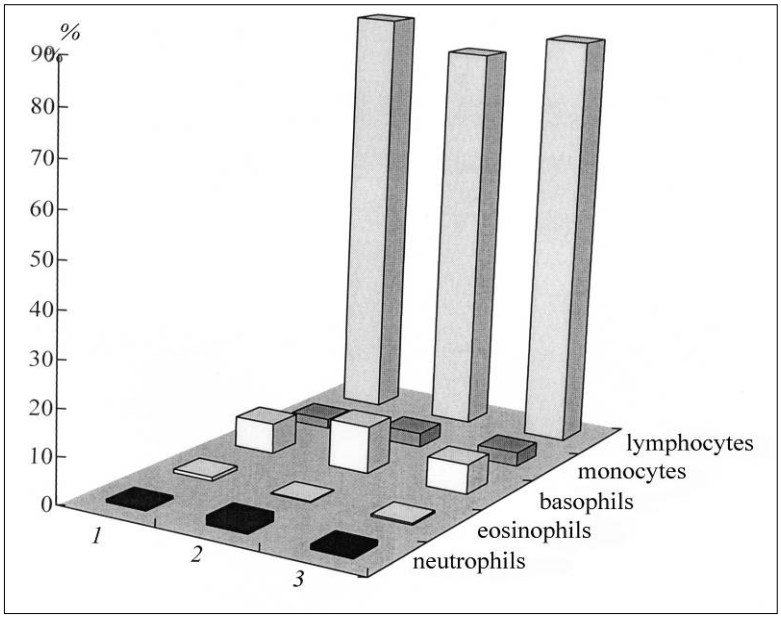

Figure 1. The dependence of the white blood cell formula of *Xenopus laevis* on the ratio of light and dark time of day (L/D, h) (in %). 1 – 0/24; 2 -12/12; 3 – 24/0.

Table 1

Dependence of the leukocyte formula of the blood of *Carassius auratus* and *Glossolepis incisa* on the illumination (in %)

Leukocytes	*Illumination, lx*				
	0	*3*	*7*	*600*	*2400*
			Glossolepis incisa		
Neutrophils	4.7	4.8	3.9	3.5*	5.9*
Eosinophils	3.5	6.0*	5.5*	4.9	5.8*
Basophils	12.4	6.6*	6.7*	7.1*	6.3*
Monocytes	2.9	3.7	4.6*	3.2	3.1
Lymphocytes	76.5	78.9	79.3	81.3*	78.9
			Carassius auratus		
Neutrophils	0	0.3	0.6	0	0.7
Eosinophils	1.9	1.2	0	0.4*	0.3*
Basophils	0	0.6	0.4	1.0	0
Monocytes	1.2	0.3*	0	0	0.3
Lymphocytes	96.9	97.6	99.0*	98.6	98.7

* - the significance of differences at the level of 95%.

Figures 2 and 3 show the dynamics of the indicators of the leukocyte formula for *C. gibelio* and *C. carpio* when exposed to different light conditions. It turned out that the number of eosinophils and monocytes increased by 30 days of growing *C. gibelio* in conditions of round-the-clock darkness, and basophils and neutrophils were not detected at all. In the same mode, the number of lymphocytes was lower than in other light modes. At 3 lx illumination, there was a gradual increase in the number of eosinophils by 30 days of cultivation, and at 2400 lx illumination, there was a decrease in the number of monocytes.

By day 30, there was an increase in the number of neutrophils, eosinophils and monocytes, a decrease in the number of basophils and white blood cells when growing *C. carpio* fingerlings in conditions of round-the-clock darkness. At 3 lx illumination, there was an increase in the number of neutrophils and lymphocytes in the blood and a decrease in the number of other blood elements. At a higher illumination of 2400 lx, there was a decrease in the number of neutrophils, basophils, and monocytes, and an increase in the number of other blood cells.

The amount of hemoglobin in *C. gibelio* significantly increased in some modes of monochromatic lighting. This increase was observed in the modes with high growth rates (green and blue light). At the same time, the number of red blood cells in any color of light changed unreliably (Table 2), and the hemoglobin content in one red blood cell was significantly higher in blue light.

Table 2

Some blood parameters of *Carassius gibelio* under different monochromatic illumination

Lighting color	*Hemoglobin, g %*	*Number of erythrocytes, million/μL*	*The content of hemoglobin in the erythrocyte, mcg*
Control	7.08±0.07	0.91±0.05	77.8±0.04
Red	6.65±0.10	0.90±0.08	73.8±0.03
Green	7.60±0.08*	0.98±0.04	77.6±0.05
Blue	7.01±0.03	0.95±0.04	73.8±0.05*
Dark blue	7.85±0.06*	0.96±0.05	81.8±0.04*
Violet	7.10±0.07	0.90±0.06	78.9±0.06

* - the significance of differences at the level of 95%.

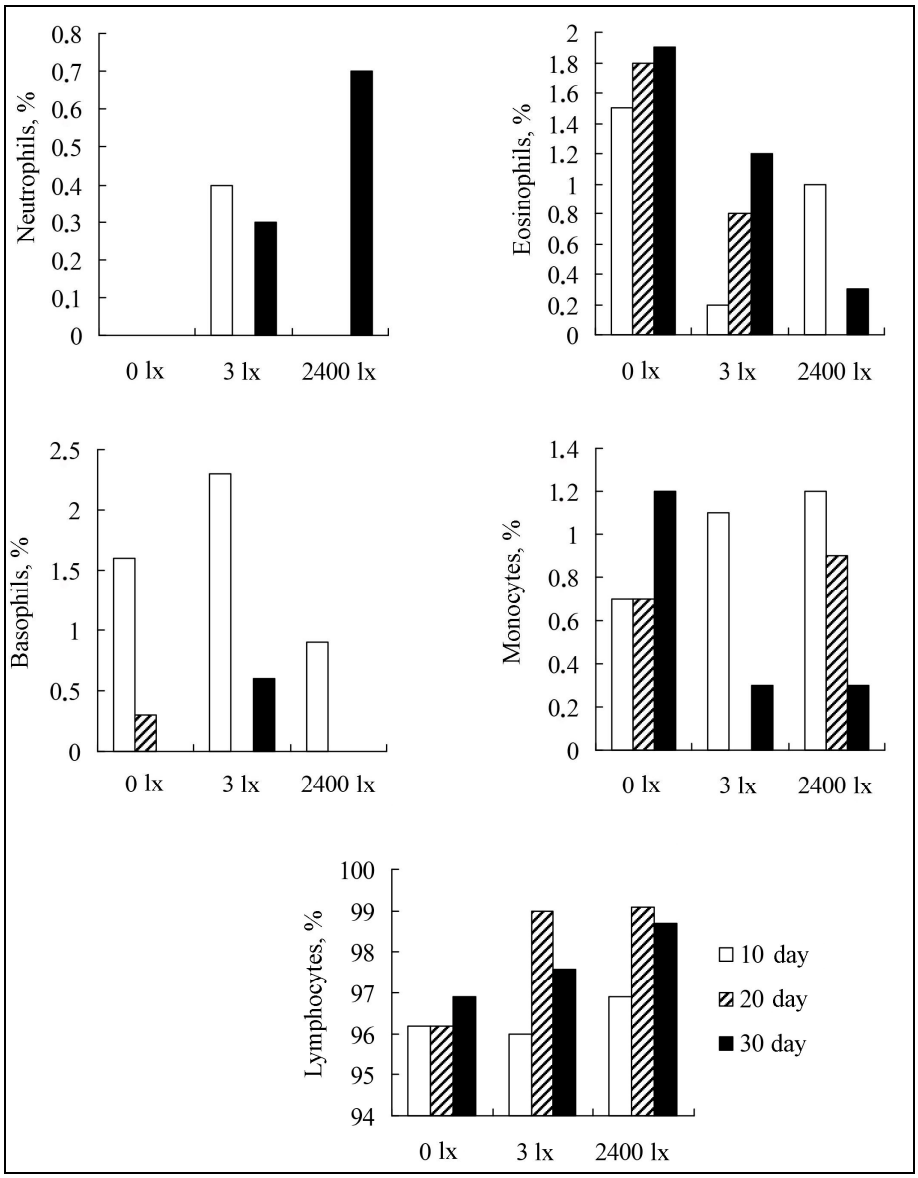

Figure 2. Dependence of the leucocyte formula of *Carassius gibelio* blood on illumination during long-term cultivation.

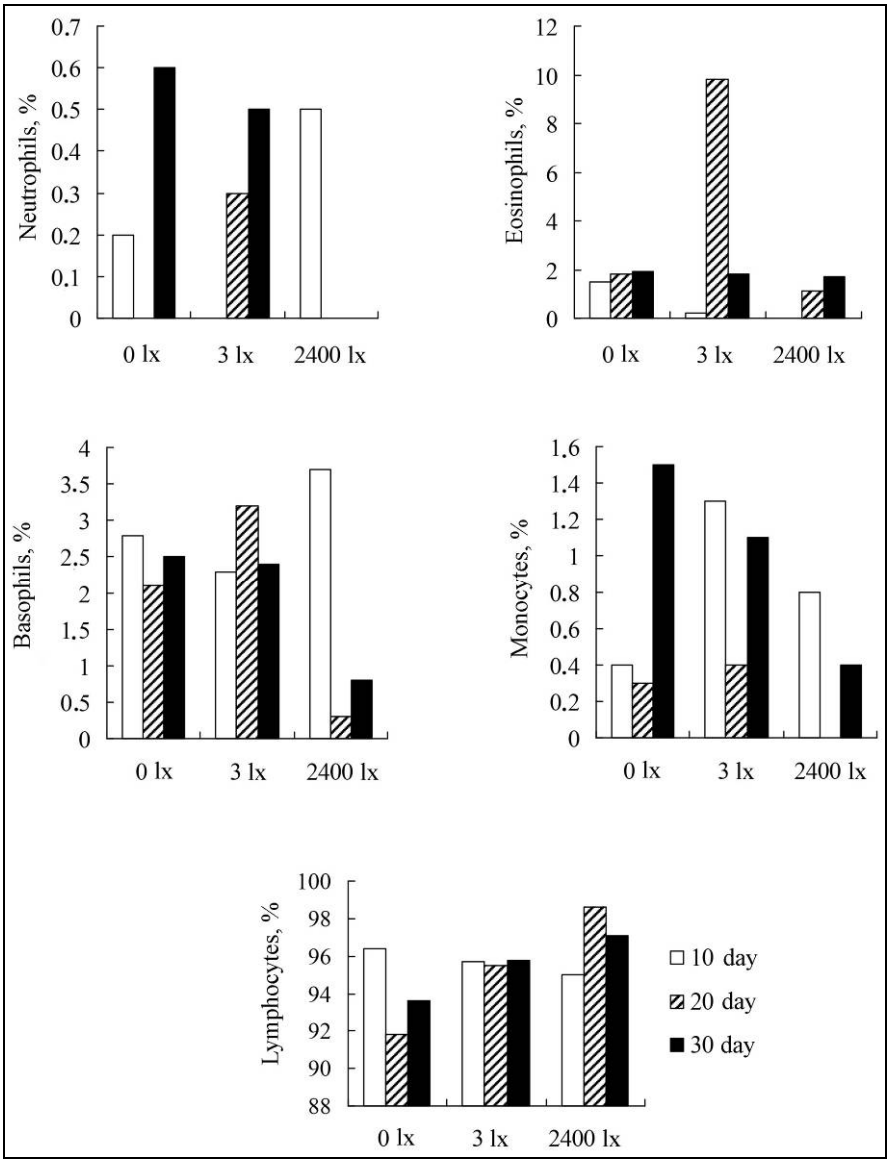

Figure 3. Dependence of the leucocyte formula of the blood of *Cyprinus carpio* on the illumination during long-term cultivation.

Discussion. When severe environmental changes occur, the well-being of organisms in aquaculture is disrupted. Fish feeding, sampling, sorting, and transportation can cause stressful reactions. Adverse environmental conditions can affect the number and shape of circulating red blood cells, but they can also alter the composition of white blood cells in circulation (Barton & Iwama 1991; Pulsford et al 1994; Segner et al 2012).

It is known that the ratio of certain populations of leucocytes in the blood can indicate the reaction of organisms to environmental influences (da Silva et al 2020). Primary stress reactions are characterized by the release of catecholamines and corticosteroids. Subsequent secondary stress responses have multiple actions in many tissues, including blood (Wendelaar Bonga 1997; Burgos-Aceves et al 2019; Kültz 2020). Stress hormones inhibit lymphocyte proliferation, granulocyte apoptosis, and the emigration of monocytes and neutrophil granulocytes from the hematopoietic tissue of the pronephros to the peripheral blood. Thus, a high number of circulating leucocytes may reflect an increase in the number of monocytes (monocytosis) and neutrophils (neutrophilia) and a concomitant decrease in the number of lymphocytes (lymphopenia) (Pickering 1984; Barton & Iwama 1991; Weyts et al 1998; Ortuño et al 2001; Davis & Maney 2018). Subsequently, as tertiary reactions, there is a slowdown in growth, a deterioration in the rate of development, a decrease in reproduction, and a decrease in

the adaptive abilities of farmed animals (von Borell 2000; Segner et al 2012; Petitjean et al 2019).

Light exposure is important for growing, since all manipulations with organisms are carried out under a certain illumination. Thus, an increase in light caused an increase in the number of immature erythrocytes and the content of hemoglobin in the blood of trout *Salmo gairdneri* (Esavkin 1979). In the leukocyte formula of Siberian sturgeon *Acipenser baerii* fingerlings, the number of neutrophils and eosinophils practically did not change in different light conditions. At the same time, the number of monocytes significantly decreased under variable illumination, and the number of lymphocytes, on the contrary, increased (Ruchin 2008). When the light intensity increased to 2000-3000 lx, the number of lymphocytes, eosinophils, basophils and monocytes in adult *Siganus sutor* increased, the number of neutrophils decreased (Shirinabadi 2013). The erythrocyte sedimentation rate in the cultivation of Nile tilapia, *Oreochromis niloticus* increases at a light intensity of 700-5600 lx (Zobova 2004). Illumination had an ambiguous effect on the leukocyte formula of *C. carpio*. The illumination of 2400 lx clearly improved the physiological status of the youngsters. At the same time, zero illumination led to the opposite results. The number of lymphocytes in the variant with illumination of 2400 lx exceeded the variant with zero illumination by 13%, and this led to a decrease in all other groups of leukocytes – eosinophils by 77%, basophils by about 2 times (Ruchin 2006). These results (Ruchin 2006) were obtained in experiments with a short duration of light exposure. However, the results presented here indicate similar conclusions for long-term cultivation of *C. carpio*.

In our previous studies, we observed that there is a tendency to improve the blood parameters of fish and amphibians (lymphophilia, an increase in the amount of hemoglobin, etc.) in the optimal lighting conditions. Previously, similar tendencies were recorded in other fish species (Ruchin 2001, 2004). The number of erythrocytes increased unreliably in the blood of Siberian sturgeon under the influence of different colors of light, but at the same time there was an increase in the amount of hemoglobin in the variants with green, dark blue and blue lighting. Consequently, this was due to an increase in the content of hemoglobin in one red blood cell, which was shown by calculations (Ruchin 2011). Sturgeon raised under red light, had a very variable number of red blood cells. Fish kept in green and blue light had a higher number of lymphocytes. At the same time, the percentage of neutrophils significantly decreased in the juvenile sturgeon in these variants. Different light colors also caused changes in the fractional composition of blood proteins of Siberian sturgeon and Nile tilapia (Ruchin & Dudko 2008; Sabri et al 2012). At the same time, with the stressful effects of the constant stay of animals in complete darkness, the opposite indicators are noted.

Conclusions. Under light conditions favorable for the growth of fish and amphibians, all hematological indicators have parameters that do not deviate from the norm. Consequently, the general condition of the animals can be assessed as quite satisfactory. In the case of deterioration of growth under certain conditions of photoperiod, illumination and monochromatic lighting, these indicators change. At the same time, there is lymphopenia, monocytophilia, neutrophilia and basophilia, the hemoglobin content decreases, the number of red blood cells decreases. In the blood of *Xenopus laevis*, the level of basophils was almost the same in conditions of constant darkness or constant lighting (6.4-6.6%). At the same time, when alternating light and darkness, an increase in their number by 60% was recorded. When *Glossolepis incisa* was grown for a month in the dark and at a significant light intensity (2400 lx), the number of neutrophils and basophils in the blood increased significantly. But well-expressed lymphophilia was observed at 600 lx illumination. When growing *Cyprinus carpio* fingerlings in round-the-clock darkness, an increase in the number of neutrophils, eosinophils and monocytes, a decrease in the number of basophils and leukocytes was observed. The amount of hemoglobin in *Carassius gibelio* significantly increased under green and blue lighting. Thus, the cultivation of fish and amphibians under certain light parameters has a positive effect on the physiological status of the animal body. From a practical point of view, the light conditions in aquaculture should be carefully thought out for each stage of the life

cycle and in accordance with the species preferences of the animals. Light should provide optimal conditions for the well-being of organisms and, ultimately, provide good quality of aquaculture facilities and their high productivity.

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