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# Reproductive Parameters of Adult Birds and Morphophysiological Characteristics of Chicks in the Pied Flycatcher (*Ficedula hypoleuca* Pall.) in Technogenically Polluted Habitats

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**Abstract**—Effects of toxic emissions (heavy metals and sulfur dioxide) on reproductive parameters of adult pied flycatchers (*Ficedula hypoleuca* Pall.) and morphophysiological characteristics of their progeny have been studied in the vicinity of the Middle Ural Copper-Smelting Plant. In polluted areas, the fecundity of adult birds is lower, mortality among the progeny is higher, and changes in the physiological parameters of birds are observed. The effects of toxic load are better manifested in nestlings than in adult birds. They include an increase in the liver index, the reduction of hemoglobin concentration, and an increase in the proportion of immature erythrocytes in the peripheral blood. The proportion of anemic birds also increases. The weakening of nestlings facilitates their infestation by the larvae of parasitic flies. Changes in the differential blood count of nestlings reflect the combined impact of toxic pollutants and parasites.

*Key words*: heavy metals, pied flycatcher, reproductive parameters, liver, red blood, differential blood count, ectoparasites, *Ficedula hypoleuca, Protocalliphora azurea*.

To make an adequate assessment of reproductive success, it is necessary to take into account the quality of progeny, which largely depends on their physiological state. The parameters of some metabolically active organs and systems that readily respond to external influences have long been used for estimating the state of animal health upon exposure to various ecological factors and for biological indication of environmental pollution (Shvarts *et al.*, 1968; Bogach *et al.*, 1988; Lebedeva, 1996). They include the indices of the liver, kidney, and some other organs, as well as hematological parameters. The importance of studies on the blood system stems from the fact that this internal medium of the body plays a key role in the maintenance of homeostasis and, in particular, provides for resistance to infections and other deleterious influences. The liver performs a number of functions and plays an important role in metabolic processes, detoxification of xenobiotics, and hematopoiesis.

Specialists have accumulated abundant data on the influence of heavy metals on the physiological state of birds, but relevant studies have been mainly performed under laboratory conditions. The biological effects of toxic exposure on birds in nature are known to differing extents. Effects at the population level have been studied better. In particular, this concerns changes in reproductive parameters, such as clutch size, reproductive success, and nestling mortality. Data on the effects of heavy metals on morphophysiological parameters of birds are less abundant. Some relevant studies on nestling pied flycatchers and great tits were performed near enterprises of the nonferrous metal industries in Scandinavia and the Kola Peninsula (Nyholm, 1995; Eeva *et al.*, 2000; Gilyazov, 2001). Their authors concentrated either on changes in the indices of internal organs (Nyholm, 1995) or on biochemical parameters (Eeva *et al.*, 2000). Blood tests were usually limited to determining hemoglobin concentration and hematocrit, whereas the multicomponent toxic effect of pollutants on the red and white blood counts of birds in nature received little attention.

The purpose of this study was to analyze the influence of toxic environmental pollution on the body weight, liver index, and main hematological parameters of nestling pied flycatchers (*Ficedula hypoleuca* Pall.) in the Middle Urals.

## MATERIAL AND METHODS

Studies were performed in the vicinity of the Middle Ural Copper-Smelting Plant (MUCSP) in 2000 and 2001. The plant is a major source of emissions containing sulfur dioxide and metal-enriched dust. In 2000, the total amount of emissions reached 62000 t, including 56300 t of sulfur dioxide (*Gosudarstvennyi doklad…*, 2001).

**Table 1.** Concentrations of metals in the soil (movable forms) and in the liver of nestling pied flycatchers in zones with different pollution levels, μg/g air-dry weight

| Substrate | Metal | Zone | | |
|---|---|---|---|---|
| | | reference | buffer | impact |
| Soil | Pb | 67.3 ± 4.9 | 283.2 ± 14.1** | 639.1 ± 43.2** |
| | Cd | 2.0 ± 0.2 | 8.0 ± 0.8** | 6.5 ± 0.4** |
| | Cu | 86.9 ± 7.6 | 862.4 ± 63.9** | 3769.6 ± 151.9** |
| | Zn | 135.2 ± 18.3 | 366.0 ± 34.2** | 241.8 ± 11.8** |
| *n* | | 32 | 42 | 47 |
| Liver | Pb | 5.5 ± 0.9 | 10.1 ± 1.7* | 21.7 ± 2.8** |
| | Cd | 1.0 ± 0.1 | 1.0 ± 0.1 | 2.6 ± 0.3** |
| | Cu | 16.1 ± 1.0 | 14.9 ± 1.3 | 20.2 ± 2.2 |
| | Zn | 92.5 ± 6.2 | 125.8 ± 11.0* | 145.3 ± 8.8** |
| *n* | | 31 | 27 | 22 |

Note: Differences from the background parameters are significant at * $p < 0.05$ or ** $p < 0.001$.

The proportion of heavy metals in the total amount of pollutants is approximately 4%.

Three zones differing in toxic load were distinguished along the pollution gradient: the impact zone (within a distance of up to 3 km around the plant), the buffer zone (3–15 km), and the reference zone (more than 15 km west of the plant, against the prevailing wind direction). Two to four test plots with artificial nestboxes were established in each zone to perform observations on hole-nesting passerine birds (the pied flycatcher was the most abundant species among them). The toxic load on the plots was estimated from the concentrations of heavy metals in the A1 soil horizon, determined in 5% $HNO_3$ extracts.

Nestboxes were regularly examined to record the numbers of eggs, hatched chicks, and fledglings in each nest. Fledglings at the age of 14 days (before leaving the nest) were weighed on a KERN electronic balance to an accuracy of 0.01 g. Two to three nestlings were temporarily taken from the nest to collect blood samples from the brachal vein. Hemoglobin concentration was determined and blood smears were prepared in the field. The smears were fixed in the May-Grunwald solution, stained according to Romanovsky, and studied under a microscope in the laboratory. We determined the proportions of mature and immature erythrocytes (per 200 cells) and took white blood counts (per 100 cells), recording the numbers of pseudoeosinophils (juvenile, band, and segmented), eosinophils, basophils, monocytes, and lymphocytes.

Nestlings were examined for the presence of parasitic fly larvae (*Protocalliphora azurea* Fll.) under the skin or for the signs of such invasion. One medium-sized nestling from each brood was sacrificed to perform morphophysiological studies and chemical analysis of the liver. Immediately after dissection, the liver was weighed to an accuracy of 0.001 g. The liver index was determined as a liver-to-body weight ratio expressed in percent. Liver tissue samples were dried and assessed for the contents of heavy metals (copper, lead, zinc, and cadmium) by atomic absorption spectrophotometry using an AAS-3 instrument.

## RESULTS

The toxic load on natural ecosystems and birds in the vicinity of MUCSP was estimated from the concentrations of heavy metals in the soil and liver tissue, respectively (Table 1). The amounts of toxic substances accumulated in the soil adequately reflect the chemical composition of emissions and the intensity of pollution of a concrete area. The greatest increase in concentration along the pollution gradient (by a factor of more than 40) was observed for copper, whereas zinc concentration increased only 1.8-fold. The concentrations of lead and cadmium in the soil near the plant exceeded the background concentrations by factors of 9.5 and 3.3, respectively. The concentrations of heavy metals in the liver increased as well. Significant differences in concentrations along the pollution gradient were revealed for lead, cadmium and zinc; the concentrations of copper in the livers of nestlings from the impact and control (background) zones differed insignificantly.

Table 2 shows the main reproductive parameters of the pied flycatcher recorded in the pollution gradient in 2000 and 2001. Differences between these parameters in the impact and reference zones are highly significant: the average values of clutch size differ by a factor of 1.5; the numbers of hatched chicks and fledglings per nest (excluding abandoned clutches), by a factor of 2; egg mortality near the plant is 3.5 times higher than in the background zone, and nestling mortality is 1.7 times higher (in this case, however, the difference is

**Table 2.** The main reproductive parameters of pied flycatchers in zones with different pollution levels

| Parameter | Zone | | |
|---|---|---|---|
| | reference | buffer | impact |
| Number of eggs per nest | 6.4 ± 0.1 | 6.3 ± 0.1 | 4.4 ± 0.3* |
| *n* | 131 | 56 | 24 |
| Number of hatched chicks | 5.9 ± 0.1 | 5.7 ± 0.2 | 3.0 ± 0.4* |
| *n* | 116 | 54 | 24 |
| Number of fledglings per nest | 5.5 ± 0.1 | 5.5 ± 0.2 | 2.8 ± 0.4* |
| *n* | 118 | 54 | 23 |
| Egg mortality, % | 7.8 ± 1.0 | 9.8 ± 1.6 | 27.0 ± 4.4* |
| *n* | 740 | 336 | 100 |
| Nestling mortality, % | 7.1 ± 1.0 | 5.1 ± 1.3 | 12.3 ± 3.8 |
| *n* | 675 | 297 | 73 |

* Differences from the background parameters are significant at $p < 0.001$.

**Table 3.** Body weight, liver index, and parameters of infestation by *Protocalliphora azurea* 14-days old larvae in nestling pied flycatchers from zones with different pollution levels

| Parameter | Zone | | |
|---|---|---|---|
| | reference | buffer | impact |
| Body weight, g | 13.25 ± 0.12 | 14.00 ± 0.13 | 13.46 ± 0.21 |
| *n* | 116 | 91 | 45 |
| Liver index, % | 4.28 ± 0.09 | 5.15 ± 0.26** | 5.72 ± 0.34*** |
| *n* | 29 | 28 | 20 |
| Proportion of infested chicks, % | 1.24 ± 0.62 | 21.43 ± 3.31** | 20.63 ± 5.10** |
| Average number of fly larvae per infested chick | 1.75 ± 0.48 | 2.58 ± 0.42 | 3.77 ± 0.73* |
| Average number of fly larvae per chick in total sample (including noninfested chicks) | 0.02 ± 0.01 | 0.55 ± 0.12*** | 0.78 ± 0.24** |
| *n* | 322 | 154 | 63 |

Note: Differences from the background parameters are significant at * $p < 0.05$, ** $p < 0.01$, or *** $p < 0.001$.

statistically nonsignificant). The parameters recorded in the buffer zone are close to the control values.

The average body weight of fledglings proved to be virtually independent of the toxic load (Table 3). Its values in the impact and reference zones were virtually the same, and fledglings examined in the buffer zone were even larger than in the reference zone.

In polluted areas, we revealed a number of nestlings infested by the larvae of parasitic flies *Protocalliphora azurea* Fll. The larvae were found under the skin mainly on the upper body part (on the head and wings), which are more easily accessible to the flies that enter the birdhouse to lay eggs. When mature, the larvae wriggle from under the skin and leave the bird. The wounds they inflict may heal, but feathers at the affected sites do not regrow. Both the proportion of infested nestlings and the intensity of infestation increased in polluted zones, with the increase in the former parameter being more distinct (Table 3): the proportion of infested nestlings was significantly greater than in the control even in the buffer zone, whereas the number of larvae per nestling significantly exceeded the control value only in the impact zone.

The liver index of the nestlings was significantly greater in the impact zone than in the reference zone: the respective average values were 6.0 ± 0.4% ($n = 9$) vs. 4.2 ± 0.1% ($n = 17$) in 2000 and 5.5 ± 0.5 ($n = 11$) vs. 4.2 ± 0.1% ($n = 17$) in 2001. The indices of individual nestlings varied from 3.4 to 5.4% in the reference zone, from 3.4 to 9.0% in the buffer zone, and from 3.5 to 9.8% in the impact zone. We revealed a strong correlation between the number of parasitic fly larvae infesting a nestling and the liver index: $r = 0.74$ at $p < 0.001$ ($n = 77$).

The results of blood tests (Table 4) revealed significant changes in the parameters of red blood along the pollution gradient. In particular, the average hemoglo-

**Table 4.** Differential blood counts of nestling pied flycatchers from zones with different pollution levels

| Parameter (%) | Zone | | |
|---|---|---|---|
| | reference | buffer | impact |
| Hemoglobin, g/l | 110.1 ± 1.9 | 101.1 ± 2.6* | 97.9 ± 2.7* |
| *n* | 55 | 52 | 45 |
| Immature erythrocytes | 9.1 ± 0.8 | 11.5 ± 0.8 | 16.9 ± 1.4 |
| Leukocytes: | | | |
| Pseudoeosinophils | 8.2 ± 1.2 | 4.5 ± 0.7 | 7.7 ± 1.3 |
| Including: | | | |
| juvenile | 0.5 ± 0.2 | 1.0 ± 0.3 | 0.8 ± 0.2 |
| band | 4.1 ± 0.6 | 1.9 ± 0.4 | 3.6 ± 0.6 |
| segmented | 3.6 ± 0.6 | 1.6 ± 0.3 | 3.3 ± 0.8 |
| Eosinophils | 3.8 ± 0.5 | 4.9 ± 0.7 | 5.4 ± 1.3 |
| Basophils | 11.2 ± 1.2 | 7.2 ± 0.8 | 10.3 ± 1.1 |
| Monocytes | 3.1 ± 0.6 | 3.5 ± 0.7 | 2.6 ± 0.4 |
| Lymphocytes | 73.7 ± 2.0 | 79.9 ± 1.8 | 74.0 ± 2.2 |
| *n* | 58 | 59 | 48 |

* Differences from the background parameters are significant at $p < 0.05$.

bin concentration was significantly lower in nestlings from polluted areas. An analysis of the distribution of nestlings with respect to hemoglobin concentration showed that this parameter in 95% of individuals in the control sample was no less than 91 g/l. This value was taken as the lower limit of the norm, and nestlings with a lower hemoglobin concentration were considered to be anemic. The proportions of such nestlings in the buffer and impact zones were 26.9 and 31.1%, with differences from the control being significant at $p < 0.01$.

A fairly strong negative correlation was revealed between hemoglobin concentration in the blood and heavy metal content in the liver of nestlings not infested by ectoparasites ($r = -0.60$ at $p < 0.001$, $n = 42$). Blood hemoglobin in infested nestlings was lower than in healthy (not infested) nestlings from the same zones: its relative content was 92% in the impact zone and 77% in the buffer zone. The average proportion of functionally inactive (immature) erythrocytes increased by a factor of 1.9 along the pollution gradient and was 1.6–1.7 times greater in infested nestlings than in healthy nestlings from the same zones.

Changes in the white blood count along the pollution gradient lacked statistical significance, but some tendencies could be observed (Table 4). Deviations from the control count were more apparent in the buffer zone. They concerned virtually all cell forms: a decrease was observed in the total content of pseudoeosinophils (due to a smaller number of functionally active forms) and basophils, whereas the proportions of eosinophils, monocytes, and leukocytes proved to be slightly increased. The ratio of these cell forms in nestlings from the impact zone was close to the reference. Nestlings infested by ectoparasites had an increased content of lymphocytes and eosinophils and a reduced proportion of basophils: in the impact zone, the proportions of eosinophils and basophils in these birds were 11.4 and 5.9%, compared to 4.0 and 11.3%, respectively, in healthy individuals. A significant linear correlation was revealed between lymphocyte content and intensity of infestation ($r = 0.20$ at $p < 0.05$, $n = 139$).

## DISCUSSION

The concentrations of heavy metals in the livers of nestling pied flycatchers change along the pollution gradient in the same way as their concentrations in the soil, but the magnitude of these changes is lower: lead concentration in the liver increases by a factor of 3.9; cadmium concentration, by a factor of 2.6; and copper and zinc concentrations, by factors of 1.3 and 1.6, respectively. This is due to the partial loss of some metals in the course of their migration along the food chains and to the presence of special mechanisms regulating the concentrations of physiologically significant elements (such as copper and zinc) in the animal body. Similar differences in the accumulation of copper and zinc, compared to arsenic, cadmium, mercury, and lead, were observed in the livers of nestling pied flycatchers from the vicinity of a metallurgical plant in Sweden (Nyholm, 1995).

The retarded growth and reduced body weight of nestlings in polluted areas have been observed in a number of bird species, including *Passer montanus* and *P. domesticus* (Pinowski *et al.*, 1993), *Parus major* (Knistautas, 1982; Eeva and Lehikoinen, 1996), and

*P. ater* (Bel'skii *et al.*, 1995). In the region of our study, we found the average body weight of fledgling pied flycatchers to be virtually independent of pollution level. The absence or inconsistency of the relationship between nestling body weight and toxic load in this species was also observed by other researchers (Knistautas, 1982; Eeva and Lehikoinen, 1996). This may be attributed to the reduction of clutch size due to a decrease in female fecundity and an increase in chick mortality; as a result, the amount of food available to each nestling in the impact zone should be greater than in the reference zone. Apparently, food supply is not a limiting factor for the pied flycatcher in degraded areas.

The liver receives special attention in studies dealing with toxic effects, because this organ is responsible for a number of vitally important functions, such as digestion, and metabolic functions, including the synthesis of proteins, lipids, polysaccharides, and some vitamins; the maintenance of immunity; and the neutralization of toxic substances. The liver plays an important role in hematopoiesis, utilizes destroyed red blood cells, synthesizes hemoglobin, and is a major blood depot (Romanenko, 1978).

The toxic effects of heavy metals on the animal body, including the liver, have been thoroughly studied under laboratory conditions (Hoffman *et al.*, 1985; Daskalova, 1989). Studies performed in nature, where a combined toxic influence is complicated by other factors, are far less numerous (Grue *et al.*, 1986; Nyholm, 1995).

The degree of manifestation of an effect depends primarily on the strength and period of the corresponding influence. A chronic toxic load exceeding the adaptive potential of a bird organism results in damage to the liver. For example, the morphophysiological disturbances observed in settled bird species living near a metallurgical combined works in Bulgaria include interstitial and granulomatous hepatitis (Donchev and Stoyanov, 1984). Our observations continued for only 14 days, as long as the chicks remained in the nest. However, even so short a period was sufficient for recording changes in the functional state of the liver, because ontogenetic processes are highly vulnerable to external influences. All organ systems of nestlings continue to develop in this period, which implies a high intensity of metabolic processes, and the input of toxic agents markedly increases the load on the liver.

The liver plays a key role in the neutralization of toxicants. In particular, metallothioneins and bile acids synthesized in the liver are responsible for the binding, transport, and removal of heavy metals from the body (Romanenko, 1978). Many organic compounds are also metabolized in the liver. The input of toxic agents activates oxygenases in liver microsomes. A high oxygenase activity entails the production of reactive oxygen species, lipid peroxidation (LPO), and an accumulation of LPO products (Archakov, 1975). Intensification of LPO was observed in nestling pied flycatchers and great tits near the MUCSP (Bel'skii and Stepanova, 1995). Activation of liver oxygenases with mixed functions was also recorded in nestling pied flycatchers in the vicinity of a copper–nickel plant in Finland (Eeva *et al.*, 2000).

Vitamins A and E are endogenous antioxidants inactivating free radicals that can damage biological membranes (Kukhtina *et al.*, 1983). The concentrations of these protectors in the livers of nestling pied flycatchers and great tits decrease under increasing technogenic load (Bel'skii and Stepanova, 1995). Depletion of their resources entails an excessive accumulation of LPO products, which may lead to membrane damage, disturbances in the enzyme systems metabolizing xenobiotics, and toxic effects.

Hyperfunctioning of the liver under a toxic load leads to an increase in its size. This was observed in mallards wintering on a polluted water body in Belarus (Kozulin and Pavluschick, 1993) and in nestling pied flycatchers in the vicinity of a metallurgical plant in Sweden, in which the liver index increased from 4.2% in the control to 4.5% near the plant (Nyholm, 1995). In the region of our study, this increase was more pronounced (Table 3).

The effects of heavy metals on the blood system have been revealed in laboratory experiments. Thus, lead and cadmium at increased concentrations cause premature erythrocyte destruction, disturbances of erythropoiesis in the bone marrow, a decrease in blood hemoglobin, and the inhibition of some enzymes (Hoffman *et al.*, 1985; Daskalova, 1989; Ochiai *et al.*, 1993). Our data also show that parameters of the red blood, such as hemoglobin content and the ratio of mature and immature erythrocytes, are highly sensitive to chemical pollution, with toxic effects being more pronounced in nestlings than in adult birds.

The increasing toxic load leads to an inhibition of respiratory processes in nestlings and their general weakening. Anemia is often diagnosed in the nestlings from polluted areas. In the impact zone, the average hemoglobin concentration decreases by more than 11%, and the proportion of anemic nestlings is six times greater than in the control. A similar decrease in blood hemoglobin under the impact of industrial emissions and automobile exhausts was observed in nestling pied flycatchers in Sweden (Nyholm, 1995) and in American kestrels (Henny *et al.*, 1994) and starlings in the United States (Grue *et al.*, 1986). The proportion of mature erythrocytes decreases because of their premature degradation. In polluted areas, this creates an additional load on the liver, as this organ is responsible for the utilization of degraded cells. To compensate the functional insufficiency of the oxygen transport system, immature erythrocytes are urgently released from the bone marrow into the peripheral blood.

The immune status of birds deteriorates upon toxic exposure, and their resistance to infections and invasions decreases. Correlations were revealed between the level of environmental pollution and the rate of nematode infestation in pheasants (Novakova and Tremilova, 1971) or the abundance of *Ceratophyllus gallinae* fleas in the nests of pied flycatchers near a metallurgical plant in Finland (Eeva *et al.*, 1994). We observed such a correlation for the degree of infestation by *Protocalliphora azurea* larvae in nestling pied flycatchers. Parasitic infestation markedly affects physiological processes, leading to a decrease in hematocrit value and body weight, retarded growth, and the eventual death of nestlings (Bakkal, 1980; Kerimov *et al.*, 1985; Heeb *et al.*, 2000). According to our observations, infested nestlings had a lower hemoglobin level and a higher proportion of immature erythrocytes in the peripheral blood than healthy nestlings. In addition, they were characterized by a reduced body weight, irregular plumage, and sluggish behavior. Such nestlings were apparently vulnerable to secondary infection by pathogenic microorganisms through the wounds inflicted by parasites. This parasitic infestation aggravated the toxic impact of pollutants on nestlings.

Leukocytes are involved in the immune response, phagocytosis, and neutralization of toxins (Kudryavtsev and Kudryavtseva, 1974). Changes in the white blood count and, in particular, the increased proportion of juvenile pseudoeosinophils in nestlings from polluted areas are evidence for the acceleration of granulocytopoiesis and the premature release of these cells into the peripheral blood. Changes in some hematological parameters in the pollution gradient lack statistical significance, which may be due to the fact that some weakened nestlings perished at early developmental stages: nestling mortality in the impact zone exceeded the control level by a factor of 1.7. Hence, "marginal" individuals were not included in the sample and had no effect on its statistical characteristics (Bezel' *et al.*, 2001).

The increased proportions of eosinophils and lymphocytes apparently reflect the response of nestlings to the combined influence of toxic pollutants and parasites. Eosinophilia is usually manifested in diseases involving an allergic reaction. Lymphocytes are the cells playing a key role in the immune response. They produce antibodies and deliver them to the foci of inflammation and inactivate various toxins (Kudryavtseva and Kudryavtsev, 1974). Their increased concentration is evidence for the presence of inflammation foci at the sites of parasitic invasion in nestlings affected by toxic pollutants.

In conclusion, it should be noted that changes in hematological parameters of nestling pied flycatchers along the pollution gradient reflect not only the direct toxic influence of pollutants, but also indirect effects related to hyperfunctioning and hypertrophy of the liver and infestation by parasites.

Thus, toxic agents in emissions from the copper-smelting plant cause serious changes in the physiological parameters of nestling pied flycatchers. Some of these changes, such as the intensification of detoxification processes and deviations in the white blood count, are within the limits determined by the adaptation potential of nestlings. Other changes are pathological. In particular, this concerns the suppression of the respiratory function of the blood (hemolytic anemia). To evaluate changes occurring in the liver, histological studies are required. Infestation of nestlings by ectoparasites is an important factor aggravating the impact of toxic pollutants.

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