## Study on the effects of migration and lead pollution on host immunity, and infection of influenza A virus in Black-Headed Gull (*Chroicocephalus ridibundus*)

Abstract of Doctoral Thesis

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Migratory birds are highly susceptible to diseases during their migration period because of the energy trade-off between immune and migration, which results in immunosuppression. This study was conducted on Black-headed gulls (*Chroicocephalus ridibundus*) with high infection rate to influenza A virus, and the basic information about their immunity and body condition, such as body mass, muscle mass, and fat mass, were unknown. The purpose of the study was to determine the effect of lead (Pb) pollution, which results in immunosuppression and changes in body condition during the migration period on the gulls' immunity, and to check if they cause increased infection rate during the migratory period.

First, a study was performed to evaluate the effect of body condition and Pb pollution on the immunity. The gulls were captured from the two areas, Tokyo-bay and Mikawa-bay, and classified based on the three seasons of migration in winter, namely autumn migration, wintering, and spring migration. Pb level in peripheral blood was significantly different in all three seasons and was highest in the wintering season, followed by spring and autumn migrations. Among immune parameters, the proportion of heterophils and lymphocytes had a significant relationship with the blood Pb level, and it was found that an increase in blood Pb level confers immunosuppression. In addition, it was clarified that a blood Pb level of 4.0  $\mu$ g/dL causes immune suppression. For analyzing the effect of body condition, body condition index (BCI) was set using principal component analysis. On analyzing its relationship with immune components tested, BCI had a positive correlation with the proportion of lymphocytes and the number of white blood cells. It could thus be considered that BCI plays a role in maintaining immune homeostasis.

We found that the proportion of heterophils and lymphocytes in the spring migration was different from that other period. These significant results indicate that there is an energy trade-off between migration and immunity during the spring migration and even during the wintering, suggesting that BCI and Pb contamination have a significant effect on immunity in this period.

Next, the infection status of influenza A was evaluated by enzyme-linked immunosorbent assay (ELISA). The qualitative test results obtained via ELISA were defined as the history of infection and the absorbance, which were measured based on the amounts of antibody. The test results showed that both the parameters increased significantly only during the spring migration. There was a significant difference between BCI and blood Pb levels depending on history of infection, and the group with low BCI and high blood Pb levels had a higher history of infection than the group with high BCI and low blood Pb levels. Finally, it was analyzed whether the BCI and blood Pb levels were related to the history

of infection and the amounts of antibodies for each period. No significant relationship was found during the autumn migration, and high blood Pb levels were seen along with an increased infection history of influenza A infection during wintering. In the spring migration, the history of infection was high whereas the blood Pb levels increased and BCI decreased, and the amounts of antibodies increased as BCI decreased. These results demonstrate the effects of both BCI and blood Pb levels on the history of infection during the spring migration. In addition, gulls' the existing findings indicate that the amounts of antibodies during the spring migration and the history of infection significantly increased in the Black-headed gulls, suggesting that the characteristics are also related.

This study is expected to contribute to conservation biology related to species diversity by providing an insight into the role of migratory birds in the infection cycle of infectious pathogens and the effects of domestic environmental pollutants through migratory birds.