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THE ROLE OF THE GREATER WHITE-FRONTED GOOSE, *ANSER ALBIFRONS* (SCOPOLI, 1769) (ANSERIFORMES: ANATIDAE) IN MAINTAINING THE NATURAL CIRCULATION OF THE INFLUENZA A VIRUS IN THE NORTHWESTERN PART OF BLACK SEA REGION (UKRAINE)

Haidash O. M.^{1,2}, Muzyka D. V.^{1,3,4}¹ H. S. Skovoroda Kharkiv National Pedagogical University, Kharkiv, Ukraine, e-mail: alevgaidash@gmail.com² Danube Biosphere Reserve, Vylkove, Ukraine.³ National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine', Kharkiv, Ukraine⁴ Linnaeus University, Kalmar, Sweden

Summary. The publication combines the results of ornithological observations of the greater white-fronted goose, *Anser albifrons* (Scopoli, 1769) during migration and wintering in the south-western part of Odesa Region of Ukraine (Bilhorod-Dnistrovskyi, Izmail, and Bolhrad districts) and the results of laboratory tests of biological samples of this species for the presence of the influenza A pathogen. The study was conducted in 2017–2019. A total of 1,591 samples of biological material (feces) of the greater white-fronted goose, collected in 48 locations in Odesa Region, were examined using polymerase chain reaction (PCR). The influenza A virus genome was detected in 23 samples. The total annual prevalence was 1.44%, in winter — 1.44%, in spring — 1.81%. During the fall migration, no influenza A virus was detected in the genome samples, which determines the wintering period as the most important stage of integration of the greater white-fronted goose in maintaining the natural circulation of influenza A in the study area. By year, the prevalence was distributed as follows: in 2017 — 1.75%, in 2018 — 1.97%, in 2019 — 0.78%. All cases of detection of influenza A virus genome in fecal samples of the greater white-fronted geese concerned areas near large bodies of water in the coastal part of the Black Sea (Sasyk Lagoon and Tuzly Lagoons). Among the factors that may determine the high involvement of the greater white-fronted goose in the circulation of influenza A in these areas are the presence of other sources of infection: numerous dense multi-species aggregations of birds in the wetland complex and favorable conditions for pathogen transfer

Keywords: epizootological monitoring, wild birds, prevalence, Odesa Region, polymerase chain reaction

Introduction. Today, studying and controlling the circulation of hazardous pathogens in a natural reservoir is vital. One of these pathogens is the influenza A virus, which threatens the health of mammals, birds, and humans. Today, this pathogen is considered to be one of those that could cause the next pandemic. Wild waterfowl are known to be the main natural reservoir of the influenza A virus in the world (Swayne, Suarez and Sims, 2013). Studies in Ukraine have also revealed widespread circulation of this pathogen among wild birds (Muzyka et al., 2012; Stegnyy et al., 2018). At the same time, the role of certain species of waterfowl and shorebirds has not been sufficiently studied, especially in Ukraine. One of these species is the greater white-fronted goose, *Anser albifrons* (Scopoli, 1769).

The greater white-fronted goose is a migratory, wintering species of the fauna of Ukraine. The wintering areas of the species are located in Azov-Black Sea Region and the lower part of the Dnipro (Fesenko and Bokotej, 2007). There are two main migratory routes of the greater white-fronted geese in Ukraine: northern and southern. Both routes are latitudinal, i. e. the general direction of flight is from east to west in the fall and from west to east in the spring (Poluda, 2009). During the

migration period, geese nesting in northern Eastern Europe, Western Siberia, and Taymyr are found in Ukraine. Birds migrating through Ukraine spend the winter in Western Europe (the Netherlands, Belgium, Great Britain), Central Europe (Middle Danube Plain), and Black Sea Region (Balkan Peninsula, Southern Ukraine — Odesa, Mykolaiv, Kherson, Zaporizhzhya regions, and AR Crimea) (Poluda, 2009). According to some ornithologists, the total number of the greater white-fronted geese migrating through Ukraine may reach more than one million individuals (Poluda, 2009).

An important ecological feature of this species is that during migration and wintering in southern Ukraine, birds form numerous aggregations in feeding and resting areas (large reservoirs, agricultural landscapes), where they come into contact both with other groups of their species and with other species of birds in the wetland complex. Such dense aggregations create optimal conditions for the exchange of pathogens, including the influenza A virus.

The number of wintering the greater white-fronted geese in Ukraine decreased from 82,953 birds in 2005 to 3,116 birds in 2017, according to the results of annual January censuses in 2005–2017 (Andryushchenko et al.,

2019). However, according to the results of similar population censuses in 2018–2021, the number of the species wintering in the mainland of Ukraine was 22,038 birds in 2018, 17,011 birds — in 2019, 17,440 birds — in 2020, 15,823 birds — in 2021, and 26,970 birds — in 2022 (Results ..., 2023).

Thus, taking into account the wide circulation of influenza A virus in the natural reservoir in Ukraine, as well as the peculiarities of the migratory behavior of the greater white-fronted goose, its migratory connections, its number during migration and wintering, this study aimed to determine the role of the greater white-fronted goose as one of the natural reservoirs in maintaining the natural circulation of influenza A virus in Ukraine based on the results of ornithological and virological monitoring.

Materials and methods. Ornithological studies were conducted in 2017–2019. Field material was collected during field visits to the territory of the south-western part of Odesa Region (Bilhorod-Dnistrovskiy, Izmail, and Bolhrad districts). The territory was surveyed by car, boat and on foot. During the survey of the study area, absolute bird counts were made of the wetland complex within the waters and adjacent areas of large water bodies (the Danube lakes, Tuzly Lagoons, Sasyk Lagoon, and the Danube Delta). In cases where absolute counts were not possible, the method of relative (point) counts was used. Counts were conducted during daylight hours, starting 30 minutes after sunrise and ending 30 minutes before sunset. During bird counts, geographical location, species composition and number were recorded. During the collection of field material, $\times 10$ binoculars, $\times 20$ – 60 telescopes, cameras with 300–500 mm lenses, GPS navigators and drones were used. The collected data were recorded in a field diary and an electronic database. The field data were processed using MS Excel and QGIS software.

The collection of biological material (feces) for laboratory tests to detect the genome of the avian influenza virus was carried out in 2017–2019 in the south-western part of Odesa Region (Bilhorod-Dnistrovskiy, Izmail, and Bolhrad districts) within the framework of monitoring studies on the circulation of particularly dangerous infections in wild birds in Ukraine under the state scientific research of the National Scientific Center ‘Institute of Experimental and Clinical Veterinary Medicine’ and the international partner project UP4 ‘Analysis of risks associated with certain particularly dangerous pathogens that can be transmitted by migratory birds in Ukraine’.

Fecal samples were collected by standard methods using sterile applicators (FLOQSwabs, Copan Flocked Swabs). Brain Heart Infusion Broth (BHIB), manufactured by Sigma-Aldrich (USA) in a mixture with antibiotics, pH (7.4 ± 0.2) was used as a transport medium (Spackman et al., 2013). After field collection, samples were immediately placed in liquid nitrogen in a Dewar vessel (-196°C). Before molecular biological studies, biological samples were stored in liquid nitrogen

at -196°C (in the field) and -80°C (in the laboratory). Sampling, transport, and storage were performed under biosafety requirements.

The RNA was extracted from fecal samples using QIAamp Pathogen Mini Kit (Qiagen). Positive AIV diagnosis was based on real-time RT PCR (qRT-PCR) using universal primers targeting the Matrix Protein (MP) gene (Spackman, 2014).

Results. Ornithological studies of the greater white-fronted goose. During 2017–2019, 106 flocks of the greater white-fronted geese with a total number of 76,053 birds were recorded during field surveys (Table 1, Fig. 1). During the observation period, flocks of the greater white-fronted geese were recorded from September to April. The highest number of flocks was recorded in winter — 47.2% ($n = 50$). The total number of recorded flocks was 39,903 birds in winter and 34,139 birds in spring, which is 52.5% and 44.9%, respectively. The highest number of birds was recorded in 2018. Flocks were recorded in agricultural landscapes (mainly winter wheat and rapeseed fields), wetlands, and in the air. In 55.7% of the cases, accompanying bird species were present in the flocks of the greater white-fronted geese or near the flocks. The localization of concentrations of the greater white-fronted geese has been linked to large bodies of water: the Danube lakes (China Lake, Katlabukh Lake, Kugurlui Lake, Yalpus Lake, Kartal Lake, Kagul Lake, etc.), Sasyk Lagoon and the group of Tuzly Lagoons (Shahany, Alibey, Burnas, etc.) (Haidash and Yakovliev, 2024)

During migration and wintering, most concentrations of the species prefer areas bordering the Black Sea. In areas far from the sea, birds were recorded mainly during migratory movements (Fig. 1).

The birds fed mainly on winter crops and rapeseed fields, which are the dominant crops in the region.

Molecular biological studies. The results presented in this article are part of a wider study of wild birds in Ukraine. Thus, from December 2016 to January 2020, 14,502 samples of biological material from 53 species of wild birds of the wetland complex were collected in the northern and southern parts of the country. Of these, 3,072 samples were collected from the greater white-fronted geese at 91 sites. In particular, in the south-western part of Odesa Region from January 2017 to December 2019 (our target region and the research period in this article) 1,591 samples were collected in 48 locations (Table 1).

The results of laboratory screening indicate the presence of influenza A pathogen among migratory and wintering groups of the greater white-fronted geese in the northwestern part of Black Sea Region. The circulation of the pathogen was recorded in winter and spring within the territories located along the Black Sea coast (Fig. 1). In total, the influenza A virus genome was detected in 23 samples of biological material from the greater white-fronted goose in the south-western part of Odesa Region (Table 2). Circulation of the influenza A virus was confirmed in 11 out of 48 flocks of this species.

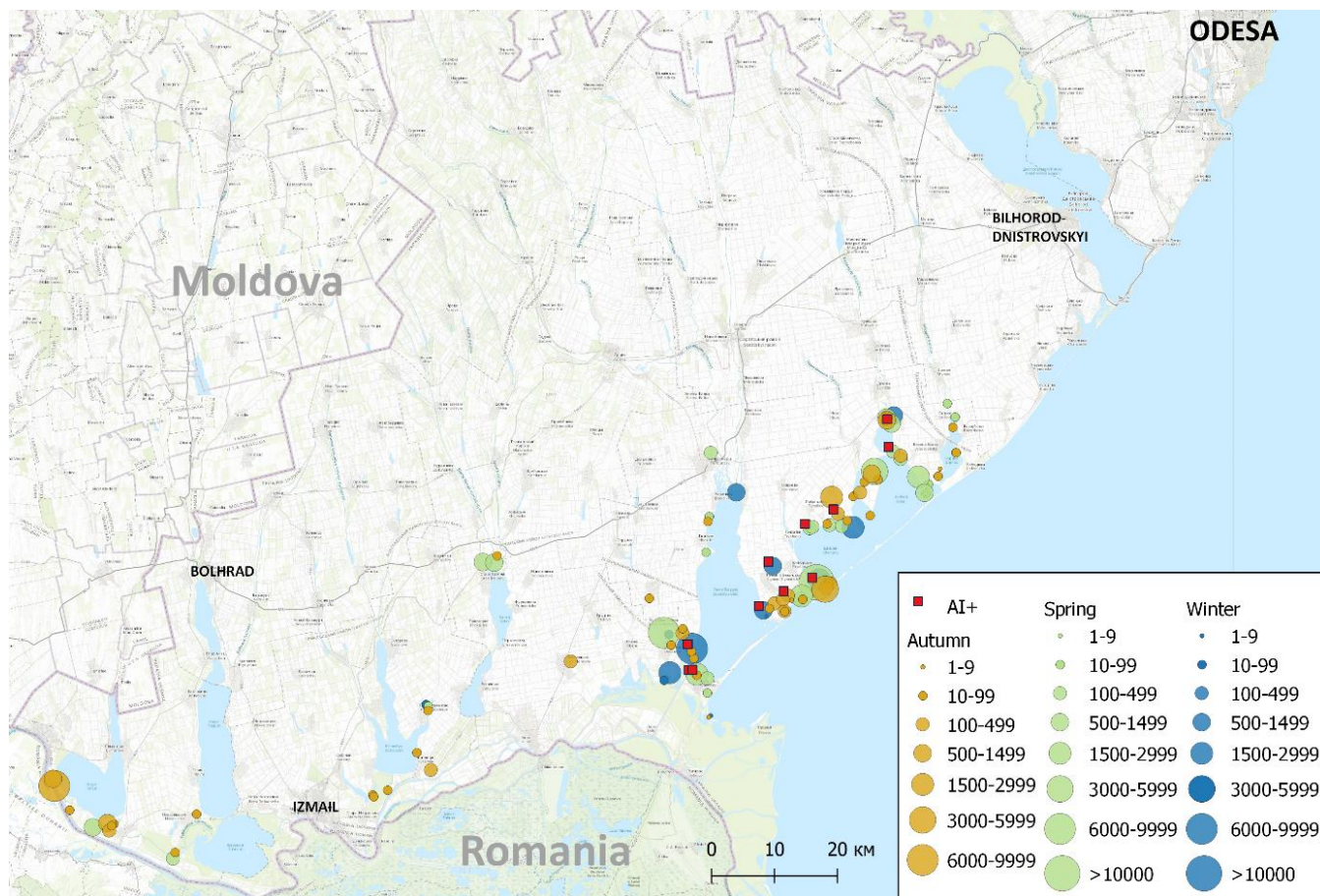


Figure 1. Location and number of flocks of the greater white-fronted goose, *Anser albifrons* (Scopoli, 1769) in the south-western part of Odesa Region in 2017–2019 and sampling sites where the influenza A virus gene was detected by PCR.

The prevalence in flocks in which the presence of influenza A virus was detected ranged from 2.00% to 13.33%. In one case the prevalence was 50.00%, but the study included biological material from only 2 geese, one of which was positive for influenza A. The average prevalence was 1.44%. The prevalence was 1.44% in winter and 1.81% in spring. No infected the greater white-fronted geese were detected in the fall period. The prevalence was distributed by years as follows: in 2017 — 1.75%, in 2018 — 1.97%, in 2019 — 0.78%.

Table 1 — Summary of the results of the registration of the greater white-fronted goose, *Anser albifrons* (Scopoli, 1769) in the south-western part of Odesa Region in 2017-2019 and the sampling of biological material

Parameters	Year			Total
	2017	2018	2019	
Identified flocks	48	38	20	106
Total number of birds counted	26,680	43,700	5,673	76,053
Flocks sampled	20	13	15	48
The total number of samples collected	398	558	635	1,591

Discussion. The results of molecular biological studies and the detection of the influenza A virus genome in the populations of the greater white-fronted geese migrating and wintering in the south-western part of Odesa Region of Ukraine indicate the involvement of the species in the process of influenza A circulation.

The absence of pathogen detection during the fall migration may indicate both insufficient sampling (101 fecal samples collected during the fall period were examined) and the fact that most birds acquire the pathogen during the wintering period, in contact with other bird species in the wetland complex in feeding, resting and roosting areas. This hypothesis is supported by some other studies (Yin et al., 2017; Ely et al., 2013).

During similar studies on the circulation of influenza A pathogen in flocks of the greater white-fronted geese in southern Ukraine in 2010–2011 during the fall migration of the virus among birds, no virus was detected, while 9 isolates were obtained from wintering birds (Muzyka et al., 2012). Similarly, a highly pathogenic avian influenza virus was isolated from the greater white-fronted geese in the Askania Nova reserve in 2016 during the wintering period (Stegniy et al., 2018). The role of the greater white-fronted goose as a vector of infection in the wintering grounds during fall migration needs to be clarified.

Table 2 — The results of molecular genetic studies of biological samples from the greater white-fronted goose, *Anser albifrons* (Scopoli, 1769) for the presence of the influenza A virus genome in the south-western part of Odesa Region

Date	The nearest settlement to the sampling site/habitat	Number of samples collected / number of positive samples / prevalence
28.01.2017	Prymorske village, Izmail district/winter crops field	2 / 1 / 50.00%
09.03.2017	Balabanka village, Bilhorod-Dnistrovskiyi district/winter crops field	15 / 1 / 6.66%
09.03.2017	Prymorske village, Bilhorod-Dnistrovskiyi district/winter crops field	50 / 1 / 2.00%
10.03.2017	Prymorske village, Izmail district/winter crops field	15 / 2 / 13.33%
16.12.2017	Lyman village, Bilhorod-Dnistrovskiyi district/winter crop field	30 / 2 / 6.66%
25.02.2018	Prymorske village, Izmail district/winter crops field	120 / 3 / 2.50%
31.03.2018	Zhovtyi Yar, Bilhorod-Dnistrovskiyi district/wetlands	35 / 1 / 2.85%
01.04.2018	Vesela Balka village, Bilhorod-Dnistrovskiyi district/winter crops field	40 / 2 / 5.00%
01.12.2018	Balabanka village, Bilhorod-Dnistrovskiyi district/winter crops field	40 / 5 / 12.50%
14.01.2019	Prymorske village, Bilhorod-Dnistrovskiyi district/winter crops field	80 / 3 / 3.75%
14.01.2019	Lyman village, Bilhorod-Dnistrovskiyi district/winter crop field	55 / 2 / 3.63%

The absence of pathogen fixation among flocks of the species during fall migration may indicate a much lower prevalence than during wintering and spring migration.

The value of wintering grounds and stopover sites during migration for the greater white-fronted geese is determined by the availability of a trophic base (mainly winter wheat and rapeseed seedlings, grain residues, corn, sunflower, and soybeans), as well as the availability of watering and resting places with minimal impact of avian disturbance factors (Andryushchenko et al., 2019). In the south-western part of Odessa Region, such places are concentrated near large bodies of water. Firstly, fodder crops are concentrated along the banks of these reservoirs, while the reservoirs themselves are safe places for watering, resting, and roosting.

Large reservoirs in the northwestern part of Black Sea Region of Ukraine are concentrated in the lower reaches

of the Danube and the Dniester. Within our study area (the south-western part of Odesa Region) such reservoirs can be divided into 3 groups:

(i) The Danube lakes are freshwater lakes located at the mouth of the Danube, far from the sea (Lake Kagul, Lake Kurgului, Lake Kartal, Lake Kotlabukh, etc.).

They are large and rather deep lakes. The total water surface of the Danube lakes is about 468.7 km², with an average depth of 0.8–2.6 m and a maximum depth of 2–7 m, depending on the level of the Danube (Shvebs and Igoshin, 2003). During severe winters, the lakes can be completely covered with ice;

(ii) The Danube Delta (Ukrainian part) is an extensive system of the Danubian freshwater channels, canals, straits, inland freshwater lakes of various sizes and depths, bays, floodplain ecosystems, and the sea coast. The total area of the Danube Delta is 2500 km², but most of the delta is located in Romania (Klymenko, 2010). During low winter temperatures, a large part of the delta's water surface freezes;

(iii) Seaside lakes include the system of salt lakes Shahany–Alibey–Burnas and others (Tuzly Lagoons), and the desalinated Sasyk Lagoon. These are large lakes, most of which are shallow: the area of Sasyk Lagoon is 210 km², with an average depth of 2 m and a maximum depth of 3 m (Shvebs and Igoshin, 2003). The total area of the Tuzly Lagoons is more than 208 km², with an average depth of 0.6–1.2 m and a maximum depth of 1.0–2.5 m (Shvebs and Igoshin, 2003). The Sasyk reservoir is desalinated and connected to the Danube. Canals connect the Dzhantshey and Malyi Sasyk lakes to both Sasyk Lagoon and the saline Tuzly Lagoons, so the salinity of these lakes can vary considerably. The Sasyk reservoir freezes in severe winters, while other lakes can be ice-free due to their high salinity.

The epizootological potential for influenza A and other infections circulating in wild bird populations within these three groups of water bodies is determined, in particular, by the species richness and number of birds using these water bodies.

The analysis of the long-term monitoring of the wintering bird fauna within these groups of water bodies within the framework of the Regional Ornithological Monitoring Program shows that the Danube and the sea lakes are equivalent in terms of the number of species and the number of birds, while these indicators are higher in the Danube Delta (Table 3).

The largest number of bird species of the water and wetland complex and their abundance in the study area is usually concentrated in the Danube delta, which creates ample opportunities for the exchange of infections in places of congregation (Table 3). However, the number of the greater white-fronted geese in this area is small and varies from 1 to 264 birds in winter (Results ..., 2011, 2017, 2023). Quantitative indicators of wintering avifauna are primarily influenced by the ice regime of the water bodies in the Delta.

Table 3 — Comparative characteristics of the qualitative and quantitative composition of the avifauna during the wintering period in different types of water bodies in the south-western part of Odesa Region (Results ..., 2011, 2017, 2023)

Group of water bodies	Period, year	Number of birds			Number of species		
		max	min	average	max	min	average
The Danube lakes	2006, 2018–2022	28,431	35	12,494.6	28	4	19.2
The Danube Delta	2006, 2009, 2011–2016, 2018–2022	50,045	3,807	19,073.1	43	13	30.3
Seaside lakes	2005–2010, 2014, 2015, 2017–2022	24,332	523	13,191.9	32	7	18.9

Species diversity and abundance of wintering avifauna within the Danube lakes also depend largely on the ice regime of the water bodies. According to our observations, wintering species in this area are usually dispersed over water surfaces, and multi-species aggregations are formed in shallow areas (coastal parts of water bodies, fish hatcheries, etc.).

First of all, the distribution of the birds in this area is determined by the large area of the Danube lakes and the homogeneity of the ecosystems. The greater white-fronted goose does not form winter aggregations here every year but regularly uses the reservoirs and surrounding agrocenoses during migration (Fig. 1, 2). The largest overnight aggregation of the species we recorded during the fall migration on Lake Kahul in 2011 consisted of about 15,000 individuals. According to our observations, the formation of winter aggregations of the species is directly related to temperature, snow, and ice conditions in the area. For example, wintering aggregations of the greater white-fronted geese have been regularly recorded in the area since 2019. During the same period, the region was virtually free of severe winters with ice cover and heavy snowfall.

The most numerous and regular wintering of the greater white-fronted geese within the study area is recorded in the Seaside Lakes (Fig. 2, Tabl. 3). This area unites the most diverse saltwater and freshwater wetland ecosystems in the northwestern part of Black Sea Region. A significant part of the water area in this area does not freeze even in severe winters due to its high salinity. The distribution of birds in the waters of these lakes is not uniform. Due to the concentration of birds in shallow waters and on the spits within the lakes, many thousands of dense multi-species aggregations of birds of the wetland complex are recorded. All this creates conditions for the formation of stable centers of pathogen transfer.

Taking into account our own and retrospective data of ornithological studies in the south-western part of Odessa Region, as well as the results of virological studies, we can conclude that the territory of the seaside estuaries (Tuzly Lagoons and Sasyk Lagoon) is both the most important area for the greater white-fronted goose

during migration and wintering, and a place where the species is involved in the process of influenza A circulation.

In our opinion, the main factors that determine the ecological role of this area for the greater white-fronted goose can be the following: availability of food resources, large non-freezing water bodies — places for watering, resting and overnighting, low disturbance factor, which is determined by the conservation status of the area.

The epizootological role of seaside estuaries for the species can be determined by the presence of a source of infection, primarily regular, numerous and multi-species wintering aggregations of birds in the wetland complex, which are the main reservoir of infection (Swayne, Suarez and Sims, 2013). First of all, these are the mallard, *Anas platyrhynchos* Linnaeus, 1758 and the Eurasian duck, *Tadorna tadorna* Linnaeus, 1758, among which the circulation of influenza A viruses has been confirmed in the southern regions of Ukraine (Muzyka et al., 2012; Stegnyy et al., 2018). Both species make up the majority of wintering ducks in the sea estuaries: mallard — from 10 to 4,992 birds, an average of 1,905 birds, Eurasian duck — from 0 to 10,468 birds, an average of 2,564 birds, according to the results of traditional winter bird counts (Results ..., 2011, 2017, 2023).

Transmission of the virus between birds occurs through direct contact between infected and susceptible birds and indirect contact via aerosolized droplets and fomites, primarily virus-contaminated droppings (Swayne, Suarez and Sims, 2013).

Therefore, of particular importance for the spread of the virus are large multispecies flocks of birds that remain in one place for long periods of time, shed the virus, and exchange infections. Such aggregations are recorded annually in the Primorsky estuaries, mainly in the shallow areas and spits of Sasyk Lagoon and Malyi Sasyk, Dzantshey, and Shagany lagoons. Such aggregations can play a special role in winter, when the birds stay in the wintering area for a long time, having daily contact at resting, sleeping and feeding places, exchanging infections.

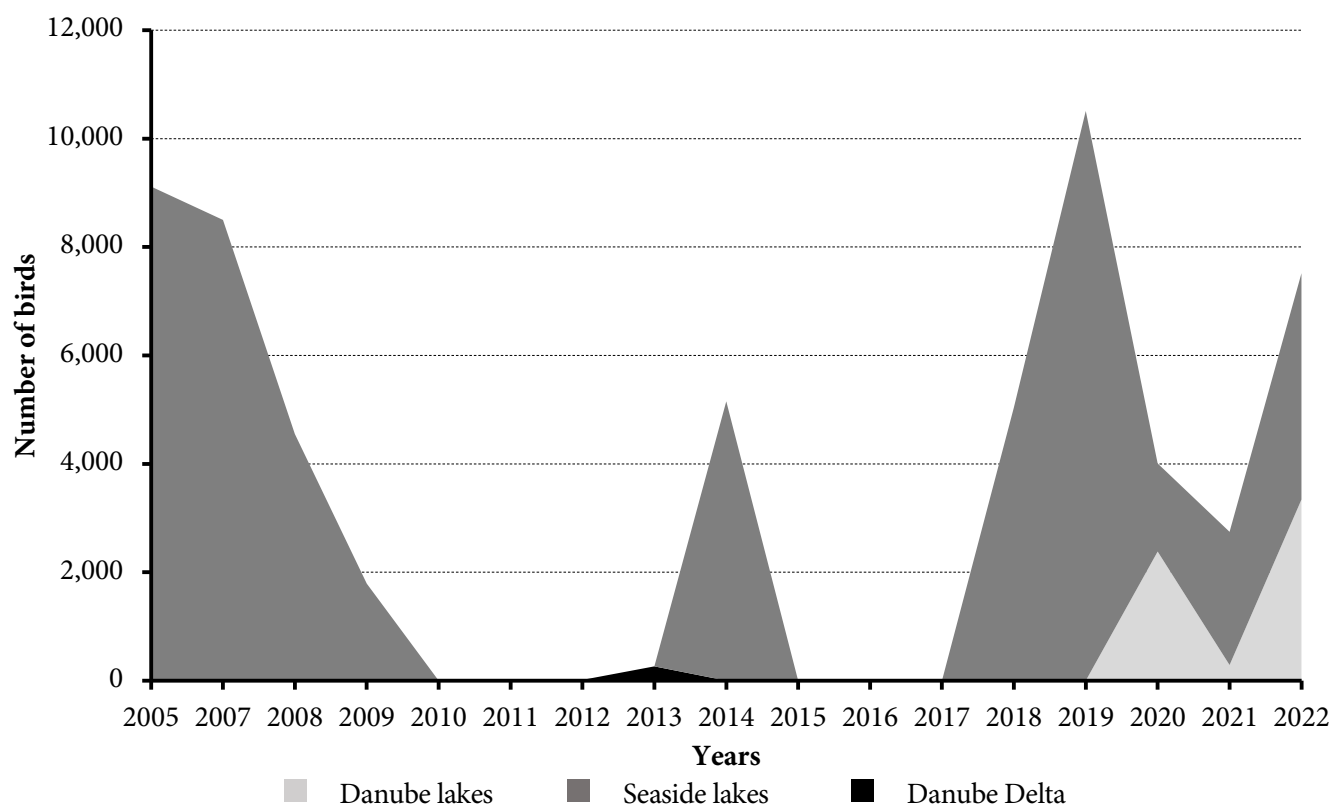


Figure 2. Wintering of the greater white-fronted goose, *Anser albifrons* (Scopoli, 1769) in the south-western part of Odesa Region according to the results of the Regional Ornithological Monitoring (Results ..., 2011, 2017, 2023).

Conclusions. Thus, the results of our research indicate that the greater white-fronted goose is involved in maintaining the natural circulation of influenza A virus in the natural reservoir in Odesa Region. The degree of involvement is likely to be most influenced by seasonality and the nature of intra- and interspecific interactions between birds in feeding, resting, and roosting areas. All positive samples were collected within the Seaside estuaries (Sasyk Lagoon and Tuzly Lagoons), which are characterized by the presence of large dense polygynous aggregations of birds localized in shallow waters, spits, etc. Such places contain valuable trophic habitats and, at the same time, watering and resting places for birds of the wetland complex. At the same time, they can be important sites for the transfer of pathogens. For the greater white-fronted goose, these are traditional wintering and migratory stopover sites for at least the last 15 years.

Our data complete the ecological picture of the avian influenza virus circulation in the greater white-fronted geese populations. At the same time, questions remain about the contact of this species with the source of infection during seasonal migrations, especially in the fall. The continuation of these studies is an important element in studying the ecology of the influenza A virus as one of the unpredictable pathogens, especially in a natural reservoir.

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