



Risk assessment of lead poisoning and pesticide exposure in the declining population of red-breasted goose (*Branta ruficollis*) wintering in Eastern Europe

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ABSTRACT

The red-breasted goose *Branta ruficollis* is a globally threatened species (IUCN Vulnerable) and the only European goose species currently in decline. Working on the wintering grounds on the Black Sea Coast, we address two potential causes of decline of this species for the first time: lead poisoning, and contamination from pesticides. We quantified the densities of spent Pb shot in three wetlands used by the geese in north-east Bulgaria, and analysed the Pb concentration in the faeces of red-breasted geese and the more abundant greater white-fronted geese *Anser albifrons*, using Al concentration as an indicator of soil ingestion. Pb shot densities in sediments were low, and we found no evidence for Pb shot ingestion in red-breasted geese. On the other hand, we found that the geese were feeding on wheat whose seeds were treated with four fungicides: thiram, tebuconazole, difenoconazole and fludioxonil, and the two first were even detected in goose faecal samples. Using data on the daily food intake, we estimated the exposure levels of the geese to these fungicides, both by measuring the concentrations remaining on seeds and by estimating the amount used to coat the seeds at the time of sowing. We found that the exposure rates estimated during the sowing period for both geese species can exceed the recognized hazardous doses for thiram, and to a lesser extent for tebuconazole, which indicates that some pesticides may be playing a previously overlooked role in the decline of red-breasted geese.

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1. Introduction

The red-breasted goose *Branta ruficollis* is a globally threatened species (IUCN category: Vulnerable) and is one of the most threatened goose species in Eurasia (Birdlife International, 2015). Red-breasted geese breed on the Taimyr, Gydan and Yamal peninsulas in Russia. In recent decades, the entire world population of this Arctic breeder has wintered on the north-western Black Sea coast in Ukraine, Romania and Bulgaria (Dereliev, 2006). The population declined dramatically in the early 2000s, dropping to fewer than 40,000 birds (Dereliev, 2006; Cranswick et al., 2012). A recent assessment concluded that this is currently the only goose species in decline in the Western Palearctic (Fox et al., 2010),

although there are some indications of recovery in recent years (Aarvak et al., 2012; Petkov, 2013). The reasons for recent declines are not very clear, although likely to include the effects of hunting, changes in land-use and climate change (see Section 4). In this paper, we study the possible impact of pollutants on this species in wintering grounds in Bulgaria. We have focused our investigation on those chemicals that may represent a risk for red-breasted goose because of their feeding habits and habitat use, namely lead poisoning and pesticide exposure.

Lead poisoning is a major conservation problem for many European Anatidae (Mateo, 2009). Lead contamination of wetlands with spent shot pellets was shown to be a serious problem in Greece (Pain and Handrinos, 1990), a neighbouring country for Bulgaria. Ingestion of spent lead shot and of lead from mining waste have been shown to affect an important proportion of the population of greylag geese *Anser anser* wintering in southern Spain (Mateo et al., 2007). However, no previous information is available about the prevalence of lead contamination in Anatidae wintering along the Black Sea Coast. Hunting in the wetlands of this area can be intensive, as in other European countries (Thomas

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and Guitart, 2010), and to date Bulgaria has only banned the use of Pb shot 200 m around wetlands (MoEW, 2007). The level of compliance of this ban is unknown and may be limited if not accompanied by an effective law enforcement (Cromie et al., 2010; Mateo et al., 2014). The AEWA action plan for the red-breasted goose highlighted the need to assess whether or not lead poisoning is a problem (Cranswick et al., 2012).

The second group of chemicals that can affect the red-breasted goose population are the pesticides currently used in agriculture and known to affect farmland birds (Mineau et al., 2001). Goose species could be especially at risk of being exposed to pesticides used for seed treatment, for several reasons (EFSA, 2009; Goulson, 2013; Lopez-Antia et al., 2016). First, geese could ingest pesticide-treated seed in recently sown fields. Second, geese feeding on early grown shoots of wheat and barley could also ingest the germinated seed remaining with the plant. Third, geese commonly grazing on cereal shoots could be exposed to systemic pesticides transferred from seeds to leaves throughout the wintering season. Because of this, pesticide exposure in geese species has frequently been reported in farmland areas (Hamilton et al., 1976; Stanley and Bunyan, 1979; Blus et al., 1984; Madsen, 1996). Seed coating, especially with insecticides, was estimated to be responsible for up to 50% of cases of lethal poisoning of wildlife caused by approved pesticides in European countries (De Snoo et al., 1999). Moreover, rodenticides can also be a threat when the treated bait (i.e. cereal seed) is spread on the soil surface for the control of vole plagues (Olea et al., 2009).

In this paper we study the Pb shot densities in the main Bulgarian wetlands used by red-breasted geese and greater white-fronted geese *Anser albifrons* for roosting. Although the geese spend most of the daytime grazing on cereals in the surrounding fields, they visit the wetlands regularly during the day for drinking, for roosting or as a refuge from disturbance. In order to study the ingestion of Pb shot in both geese species, and because of the difficulties of capturing them in large open fields, we used a non-invasive method based on the analysis of geese excreta as used in previous studies (Mateo et al., 2006; Martínez-Haro et al., 2013; Aloupi et al., 2015). This method involves measuring Pb concentration in faeces and relating it to aluminium content (Al), which is a good marker of soil ingestion (Martínez-Haro et al., 2010). The relationships between Pb and Al levels in excreta and in soil samples from the study area are used to identify faecal samples in which other sources of Pb (i.e. Pb shot) are likely to explain observed Pb levels. At the same time, samples of the winter wheat grazed by geese were taken for pesticide analysis, in particular the root containing the remains of the seed (often with an evident red staining indicative of pesticide coating). Moreover, the non-invasive sampling of faeces was used to monitor pesticide exposure in geese. The potential effect on geese of the detected pesticides was evaluated according to the expected concentration in seeds at sowing time and to the concentrations measured in plants in winter.

2. Material and methods

2.1. Study area

Bulgaria is situated at the southern end of the red-breasted goose flyway, forming a major part of its wintering range. The core wintering range is located around the N and NW Black Sea Coast, covering several large lake complexes in Ukraine (Sivash lake complex, Danube Delta and Azov sea area), Romania (the Danube Delta and some polder areas around the Danube) and Bulgaria (the so called Coastal Dobrudzha). Dobrudzha in NE Bulgaria comprises a major winter site for this species, with roosts at the coastal

lagoons of Shabla and Durankulak and on the adjacent Black Sea (Dereliev et al., 2000). In recent decades, the highest winter concentrations of the species have been recorded in this area. Total goose numbers at these roosts peak at up to 300,000 individuals in some years (mainly greater white-fronted geese, Kostadinova and Dereliev, 2001; BSPB, unpublished data). Both Shabla and Durankulak lakes are Special Protection Areas (SPAs) and part of the Natura 2000 network in Bulgaria. When geese numbers are particularly high they also roost in the sea bays along the Black Sea coast in the Kaliakra SPA. Our study area included arable fields used for grazing by the geese around Durankulak Lake SPA, Shabla Lake Complex SPA and Kaliakra SPA (Fig. 1). Larger numbers of greater white-fronted geese were also grazing in these fields. At the time of our sampling (17–20 January 2012), 1000 red-breasted geese and 7000 greater white-fronted geese were present in the study area.

2.2. Sampling

Lead shot densities were studied in shallow water areas (where geese could reach the sediments and be at risk of Pb shot ingestion) near the shorelines of Durankulak Lake, the adjoining Eagle Marsh and Shabla-Tuzla Lake (Fig. 1). For this purpose, sediments at 5–15 points separated at least 10 m apart in each site were sampled along transects with a metallic corer of 6 cm diameter. At each point, 5 samples were collected from the upper 15 cm of sediment, washed and sieved in situ with a 1 mm mesh, and the retained material was pooled and stored in a Ziploc plastic bag until further laboratory examination and metal analysis.

In order to interpret the results of Pb and Al analysis in geese faeces we collected sediment and soil samples of the sites used by geese. Sediment samples of Durankulak Lake ($n=3$) and Eagle Marsh ($n=3$) were collected with the corer (upper 5 cm) to determine Pb and Al levels. Sediment samples from Shabla-Tuzla Lake were not taken for Pb and Al analysis because geese were not using this wetland during our sampling period. Soil samples ($n=16$) were collected with a small shovel at 5 winter cereal fields where the red-breasted geese were grazing (Fig. 1). Samples were taken at 3 points along a transect in each field with a separation of 20 m between points. At each point, 5 soil samples were taken and pooled in a plastic bag to reach an overall mass of approximately 100 g. One additional soil sample was collected in a cereal field around Durankulak Lake.

Faecal samples of greater white-fronted and red-breasted goose were collected for metal analysis (Pb and Al) at fields where monospecific flocks could be detected. Fifty samples from each species were individually taken in plastic bags and stored frozen at -20°C until metal analysis. In order to avoid repeated sampling of faeces from the same bird, samples were taken at a minimum distance of 5 m from each other.

During the collection of faecal samples we could observe that cereal shoots (mostly wheat) being eaten by geese still showed the seed attached to the root with the red-staining characteristic of pesticide treated seeds. Therefore, in order to evaluate the pesticide exposure in geese we plucked out cereal shoots from different fields. As the amount of seeds remaining in the shoots was very limited we had to make a pooled sample from different fields composed of 102 germinated seed remains with a total mass of 1 g. Another ten shoot samples from individual fields, including leaves, roots and a few seeds were analysed individually (mass from 0.5 to 1 g). Moreover, 66 additional geese faecal samples were collected in fields where both species were feeding in order to determine the presence of cereal seed remains with a binocular microscope ($\times 45$) that could confirm seed ingestion in winter; and these samples were also used to determine the presence of pesticide residues. Plants and faeces were stored frozen at -20°C

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