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Impact of Usutu virus (USUV) on bird populations in Belgium

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Abstract. Usutu virus (Usuv) was first diagnosed in two corpses in Belgium in 2012. The first widespread outbreak affected NE Flanders in late summer 2016. A much larger outbreak followed in summer 2017, and again in summer 2018, each year expanding further west. Many sick and dead Eurasian Blackbirds *Turdus merula* were reported by the public to the data portal www.waarnemingen.be / www.observations.be.

We evaluated the impact of Usuv at population level using data from Common Bird Monitoring (CBM). We looked for contrasting changes in abundance from areas inside/since the outbreaks with outside/before the outbreak. In Flanders, of 60 species, Eurasian Tree Sparrow *Passer montanus* (–85% inside/+38% outside), Common Moorhen *Gallinula chloropus* (–60%/+57%), Song Thrush *T. philomelos* (–19%/+16%), Eurasian Blackbird (–11%/+24%), Northern Wren *Troglodytes troglodytes* (–13%/+22%), Dunnock *Prunella modularis* (–13%/+28%) and Mistle Thrush *Turdus viscivorus* (–62%, –20%) fitted the expected contrasting patterns of different dynamics between affected and unaffected areas exactly. Overall, in 31 of the 60 species annual changes were >20% worse inside than outside affected areas (11 species were >50% worse). Only 17 species were >20% better inside.

Declines were habitat specific: more marked in areas dominated by (sub)urban, agriculture or wetlands, but not in woodland. This pattern fits the ecology of Usuv: thermophilic, faster mosquito (vector) cycles in small water bodies on artificial substrates, and density dependent risks of outbreaks. In the less urbanized, more wooded and hilly parts of Wallonia, no strong declines fitting Usuv were found, despite the virus being widespread.

Evidence indicates that:

- Usuv is more widespread than mass mortality in birds indicates.
- mortality outbreaks happen under specific, local (micro)climatic conditions; particularly urban areas, agriculture habitats and wetlands are at risk.
- USUV affects many bird species at population level, some worse than Eurasian Blackbird.
- mortality of other sensitive species does not necessarily coincide with mortality of Blackbirds, and may happen unnoticed.

Introduction

Usutu virus (Usuv) is an encephalitic flavivirus transferred by mosquitos (Weissenböck et al. 2002, Nikolay 2015, Engel et al. 2016, Benzarti et al. 2019b). It originated in Africa and different strains reached Europe independently many times during the last 50 years, possibly imported by migratory birds (Engel et al. 2016). The first epozootic outbreak killing birds in Europe was documented in 1996 in Italy (Weissenböck et al. 1996); later in 2001 in Austria (Weissenböck et al. 2002, Rubel et al. 2008, Steiner & Holzer 2008) and in 2005 in Hungary (Bakonyi et al. 2007). Usuv was found in 93 bird species and was lethal to birds of 36 species, but Eurasian Blackbirds (*Turdus merula,* hereafter Blackbird) have suffered particularly high mortality (Chvala et al. 2007, Bosch et al. 2012, Tietze et al. 2014, Nicolay 2015, Lühken et al. 2017, Walter et al. 2018, Benzarti et al. 2019b). Meanwhile outbreaks have been documented from some 10 countries, mostly in southern and central Europe. Closest to Belgium, there was a large outbreak in the Rhine valley in southwestern Germany (Frankfurt-Karlsruhe) in 2011–2013 (Ziegler et al. 2015, 2016, Lühken et al. 2017) of the same strain as some ten years earlier in Italy, Austria and Hungary (Becker et al. 2012, Lühken et al. 2017). However, the major outbreak in Belgium and The Netherlands in 2016 (Rijks et al. 2016) followed an outbreak just across the border in Germany in 2014–2016, and was caused by a different virus strain (Cadar et al. 2017).

In Belgium, Usuv was first diagnosed in two corpses in 2012: a captive Bullfinch (*Pyrrhula pyrrhula*) and a Great Spotted Woodpecker (*Dendrocopos major*) (Garigliany et al. 2014). Judging by the many Blackbirds reported ill and dead by the public to the online portal www.waarnemingen.be/www.obervation.be in the summers of 2016, 2017 and 2018, a large outbreak spread from East to West across northern Belgium, but this was a different virus strain than the earlier two cases (Cadar et al. 2017, Garigliani et al. 2017).

In this paper we map the extent of the Usuv outbreak based on citizen science data of dead and ill Blackbirds. We screen the impact at population level of the Usuv outbreak on other common birds in Belgium and look at the particular climatic context of the outbreak.

Methods

Identification of affected areas

In the online portal www.waarnemingen.be/www.obervation.be, we routinely collect citizen science data from the public on biodiversity in general, including dead and ill birds (also road victims). When increased mortality of Blackbirds was reported (and Usuv suspected), we placed a call to the public through the press (in Flanders) in summer in each of the years 2016–2018 to report dead and ill birds. The areas heavily affected by Usuv in Flanders (northern part of Belgium) clearly stood out on maps of Blackbirds reported ill or dead. From this, we mapped the areas of virus outbreak in 2016 and 2017 (Fig. 1). The first outbreak in 2016 encompassed some 6,000 km^2 in the northeast of the country (Fig. 1A). In the next summer heavy mortality of Blackbirds was reported from a much larger area (12,000 km^2), moving further west. The remaining 3,000 km^2 of Flanders further west up to the coast were affected in summer 2018. The Brussels capital region was just at the edge of the outbreak in 2016, but became affected in 2017. No increased mortality of Blackbirds was reported from Wallonia (except for some cities) and it appears that it was spared from widespread Usuv mortality.

Because mortality occurs in summer (July-September), the effects of the outbreak in 2016 on breeding bird surveys could only be detected for the first time in spring 2017. This nevertheless results in a pattern of three areas consecutively affected from different years onwards, giving the opportunity of a clear comparison of areas before *versus* after and inside *versus* outside the virus outbreak (Fig. 2). If Usuv mortality affects bird populations, the hypothesis is that species sensitive to Usuv will have declined in the year(s) after the outbreak compared to before or to unaffected areas (Fig. 2).

Bird monitoring data

We used data from the Common Bird Monitoring (CBM) in Flanders (Vermeersch et al. 2007, 2019), Brussels (Weiserbs & Paquet 2010) and Wallonia (Derouaux & Paquet 2018) to screen as many species as possible for impact at population level. In the end 60 species were sufficiently abundant (with enough data available from theCBM) to be evaluated. For Flanders, we lumped the CBM counts in different 'Usuv areas' (Fig. 1) by year and calculated changes in abundance between years. Sample size varied between 184–197 counted grid-cells (km-square) annually in 2016–2018, and 25–94 grid-cells per area per year. We conducted two analyses: (1) identifying those species that fitted exactly the pattern of systematically declining in each of the three affected areas compared to each of the three unaffected areas (before or elsewhere) (see Fig. 2), and (2) comparing the population changes between affected and unaffected areas for all areas lumped. We calculated changes from the breeding season before the outbreak (2016) to the first (2017) and second (2018) year of the outbreak and averaged

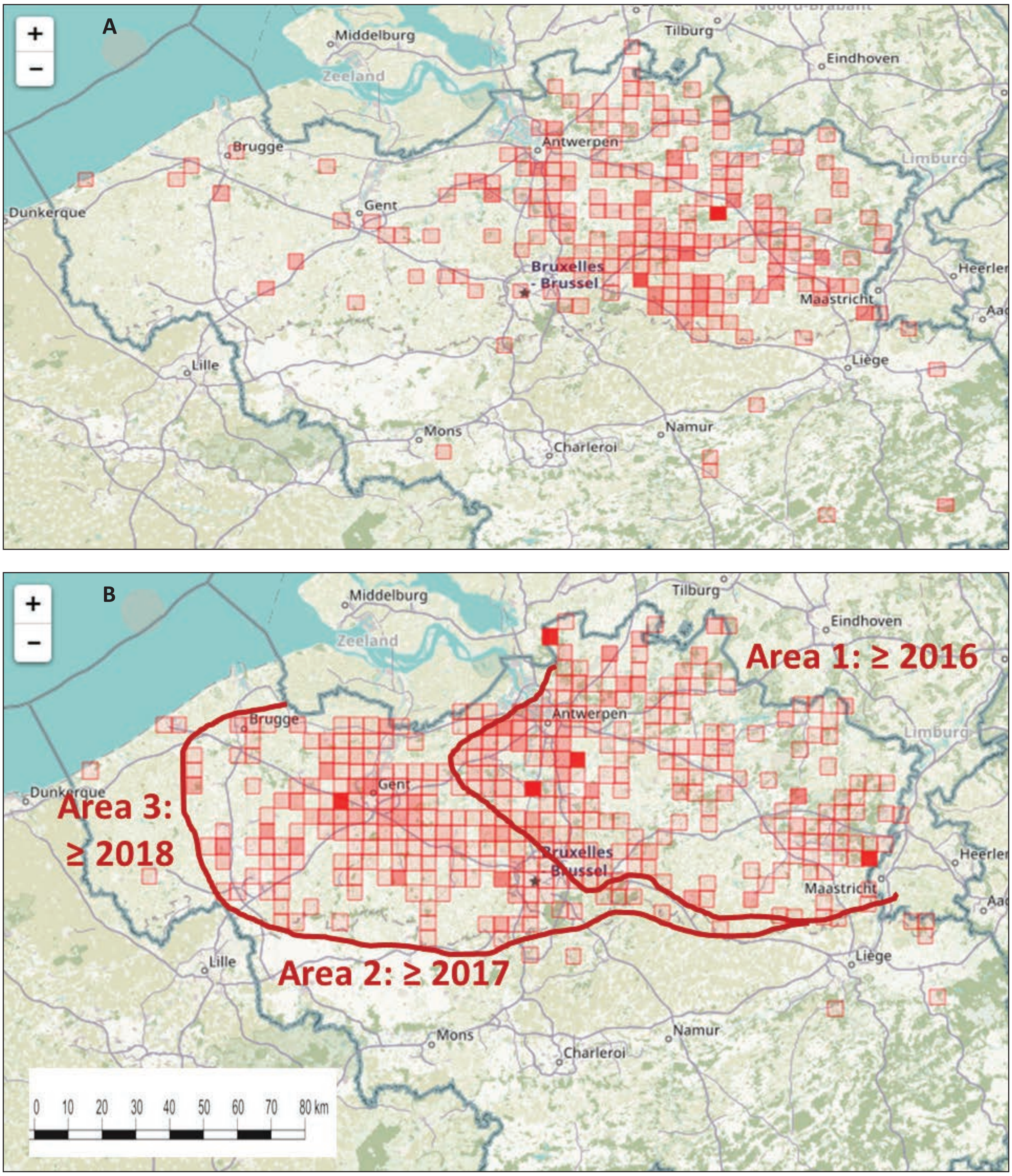

Figure 1. (A) Map of ill and dead Eurasian Blackbirds (*Turdus merula*) reported to the online portal www.waarnemingen.be/www.obervation.be between July and September 2016.
(B) Delineation of the spread of Usuv outbreaks over the years in Flanders based on maps of ill and dead Eurasian Blackbirds (*Turdus merula*) reported to the online portal www.waarnemingen.be/www.obervation.be between July and September each summer. Area 1 affected from 2016 onwards, area 2 from 2017 onwards and area 3 from 2018 onwards.

the values for areas outside and inside the outbreak (Fig. 3). The total difference between the average population change in affected and unaffected areas was added to calculate the "population change contrast": e.g. an average increase of +12% outside and +26% inside results in a contrast of +14, but an increase of +28% outside and a decrease of –64% inside results in a contrast of –92. When populations inside affected areas fared better, the contrast is positive, when they do worse than outside, the contrast is negative.
The CBM surveys in Flanders are randomly stratified for the habitat that is dominant in the km-square grid cell. Habitat of the surveyed cells

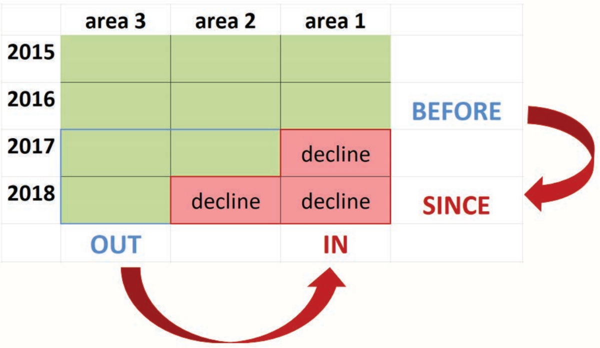

Figure 2. Schematic pattern of outbreak areas and expected effects (if any) on breeding birds compared to years before the outbreaks and areas outside of it. Green=breeding birds unaffected, Red=breeding season following Blackbird mortality in the previous summer.

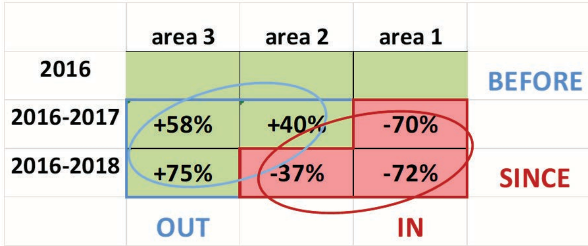

Figure 3. Example for Common Moorhen (*Gallinula chloropus*) of calculation of contrast between the average population change in affected and unaffected areas: first calculation of average population change outside (+57%) and inside (–60%) affected areas, then distance between these two changes (–117).

Table 1. Average population changes (%) outside and inside areas affected by Usutu virus in Flanders (Belgium) 2016–2018 for seven species fitting the pattern in Figure 2.

	outside	inside
Tree Sparrow (*Passer montanus*)	+38%	–85%
Moorhen (*Gallinula chloropus*)	+57%	–60%
Dunnock (*Prunella modularis*)	+28%	–13%
Songthrush (*Turdus philomelos*)	+16%	–19%
Eurasian Blackbird (*Turdus merula*)	+24%	–11%
Northern Wren (*Troglodytes troglodytes*)	+22%	–13%
Mistle Thrush (*Turdus viscivorus*)	–20%	–62%

were selected to belong to one of the six categories: forest, agriculture, suburban, urban, heathland or wetland. Hence, for common species trends can be calculated and compared by habitat (for methods and results about multimput in R, see Onkelinx et al. 2017, Onkelinx & Vermeersch 2019).

Results

Victims reported

The number of dead or ill Blackbirds (excluding road victims) reported to the online portal increased sharply in Flanders from summer 2016 onwards: from an average of 8 (max 11) during July-September each year between 2009–2015 to 396 in 2016, 1202 in 2017 and 397 in 2018. Calls to the public made reports the following week double to quadruple.

Impact on bird populations

Six species out of 60 (see supporting information Table S1 for species list) fitted exactly the expected pattern (Fig. 2), i.e. strongly declining in areas with important mortality of Blackbirds and doing much better in (as yet) unaffected areas (Table 1): Tree Sparrow *(Passer montanus),* Common Moorhen *(Gallinula chloropus),* Dunnock *(Prunella modularis),* Song Thrush *(Turdus philomelos),* Blackbird and Wren *(Troglodytes troglodytes)* (in order of decreasing contrast between affected and unaffected areas). Mistle Thrush *(Turdus viscivorus)* also fitted the pattern, except that it was also declining in unaffected areas, but much less so than in affected ones (Table 1). Not a single species showed the reverse pattern, i.e. consistently doing better in each of the three affected areas of Fig. 2 than in each of the three outside. Other common “species to watch”, which showed large negative overall contrasts between affected and unaffected areas and almost fitting the pattern of Fig. 2 (at most one of the six cells at odds) are Northern House Martin (*Delichon urbicum*) (total contrast –68), Grey Partridge (*Perdix perdix*) (–47), Stock Dove (*Columba oenas*) (–41), Common Pheasant (*Phasianus colchicus*) (–37), Eurasian Magpie (*Pica pica*) (–34), Common Kestrel (*Falco tinnunculus*) (–29) and Common Starling (*Sturnus vulgaris*) (–25).

When we plot the total contrast between the average population changes outside and inside affected areas for all 60 species (irrespective whether or not they fit the pattern in Fig. 2), the distribution is shifted to negative values (Fig. 4). 31 species had a total contrast >20 units worse inside than outside (11 species even >50 units worse). Only 17 species did >20 units better inside (8 species >50 units better) than outside affected areas.

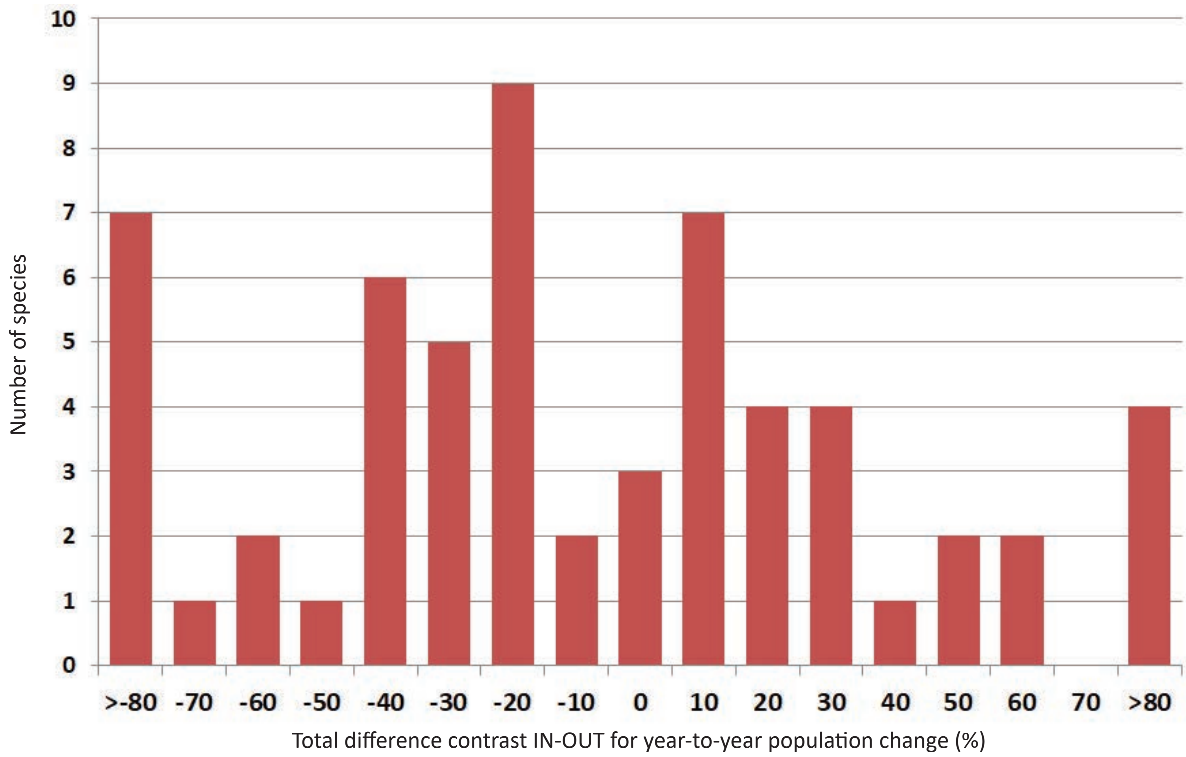

Figure 4. Distribution pattern for 60 species of total contrast between population changes outside *versus* inside areas affected by Usuv: negative values indicate that populations inside fared worse than outside, positive the reverse.

Habitat specific impact

Of the seven species considered very sensitive to Usuv (Table 1), all six with sufficient data showed significantly different trends according to habitat in Flanders, with strong declines recently in squares dominated by (sub)urban, agriculture or wetland, but stable or increasing populations in forest or heathland. Population models for Blackbird e.g. showed significant trends in three habitats, with increases over the last decade of +28% in squares dominated by forest, but declines by –23% in agriculture and –30% in squares dominated by urban areas (Onkelinx & Vermeersch 2019).

Discussion

Virological evidence

When the Usuv epidemy became obvious, virological screening of corpses was undertaken in Belgium by the University of Liège. Analyses of 131 birds of 40 species found dead in 2017–2018 showed 37 birds of 11 species to be positive for Usuv, originating from a wide area of southern Belgium (Benzarti et al. 2019a, Benzarti et al. 2019c), raising fears for impact on bird populations (Garigliany et al. 2017).

Identification of affected areas by citizen science

The density of citizen science bird data is high in Belgium: for Flanders (13,522 square-km), the online portal www.waarnemingen.be received 4.6 million bird records for the years 2016–2018 from ca. 7,000 persons annually. The data flow from Wallonia is about half of that in Flanders — substantially less, but not so low to account on its own for the virtual absence of reported Blackbird mortality. Therefore, the increase in dead and ill Blackbirds reported to the portal during the epizootic emergence of Usuv allowed us to map the boundaries of the outbreak using citizen science reports of dead birds. The same approach has already been used to map an outbreak of the closely related West Nile virus in the US (Eidson et al. 2001).

Impact on bird populations

The seven species listed in Table 1 may be particularly sensitive to Usuv. It is interesting that Blackbird, which has widely been reported as highly sensitive to Usuv, is by far not the worst affected species in this list. Furthermore, the shift towards more negative population changes inside affected areas in more than halve the species suggests that Usuv might have more widespread negative effects on bird populations, well beyond Blackbirds and a handful of other very sensitive species.

Owls, some songbirds (e.g. Turdidae, Corvidae) and woodpeckers have been found to be particularly affected by Usuv (Becker et al. 2012, Bosch et al. 2012, Nikolay 2015, Rijks et al. 2016, Benzarti et al. 2019b). We did not include any owls in this screening, and none of the three woodpeckers investigated had population trends worse inside than outside affected areas, but Eurasian Magpie declined inside while increasing outside. Among small passerines, House Sparrow (*Passer domesticus*) and Blackcap (*Sylvia atricapilla*) were reported to have a high percentage of positive cases for Usuv (Bosch et al. 2012, Nikolay 2015, Benzarti et al. 2019b). Population changes in both species were indeed also far worse inside affected areas in our study than outside, but the patterns of decline were not consistent with the advancing Usuv-front according to Blackbird mortality.

That raises the question, however, whether or not all species are necessarily affected in the same way and synchronously with Blackbirds? There may be different scenarios for an epizootic outbreak of Usuv, simplified: (1) when Usuv arrives in an area, it may affect all the sensitive species synchronously, as exemplified by mass mortality of Blackbirds, or (2) when Usuv arrives in an area, it may mostly remain unnoticed and may or may not affect individuals and populations depending on many local conditions (habitat, vectors, temperature, rainfall, host density, direct transmission?). Mortality of several species can be local and undiscovered until at some moment conditions are right for a large scale epizootic outbreak in a more common species like Blackbird. Blackbirds are so common in most parts of Europe and live so conspicuously close to people that mass mortality becomes a prominent and easily noted event when it happens in this species.

There is evidence that the second scenario applies more than the first. In Germany, Italy and Belgium, Usuv was already diagnosed before a large epizootic emergence occurred, or outside regions where elevated mortality was recorded (Jöst et al. 2011, Salvini et al. 2011, Garigliany et al. 2014). Many healthy birds (including Blackbirds) have been found carrying Usuv (Benzarti et al. 2019b, de Jong 2019). Outbreaks seem to coincide with particularly wet and warm conditions in spring and summer: enhanced mosquito (vector) and virus cycles under these conditions increase the risk for an outbreak (Rubel et al. 2008, Becker et al. 2012, Rijks et al. 2016). This also means that climate disruption (particularly warming (Brugger & Rubel 2009), but also excessive rainfall) constitutes an extra risk for Usuv outbreaks.

Usuv was first discovered in Belgium in 2012, but a large scale outbreak only happened from 2016 onwards. What was peculiar then in 2016 and afterwards? Rainfall in June was more than two times higher than the average value in the north-eastern part of Flanders (KMI url 1), closely coinciding with the area of mortality of Blackbirds later that summer. Rainfall remained high in that area throughout summer (KMI url 2). September 2016 was the hottest in Belgium since 2006 (KMI url 3) and the summers of 2017 and 2018 were both exceptionally hot (KMI url 4). The habitat specific trends in Blackbird further indicate that particular conditions in (sub)urban areas and in the agricultural landscape were favourable for the local outbreak. The urban heat effect and the larger areas of artificial substrates retaining shallow and fast warming water in these habitats may have played a key role. Blackbird population trends in cooler forested areas are not affected. This is supported by the records from the Netherlands, where survival rates of urban Blackbirds have become lower than in the countryside since the outbreak of Usuv (de Jong 2019).

Usuv risks can quite well be modelled, and unusually warm temperatures are an important predictor (Brugger & Rubel 2009, Cadar et al. 2016, Lühken et al. 2017, Walter et al. 2018). In all these models, the cooler, more forested higher areas in southern Belgium (most of Wallonia), which are also less suitable for mosquitoes (Versteirt et al. 2009), have a much lower risk for Usuv. The patterns of the recent outbreaks in Belgium fit these risk models very well, with no mass mortality reported from these areas so far and no detectable impact on bird populations.

Available evidence therefore indicates that:

- Usuv is more widespread in Europe than epizootic emergences and documented mass mortality in birds indicate
- Usuv does not primarily affect Blackbirds; it affects many species, also at population level, and some do far worse than Blackbirds
- massive outbreaks in Blackbirds happen under specific, local (micro)climatic conditions,

where particularly urban and agriculture areas and wetlands are at risk.
- mortality of other sensitive species does therefore not necessarily coincide with mortality of Blackbirds. Mass mortality may also happen unnoticed.

With this paper and the formulated hypotheses, we want to stimulate more detailed research on the impact of Usuv on bird populations (also beyond Blackbirds).

Acknowledgements

We are particularly grateful to all the volunteers that conduct the common bird monitoring and to the public for helping to map Blackbird mortality.

Supporting information

Table S1. List of all 60 screened species with sufficient data (names follow Handbook of the Birds of the World).

Grey Partridge	*Perdix perdix*	Common Chiffchaff	*Phylloscopus collybita*
Common Pheasant	*Phasianus colchicus*	Long-tailed Tit	*Aegithalos caudatus*
Canada goose	*Branta canadensis*	Eurasian Blackcap	*Sylvia atricapilla*
Egyptian goose	*Alopochen aegyptiaca*	Garden Warbler	*Sylvia borin*
Mallard	*Anas platyrhynchos*	Common Whitethroat	*Sylvia communis*
Stock Dove	*Columba oenas*	Short-toed Treecreeper	*Certhia brachydactyla*
Common Wood-Pigeon	*Columba palumbus*	Eurasian Nuthatch	*Sitta europaea*
Eurasian Collared-Dove	*Streptopelia decaocto*	Northern Wren	*Troglodytes troglodytes*
Common Moorhen	*Gallinula chloropus*	Common Starling	*Sturnus vulgaris*
Common Coot	*Fulica atra*	Mistle Thrush	*Turdus viscivorus*
Northern Lapwing	*Vanellus vanellus*	Songthrush	*Turdus philomelos*
Eurasian Buzzard	*Buteo buteo*	Eurasian Blackbird	*Turdus merula*
Great Spotted Woodpecker	*Dendrocopos major*	European Robin	*Erithacus rubecula*
Eurasian Green Woodpecker	*Picus viridis*	Bluethroat	*Luscinia svecica*
Black Woodpecker	*Driocopus martius*	Black Redstart	*Phoenicurus ochruros*
Common Kestrel	*Falco tinnunculus*	Common Stonechat	*Saxicola torquatus*
Eurasian Magpie	*Pica pica*	Goldcrest	*Regulus regulus*
Eurasian Jay	*Garrulus glandarius*	Dunnock	*Prunella modularis*
Eurasian Jackdaw	*Corvus monedula*	House Sparrow	*Passer domesticus*
Carrion Crow	*Corvus corone*	Eurasian Tree Sparrow	*Passer montanus*
Coal Tit	*Peripatus ater*	Tree Pipit	*Anthus trivialis*
Crested Tit	*Lophophanes cristatus*	Meadow Pipit	*Anthus pratensis*
Eurasian Blue Tit	*Cyanistes caeruleus*	Western Yellow Wagtail	*Motacilla flava*
Great Tit	*Parus major*	White Wagtail	*Motacilla alba*
Wood Lark	*Lullula arborea*	Common Chaffinch	*Frincilla coelebs*
Eurasian Skylark	*Alauda arvensis*	European Greenfinch	*Chloris chloris*
Common Reed Warbler	*Acrocephalus scirpaceus*	Common Linnet	*Linaria canabina*
Northern House Martin	*Delichon urbicum*	European Goldfinch	*Carduelis carduelis*
Barn Swallow	*Hirundo rustica*	Yellowhammer	*Emberiza citrinella*
Willow Warbler	*Phylloscopus trochilus*	Reed bunting	*Emberiza schoeniclus*

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