INTRODUCTION

Pathogens play a significant role in shaping the dynamics of wildlife populations, and those that are highly virulent can rapidly and extensively decrease population numbers (e.g., Delibes-Mateos et al., 2014). Moreover, a disease-induced population decline of one species usually influences changes in the population numbers of other species from various trophic levels, and causes—through the cascade effect—large modifications in the structure of the
ecosystem (e.g., Delibes-Mateos et al., 2014; Lindström et al., 1994). Less virulent pathogens may alter host behaviour, fitness (e.g., reduced body condition and/or reproduction) and vulnerability to predation (Beldomenico et al., 2008; Prenter et al., 2004). In addition, the host immune response reduces the infection rate and its negative impact, although in various demographic groups (sex or age) and/or under poor environmental conditions (e.g., scarcity of food), hosts may prioritize growth or self-maintenance over an immune response. Therefore, a pathogen’s influence on population dynamics may vary temporally. Finally, low virulent strains of pathogens may have no effect on the host (its condition and/or reproduction) and on host population numbers.

The role of pathogens in shaping the population dynamics of invasive non-native species (INNS) is particularly important for understanding their success in establishing novel populations and colonizing new areas (Armstrong & Seddon, 2008). One of the hypotheses explaining the successful introduction and spread of INNS is the Enemy Release Hypothesis, which suggests that invasive species often lose their enemies (e.g., pathogens) and accumulate relatively few new pathogens in colonized habitats (Laurimaa et al., 2016; Torchin et al., 2003). The lack of enemies in an invaded range may increase INNS fitness (e.g., fecundity and/or survival) and accelerate an increase of their density and further expansion. However, in some cases pathogens are introduced with INNS and the benefits of enemy release are limited. Co-introduced pathogens may affect INNS in the new range and their hosts may become reservoirs from which the pathogen can spill over to native species (Callaway & Ridenour, 2004).

The American mink (Neovison vison) is a medium-sized carnivore, recognized as one of the most invasive non-native mammals (Nentwig et al., 2010). The species originates from North America and has been introduced to Europe, Asia and South America both on purpose (e.g., in the former Soviet Union), and accidentally (having escaped from fur farms established in many countries in the 1920s; Bonesi & Palazon, 2007). The first feral populations in Europe were recorded in the 1930s and in the following decades, they gradually colonized large parts of the continent. Due to the large-scale and long-term nature of mink farming, the number of escapees, which started to breed in the wild and became the founder individuals of feral populations, has been high (Bonesi & Palazon, 2007; Zalewski et al., 2010, 2011). In many regions where mink populations were derived from farm escapees, they are still well supplied by ongoing introductions. In Poland, it has been documented that a colonization wave, originating from the east, crossed the Polish-Belarusian border at the end of the 1970s and spread westwards over subsequent years (Brzeziński et al., 2019). However, developing mink farming in Poland resulted in farm escapees giving birth to many local populations (mostly in the northwest of the country), which increased in number and joined together (Zalewski et al., 2011). The present geographic range of feral mink covers all of northern and central Poland and is continuous (Brzeziński et al., 2019). Multiple and ongoing introductions that shaped the genetic structure of feral mink in Poland (Zalewski et al., 2010) may have increased the spread of pathogens and increased pathogen diversity in these populations.

Aleutian mink disease (AMD) is one of the pathogens that was introduced to Europe with the American mink and was first recognized in farmed mink in the 1940s (Hartsough & Gorham, 1956). It is caused by a highly contagious parvovirus belonging to species Carnivore am-doparvovirus 1—the Aleutian mink disease virus (AMDV). AMDV is a single-stranded DNA virus of about 4.7 kb belonging to the genus Amdoparvovirus and family Parvoviridae (Bloom et al., 1988; Canuti et al., 2015). The virus may be transmitted vertically and horizontally by various routes (urine, faeces, saliva, blood; Farid et al., 2015; Gorham et al., 1964; Jensen et al., 2014). Several strains of AMDV have been described, ranking from non-virulent to highly virulent (Alexandersen et al., 1994; Bloom et al., 1988). In the American mink, virulent strains of AMDV can cause severe progressive disease with multiple clinical syndromes, such as acute, usually fatal, interstitial pneumonia depending on host factors, such as age or genotype (Alexandersen et al., 1994; Bloom et al., 1994). The classic form of AMD is characterized by a persistent viral infection, the development of plasmacytosis, hypergammaglobulinemia, and immune complex-mediated glomerulonephritis and arteritis in the adult mink (Bloom et al., 1994; Porter, 1986). In addition, some animals can have a chronic infection, while others can clear the infection (Hadlow et al., 1983); although it is uncertain if this is a consequence of host susceptibility or the particular isolate of the infecting virus (Fox et al., 1999). Depending on AMDV strain, infected mink lose all of their body fat and some organs, particularly the liver and spleen, may become enlarged and turgid (Henson et al., 1976; Persson et al., 2015). The kidneys are larger in the early phase of the disease and later become pale, shrunken and irregular. Therefore, an indicator of AMD influence on feral mink would be their body condition and organ size.

Serologic evidence of AMDV has been found both in native mink in Canada, as well as in feral mink in many European countries, and the prevalence of AMDV in farmed mink has reached up to 90% in some locations (Mañas et al., 2016; Nituch et al., 2011). The virus is also widespread in mink kept on Polish farms (Kowalczyk et al., 2019; Reichert & Kostro, 2014). AMDV transmission from farms to feral populations is caused by direct contact between farmed and feral mink, but also through contact by feral mink with infected carcasses and waste, or through aerosol dispersal (Nituch et al., 2011). Another possible way of transmission may also be the mosquito (Aedes itchii), which can carry AMDV for up to 35 days after an infectious blood meal, indicating the possibility of the vector-borne transmission of AMDV (Shen et al., 1973). Therefore, the spillover of AMDV from farmed to feral mink is highly probable. Moreover, the virus may be transmitted from mink to native predators. In Europe, serologic evidence of AMDV has been found in the European mink (Mustela lutreola), European polecat (Mustela putorius), stone marten (Martes foina), pine marten (Martes martes), river otter (Lutra lutra), European badger (Meles meles) and common genet (Genetta genetta; Fournier-Chambrillon et al., 2004; Knuuttilla et al., 2015; Mañas et al., 2001; Yamaguchi & Macdonald, 2001). The analyses of AMDV prevalence in native mink in Canada and feral mink in Europe suggest that mink farms are sources of the virus in wild populations (Knuuttilla et al., 2015; Nituch et al., 2011). Feral populations of American mink are still
augmented by escapees from mink farms (Zalewski et al., 2010); therefore, it is likely that they will become infected with AMDV from farmed mink. As the number of escapees correlates with the number of farmed mink (Zalewski et al., 2010), we may expect a significantly higher prevalence of AMDV in areas with a high number of mink farms. The level of infection of AMDV at farms varies temporally (Farid et al., 2012), so the virus transmission and its prevalence in feral mink populations may also vary over the years (Knuuttila et al., 2015). However, there are no data describing the temporal and spatial variation of AMDV prevalence in native and feral mink, and there is only fragmentary evidence regarding the influence of AMD on feral populations of mink (Persson et al., 2015).

In this study, we analysed mink farm distribution and stock size in Poland. Next, we analysed the temporal variation of AMDV prevalence in feral mink, the influence of farming intensity on spatial prevalence variation, as well as the influence of the infection on feral mink body condition. First, we expected that AMDV prevalence in feral mink populations would vary in consecutive years and be higher in areas with a high number of farmed mink. Second, we expected that individuals infected by AMDV would have poorer body condition and such symptoms of AMD as an enlarged spleen, liver and kidney.

2 | MATERIALS AND METHODS

2.1 | Distribution and size of mink farms in Poland

To evaluate the influence of mink farming intensity on the prevalence of AMDV in Poland, we combined data on the location, number of farms and number of mink farmed in 2013 from various sources. The data were obtained by queries send to the Voivodship or County Veterinary Inspectorates, responsible for supervising mink farms. We combined these data with the register of entities conducting supervised activities published by the General Veterinary Inspectorate. The location of each farm was verified on satellite images, where the farm was identified based on the characteristic structure of buildings with mink cages. This approach allowed us to precisely compile information about the number of farms and their size, although we know that the data may have contained some uncertainty due to the not always precise estimates of mink numbers kept on farms. In addition, to account for the potential influence of mink farms located in the border zone of neighbouring countries, we investigated (basing on satellite images) the presence of mink farms in the vicinity of Białoświęta Forest on the border with Belarus and the Warta Mouth National Park on the border with Germany.

2.2 | Mink collection and dissection

We collected 1,153 feral mink carcasses (692 males and 461 females) from two seasons (non-breeding—September-January, and breeding—February-August) between 2006 and 2017. Mink were collected at nine sites: Białowieża Forest (BF), Biebrza National Park (BNP), Narew National Park (NNP), Vistula River (VR), Gwda River (GR), Drawa National Park (DNP), Warta Mouth National Park (WMNP), Słowiński National Park (SNP), and Modla Lake and surrounding area (ML; Figure 1). Most of these sites are well-preserved ecosystems, with various aquatic habitats: large rivers (VR, WMNP), medium-sized rivers (BNP, NNP, DNP, GR), small rivers (BF), lakes (SNP, ML) and both rivers and lakes in some sites (ML, DNP). The predominating habitat types of the study sites are open river valleys (BNP, NNP, WMNP), forests (DNP, BF) or a mix of the two (VR, SNP). The sites were combined into three regions, which cover the areas of the main river basins: west (W)—Oder River (WMNP, DNP, GR), east (E)—Vistula River (VR, NNP, DNP, BF) and north (N)—Baltic Sea tributaries (SNP, ML). In all these sites, mink were eradicated to implement nature protection plans within the scope of bird conservation projects, under permission granted by local and government authorities. Some mink were killed by hunters or collected on the road as roadkills.

Mink carcasses were frozen and stored at −20°C before dissection. Measurements of mink bodies (body mass and body length without tail) were taken to estimate the body condition index. Next, the mink were dissected and the muscle tissue, heart, liver, kidneys and spleen were collected. The liver, kidneys and spleen were weighed and the relative weight (to body mass) of these organs was used to compare their size in AMDV infected and non-infected mink. The canines of the mink were also extracted from the skulls to obtain the age of the mink on the basis of dental analyses. We determined the age of the mink by analysing the upper canines in two steps. First, the mink canines were x-rayed, and two age classes (young and older than 10 months) were defined according to the proportion of pulp in the teeth (Dix & Strickland, 1986). Next, the age of mink older than 10 months was determined precisely by a cementum analysis of the canines, performed at Matson’s Laboratory. In these analyses, we grouped the mink into two age classes: sub-adults (<1st year of life) and adults (>1st year of life).

2.3 | Serological detection of AMDV antibodies in American mink

Blood from the heart or spleen was absorbed by filter paper strips that were air-dried and stored at −20°C. A circular piece (5 mm) was used to screen animals for AMDV antibodies with AMDV VP2 ELISA (Knuuttila et al., 2009). The filter paper pieces were incubated o/n in 100 µl of dilution buffer (PBS + 0.5% BSA + 0.05% Tween 20) and used in ELISA. Goat anti-ferret IgG (H + L) secondary antibody (Novus) with 1:20,000 dilution or peroxidase-conjugated AffiniPure Goat Anti-Cat IgG (H + L) (Jackson ImmunoResearch) with 1:4,500 dilution was used as a conjugate. The ELISA cut-offs were determined by testing a panel of 10 negative samples in seven replicates and adding two standard deviations to the mean absorbance. All analyses were performed at the University of Helsinki, Department of Virology and Department of Veterinary Biosciences.
2.4 | Statistical analyses

First, we analysed the overall variation in the prevalence of AMDV between regions using the generalized linear model GLM with a binomial family and four explanatory variables: sex, age, season (breeding and non-breeding) and region, which reflects the area of a main river basin (west—Oder River, east—Vistula River, and north—Baltic Sea tributaries).

Next, we analysed the temporal pattern of AMDV infection in mink populations in relation to the abundance of mink farms. We predicted a non-linear temporal variation in AMDV prevalence with time and a higher prevalence in areas with a higher number of farmed mink. To determine this prediction, we performed generalized additive models (GAM1). GAMs are an approach for the estimation of non-linear and not monotonic relationships between response and explanatory variables. Five explanatory variables were included: year of mink carcass collection, number of farmed mink within a 60 km radius from the site centre, sex, age (sub-adult and adult) and season (breeding and non-breeding). As the mink were trapped or hunted mainly from September to March, the year indicates the period lasting from the autumn of one year to the spring of the following year. To test our hypotheses of a non-linear variation of AMDV prevalence in time, year was fitted with thin plate regression splines with $k = 9$ (the level of smoothing). We also tested for two-way interactions between three categorical variables (sex, age, and season), but the interactions were insignificant and thus excluded from the final models. To test the influence of the number of farmed mink, we calculated the number of mink at all farms located in a buffer zone of a 60 km radius around the centre of a site using ArcGis. We selected a 60 km buffer around the site because mink can disperse more than 50 km (Oliver et al., 2016).
To analyse how the temporal dynamics of AMDV prevalence change at the local scale in relation to mink farming intensity, we used a subset of data from 2009 to 2016 and sites with high mink farming intensity (WMNP, n = 307) and low farming intensity (NNP and BNP, n = 607). From these years and sites, we collected the largest number of samples. We combined two sites: NNP and BNP as these sites were located in close vicinity and genetic studies have shown a high dispersal rate between BNP and NNP (Zalewski et al., 2010, Zalewski, unpublished data). We used generalized additive models (GAM2) with year of mink carcass collection, sex, age (subadult and adult) and season (breeding and non-breeding) as explanatory variables. We also fitted the effect of year separately for the two sites (WMNP vs. NNP and BNP) to test the differences in the temporal dynamics of AMDV prevalence between those sites. We used the 'mgcv' package (Wood, 2017) implemented in R (R Core Team, 2018) for the performed GAM with binomial distributions and logit link. We then checked the residuals and the overdispersal of the model using the DHARMa package (Hartig, 2018).

We tested the hypothesis that AMDV infection affects the body condition of mink. The most common method used to measure condition involves regressing body mass on some linear index of body size (e.g., body length) and using the residuals from this regression as an index of body condition. An individual with a positive residual is considered to be in better condition than an individual with a negative residual (Schulte-Hostedde et al., 2005). This method gives a good predictive index of body condition for mink (Mustonen et al., 2015). As some mink provided by hunters or conservationists were skinned, we obtained data on body size for 646 males and 441 females. The relationship between body mass and body length (log-transformed) was linear ($R^2 = .776, n = 1,087, p < .001$) with the residuals evenly distributed across the range of body sizes. Next, we fitted a simple linear model with residuals of body mass as the response variable and the following categorical explanatory variables: status of AMDV infection, sex, age and season. Similarly, we estimated the residuals of spleen, kidney and liver size in relation to body mass (log-transformed) and fitted linear models with the four explanatory variables described above to estimate the relation of AMDV status and the relative size of the three organs. The relationship between organ mass and body mass was significant (for spleen $R^2 = .375, n = 1,087, p < .001$, for liver $R^2 = .736, n = 1,087, p < .001$, and for kidneys $R^2 = .735, n = 1,015, p < .001$).

3 | RESULTS

3.1 | Number of farms and mink farmed in Poland

Based on the inventory of the number and distribution of mink farms in Poland, their number in 2013 was estimated to be 361. In total, 1,170,219 mink were farmed before breeding and 5,689,792 after parturition.

For the three regions we distinguished (see Material and methods), 197 (54%) farms breeding 4,833,751 mink (85%) were located in the west (W) region, 60 (17%) farms breeding 300,920 mink (5%) in the north (N) region and 104 (29%) farms breeding 555,121 (10%) in the east (E) region (Figure 1). The number of mink kept on farms located within a 60 km radius buffer around the study site varied from 5,000 around BF to 691,000 around WMNP (155,493 mink on average, $SD = 189,032$) and the highest number of farms in the buffer was 23 (11 farms on average, $SD = 7$).
TABLE 1 Parameter estimates (coefficients) and SE from the generalized linear model (GLM) investigating the relationships between regions, sex and age, season, year and Aleutian mink disease virus prevalence in the American mink

| Variables                  | Estimate | SE   | Z value | p Value | Odds ratio |
|----------------------------|----------|------|---------|---------|------------|
| Intercept                  | 0.9148   | 0.1641 | 5.576   | <.0001*** |           |
| Region (W vs. N)           | -1.7814  | 0.2945 | -6.050  | <.0001*** | 0.17       |
| Region (W vs. E)           | -1.0665  | 0.1518 | -7.024  | <.0001*** | 0.34       |
| Region (N vs. E)           | 0.7150   | 0.2784 | 2.568   | .01       | 2.04       |
| Sex (M)                    | 0.3242   | 0.1411 | 2.297   | .0216     | 1.38       |
| Age (Adult)                | 0.7952   | 0.1482 | 5.365   | <.0001*** | 2.21       |
| Season (breeding)          | 0.5214   | 0.1510 | 3.452   | <.0001*** | 1.68       |

Note: For Region the first value in parenthesis is used as a reference level.
* p<.05, ** p<.005, *** p<.001.

TABLE 2 Parameter estimates (coefficients) and SE from the generalized additive mixed model (GAM1) investigating the relationships between the sex and age of mink, season, year and Aleutian mink disease virus prevalence in the American mink

| Variables                  | Estimate | SE   | Z value | p Value |
|----------------------------|----------|------|---------|---------|
| Intercept                  | -8.603   | 1.075| -8.000  | <.001***|
| N mink on a farm           | 1.673    | 0.202| 8.290   | <.001***|
| Sex (M)                    | 0.399    | 0.148| 2.699   | .007**  |
| Age (Adult)                | 0.921    | 0.157| 5.864   | <.001***|
| Season (breeding)          | 0.480    | 0.162| 2.955   | .003**  |

Note: edf—effective degree of freedom indicates the amount of non-linearity of the smooth in GAM (1—indicative of a linear pattern of relation, >1 denoted a non-linear pattern of relation).
* p<.05, ** p<.005, *** p<.001.

3.2 | Variation in AMDV prevalence

The presence of antibodies (AMDV-positive) was detected in all study sites, in 803 (69.6%) animals but its prevalence varied between regions, sex, age and season (Figure 2, Table 1). Estimated AMDV prevalence based on the GLM model showed a high variation between the three regions, with the highest prevalence in the west region (0.826, CI 95% = 0.799–0.867), lower in the east region (0.637, CI 95% = 0.597–0.674) and the lowest in the north region (0.461, CI 95% = 0.338–0.589). Males were infected more frequently (0.745, CI 95% = 0.709–0.778) than females (0.679, CI 95% = 0.631–0.723) and adults were infected more frequently (0.804, CI 95% = 0.764–0.839) than subadults (0.649, CI 95% = 0.610–0.687). Mink were more often infected in the breeding season than in the non-breeding season (Figure 2).

The GAM1 model showed that the prevalence of AMDV in feral mink was significantly associated with the year, number of farmed mink, season, sex and age (Table 2). The prevalence changed non-linearly in consecutive years: it was high in 2006 and 2007, decreased in 2008, increased in 2009 and 2010, decreased again in 2011, remained on a similar level until 2015, started to increase in 2016 and reached a very high level in 2017 (Figure 3). In general, the peak of AMDV prevalence was every 3–4 years. The probability of AMDV presence was low at sites where the number of farmed mink was low and increased linearly with the increase of the number of mink kept on farms (Figure 3). The predicted AMDV prevalence was higher in adult mink compared to subadults, and higher in males than females. This parameter was also higher in the breeding season compared to the non-breeding season (Table 2). In years of low AMDV prevalence, the relation between presence of AMDV in adult and subadult mink and the intensity of mink farming was similar (Figure 4). However, in years of high AMDV prevalence, the presence of AMDV in adult mink in relation to the intensity of mink farming was much higher than in subadult mink (Figure 4). The analyses of the subset of data by GAM2 showed that the predicted AMDV prevalence within the 60 km buffer zone in the site with few farmed mink (NNP and BNP) strongly varied between years, whereas the predicted prevalence did not change significantly in the site with a high intensity of mink farming (WMNP) (Table 3, Figure 5). For example, in NNP and BNP, the prevalence in adult males changed from 0.59 (CI 95% = 0.48–0.71) in 2012 in the non-breeding season to 0.94 (CI 95% = 0.89–0.99) in 2010 (Figure 5). In contrast, in WMNP, the prevalence varied from 0.87 (CI 95% = 0.77–0.97) to 0.92 (CI 95% = 0.85–0.99).

3.3 | Influence of AMDV on mink condition and organ size

Mink body condition was associated with the animal’s AMDV status as well as its sex and age. AMDV-positive mink were in worse condition than AMDV-negative mink (Figure 6, Table 4). The body condition index was higher in males and adults compared to females and subadults, respectively (Figure 6). The three tested organs (spleen, liver and kidneys) were relatively larger in AMDV-positive mink than in negative mink (Figure 7). The spleen was also larger in females than in males and in the breeding season than in the non-breeding season. The liver was larger in females and subadults than in males and adults, respectively, and the kidneys were larger in adult than in subadult mink.
FIGURE 3  Relative changes in the prevalence of Aleutian mink disease virus in the American mink as a function of year and the number of mink kept on farms located within a 60 km radius buffer around the study site, as predicted by the GAM1 summarized in Table 1. Curves represent the estimated smooth function, and shading denotes the 95% confidence intervals. The y-axis presents the partial residuals of the model after removing the effects of the other covariates. The rug plots along the x-axis show the data distribution. The year indicates the period lasting from the autumn of one year to the spring of the following year (e.g., year 2006 is the trapping season 2006–2007). The number on the top of the first panel indicates sample size for each year.

FIGURE 4  Aleutian mink disease virus (AMDV) prevalence in mink males predicted from the GAM1 model summarized in Table 2 in relation to mink farming intensity within a 60 km radius buffer around the site. Predicted values estimated for subadult and adult males, for non-breeding season in year of high (2010) and low (2012) AMDV prevalence. Curves represent the estimated prediction, and shading denotes the 95% confidence intervals [Colour figure can be viewed at wileyonlinelibrary.com]
This study provided the first comprehensive investigation of the spatial and temporal dynamics of AMDV occurrence in feral mink populations in relation to the propagule pressure of farmed mink. It is also the first complex attempt to evaluate the influence of the virus on its host by evaluating AMD symptoms in feral mink. The analyses showed that feral mink were infected with AMDV in all sites, but the prevalence and temporal dynamics varied in relation to farming intensity. The prevalence values showed regular fluctuations in time. Infection with AMDV affected mink body condition and caused an increase in the size of the spleen, liver, and kidneys.

### Table 3

Parameter estimates (coefficients) and SE from the generalized additive mixed model (GAM2) investigating the relationships between the sex and age of mink, season, year at site of high (WMNP) and low (NNP and BNP) intensity of mink farming, and AMDV prevalence in the American mink.

| Variables       | Estimate | SE   | z value | p Value |
|-----------------|----------|------|---------|---------|
| Intercept       | −0.297   | 0.155| −1.923  | .055    |
| Site (WMNP)     | 1.263    | 0.186| 6.789   | <.001***|
| Sex (Male)      | 0.409    | 0.165| 2.477   | .013*   |
| Age (Adult)     | 0.834    | 0.178| 4.684   | <.001***|
| Season (breed)  | 0.718    | 0.199| 3.601   | <.001***|

Spline fit

| edf | Ref. df | Chi sq | p Value |
|-----|---------|--------|---------|
| Year: site (NNP + BNP) | 6.563 | 6.939  | 39.030  | <.001*** |
| Year: site (WMNP)   | 1.000 | 1.000  | 0.450   | .503    |

Note: edf—effective degree of freedom indicates the amount of non-linearity of the smooth in GAM (1—indicative of a linear pattern of relation, >1 denoted a non-linear pattern of relation). * p<.05, ** p<.005, *** p<.001.

### Figure 5

Aleutian mink disease virus prevalence in mink males in consecutive years predicted from the GAM2 model summarized in Table 3. Predicted values were estimated for subadult and adult males in the non-breeding season at the site with high intensity (WMNP) and low intensity (NNP and BNP) of breeding farming. Curves represent the estimated prediction and shading denotes the 95% confidence intervals. The year indicates the period lasting from the autumn of one year to the spring of the following year (e.g., year 2006 is the tapping season 2006–2007). The number on the top of the first panel indicates sample size for site with high mink farming (red) and low mink farming (blue) intensity in each year [Colour figure can be viewed at wileyonlinelibrary.com]

### 4 | Discussion

Feral mink infected with AMDV had a lower index of body condition and enlarged organs (spleen, liver and kidneys), indicating that AMD reduces mink body condition and fitness. The observed symptoms were similar to those described in infected farmed mink (Henson et al., 1976; Persson et al., 2015). In earlier studies, experimental infection of farmed
mink with AMDV caused the development of various histopathological changes in the liver, kidneys, brain and spleen during 24 weeks of observation, such as infiltrations of mononuclear cells in the liver and kidneys, and increased numbers of plasma cells in the spleen (Jensen et al., 2016). The majority of clinical signs were observed in the first two months of the experiment (Jensen et al., 2016). In contrary to other viral diseases recorded in mink, the symptoms of AMDV infection occur over a relatively long time (e.g., the decrease of body mass in mink infected with the Avian Influenza virus was observed only 5–11 days after infection; Zhang et al., 2015). Therefore, introduction of a pathogen with INNS may affect its population dynamics contrary to the Enemy Release Hypothesis. However, the obtained results do not answer the question of whether the infection affects feral mink density in a highly infected population. The decrease in mink density observed ca. 12 years after the establishment of the feral population (Brzeziński et al., 2019) may suggest that pathogens (diseases or parasites) reduce population density. However, other environmental factors, such as a reduction of food availability, may also affect mink population dynamics; therefore, this hypothesis requires further study.

The prevalence of AMDV infection among various demographic groups of mink differed. In our study, as well as in other studies, the prevalence of AMDV in subadults was lower than in adults (Mañas et al., 2016; Persson et al., 2015; Yamaguchi & Macdonald, 2001). This may be related to the fact that the probability of contact with another mink increases with time. Therefore, in such a chronic disease as AMD, virus prevalence increases with age (Mañas et al., 2016). Moreover, contrary to other studies where the prevalence in males and females was on a similar level (Farid, 2013; Fournier-Chambrillon et al., 2004; Mañas et al., 2016; Yamaguchi & Macdonald, 2001), the results of our study show that males had a higher AMDV prevalence than females. This could be related to the larger home ranges utilized by males than by females (Melero et al., 2008; Zabala et al., 2007) and the higher number of individuals that had interacted with others. Furthermore, males disperse over larger areas than females (Oliver et al., 2016; Zalewski et al., 2009) and may have contact with more individuals during dispersal. The recorded higher AMDV prevalence in the breeding season compared to the non-breeding season can be explained by the increased number of contacts between individuals of both sexes in this period. In the non-breeding season, mink are solitary, making the probability of a direct encounter rather low (e.g., Zabala et al., 2007). Therefore, the transmission of the virus is related to the contact rate between individuals, which is higher in adults, males, and during the breeding season.

### TABLE 4
Parameter estimates and test statistics from the linear models explaining the index of body condition and organ size in the American mink in relation to infection status

| Coefficients | Estimate | SE   | t Value | p Value |
|--------------|----------|------|---------|---------|
| **Body condition** |          |      |         |         |
| Intercept    | -0.0191  | 0.0102 | -1.877  | .061    |
| AMDV (positive) | -0.0272  | 0.0097 | -2.793  | .005**  |
| Sex (M)      | 0.0428   | 0.0093 | 4.582   | <.001***|
| Age (Adult)  | 0.0329   | 0.0095 | 3.466   | <.001***|
| Season (Breed) | -0.0041  | 0.0096 | -0.429  | .668    |
| **Spleen**   |          |      |         |         |
| Intercept    | -0.1207  | 0.0253 | -4.779  | <.001***|
| AMDV (positive) | 0.2083   | 0.0241 | 8.636   | <.001***|
| Sex (M)      | -0.1244  | 0.0232 | -5.372  | <.001***|
| Age (Adult)  | 0.0208   | 0.0235 | 0.883   | .378    |
| Season (Breed) | 0.1232   | 0.0237 | 5.194   | <.001***|
| **Liver**    |          |      |         |         |
| Intercept    | -0.0112  | 0.0136 | -0.821  | .412    |
| AMDV (positive) | 0.0602   | 0.0130 | 4.631   | <.001***|
| Sex (M)      | -0.0357  | 0.0125 | -2.858  | .004**  |
| Age (Adult)  | -0.0305  | 0.0127 | -2.401  | .016*   |
| Season (Breed) | 0.01094  | 0.0128 | 0.855   | .392    |
| **Kidneys**  |          |      |         |         |
| Intercept    | -0.0558  | 0.0117 | -5.022  | <.001***|
| AMDV (positive) | 0.0521   | 0.0106 | 4.913   | <.001***|
| Sex (M)      | 0.0105   | 0.0100 | 1.046   | .2960   |
| Age (Adult)  | 0.0388   | 0.0102 | 3.795   | <.001***|
| Season (Breed) | -0.0088  | 0.0103 | -0.858  | .391    |

Note: * p<.05, ** p<.005, *** p<.001.
Aleutian mink disease virus prevalence in mink populations can be highly varied. In Canada, the prevalence of AMDV in native mink varied from 25% to 94%, whereas in Europe in non-native mink, it ranged from 3% to 67% (reviewed by Mañas et al., 2016). Our data showed that in Poland, the prevalence of AMDV in feral mink in 2006–2017 was relatively high and varied regionally, from 46% to 83%. The recorded high prevalence is related to the large number of mink farms and high production of mink pelts, as Poland produced over 5 million mink pelts in 2015 (our data) and 8 million pelts in 2017 (http://www.fureurope.eu). Our results showed that AMDV prevalence in feral mink was related to farm abundance and the number of mink kept on farms in the vicinity of the study site. As the proportion of mink escapees from farms is related to their abundance (Bowman et al., 2017; Kidd et al., 2009; Zalewski et al., 2010), the infection intensity of the feral population is largely related to farming intensity. We used data about the number of mink kept on farms since 2013 but the number of mink farms in Poland constantly increased during the study period which may bias the results. However, we expected that in a buffer zone of a 60 km radius around the site centre, this variation was not very high and only slightly affected the results. Results similar to ours were obtained in Canada: the prevalence in wild-living mink was significantly higher in areas close to mink farms than in mink located further from the farms (Nituch et al., 2011). In Spain, the highest AMDV prevalence was recorded in Galicia, which harboured 80% of Spanish mink farms (Mañas et al., 2016). On the other hand, the results of genetic analyses of AMDV strains are ambiguous. The analyses of Finnish strains showed that farm strains and feral strains were mixed in the phylogenetic trees, suggesting

**FIGURE 7** The relative size of the spleen, liver and kidneys of mink in response to Aleutian mink disease virus infection (neg—negative and pos—positive for the virus), sex (F—female, M—male), age (subadult or adult) and season (non-bre—non-breeding season, breed—breeding season). The bars are the confidence intervals at 95%
virus transmission between farms and feral populations (Virtanen et al., 2019). Analyses of Polish strains, however, showed high genetic dissimilarity between the AMDV from both groups (Jakubczak et al., 2017). AMDV strains recorded on farms differ geographically (Kowalczyk et al., 2019), and this fact should be considered when analysing the circulation of the virus between farmed and feral mink: samples should be compared rather from the same region and not across the whole country.

The impact of mink farming as a source of AMDV in feral mink populations is probably related to the proportion of infected mink at a farm. In Denmark, which is the country with the highest density of mink farms, the prevalence of AMDV on mink farms was low—about 5% in 2001–2015 (Hjulsager et al., 2016; Ryt-Hansen et al., 2017). This low prevalence was sustained by a programme of AMDV reduction on the farms, implemented since 1976 and legislated in 1999 (Ryt-Hansen et al., 2017). As a consequence, AMDV prevalence in feral populations was also low—about 3% (Jensen et al., 2012). In contrast, there is no national level programme of eradication of this disease at farms in China. The extensive review of AMDV in Chinese farms showed that the levels of prevalence in farmed mink ranged from 7% to 84%. It increased from an average of 48% in 1981–2009 to 61% in 2010–2017 (Gong et al., 2020).

In Poland, the programme of AMDV control on farms was implemented selectively. Farms where the programme was implemented very early have not recorded AMDV. The virus prevalence reached 70% on farms where the programme was implemented a few years before the study and 93% on farms that had not implemented the programme (Siemionek et al., 2017). However, these data come from only 6 farms and there is no information on how many farms in Poland (from about 360) implemented this programme. Other data showed that the prevalence of AMDV on 11 farms in Poland varied from 40% to 90% (average 69%; Kowalczyk et al., 2019). Thus, it seems obvious that AMDV occurs on mink farms in Poland, the prevalence can be high, and it can vary spatio-temporally. We expect that non-infected and highly infected farms were located near our study sites, but data on the AMDV prevalence in their stocks were unavailable.

4.3 Temporal variation of AMDV prevalence

Our results showed fluctuations of AMDV prevalence over time, with a peak every 3–4 years. In the three regions, the prevalence fluctuations were similar and the peaks occurred synchronously (based on the inspection of raw data), suggesting the spread of AMDV across Poland. However, the level of changes over the years differed in particular regions. Therefore, the model showed that at sites with high farming intensity, AMDV prevalence was higher and no between-year fluctuation was observed, whereas at sites with low farming intensity AMDV prevalence was lower and largely fluctuated over time. This suggests a constant flow of the virus from farms to feral mink populations, which is related to the number of mink escapees. In western Poland, the number of escapees is much higher than in eastern Poland (Zalewski et al., 2010), and this may explain the lack of a between-year variation of AMDV prevalence in feral mink in WMNP. We may, however, expect that the dynamic of AMDV prevalence may also be related to natural processes of the virus spreading in the feral population and mink population dynamics. A low virus prevalence in 2012–2014 may be related to the very extensive control of mink in NNP and BNP, which reduced the density of mink in these sites (Niemczynowicz et al., 2017). Beside the reduction of density, which may be one of the main factors affecting the spread of the disease, the proportion of subadult mink in the population increased and—as was shown in our study—this age class generally has lower AMDV prevalence. In years when high AMDV prevalence was recorded, the proportion of infected adult mink increased.

Aleutian mink disease virus was maintained in feral populations throughout the whole study period at a relatively high level; therefore, it is possible that this virus was transmitted in both directions: from farms to feral mink and from feral mink to farms. In Iceland, after removing AMDV from farms, farmed mink became re-infected by feral mink (Gunnarsson, 2001). On the other hand, genetic analyses of AMDV during an outbreak in Denmark showed that the virus strains recorded in farmed mink differed from the strains in feral mink; therefore, it was unlikely that feral mink could be the source of the AMDV at the Danish farms (Ryt-Hansen et al., 2017). However, this result is based on analyses of only a few feral mink and AMDV prevalence in the feral population in Denmark was relatively low.

In Poland, AMDV prevalence in feral mink is high, and a spillback infection is more likely. It is also possible that feral mink infect other mustelids, for example the stone marten, which is synanthropic (Wereszczuk & Zalewski, 2015) and can easily visit mink farms in its search for food. Our preliminary results on AMDV prevalence in native mustelids showed that stone martens were also highly infected (unpublished data). Similarly, genetic analyses of AMDV suggested that the raccoon (Procyon lotor) may have played a role in the transmission of AMDV to farmed mink during an outbreak in the USA (Oie et al., 1996). Another piece of evidence for the possibility of the virus’s transmission from wildlife to farms is the outbreak of the canine distemper virus on mink farms in Denmark in 2012, when farmed animals probably were infected by wildlife species (Trebbien et al., 2014). Therefore, the system maintaining a high level of virus prevalence on farms and in wildlife populations seems to be complex. The transmission of the virus between two partly separated mink groups and other wild-living native mustelids maintains a high level of infection in these groups. Similar results of the between-year fluctuation of the canine distemper virus transmitted between wildlife and animals accompanying humans were described in Africa (Viana et al., 2015). The outbreak of canine distemper virus in the lion (Panthera leo) population was partly related to the transmission of the virus from dogs, as well as from other animals (Viana et al., 2015). Our data provide another example of disease transmission between animals bred by humans (livestock) and wildlife species. Taking into account that cases of AMD have been described in mink farmers in Denmark (Jepsen et al., 2009), the
zoonotic potential of AMDV should also be considered. However, human AMDV infection is an exceptionally rare event or rarely diagnosed. In general, increasing numbers of animals kept on farms (such as mink), pets (dogs and cats) and wildlife species co-habiting with humans due to developing synurbization (e.g., red fox [Vulpes vulpes], raccoon and stone marten; Bartoszewicz et al., 2008; Duduš et al., 2014; Wereszczuk et al., 2017) largely increase the probability of AMD transmission from livestock to wildlife and its spillover even to humans.

The development of farming increases the possibility of interactions between livestock and wildlife. In the last 120 years, the biomass of domestic animals has increased about 3.5 times (Pozio, 2020). The probability of domestic animal pathogens spilling over to wildlife species, including INNS, has highly increased. Conversely, the pathogens from native wildlife species or INNS feral populations can be transmitted to domestic animals, causing losses in animal production and conflicts between humans and nature conservationists. These two groups of animals create a complex system affecting temporal and spatial pathogen maintenance in the environment. Two-way pathogen transmission may cause disease outbreaks more often and/or a higher level of maintained pathogen prevalence in both livestock and wildlife. If farmed mink play an important role in AMDV transmission from farms to feral mink, they probably can also transmit other viruses in a similar way, especially that the number of farms and number of mink kept on farms can be very high locally. The viruses prevalent in farmed mink are as follows: canine distemper virus, influenza A virus H1N2 or H1N1, avian influenza virus H9N2, or recently even COVID-19 (Äkerstedt et al., 2012; Gagnon et al., 2009; Oreshkova et al., 2020; Trebbien et al., 2014; Yong-Feng et al., 2017; Zhang et al., 2015). Farmed mink can also be a reservoir for other pathogens, for example, methicillin-resistant Staphylococcus aureus (MRSA), which is also transmitted from mink to humans (Fertner et al., 2019; Hansen et al., 2020). Therefore, the main goal to mitigate the transmission of the viruses or other pathogens is to analyse the pathways of farm-wildlife and wildlife-farm pathogen transmission and further increase epidemiological surveillance on farms. The development of mink farming without reducing AMDV occurrence on farms affects feral mink which may be transmitting this disease to native species.

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CONFLICT OF INTEREST

All authors declare that they have no competing interests.

ETHICAL APPROVAL

The authors confirm that the ethical policies of the journal, as noted on the journal’s author guidelines page, have been adhered to. According to the opinion of the Local Ethics Commission, there is no need to obtain permission to use tissue samples from animals acquired in the course of other projects, especially during eradication programmes of invasive species as part of a national park or Nature 2000 conservation plan.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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