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Cheilospirura hamulosa in the Rock Partridge (*Alectoris graeca saxatilis*): Epidemiological Patterns and Prediction of Parasite Distribution in France

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Abstract: The rock partridge (*Alectoris graeca saxatilis*) is an alpine Galliform with high conservation value. Several factors, including parasitic helminths, play a role in population dynamics, and consequently in the conservation management of wild Galliformes. The aim of this study was to assess the epidemiological characteristics of *Cheilospirura hamulosa* (Nematoda, Acuarioidea) in the Rock partridge population in France. Machine learning modeling algorithms were applied to identify the environmental variables influencing parasite occurrence, and to map parasite presence probability. The present work is based on a long-term sampling (1987–2019) conducted in the French Alps. *C. hamulosa* was found with a prevalence (P) of 39% (Confidence Interval—CI 95%: 34–43), and mean intensity of 7.7 (7.8 sd). The highest prevalence (P: 67%, CI 95%: 54–80) was detected in the period 2005–2009. Latitude was the most important variable shaping the parasite distribution, followed by altitude, annual mean temperature, temperature seasonality, and the amount of precipitation of the coldest quarter. The area suitable for parasite presence included 73% of the French Alps. This work represents the first epidemiological surveillance on *C. hamulosa* infection in the rock partridge. It provides evidence of a high level of infection and identifies priority areas at higher infection risk, where a close monitoring of the rock partridge populations ² should be carried out.

Keywords: rock partridge; *Alectoris graeca saxatilis*; *Cheilospirura hamulosa*; French Alps; random forest; predictive maps

1. Introduction

The rock partridge (*Alectoris graeca saxatilis*) is an alpine Galliform inhabiting the prairies and rocky meadows from the French Alps to Slovenia [1]. Like other alpine Galliformes such as the rock ptarmigan (*Lagopus muta*), it has recently suffered a significant decline in the Alps [2], (and it is now listed in Annex I of the “Bird” Directive 2009/147/EC, aiming to preserve species and their habitats. The species *A. graeca* is classified as “Near threatened” (NT) in the last update of the IUCN Red list, in view of the fact that the population is significantly declining, particularly in the Eastern part of its range [3]. Most of its global population have suffered a reduction exceeding 30% of the estimated consistency over the last three generations [4]. The same classification (NT) appeared in the last update of the French National Red book [5].

The species’ long-term conservation is negatively influenced by several factors, including habitat degradation [6], reduction of habitat suitability due to changes in traditional agro-pastoral activities [7], and fragmentation of habitat, with decreased connection among metapopulations [8]. Pathogens are

also considered among the factors threatening species conservation [9,10]. Infectious diseases have been listed among the top five causes of wildlife extinctions and endangerment [11], but few studies have assessed the role of pathogens in rock partridge population dynamics [2,9,10]. Indeed, most of the research about the potential threats to the rock partridge focus on human disturbance, habitat changes, and over-hunting [6,12,13]. In addition, invasive species and translocated populations may pose a risk to native wildlife populations by hybridization and the introduction of new pathogens [14–17]. This is the case for partridges in Europe for which concerns have been raised considering the risk of genetic pollution with captive-reared hybrids [18].

Helminths may play an important role in determining changes in grouse numbers [10,19,20], through direct mortality or causing a reduction in reproductive success. In particular, spirurid worms, and to a less extent cestodes, have been suspected to regulate the rock partridge population in the French Alps [21]. Spirurid-related morbidity and mortality has been widely described in captive birds [22], but only a few reports exist for wild birds. These include studies evaluating the role of infection connected with population decline in avian species [23,24].

Among the spirurids, *Cheilospirura hamulosa* (Nematoda, Acuarioidea), is a parasite of great importance, due to its high pathogenicity in Galliformes. It may cause severe lesions, such as granulomas and nodules in the gizzard, which lead to anemia and significant mortality [25]. Birds get infected by eating contaminated arthropods containing third-stage larvae. Many intermediate hosts are involved like grasshoppers (*Melonopus* sp., *Coenocephalus* sp., *Oedaleus* sp., *Oxya nitidula*, or *Spathosternum prasiniferum*), beetles (*Tribolium* spp.), sandhoppers (family *Talitridae*), and weevils (superfamily *Curculionoidea*) [26]. Sanitary monitoring, and the evaluation of the effect of pathogens, in wildlife needs an approach different from the “classic” clinical evaluation of the single animal [27]. Sanitary surveillance in free roaming populations is carried out at population level, and the data needs to be aggregated and analyzed using advance statistical approaches. In the context of wildlife sanitary surveillance, the application of spatio-temporal analysis techniques has provided valuable insights [28,29]. Additionally, spatial modelling has increasingly been employed as a support for decision makers, for population management and surveillance, predicting parasite distributions [30–33], and allowing the prioritization of the zones where conservation actions need to be applied. Different techniques are available to build predictive maps, combining data on parasite occurrences with the potential drivers (i.e., climatic, environmental factors) impacting on intermediate—host populations and parasite survival and development [34].

More recently, machine-learning (ML) methods have been used in distribution modeling with promising results and better performance than traditional algorithms [35,36]. In particular, ML models effectively tackle issues such as non-linearity of the data, the presence of zero values, and a reduced sample size. Thus, they have demonstrated a high potential for obtaining accurate predictive models using complex and noisy data [37].

The monitoring of disease occurrence and epidemiological characteristics, in addition to environment preservation, is one of the most important actions to protect biodiversity. For this purpose, we assessed the basic epidemiological characteristics (prevalence, abundance, and intensity) of *C. hamulosa* in the French rock partridge, based on long-term sampling (1987–2019). Additionally, we used ML models to identify the environmental variables influencing parasite occurrence, and to map their presence probability in the French Alps.

2. Materials and Methods

2.1. Field Sampling and Study Area

Four-hundred and sixty-nine rock partridges were collected in the French Alps from 1987 to 2019 (no samples collected during the period 1997–1999). For more details about the sampled animals please refer to Supplementary materials—Tables S1 and S2. Most of the animals derived from birds killed during hunting activity, with a few others found dead for other reasons (impact with cables,

or predation). All the samples were geo-referenced, with the exception of eight animals for which it was not possible to obtain accurate spatial information (Supplementary materials—Figure S1).

2.2. Parasitological Analysis

The gizzard of each partridge was examined following the common parasitological standard techniques [38]. Adult worms were counted and collected under a stereoscopic microscope, and identified using a light microscope. The morphological identification of the nematodes was done according to the keys described by Skrjabin et al. [39] and Ebrahimi et al. [40].

2.3. Epidemiological Descriptors

Epidemiological indexes of prevalence (number of infected animals/total animals) and intensity (number of parasites/positive animals) were computed for the whole population, by sex and age. Afterwards, prevalence data were compared using Fisher's exact test, and frequency distribution of parasite intensity and abundance using the Kruskal–Wallis test [41]. To evaluate and represent the temporal trend of infection, a five-year aggregate prevalence was calculated and plotted. Data were aggregated at five-year intervals to minimize the unbalanced distribution of samples among years, and to derive a more robust trend.

2.4. Environmental Variables

The variables used to build the models are reported in Table 1. Spatial dataset layers include bio-climatic [42] and topographical factors, represented as raster data. All the rasters were clipped using the French Alps shapefile as a mask layer [43], considered as a proxy for the host species' potential distribution. The rasters were rescaled at a resolution of 1 km², aligned, and re-projected using the same coordinate reference system (CRS-WGS 84/UTM zone 32N). All spatial data manipulation and representation were done using QGIS 3.6 [44].

Table 1. Environmental layers used to model *Cheilospirura hamulosa* distribution (Source for bioclimatic variables: WorldClim <https://www.worldclim.org/bioclim>, Source for DEM European Environment Agency <https://www.eea.europa.eu/data-and-maps/data/digital-elevation-model-of-europe>).

Variable	Description
BIO1	Annual Mean Temperature
BIO4	Temperature Seasonality (standard deviation × 100)
BIO8	Mean Temperature of Wettest Quarter
BIO10	Mean Temperature of Warmest Quarter
BIO11	Mean Temperature of Coldest Quarter
BIO12	Annual Precipitation
BIO15	Precipitation Seasonality (Coefficient of Variation)
BIO17	Precipitation of Driest Quarter
BIO18	Precipitation of Warmest Quarter
BIO19	Precipitation of Coldest Quarter
DEM	Digital elevation model (DEM) describing the altitude for each pixel
Latitude	Raster describing the latitude in UTM coordinates of each pixel

2.5. Modelling Approach

The modeling approach was set in two steps with the following objectives: (i) identify and interpret the relationships between parasite occurrence (parasite presence) and environmental covariates, and (ii) build a spatial model to predict the parasite habitat suitability across the French Alps (39,372 km²). In the first step, a tree classification model was built with the CARET package [45] in the R statistical framework [46]. Data was split into training (70% of points) and testing (30% of points) datasets. Resampling consisted in 10-fold cross-validated repeated three times and information gain was used as a splitting criterion. Although basic decision tree models are straightforward to interpret, they poorly

predict the dependent variable for new values of the covariate. This limitation is due to the fact that they provide only a single description on the relationships between response and predictors [30]. Thus, in the second step, we built a random forest model (as implemented in the R package “randomForest” [47] to make more robust predictions. The following parameters were used to define the random model: (i) type of random forest = regression, (ii) number of trees = 500, (iii) number of variables tried at each split = 4. Variable importance was assessed based on mean decrease in accuracy, and the area under the curve value (AUC) was computed to evaluate model performances. AUC compares the model sensitivity (true positives) against “1–specificity” (false positives) over the entire range of the threshold. An excellent model has an AUC close to 1, whereas a poor model has an AUC close to 0 [48]. Model outputs were used to map the probability of occurrence of *C. hamulosa*. This first output was then converted into a binary surface using a threshold value maximizing both sensitivity and specificity. The percentages of suitable and unsuitable areas were computed, and a final map was created in QGIS v 3.6 [44]. Model outputs were reviewed and validated by wild Galliformes experts.

3. Results

C. hamulosa was found in 182/469 birds (P: 39%, CI 95%: 34–43), with a mean intensity of 7.7 (7.8 sd). The prevalence in the female sample (P: 28%, CI 95%: 20–35) was not more statistically significant than in the male sample (P: 34%, CI 95%: 27–42) (p -value = 0.2516), whereas the mean intensity was significantly higher in females, 11.5 (9.7 sd), compared to males, 7(6 sd) (p -value = 0.02237). The Fisher test showed that the proportion of adult birds infested by the parasite was (P: 43%, CI 95%: 36–51), significantly higher than in the juveniles (P: 32%, CI 95%: 26–38) (p -value = 0.01779), whereas the mean intensity was not statistically different according to age (adults: 7.7, 7.1 sd and juveniles 7.9, 8.8 sd) (p -value = 0.3808).

The 5-year aggregated prevalence trend is reported in Figure 1. Prevalence was almost duplicate in the period 1992–1996 to 2005–2009, when the highest prevalence (P: 67%, CI 95%: 54–80) was detected. This was followed by a 70% reduction in the most recent period of study (Figure 1). Detailed information about (i) prevalence with 95% CI, and (ii) mean parasite intensity with standard deviation during the period, is available in the Supplementary materials—Table S3.



Figure 1. Five-year aggregated prevalence of *C. hamulosa* in the rock partridge during the period 1987–2019 (1997–1999 excluded due to the absence of samples) in the French Alps. Years are represented on the x -axis, and prevalence values on the y -axis.

The tree classification model indicates that few variables could discriminate parasite presence. Latitude was the most important predictor, with higher parasite presence probability at lower latitude. At sites with higher latitude, temperature seasonality (bio4) and altitude (dem) were important in further characterizing the distribution. Annual mean temperature (bio1), the amount of precipitation of the coldest quarter of the year (bio19), and altitude (dem) played an important role at lower latitude (Figure 2).

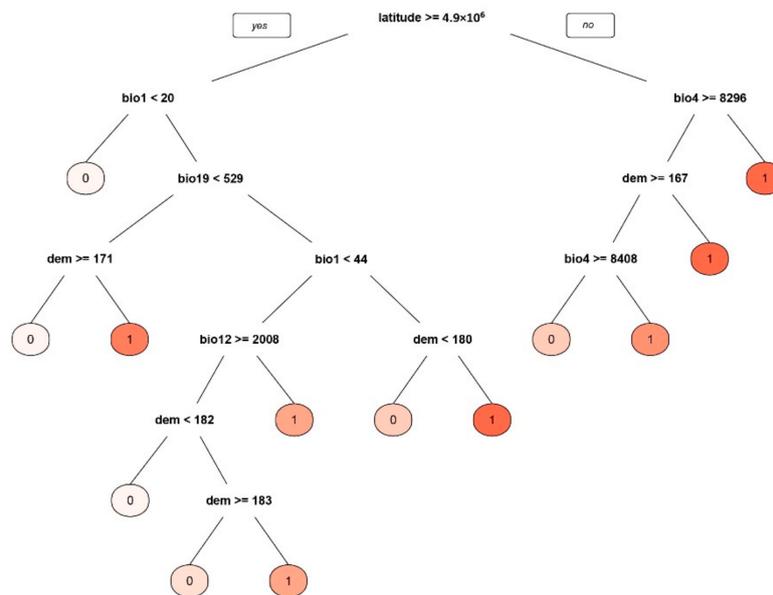


Figure 2. Classification tree describing the relationships between the predictors and parasite presence. Models are read from the top down beginning at the root node, which contains all data. At each subsequent decision node, data that satisfies the splitting rule moves to the left branch and all other data moves to the right branch. Observations in terminal nodes are classed as present (1) or absent (0).

In agreement with the classification tree, the random forest model identified latitude as the most important factor. Altitude (dem), temperature seasonality (bio4), and the amount of precipitation of coldest quarter (bio19) were also important factors selected in this model framework (Figure 3).

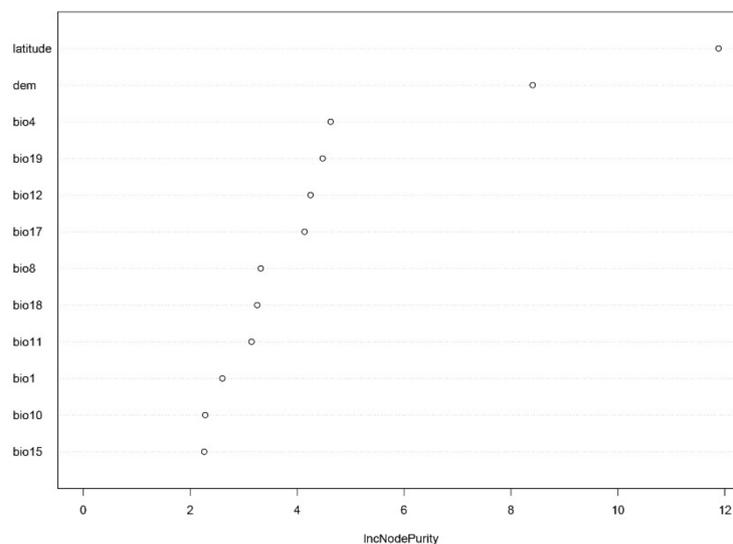


Figure 3. Importance scores for each environmental variable used as input in the random forest algorithm model. The increase in node purity is calculated based on the reduction in sum of squared errors whenever a variable is chosen to split.

Based on the results of the models, the probability of *C. hamulosa* occurrence was plotted, showing a decreasing trend, with a south-to-north and west-to-east gradient. The area with the lowest probability of parasite occurrence was located in the central part of the study area, at the border with the Italian Alps (Figure 4).

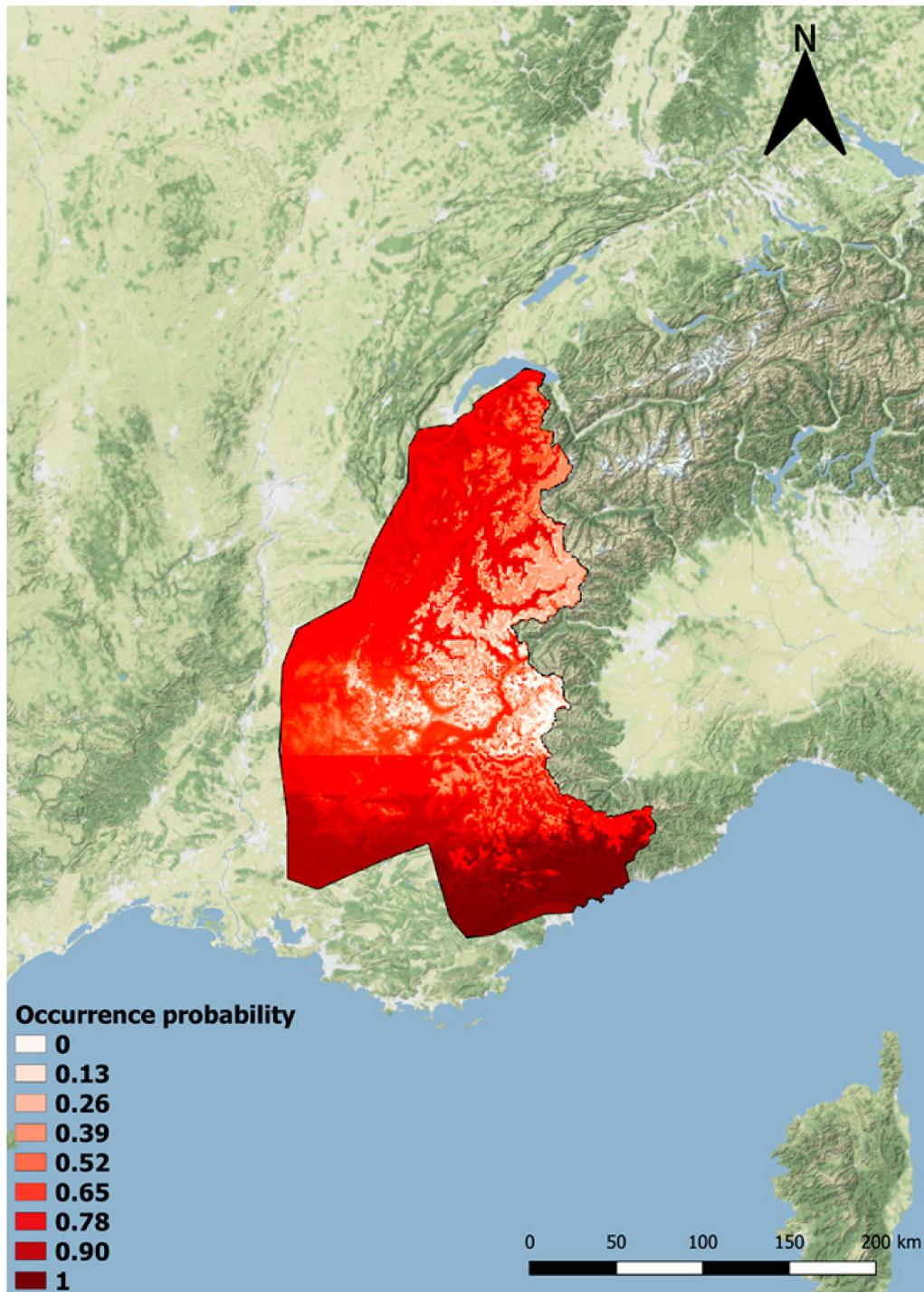


Figure 4. Species distribution model of *C. hamulosa* using a random forest model. The presence probability is represented on a scale from 0 to 1.

As shown in Figure 5, the application of a cut-off discriminant value depicted the area considered suitable for parasite occurrence. The total area suitable for the parasite development consists of 73% (28,797 km²/39,371 km²) of the French Alps, mostly located in the southern part of the study area. As regards model accuracy, both models performed quite well (decision tree AUC: 0.66, random forest AUC: 0.79).

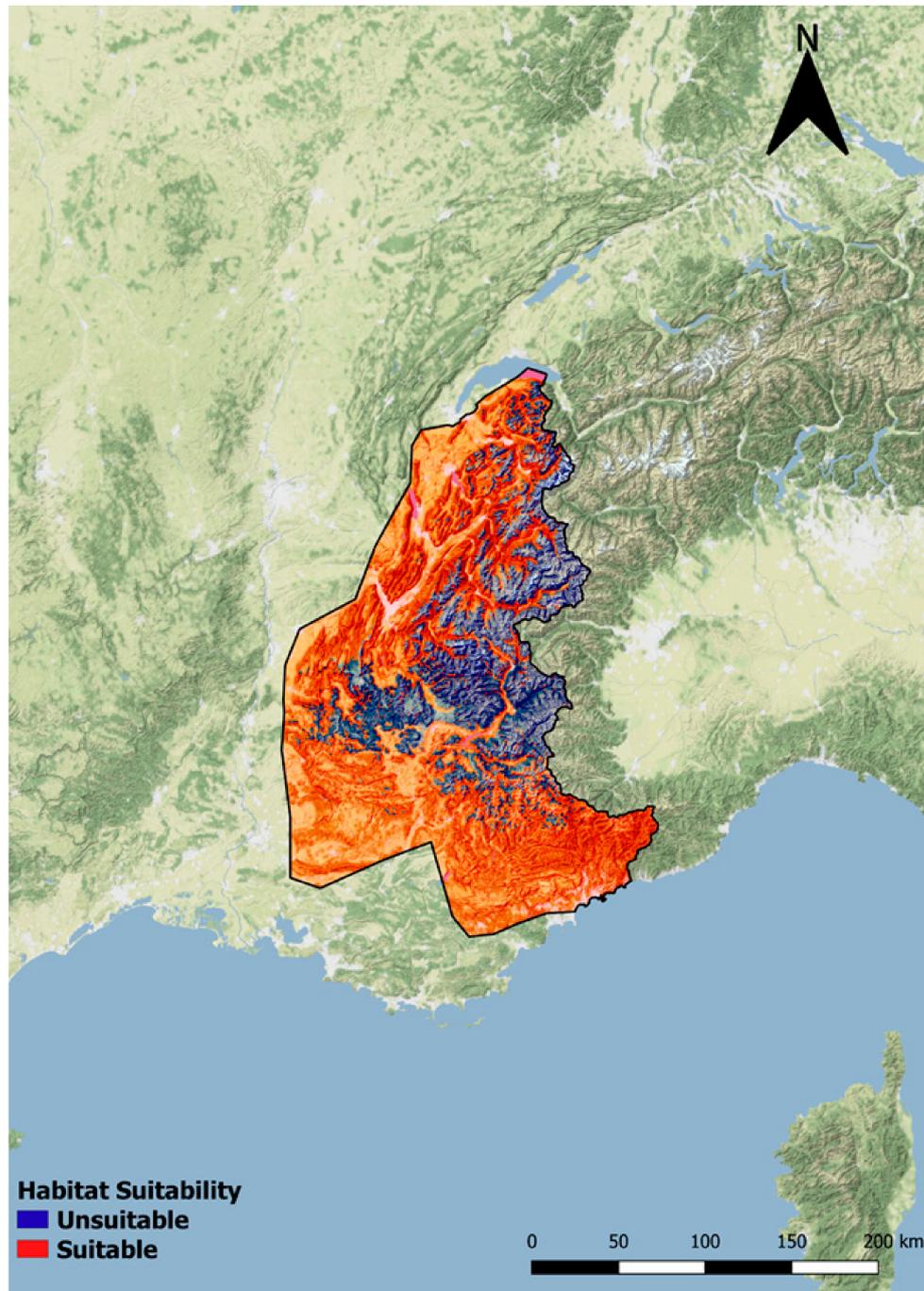


Figure 5. *C. hamulosa* habitat suitability in the French Alps.

4. Discussion

This study presents the first epidemiological data available on *C. hamulosa* infection in the rock partridge. Both prevalence (P: 39%), mean abundance (3), and mean intensity (7.7) values were quite high, with a peak in prevalence for the period 2005–2009. Our models depicted a high probability of *C. hamulosa* occurrence in the southern part of the French Alps, identified as the areas with the most suitable conditions (high probability of occurrence) for the parasite. Both models in our analysis highlighted the importance of latitude in explaining the observed parasite distribution patterns. Secondary, but still relevant, factors were altitude and bioclimatic variables (bio4, bio1, bio19—see Table 1).

Rock partridge (*A. g. saxatilis*) is an important gamebird, and at the same time a species with significant conservation value, given its recent global population decline [4]. However, information about the sanitary status of this Galliform in the Alps, including its parasite community, is still scarce. Among the available studies it is worthwhile citing the following surveys: Rizzoli et al. [49] in Trentino (Eastern Alps) in the 1990s, the most recent works of Formenti et al. [50] and Fanelli et al. [51] in the Western Italian Alps. A few other works have described the parasite community of the rock partridge [52], but *C. hamulosa* has never been reported on the Italian side of the Alps.

This nematode is found in the gizzards of different families of birds, mainly in the region of the caudal blind sac [25]. Comparable to our study, a similar *C. hamulosa* prevalence (P: 30%) was found by Ebrahimi et al. [40] in wild partridges (*Alectoris chukar*) in Iran; whereas studies on domestic birds reported a lower prevalence: 3.6% in chickens (*Gallus g. domesticus* L., 1758) from India [26], 4% in fowls from Iran [53], and 14.3% in backyard pheasants and 26.7% in backyard chickens from Brazil [54]. The large variation of prevalence values is mainly related to the origin of the samples, with the risk of infection being higher in wild Galliformes than poultry [55].

In the host population, parameters of occurrence of parasitic nematodes may vary significantly in relation to their age and sex. Generally, males experience a greater degree of parasitism than females, although these differences can be small [56,57]. Results from this study demonstrate that this is not necessarily true, as the *C. hamulosa* load was significantly higher in females than in males. This study also clarified the age–prevalence relationship of the parasite, which increased with age, the prevalence being significantly higher in adults. It is difficult to explain this result considering that the diet of young and adults is similar.

The trend of the five-year aggregated data shows a large variation in the prevalence along the study period. Even if our data do not support any cause–effect hypotheses, this finding might be linked to several factors, including a change in the rock partridge population density. The spread of parasites at population level is usually related to host abundance, with a direct relationship between the size of host population and parasite prevalence [58]. This is in line with the data reported by the Mountain Galliformes Observatory, who described a sharp increase in the rock partridge numbers in the French Alps during the period 2003–2007, followed by a population reduction (Observatoire des Galliformes de Montagne—<http://www.observatoire-galliformes-montagne.com/>, accessed on June 2020). From this perspective, even if more robust data should be obtained, this observation might indicate an effect of *C. hamulosa* in rock partridge population dynamics. Rock partridge is a species with demonstrated cyclic fluctuations [1], and the factors driving the cycles are still to be further studied. In other Galliformes, *Trichostrongylus tenuis* has been demonstrated to cause cyclic fluctuations of red grouse *Lagopus lagopus scoticus* [59]. Even if this hypothesis is yet to be demonstrated, it is possible that something similar occurs in rock partridges. The work of Rizzoli et al. [49] has in fact demonstrated that parasites may reduce partridge fecundity, and that the intensity of parasites is greater in cyclic than the noncyclic populations [10].

Another possible factor explaining the observed trend for large variation of the prevalence through the study period might be related to climatic factors. In fact, temperature and humidity have been recognized as major drivers of parasite presence and distribution [60]. Our work also provides insights into the spatial patterns of *C. hamulosa*. According to our estimates, about 73% of the French Alps is

suitable for the development of the parasitic cycle. Latitude seems to be the environmental factor with the highest contribution to shaping the parasite distribution. Interestingly, another study carried out in the French Alps has recently associated the occurrence of helminths in the rock ptarmigan with latitude, interpreting this finding as a proxy of climatic and environmental conditions required for the development of free-living stages of the parasite. In contrast with our results, this work found an opposite gradient with the Northern part of the French Alps considered at higher risk of infection. However, the results in this case were related to a different host species (*Lagopus muta*) and a different parasite (*Capillaria caudinflata*) [29]. Indeed, it is important to highlight that parasite species distribution is largely determined by their hosts, and the variation in host abundance may influence parasites' geographical range [61]. Additionally, *C. hamulosa* occurrence is strictly influenced by the density and activity of the arthropod intermediate hosts [26], with complex interactions among environmental and climatic factors. Unfortunately, this information (definitive and intermediate host distribution and density), which could be valuable to improve the performance of our predictions, is largely incomplete or missing.

To take into account host species, parasites, intermediate hosts, and environmental interactions, we used machine learning algorithms to better fit the complexity of ecological systems [62]. Model results should be interpreted on the base of the non-linear associations between the response (parasite occurrence) and the explanatory variables. In this sense, the occurrence of *C. hamulosa* at identified high altitude cut-off values might reflect the distribution of the intermediate hosts, which are mostly found in the high mountain pastures [63].

Remarkably, in all the splits of the model the presence of the parasite was associated with higher values of annual mean temperature (bio1) and temperature seasonality (bio4), whereas high amounts of precipitation in the coldest quarter negatively (bio19) affected its presence. The precipitations in this case have to be considered as the amount of snow, and its permanence on the soil. These results corroborate the importance of climatic variables on the distribution of parasites with arthropods as intermediate host. In fact, seasonal climatic variation has been shown to influence the activity and abundance of the intermediate hosts, while lower temperature in winter has been claimed to induce arrested development of parasites in both the intermediate and final hosts [26].

To our knowledge, this is the first study investigating the infection of *C. hamulosa* in the rock partridge. Based on our results, we concluded that this parasite shows a large distribution in the sampled animals, and a potential wide distribution in the French Alps, based on the results of our model. Considering the potential impact of the parasite on population dynamics, the presence of *C. hamulosa* in the rock partridge in the French Alps must be taken into account when planning conservation strategies of this threatened species. Considering the scarce literature available on *C. hamulosa*, our work highlights the need for close sanitary monitoring in the areas with a higher risk of infection.

5. Conclusions

It is worth noting that data on hunting wildlife provide valuable information on diseases circulating among the animal's populations. However, studies using hunted animals might be subjected to bias due to the fact that disease also drastically increases the risk of being hunted. Despite this, we believe that such bias is minimal in our study, and that the birds sampled in this study did not differ much from the bulk of the population. This is due to the fact that this work covered a wide part of the species range in France, and was characterized by a long-term sampling (1987 to 2019).

Supplementary Materials: The following are available online at <http://www.mdpi.com/1424-2818/12/12/484/s1>, Figure S1: Map showing the locations where the birds were sampled, Table S1: Number of birds sampled per year from 1987 to 2019, Table S2: Sample characteristics: age and sex of the birds sampled, Table S3: Five-year aggregated: Prevalence (CI 95%) Mean Intensity (SD) of *C. hamulosa* in the rock partridge during the period 1987–2019 (1997–1999 excluded due to the absence of samples) in the French Alps.

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