

# Breeding success of red-legged partridges *Alectoris rufa* in relation to landscape mosaic

## Abstract

Red-legged partridge restocking is a common practice in hunting management in Italy, as the species has become very uncommon in its native range, mostly following habitat loss. Therefore, habitat suitability plays a key role in the successful Reintroduction and especially in the breeding success of this game species. A landscape mosaic can affect the persistence and the reproductive success of partridges. We performed spring and summer censuses, in two consecutive years, to investigate the reproductive success and possibility to increase density of red-legged partridges in the province of Massa-Carrara, following programmes of sustainable habitat management and the creation of landscape mosaic.

**Keywords:** *Alectoris rufa*, breeding success, environmental improvement, habitat management, restocking

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## Introduction

Small game species have shown a progressive decline in the last decades, especially during the second half of the 20<sup>th</sup> Century.<sup>1,2</sup> In general, long-term declines in game species populations is mostly due to changes in agricultural management practices, resulting in the loss and fragmentation of landscape diversity which may affect feeding habits.<sup>3,4</sup> Predation by native and introduced carnivores, as well as disease outbreaks, may increase local mortality.<sup>3,5</sup> Moreover, the use of captive-bred hybrid individuals in restocking programmes may have locally led to a remarkable erosion of the native gene pools.<sup>6,7</sup>

In particular, the red-legged partridge *Alectoris rufa* (Aves, Galliformes) has suffered a serious decline throughout its range over recent decades.<sup>1,8-10</sup> This decline is motivated by various factors, among which we would highlight changes in habitat,<sup>11</sup> increased predation,<sup>12</sup> genetic contamination<sup>6</sup> or excessive hunting pressure.<sup>14</sup> In the case of habitat changes, there are two opposite circumstances but with equally negative effects. On the one hand, the most productive areas have experienced strong agricultural intensification, going from a mosaic landscape of high quality for the species<sup>13,14</sup> to a very monoculture of large plots, without boundaries and with a high use of phytosanitary products.<sup>15</sup> On the opposite side, areas of lower agricultural quality or more difficult to manage have been progressively abandoned, being replaced by scrubland and forest, a habitat with much less capacity to accommodate the red-legged partridge.<sup>13,14</sup> In some European countries, e.g. France, UK and Hungary, the European Agricultural Policy has favored an intensification and modernization of agriculture, creating a landscape without adapting site to recovery and refuge areas for *Alectoris* partridges.<sup>16-20</sup>

However, most threats to breeding bird populations should be observed at the extreme borders of their extent of occurrence, i.e., in the case of *A. rufa*, in central Italy.<sup>21,22</sup> In this area, little is known about conservation status of this widely-restocked species.<sup>22</sup> Accordingly, the red-legged partridge is listed as “Data Deficient” in the most recent Italian red list of Vertebrate Species.<sup>23</sup> It has been shown that genetic

pollution with the chukar partridge *Alectoris chukar* occurs throughout the Apennines.<sup>6,24-26</sup> However, also bad management practice of farmlands is reported as a threat to this species in Italy.<sup>22</sup> In the northern Apennine, an increase of covered areas with respect to open areas and a progressive decline of foraging areas for wild galliformes is ongoing.<sup>27,28</sup> The hunting interest for the red-legged partridge has developed both programs of reintroduction and environmental improvement, which has favored the resumption of land for cereal cultivation, as well as programmes of maintenance of meadows and pastures, managed by local hunting offices (hereafter, ATC: “Ambito Territoriale di Caccia”) and Council Administrations.<sup>22,29</sup> On the other hand, programs of reintroduction have become an important conservation method to restore locally game species,<sup>30,31</sup> even if many reintroductions have been compromised by highly predation or breeding failure.<sup>30,32,33</sup> Some authors linked the failure of reintroduction to others factors such as genetic deterioration of the captive stock,<sup>24,25,34</sup> modifications of physiological and morphological characteristics<sup>35</sup> and changes in behavior.<sup>36-38</sup> Moreover, the habitat suitability plays a key role for successful species reintroduction<sup>13,39,40</sup> and breeding success.<sup>17,41</sup> Ponce-Boutin<sup>42</sup> reported high densities of red-legged partridges in agricultural areas dominated by vineyards and/or cereal crops and Meriggi.<sup>43</sup> considered unsuitable for red-legged partridges in Italy areas with more than 50% of woods, scrublands, and urbanized land. Besides the percentage of open areas and presence of cultivations, the vegetation composition, the structure and the landscape pattern are essential for avian communities and for breeding birds.<sup>44-46</sup>

On intensively farmed agricultural land, Bro<sup>47</sup> showed that environmental enhancements meant to generate a landscape mosaic were ineffective for grey partridge *Perdix perdix* restoration. The use of cereal crops controlled with strip release (a common management technique in Europe) could result in species buildup (i.e., red-legged and grey partridges, common pheasants *Phasianus colchicus*, European brown hares *Lepus europaeus*), but it makes game species easy prey for raptors.<sup>47</sup>

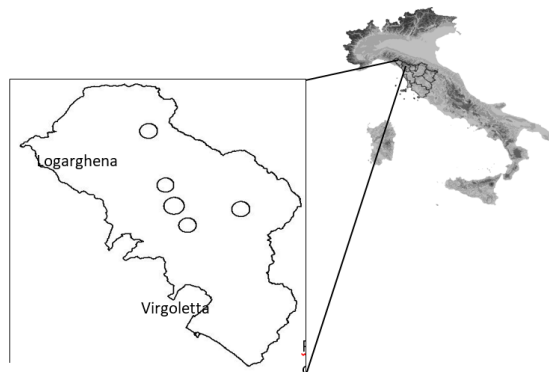
In our study, we investigated the reproductive success and the potential for population density increases of red-legged partridges in the north-eastern Apennines, following habitat management and creation of landscape mosaic. We predicted successful breeding of this species due to the environmental improvement, and the potential for a local population increase.

## Material and methods

### Study area

The study was performed on the province of Massa-Carrara (Tuscany, Italy) using 5 sample areas identifiable with the nearest villages named respectively Amola, Logarghena, Olivola, Pognana and Virgoletta (Figure 1). In particular, the local ATC released red-legged partridge pairs in these 5 sample areas on February. Precisely 24 pairs were released at Amola,<sup>10</sup> at Logarghena,<sup>15</sup> at Pognana,<sup>10</sup> at Virgoletta, both in 2011 and 2012. At Olivola site, 10 partridge pairs were released in 2011 and, after environmental improvements measures, 13 pairs in 2012.

In each sample areas we created a circular buffer area to investigate habitat use. The radius of sample area was related both to number of red-partridges released and to environmental characteristic of each area (Figure 1). The climate is classified as sub-Mediterranean, typical of the Apennines, with a rainfall averaging 921.7mm/year (min. in Amola site with 1920.0mm/year; max. in Logarghena site with 2605.1mm/year) and the annual temperature averaging 18.6° C (min. in January 8.0° C; max. in July 31.1° C<sup>27</sup> (Figure1).



**Figure 1** The province of Massa-Carrara and study sites.

### Spring count

In spring 2011 and 2012, from March 1 to April 30, according to the highest calling activity of *Alectoris rufa*<sup>48–50</sup> and a stabilization of territorial activity males,<sup>49</sup> we performed a count using a playback stimulus to individuate males.<sup>21</sup> Calling counts were conducted on three transect per area, with different length, where points were individuated at least 500 meters of successive distances.<sup>21</sup> Playback session consisted of a 30sec tape of male mating call run four times toward each cardinal wind direction, using a tape player connected to a linear amplifier with an output of 4 watts and an exponential horn with high directionality of emission. Following Karsprzykowski and Golawski,<sup>51</sup> we defined a circle of a 500m radius around every study point as “listening point”. Trials were carried out for two hours before and after dusk and dawn with low wind and no rain.

### Summer count

From May to July (2011 and 2012), pairs and broods of red-legged partridges were counted with observations from advantage points and from transects, using standard methods already used for

this species,<sup>2,52</sup> to estimate post-breeding consistence, and breeding success as percentage of successfully reproduced pairs. During this period, four replicates for each session were made by observers for two hours before and after dusk and dawn. The number of partridges observed were collected on a topographical map (1:10.000) and on count cards too. In our study areas every year red-legged partridges were released for the local game management programme. Therefore, we identified every male in reproductive activity, which could be a male released early before or a male survived from previous hunting season. In this way we could know the actual number of reproductive males present in each study area, as well as the number of released males in each year.

### Environmental improvements

In Olivola site, where environmental improvements have been intensive, patches of millet, panic and sorghum in autumn were created with spring sowings (sowed in spring), and wheat during summer with autumn/winter sowings (sowed in winter). Therefore, we created a landscape mosaic of cultivate areas, mixed with meadows and scrublands. This objective was reached with effort to create and/or to re-create open spaces with meadows and pasture.

### GIS database development

On the basis of color aerial photograph and direct inspections by authors, we assessed landscape changes in relation to agricultural practices and woodland surface. In particular, we registered on the field the changing on Olivola site, which had the most important environmental improvement measures.

All polygons, that constituted landscape mosaic, were manually delineated using ArcMap 9.2 GIS software (ESRI) and were classified into 8 land cover categories as follow: urban areas, woodland, orchards (principally vineyards and olive groves), scrublands, pasture and meadows, agricultural fields (principally cereals and Lucerne), rivers and lakes, roads.

### Statistical analysis

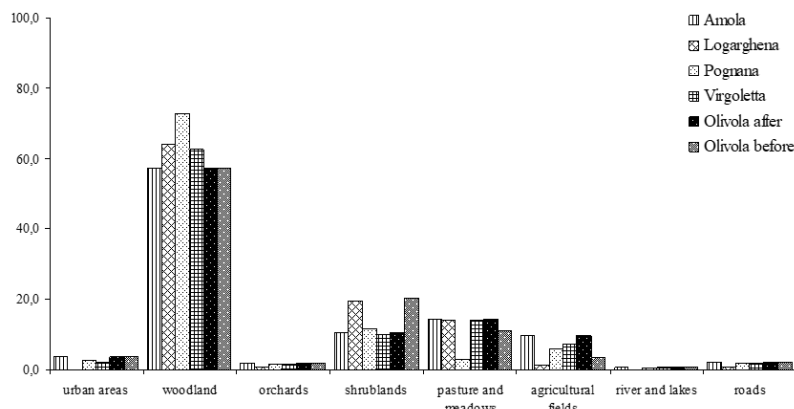
Each sample area was analyzed with the ArcMap Patch Analyst (ESRI) tool that considered many variables as follow: Number of Patches (NP); Mean of Patch Size (MPS); Standard Deviation of Patch Size (SDPS); Patch Size Coefficient of Variance (PSCV); Total edge (TE); Edge density (ED); Mean Patch Edge (MPE); Mean Shape Index (MSI); Area Weighted Mean Shape Index (AWMSI); Mean Perimeter-Area Ratio (MPAR); Mean Patch Fractal Dimension (MPFD); Area Weighted Mean Fractal Dimension (AWMPFD); Shannon’s diversity index (SDI); Shannon’s Evenness Index (SEI). Moreover, for each sample area we calculated the distance between patches of the same habitat typology and tested it with the Wilcoxon non-parametric test.

To compare spring abundances across areas, we used a chi-square test considering the number of partridges counted and the number of partridges released in each sample area. Following Gortazar,<sup>53</sup> we used the Kruskal-Wallis non-parametric ANOVA test to compare difference in summer densities of partridges.

## Results

### Landscape structure

The habitat composition was comparable amongst all study areas by considering the surface of habitat typology present (Figure 2). Biodiversity indices were similar in each area; habitat characteristics analyzed with Patch Analyst ArcMap tool are showed in Table 1.



**Figure 2** Percentage of different habitat types present in each ample area. We considered Olivola area before and after environmental improvement measures respectively.

**Table 1** Spatial analysis of different sample areas, considering all patches i.e. river, lake and roads

|                      | Amola     | Logarghena | Pognana   | Virgoletta | Olivola before | Olivola after |
|----------------------|-----------|------------|-----------|------------|----------------|---------------|
| Size area (hectares) | 844,81    | 645,73     | 666,85    | 588,75     | 628,46         | 628,46        |
| NP                   | 479,00    | 149,00     | 206,00    | 446,00     | 385,00         | 414,00        |
| MPS                  | 1,76      | 4,33       | 3,24      | 1,32       | 1,63           | 1,52          |
| SDPS                 | 13,83     | 20,69      | 18,56     | 9,55       | 12,90          | 12,45         |
| PSCV                 | 784,34    | 477,39     | 573,44    | 723,78     | 790,52         | 819,94        |
| TE (meter)           | 394393,23 | 184826,66  | 198394,33 | 309798,04  | 240534,96      | 244786,28     |
| ED                   | 466,84    | 286,23     | 297,51    | 526,20     | 382,74         | 389,50        |
| MPE                  | 823,37    | 1240,45    | 963,08    | 694,61     | 624,77         | 591,27        |
| MSI                  | 1,79      | 2,10       | 1,81      | 1,84       | 1,61           | 1,58          |
| AWMSI                | 6,01      | 3,91       | 4,06      | 5,05       | 4,64           | 4,63          |
| MPAR                 | 994,49    | 1102,59    | 946,32    | 1171,67    | 943,88         | 948,40        |
| MPFD                 | 1,42      | 1,44       | 1,41      | 1,44       | 1,41           | 1,41          |
| AWMPFD               | 1,44      | 1,40       | 1,39      | 1,45       | 1,42           | 1,42          |
| SDI                  | 4,65      | 3,92       | 3,98      | 4,78       | 4,75           | 4,84          |
| SEI                  | 0,75      | 0,78       | 0,75      | 0,78       | 0,80           | 0,80          |

NP, number of patches; MPS, mean of patch size; SDPS, standard deviation of patch size; PSCV, patch size coefficient of variance; TE, total edge; ED, edge density; MPE, mean patch edge; MSI, mean shape index; AWMSI, area weighted mean shape index; MPAR, mean perimeter-area ratio; MPFD, mean patch fractal dimension; AWMPFD, area weighted mean fractal dimension; SDI, shannon’s diversity index; SEI, shannon’s evenness index

In Olivola site, where management measures were improved, we recorded an increase in agricultural land and of meadows and pasture with respect to scrublands, from spring 2011 to spring 2012 (Table 2). This increase of habitat fragmentation determined a statistic difference on distance between open habitat, especially between patches of “agricultural land” and “meadows and pasture” (Wilcoxon test for agricultural land:  $Z = -2,223$ ,  $P = 0,026$ ; for meadows and pasture:  $Z = -4,488$ ,  $p < 0,001$ ).

**Table 2** Landscape structure in different sample areas, in relation to habitat categories and considering Olivola site before and after improvement management measures. Mean and standard deviation (S.D.) of every patches’ surface are indicated in hectares

| Categories         | Amola      |       |       | Logarghena |       |       | Pognana    |       |       |
|--------------------|------------|-------|-------|------------|-------|-------|------------|-------|-------|
|                    | n. patches | mean  | S.D.  | n. patches | mean  | S.D.  | n. patches | mean  | S.D.  |
| Urban area         | 29         | 1,06  | 3,62  | 4          | 0,09  | 0,09  | 15         | 1,25  | 3,47  |
| Woodland           | 21         | 23,02 | 63,51 | 19         | 21,73 | 55,25 | 9          | 53,83 | 76,19 |
| Orchards           | 57         | 0,26  | 0,25  | 4          | 0,77  | 0,42  | 36         | 0,29  | 0,22  |
| Scrubland          | 115        | 0,77  | 0,72  | 57         | 2,20  | 3,74  | 66         | 1,17  | 1,20  |
| Meadows & pasture  | 131        | 0,93  | 1,30  | 36         | 2,55  | 6,16  | 33         | 0,61  | 0,61  |
| Agricultural field | 120        | 0,67  | 0,93  | 27         | 0,03  | 0,31  | 43         | 0,95  | 0,98  |

Table 2 Continued...

| Categories         | Virgoletta |       |       | Olivola before |       |       | Olivola after |       |       |
|--------------------|------------|-------|-------|----------------|-------|-------|---------------|-------|-------|
|                    | n. patches | mean  | S.D.  | n. patches     | mean  | S.D.  | n. patches    | mean  | S.D.  |
| Urban area         | 19         | 0,68  | 1,16  | 29             | 1,06  | 3,62  | 29            | 1,06  | 3,62  |
| Woodland           | 52         | 46,04 | 58,69 | 21             | 23,02 | 63,51 | 21            | 23,02 | 63,51 |
| Orchards           | 9          | 0,18  | 0,16  | 56             | 0,26  | 0,25  | 57            | 0,26  | 0,25  |
| Scrubland          | 124        | 0,47  | 0,53  | 89             | 0,74  | 0,82  | 109           | 0,77  | 0,72  |
| Meadows & pasture  | 132        | 0,62  | 0,90  | 115            | 0,59  | 0,66  | 124           | 0,93  | 1,30  |
| Agricultural field | 108        | 0,39  | 0,40  | 59             | 0,48  | 0,50  | 122           | 0,67  | 0,93  |

### Abundance of red-legged partridge

In spring 2011, we found a number of males that was an average 30.5% of released males, while during spring 2012 this percentage was increased to 36.4% of released males. Even if the difference was negligible, we found the highest difference in Olivola site, which changed from 5% on 2011 to 34.6% on 2012, i.e. after environmental improvements. In all the other areas, we found a similar percentage, during spring 2011 and 2012. If we consider the number of males in reproductive activity with respect to released males, in 2011 and 2012, we did not record a significant difference by count data ( $\chi^2_{\text{spring 2011}} = 5.127$ ,  $df = 4$ ,  $p > 0.05$ ;  $\chi^2_{\text{spring 2012}} = 0.222$ ,  $p > 0.050$ ). However, we recorded an increase in Olivola site from 1 male to 9 males during calling activity respectively in 2011 and 2012 (Table 3). In the other areas, we recorded a variation in calling males of 2 individuals at maximum (Amola site – Table 3).

The relative densities of each sample area differ only in summer 2011 (Kruskall-Wallis test:  $\chi^2 = 19.574$ ,  $d.f. = 4$ ,  $P = 0.001$ ), when, in Olivola site, no partridge was found (Table 4). Instead during summer

**Table 3** Results of red-legged partridge males released and counted during both spring 2011 and 2012

| Spring 2011 |                          |                         |
|-------------|--------------------------|-------------------------|
| Sample Area | Number of males released | Number of males counted |
| Amola       | 24                       | 18                      |
| Logarghena  | 10                       | 8                       |
| Olivola     | 10                       | 1                       |
| Pognana     | 15                       | 13                      |
| Virgoletta  | 10                       | 7                       |
| Spring 2012 |                          |                         |
| Sample Area | Number of males released | Number of males counted |
| Amola       | 24                       | 16                      |
| Logarghena  | 10                       | 8                       |
| Olivola     | 13                       | 9                       |
| Pognana     | 15                       | 12                      |
| Virgoletta  | 10                       | 8                       |

2012, red-legged partridges were found in every sample area (Table 4) and the statistic test showed a not significant difference in densities values (Kruskall-Wallis test:  $\chi^2 = 6.277$ ,  $d.f. = 4$ ,  $P = 0.179$ ).

**Table 4** Number of observation point (OB), mean and standard deviation (S.D.) of densities (upper panel) and of group size (lower panel) during both summer 2011 and 2012

| Sample Area | Red-legged partridges/100ha (summer 2011) |      |      | Red-legged partridges/100ha (summer 2012) |      |      |
|-------------|---|------|------|---|------|------|
|             | N° OB                                     | mean | S.D. | N° OB                                     | mean | S.D. |
| Amola       | 14  | 31,9 | 12,0 | 14  | 29,5 | 12,8 |
| Logarghena  | 7   | 21,7 | 8,5  | 7   | 22,8 | 4,8  |
| Pognana     | 8   | 31,2 | 23,5 | 8   | 33,2 | 21,1 |
| Virgoletta  | 9   | 22,2 | 15,6 | 9   | 22,1 | 12,3 |
| Olivola     | 9   | 0,0  | 0,0  | 9   | 21,2 | 5,2  |
| Sample Area | Group size (summer 2011)                  |      |      | Group size (summer 2012)                  |      |      |
|             | N° OB                                     | mean | S.D. | N° OB                                     | mean | S.D. |
| Amola       | 14  | 8,6  | 2,0  | 14  | 8,1  | 3,0  |
| Logarghena  | 7   | 6,9  | 2,2  | 7   | 7,3  | 1,0  |
| Pognana     | 8   | 6,3  | 4,2  | 8   | 6,8  | 3,5  |
| Virgoletta  | 9   | 5,3  | 4,0  | 9   | 5,3  | 2,7  |
| Olivola     | 9   | 0,0  | 0,0  | 9   | 6,9  | 1,5  |

Table 4 Number of observation point (OB), mean and standard deviation (S.D.) of densities (upper panel) and of group size (lower panel) during both summer 2011 and 2012.

Group size ranged from 3 to 13 individuals in both summer 2011 and 2012, with an average of  $5.7 \pm 4.1$  partridges per group in 2011, and an average of  $7.0 \pm 2.7$  partridges per group in 2012. Also, for the group size analysis, the statistic test confirmed a difference only for summer 2011 (Kruskall-Wallis test:  $\chi^2 = 22.365$ ,  $d.f. = 4$ ,  $p < 0.001$ ). The percentage of successfully reproduced pair's averaged  $47.7 \pm 29.3\%$  (range 0-70.0) in 2011 and it increased till an average of  $64.8 \pm 12.7\%$  (range 46.7-80.0) after habitat improvement.

### Discussion

Red-legged partridge restocking is a common practice in hunting management in Italy, as native populations are declining.<sup>24,47</sup> Therefore, releases are necessary to preserve reproductive populations, as they are finalized to maintain traditional hunting yield levels not throughout the extent of occurrence of this species.<sup>54</sup> Thus, releases are more or less proportional to the hunters' expectancy of hunting success, which, in turn, are related to favorable conditions for red-legged partridges. On the other hand, the release success is conditioned by environmental factor, mostly by resource availability, presence of refuge areas,<sup>41,55-57</sup> and water availability.<sup>58</sup> Our study showed that also landscape mosaic

and, consequently, resources distribution constitute an important factor for breeding success and population restock.

In Olivola site, the population increase of red-legged partridge was connected to the increase of cereal-producing plots of land and to the recovery of open areas used as meadows. These environmental improvements allowed the local adaptation of released partridges and, consequentially, the opportunity to create reproductive couples in the area. The spring count conducted in 2012 showed a number of reproductive males notably higher than that of the previous spring. The chance to find a favorable environment allows the males to stay in the release area and to defend their reproductive areas. Alonso et al.<sup>59</sup> showed that female red-legged partridges chose males of higher weights and that the alert and vigilance behavioral patterns of red-legged partridge are closely related to feeding and cohesive behavioral patterns, in turn increasing the reproductive success of the breeding pair.

Nevertheless, these behavioral patterns can be mostly showed in environments, which offer higher opportunities of trophic resources finding, with respect to our study sites. The local food availability is not the only parameter to works in that sense as other areas considered in this study showed an adequate number of trophic resources. However, resource distribution in the habitat of the red-legged partridges plays a key role for the conservation of this species.<sup>60</sup> A real mosaic is a scheme that guarantees the opportunity to reproduce and to reach good breeding levels for the partridges introduced.

Red-legged partridge nest density was correlated to grain fields and mostly to herbaceous strips among fields, so that better management of agricultural areas, increasing the availability of herbaceous strips and slightly delaying cereal harvesting,<sup>56</sup> may improve partridge breeding success. Our study confirmed the importance of the vegetation strips, not only herbaceous but also shrubby, interposed between cultivated fields. Thus, if the red-legged partridge densities are higher in areas with higher fragmentation and edge abundance,<sup>41,61–63</sup> it is pivotal to properly improve the environment before any release. Thus, introduced individuals may remain in the introduction area, where they can build self-sustaining populations. The study areas, which were not subject to environmental improvements, maintained the same introduction success and the same population density during the two-year period considered. The presence of both summer and autumn crops guaranteed without doubt a good trophic availability. Accordingly, cereals represent a good contribution for summer diet of red-legged partridges (substantially based on seeds), and leguminous plants (*Fabaceae*) favor the presence of nitrogen fixing bugs, which could represent a trophic resource during fall and winter.<sup>64,65</sup> Therefore, our study highlighted that red-legged partridge management should consider the need to create landscape mosaics, as a requirement for the reproductive success.

Areas subjected to intensive agriculture, where vast monoculture lands (mainly wheat) occur, represent a limitation to the survival and to the permanence of the red-legged partridges, in such an extent that is required to realize strips to create refuge areas.<sup>9</sup> Likewise, in heavily forested lands, as those of the northern Apennines, it is important to plan plots of open areas and of various cultures, to increase the trophic availability needed to the partridges.

To conclude, our research suggests that by implementing specific habitat management plans along with calculated sampling, it will be possible to ensure proper hunting satisfaction while also improving the likelihood that natural populations of red-legged partridge can be re-created using released individuals.

## Conclusion

This study provides the first evidence that landscape management, particularly creating a mosaic of agricultural land and meadows, is able to strongly enhance the reproductive success and population stability of red-legged partridges in the northern Apennines. Our results indicate that habitat enhancements, such as creating herbaceous strips and opening up foraging space have a positive effect on the species' breeding success and density, in particular in areas with intense environmental improvements. The findings showed that habitat fragmentation with diverse vegetation types, along with open areas, was vital for the incorporation and sustainability of released partridges, further delineating the importance of habitat quality over resource availability.

This is further emphasized by the findings of the study, which demonstrate that good agricultural land management could effectively offer better opportunities for self-sustaining populations of red-legged partridges by managing a balance between crop cultivation and refuge area creation. In contrast, large-scale monoculture and intensive forestation do not have the structural complexity required for the survival and reproduction of the species. The development of landscape mosaics in future conservation plans should thus be prioritized, combining natural and agricultural elements that can support both native and reintroduced populations.

In conclusion, integrating habitat management into red-legged partridge reintroduction programs could substantially improve their conservation outcomes, ensuring long-term population stability while maintaining the cultural and economic values associated with sustainable hunting practices.

## Ethical approval

None of the animals considered in this study were ever handled or otherwise altered. The partridges were grown on accredited farms in accordance with local and national laws.

## Conflicts of interest

The authors haven no competing interests.

## Author contribution

B.P., U.S., and D.F.M. data collection, data analysis, wrote the main manuscript text; M.E. and F.M.C. wrote the main

Manuscript text; B.P., D.F.M., and F.M.C. prepared all figures and tables. All authors reviewed the manuscript.

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## Availability of data and materials

Not available.

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