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Spread of the invasive yellow-legged hornet *Vespa velutina* (Hymenoptera: Vespidae) in Italy

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Abstract

The yellow-legged hornet *Vespa velutina* Lepeletier, accidentally introduced into France in 2004, is rapidly colonizing other European countries. In Italy the species is spreading throughout the northwest part of the country.

Setting up management plans for controlling invasive alien species requires the understanding of the spread modalities and distribution range of the species, information currently not available for the yellow-legged hornet in Italy. Aims of this work are to reconstruct the spread of the yellow-legged hornet from its first arrival in the country, evaluating its distribution range and spread modalities.

The area occupied by the species increased from 205 km^2 in 2013 to 930 km^2 in 2015. In 2015 the frontline of the species was at 55 km along the coast from the French border, with a linear spread of 18.3 ± 3.3 km/year. A human-mediated dispersion could be recognized in different occasions. A cluster analysis of the range allowed the identification of 17 core areas used by the species, with a mean nest density of 2.9-3.5 nests/km^2. These information are fundamental to improve control plans and to establish an early warning and rapid response system for the yellow-legged hornet in Italy, and therefore setup an effective management plan for the species.

Keywords

invasive species, beekeeping, species distribution, range analysis, cluster analysis

Introduction

Introduced species become invasive when they spread over large areas, producing negative impacts to biodiversity, human activities and well-being (Simberloff et al. 2013; Bellard et al. 2016). To reduce these threats, it is necessary to develop a management strategy with the aim to spatially contain the species or to reduce the population abundance at a level where impacts are limited. The possibility to effectively control or contain populations increases if actions start when the species are not yet spread over large areas. Therefore, it is fundamental to monitor the spatial dynamic of species known or suspected to be invasive since their arrival in the colonized country. Collecting data on their distribution could also provide information that will help building an efficient management plan (Braysher 1993; Bertolino et al. 2005) and form the necessary basis to evaluate any impact to native species and ecosystem processes (Bertolino et al. 2014).

The yellow-legged hornet *Vespa velutina* Lepeletier (Hymenoptera: Vespidae) is a social wasp native to tropical and subtropical areas of south-east Asia (Archer 1994; Martin 1995; Carpenter and Kojima 1997). Introduced populations of the species are established in South Korea (Choi et al. 2012, 2013), Japan (Ueno 2014) and different European countries (Villemant et al. 2011a, b). In Europe the yellow-legged hornet was introduced in France probably in 2004 (Haxaire et al. 2006; Villemant et al. 2011b) and then the species colonized other countries such as Spain (Castro and

Pagola-Carte 2010; Lopéz et al. 2011), Portugal (Grosso-Silva and Maia 2012), Belgium (Bruneau 2011; Rome et al. 2013), Italy (Demichelis et al. 2014) and Germany (Witt 2015). The yellow-legged hornet is considered invasive for its impact on biodiversity, apiculture and human well-being. Adult wasps prey on bees and contribute to the loss of honeybee colonies (Monceau et al. 2013, 2014); there are also indications that the species could exert a negative effect on ecosystems by preying on wild insects (Beggs et al. 2011). Colonial nests are often established in urban areas, therefore attacks to humans are possible (De Haro et al. 2010; Villemant et al 2011a; Liu et al. 2015; Tabar et al. 2015).

The spread of the yellow-legged hornet in Europe was characterized by a mix of a natural dispersal with a diffusion-like process (Suarez et al. 2001; Villemant et al. 2011a) and sudden jumps due to the passive transport of inseminated queens with commodities and vehicles (Marris et al. 2011). Discriminating the effects of these two sources of dispersal is difficult, though it would be helpful for the establishment of an early warning system and to develop control plans. Human-mediated transportation is connected to the use of vehicles and other means of transport and therefore traditionally difficult to predict. On the other hand, natural dispersal determines the progressive spread of colonial nests and could be evaluated and modeled from data on the diffusion over successive years.

The arrival of the yellow-legged hornet in Italy was predicted by the progressive expansion of the species in France and modeling scenarios (Villemant et al. 2011a). Therefore, a monitoring system was established in 2007 in the north-west of Italy close to the French border, in Liguria and Piedmont regions, with the aim to detect the first animals entering the country (Demichelis et al. 2014; Porporato et al. 2014). The first hornet was trapped in 2012 in Loano (Liguria) (Demichelis et al. 2014), while nests were found in 2013 in Liguria and Piedmont (Porporato et al. 2014). The rapid diffusion of information to researchers, beekeepers, other stakeholders and the general public led to the establishment of a monitoring system based on the use of bait-traps and the visual observation of colonial nests and animals.

Aim of this paper is to use information from this monitoring system to 1) reconstruct the spread of the yellow-legged hornet in Italy since its arrival in the country, 2) evaluate the mean diffusion rate of the species in Italy, 3) use spatiotemporal data to discriminate the natural diffusion of the species from jumps in the distribution probably connected to human-mediated transportation, and 4) discuss how this information could be integrated in a control plan and therefore build up an early warning and rapid response system at a national scale.

Materials and methods

The yellow-legged hornet in Italy has been commonly observed in the western part of Liguria region since 2013, while in Piedmont the species was observed in few localities in year 2013, and then rarely observed in the following years. Observations of the yellow-legged hornet were reported by public warnings of beekeeper associations, local authorities and general public; in addition observations have been collected since 2015 by a monitoring team involved in the field survey. The team monitoring activities were fundamental to discover nests farther away from urban areas; in fact, most of the observations reported by the general public might be correlated with human activities (Monceau et al. 2014, Monceau and Thiéry 2016). The observations reported by the public were considered if they were verified by experts or accompanied by photographic documentation, given the possibility of misidentification (Rome et al. 2011). Moreover, in winter 2015-2016, the survey of the monitoring team continued till February, so to take advantage of months with no leaves on the trees for a more effective search of the nests.

Since the range analysis requires the availability of spatiotemporal data, only georeferenced observations were considered. The evaluation of the range and spread of the species was performed

considering only nest observations, because adult observations may not indicate the establishment of the species in new areas.

The nests are often difficult to be observed, especially when covered by the vegetation or far away from urban areas. Moreover the probability that nests were observed and reported is higher with high nest densities; in the western part of Liguria region, where the species arrived before, nest density is probably higher than the one of the eastern part of the distribution range, where the species is currently colonizing new areas. Because of the non-homogeneity of both the sampling effort and the probability to detect nests, spatiotemporal data of nests were transformed before performing the range analysis. A vector grid of 1 km^2 enclosing all nests was build, each cell containing a nest selected and the centroids extracted. The range analysis was then performed considering the extracted centroids of the grid with the kernel method of the package AdehabitatHR (Calenge 2006) of the software R (R Core Team 2015; R Studio Team 2015). With this technique the estimation of the ranges is not affected by clustered nests and, in a certain extent, by undetected nests. The resulting vectors were then exported in a GIS software (QGIS Development Team 2015) for the evaluation of the area of the ranges, the spread rate and the modalities of diffusion, natural or human-mediated. The spread rate of the species was evaluated considering both the increasing of the area occupied and the maximum distance of the frontline from a possible source of diffusion of the previous year, such as French nests or the range of the species in the previous year. Georeferenced nests in France near the Italian border were available only from 2015, although the species was recorded in the Département des Alpes-Maritimes since 2010 (Villemant et al 2011b); for this reason we used the centroids of a vector grid of 1 km^2 built around the French nests as a proxy of nests that could act as a source site. Hypothesis on the modalities of diffusion (natural or human-mediated) of the observations recorded outside the estimated range were made comparing the distances of these observations from a source site with the estimated mean spread rate in Liguria: distances less than the mean estimated spread rate could indicate a natural diffusion, otherwise a human-mediated transportation was considered.

Further analyses on the nests distribution in Liguria in 2015 were performed with both a single-linkage cluster analysis and a kernel analysis to identify the structure of the range and the core areas of the species with aggregate nests. Core areas were identified by analyzing the surface of the clusters at progressive sizes on the basis of progressive percentages of observation. For this secondary analysis nest coordinates have been considered instead of the extracted centroids, to evaluate the areas where the yellow-legged hornet could produce the higher impacts.

To further evaluate if nest sample size could influence the results of our analyses, we performed additional cluster analyses considering the nests observation grouped in five periods with a progressive increasing of the sample size: all nests produced in 2015 and observed till the end of October 2015, November 2015, December 2015, January 2016, and March 2016. Afterwards the areas covered by the clusters with progressive percentages of nests were compared between the five groups, to evaluate if the increasing of the sample size could modify the estimated structure of the range. Nests recorded in 2016 till March are ~~secondary~~ nests built in 2015, so they were included in the analyses.

Results

The yellow-legged hornet entered in Italy in 2012 (2 adults observed) and reproductive colonies were first found in 2013 (7 nests, 5 in Liguria and 2 in Piedmont). In the following years many nests were observed in Liguria (50 nests in 2014 and 221 in 2015) while no nests were discovered anymore in Piedmont.

The area occupied by the species in Liguria increased from 205 km^2 in 2013 to 930 km^2 after two years in 2015 (Table 1). Single adults were observed in Liguria outside these ranges (Fig. 1). Adult animals were repeatedly observed in Piedmont, however only one nest was found in 2013 and no

others in the following years; it is assumed the presence of another nest in 2013 because of the repeated attacks by many worker hornets to an apiary for a long period, but no nest was discovered nearby. In 2015, the frontline of the species was at 54.8 km along the coast from the French border (Fig. 1), with a linear spread of 18.3 ± 3.3 km/year (Table 1).

Six records of isolated animals and two nests were located in Liguria outside the distribution range of the species for that year at 3.2-79.2 km from a possible source of dispersal (i.e. French nests or species range of the previous year). Twelve records related to one nest and eleven observations of one or more isolated animals were located in Piedmont at 36.9-149.5 km from a possible source of dispersal. The distances of these observations from possible sources of dispersal are reported in Table 2, together with the hypothesis of diffusion's modalities.

The structure of the range occupied by the yellow-legged hornet in Liguria in 2015 was investigated progressively adding a higher percentage of nests in a single-linkage cluster analysis. The curve with the areas covered by the clusters with a progressive percentage of records is reported in Fig. 2. The curve has an inflection point when 90% of nests are included in the analysis. At this threshold, 17 separate core areas composed by three or more clustered nests were identified (Fig. 3); these core areas covered overall 56.5 km^2. Beyond this threshold there was a change in the slope of the curve and the surface included in the clusters rapidly increases. The clusters progressively included more isolated nests and core areas, with a steep increase in the overall surface (Fig. 2): i.e. 83.2 km^2 with 93% of records, 156.5 km^2 with 95%, 395.5 km^2 with 97%, 448.8 km^2 with 99% and 733.4 km^2 including 100% of records. The maximum area estimated with the cluster analysis (733.4 km^2) does not correspond to the maximum area estimated with the range analysis in 2015 (930 km^2) because of the different methodology.

Mean nest density was estimated in the core areas. Considering all the 17 core areas we estimated 3.5 nests/km^2, but 11 of them were smaller than 1 km^2. Instead, considering only the core areas larger than 1 km^2 we estimated 2.9 nests/km^2. The kernel method was used to highlight the areas where more nests were observed; interestingly, most of the nest were located along the valley bottoms (Fig. 3).

The increasing of the sample size considering the five groups of nests, always generates a variation in the structure of the clusters only above the threshold of 90% of observations (Fig. 4): till this limit the number of core-areas changed according to the sample size, but not the percentage of the area covered by these core-areas that after the first period stabilizes at 5-8% of the total (Table 3).

Discussion

The yellow-legged hornet entered Italy for the first time in 2012 and first nests were found the following year. Since then, the hornet spread in two more years over an area of 930 km^2 in Liguria. The data here reported show how the species adapted to this part of north-western Italy, spreading over a relatively large area.

The linear spread of the yellow-legged hornet in Italy at about 18.3 km/year is much lower than the mean spread rate of about 60-100 km/year reported in other parts of Europe (Anonymous 2011, 2014; Balmori 2015). Conversely, our estimated values are similar to the spread rate of the species in South Korea (Choi et al. 2012). The low spread rate in South Korea could probably be related to the richer hornet community, with six other species that could compete with the yellow-legged hornet; instead the part of Europe so far invaded by the yellow-legged hornet host only the native *Vespa crabro* L. (Rome et al. 2009; Villemant et al. 2011a; Choi et al. 2012).

Considering the range of the species, Rome et al. (2009) reported that the yellow-legged hornet in France has spread across about 130,000 km^2 within 5 years (2004-2008). This spread rate is probably linked to a mixture of natural and human-mediated dispersal. In fact, if we predict the

natural spread of the yellow-legged hornet in Liguria 5 years after its arrival in Italy from our data on the expansion, we could estimate a much lower value of about 3,900 km^2.

The low spread rate recorded in Italy is probably related to the presence of mountains acting as barriers at the border between France and Italy. All nests found in Liguria till now were at an altitude lower than 620 m a.s.l.; only one nest was found in Piedmont at 906 m a.s.l., but the next year the species was not present anymore in the area, indicating the probable non-adaptation to the local climatic condition. The border between France and Italy is at less than 1,000 m a.s.l. only for 14.5 km and at less than 1,500 m for 42.8 km, therefore the species entered Italy through a narrow corridor close to the seaside. This corridor is also highlighted in the climatic model presented by Rome et al. (2011) and the coastline could be considered a preference pathway of expansion, due to the presence of many food resources (Monceau and Thiéry 2016). Human-mediated transportation of animals could help the species overcome bottlenecks in the landscape; however, till now long distance movements have led the species to unsuitable areas in Piedmont or along the corridor close to the seaside in Liguria. Another limitation to the spread of the species over larger areas was the control activity, quickly started by beekeepers and other volunteers after the discovery of first nests, which led to the neutralization of hundreds of nests in the last two years.

Landscape characteristics (e.g. altitude, presence of rivers and water supplies, climate conditions) might facilitate or limit the dispersal of a species. According to the predictive map produced by Villemant et al. (2011a) using a climatic suitability model for the species, the area in Liguria where the species is spreading is highly suitable for the yellow-legged hornet, at least for its mild climate. On the contrary, the species has not adapted till now to the north side of the Ligurian Alps, in Piedmont, where groups of animals and at least one nest were found in these years, apparently without the establishment of the species.

The analysis of the structure of the range in Liguria in 2015 identified 17 separate core areas composed by clustered nests. These nests were probably originated by queens naturally dispersed from nearby sites the previous years. Mean nest density estimations would be useful for a better planning of control activities; however, few reference values are available for the yellow-legged hornet because of the difficulty of finding all nests in a given area. From an extensive search of nests performed in Andernos-les-Bains in France (Monceau and Thiéry 2016) mean nest density in urban areas varied from 0.44 nests/km^2 in 2007, when first nests were observed, up to 12.26 nests/km^2 in 2014. However our estimated values of nests density (2.9-3.5 nests/km^2) were obtained considering the real area, without discriminating in urban, suburban or natural environment. Therefore, the following different hypotheses should be considered: nest density has not reached the carrying capacity so densities might increase in the future; many nests were not recorded, especially in suburban and natural environments; the suitability of forested areas is lower for the species. Unfortunately at the moment there are not enough data in support of one of these hypotheses.

The cluster analyses performed on nests grouped into the five periods, simulating a different sample size, clearly show that the increase of nests should not modify substantially the structure of the range. In the different scenarios the area covered by the clusters is approximately equal below the threshold of 90% of observations. Therefore the undetected nests should not substantially modify the estimated ranges and core areas surface.

Data on the flight distance of the species are still lacking. Marris et al (2011) reporting unpublished data, suggested that males can fly for dozens of kilometers and queens are believed to be even more efficient flyers. Ongoing experiments show that queens can fly about 30 km in one day (Rome et al. unpublished data, in Beggs et al. 2011). However, the average distances flown by queens during dispersal would be more useful than extreme values.

Distances of the yellow-legged hornet recorded outside the continuous distribution range in Liguria were all except five at 36.9-149.5 km from a possible source of diffusion and could be attributed to human-mediated dispersal. Luckily, in all cases except two there were single individuals or groups of animals that did not settle down in the areas; also the nest found in 2013 in Piedmont at 60 km from French nests apparently did not give rise to other colonies in the

subsequent year. However, the reported data confirm that the human-mediated dispersal of animals is not uncommon. While in most of the cases, transported animals were probably workers, in at least three cases inseminated queens originated a nest at distances over 20 km from source areas. Similar long-distance travels of queens that established the species in new areas far away from the previous range were already reported in France (Rome et al. 2009), Belgium (Rome et al. 2013), and Portugal (Balmori 2015).

A possible bias in evaluating distances of nests from possible sources of diffusion may be the non-complete record of nests. Till now there are no methods to evaluate the percentage of nests located in respect to those actually present in an area. It is therefore possible that our analysis may have overestimated the distances of isolated nests from the continuous range of the species. This analysis had the aim also to evaluate possible long-distance movements of hornets that may be due to human-mediated dispersal. We acknowledge that possible intermediate nests would reduce the distances recorded decreasing the importance of human-mediated movements. Increasing the efficiency of nest search with new tools (Milanesio et al 2016), a goal of a LIFE project started in 2015, will increase the reliability of the approach here proposed.

The yellow-legged hornet invaded the western part of Liguria with a mixture of progressive natural dispersal and jumps bringing the species into new areas. Within limits still to be defined, some of these jumps could be due to the natural long-distance dispersal of queens. However, only human-mediated movements can take the animals to areas many tens of kilometers from the front of the species spread. Nests of the yellow-legged hornet present in the continuous range of the species could be found and neutralized allowing therefore a removal of the animals, although the main limit for an effective control is the ability to find all nests present in the area (Monceau et al. 2014). Evaluating how nests are dispersed within the species range, and identifying number and distribution of nests clustered in core-areas, will help improving the effectiveness of control operations. On the other hand, the human-mediated dispersal is unpredictable and can only be managed by implementing a nationwide early detection and rapid response system that could allow the eradication of emerging sub-populations once detected (Homans and Horie 2011).

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Table 1 Spread of the yellow-legged hornet in Liguria, for each year is reported: the new area colonized, the cumulative area of colonization, the distances of the frontline from a source of diffusion of the previous year and the cumulative distances of the frontline

Year	New colonized area (km^2)	Cumulative area (km^2)	Distance of the frontline (km)	Cumulative distance of the frontline (km)
2013	205	205	15.6	15.6
2014	141	346	17.2	32.8
2015	584	930	22.0	54.8

Table 2 Distances of the observations of the yellow-legged hornet outside the estimated distribution range in Liguria. Region of observation (Liguria or Piedmont) and municipality, typology of observation (nest or adult), shortest distance from a possible source of diffusion (French nests or estimated range for the species) and probable modalities of diffusion, natural diffusion (N.D.) or human-mediated (H.M.), are reported

Year	Region	Municipality	Typology	Shortest distance from a source (km)	Possible Source	Modalities of diffusion
2012	Liguria	Ventimiglia	Adult	3.2	French Nests	N.D.
2012	Liguria	Loano	Adult	68.7	French Nests	H.M.
2013	Liguria	Sanremo	Adult	19.7	French Nests	possible N.D.
2014	Liguria	Triora	Nest	13.9	Range 2013	possible N.D.
2014	Liguria	Savona	Adult	79.2	Range 2013	H.M.
2015	Liguria	Andora	Nest	21.1	Range 2014	possible N.D.
2015	Liguria	Pieve di Teco	Adult	18.3	Range 2014	possible N.D.
2015	Liguria	Finale Ligure	Adult	51.3	Range 2014	H.M.
2013	Piedmont	Monasterolo Casotto	Nest	60.0	French Nests	H.M.
2013	Piedmont	Vicoforte	Nest*	57.0	French Nests	H.M.
2013	Piedmont	Borgo San Dalmazzo	Adult	37.1	French Nests	H.M.
2013	Piedmont	Mondovì	Adult	58.0	French Nests	H.M.
2013	Piedmont	Bastia Mondovì	Adult	65.1	French Nests	H.M.
2013	Piedmont	Gavazzana	Adult	149.5	French Nests	H.M.
2014	Piedmont	Roccasparvera	Adult	36.9	French Nests	possible H.M**.
2014	Piedmont	Roccasparvera	Adult	37.0	French Nests	possible H.M**.
2014	Piedmont	Borgo San Dalmazzo	Adult	37.3	French Nests	possible H.M**.
2014	Piedmont	Caraglio	Adult	45.9	French Nests	possible H.M**.
2014	Piedmont	San Michele Mondovì	Adult	56.1	Range 2013	possible H.M**.
2015	Piedmont	San Michele Mondovì	Adult	51.9	Range 2014	possible H.M**.

* In this case a nest was not observed, but repeatedly attacks by worker hornets in an apiary for a long period of time was recorded, so the presence of a nest nearby was assumed.
** In these cases a possible human-mediated diffusion were hypothesized; however a nest was discovered in Piedmont in 2013, so natural diffusion should not be excluded.

Table 3 Number and surface (effective and percentage upon the total) of the core areas extracted at the threshold of 90% from a cluster analysis, considering the five groups of nests at a different level of sample size

Groups	N° core areas	Surface core areas (km^2)	% core areas
October 2015	3	74.9	16
November 2015	9	26.7	5
December 2015	13	39.0	7
January 2016	16	30.7	6
March 2016	17	56.5	8

Fig. 1

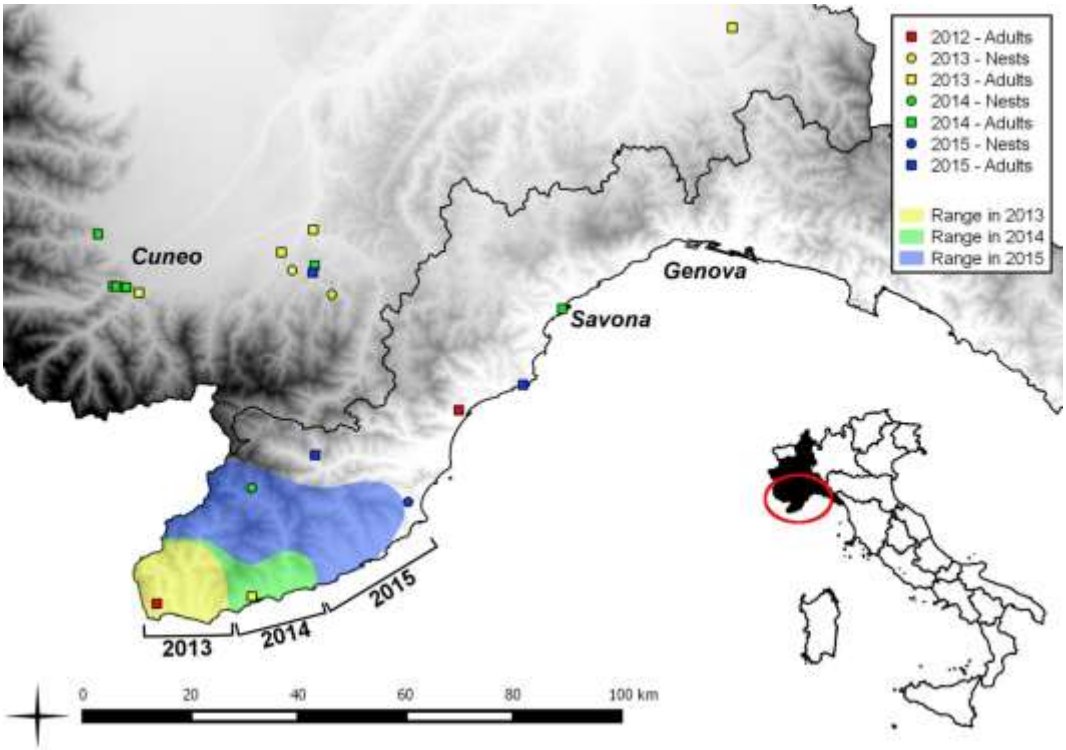

2012 - Adults
2013 - Nests
2013 - Adults
2014 - Nests
2014 - Adults
2015 - Nests
2015 - Adults
Range in 2013
Range in 2014
Range in 2015
Cuneo
Genova
Savona
2013
2014
2015
0
20
40
60
80
100 km

Fig. 2

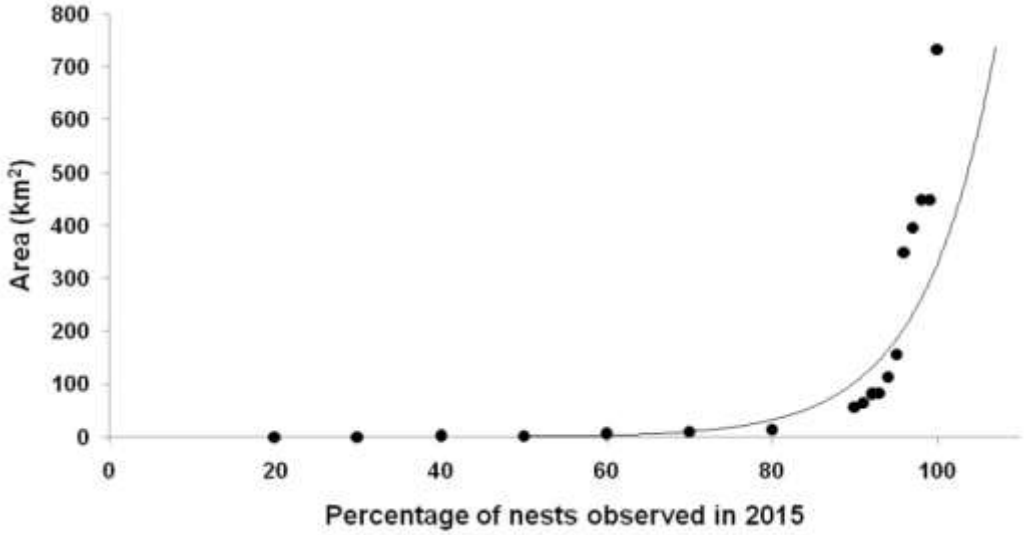
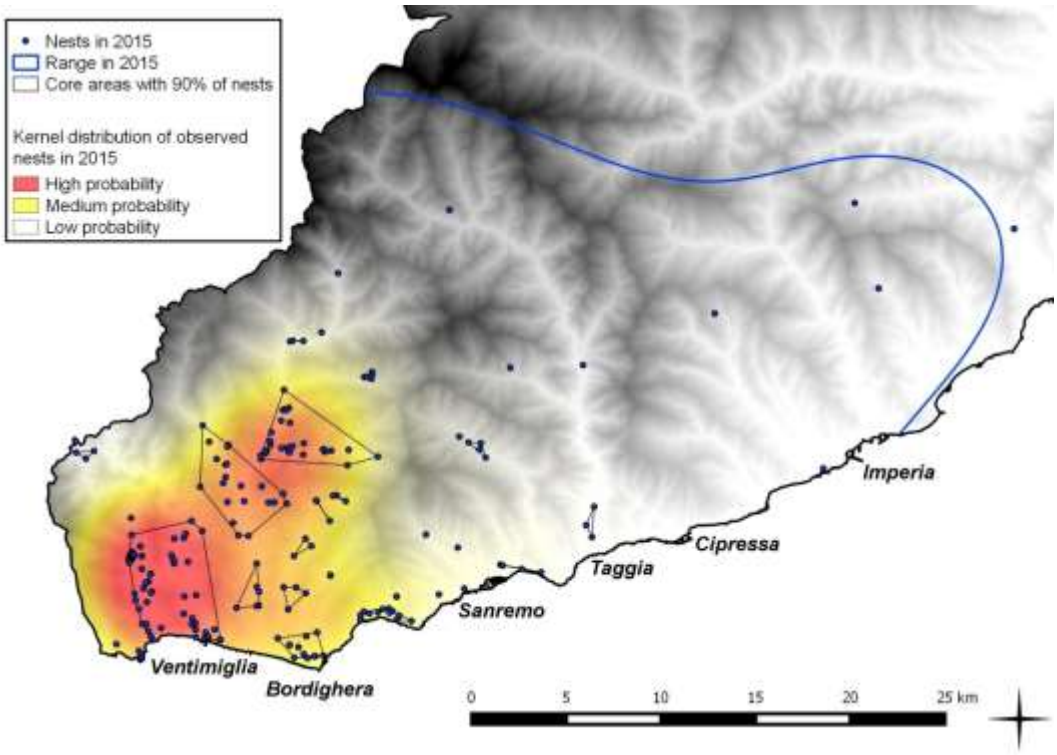
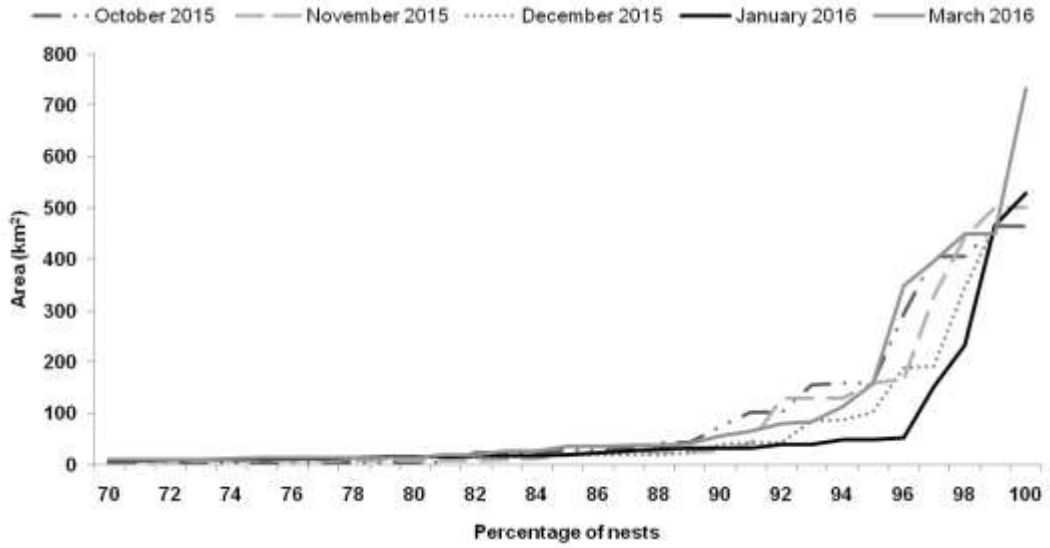

800
700
600
500
400
300
200
100
0
Area (km²)
0
20
40
60
80
100
Percentage of nests observed in 2015

Fig. 3


Nests in 2015
Range in 2015
Core areas with 90% of nests
Kernel distribution of observed nests in 2015
High probability
Medium probability
Low probability
Imperia
Cipressa
Taggia
Sanremo
Ventimiglia
Bordighera
0
5
10
15
20
25 km

Fug. 4


October 2015
November 2015
December 2015
January 2016
March 2016
800
700
600
500
400
300
200
100
0
Area (km²)
70
72
74
76
78
80
82
84
86
88
90
92
94
96
98
100
Percentage of nests