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Abstract. Thirty three maternity roosts of *Rhinolophus hipposideros* were monitored from 2000 to 2018 in an area of north-western Italy, between the Piedmont (13 roosts) and Liguria regions (20 roosts), and data were analysed through a TRIM statistic to calculate a robust estimation of reproductive population trends. Throughout the 19-years-long monitoring period, a total of 5135 *Rhinolophus hipposideros* were counted in maternity roost, with a mean of 273 (SD = 198.1) per year (mean Piedmont = 73, SD = 85.9; mean Liguria = 200, SD = 164.6). The population analysis model shows a significant overall gradient of 1.047, which corresponds to an average annual increase of 4.7 % over the period considered. The analysis suggests that in the study area, the reproductive population of *Rhinolophus hipposideros* monitored in the maternity roost has increased throughout the period from 2000-2018, with large increases after 2010 and different trends occurring in the two regions considered, including a significant moderate increase in Liguria and a stable trend in Piedmont. The results agreed with the recently observed trends in other European countries for this species.

Key word: Chiroptera, *Rhinolophus hipposideros*, population trend, TRIM

Introduction

Among the European bats which declined markedly in the middle of the 20th century, the lesser horseshoe bat, *Rhinolophus hipposideros* suffered the most spectacular population crash (Feldmann 1967). Populations in continental north-western Europe have not yet recovered from the strong decreases and range contractions which started in the 1940s (Netherlands – Van Vliet & Mostert 1997, Dekker et al. 2011; Luxembourg – Harbusch et al. 2002; Belgium – Verlinde 2003, Kervyn et al. 2009; Germany – Kock & Altmann 1994, Kulzer 2003, Zahn & Weiner 2004, Wissing 2007; Liechtenstein – Güttinger 2011; Switzerland – Bontadina et al. 2000). The species is currently considered Near Threatened with declining populations in the European IUCN Red List (Hutson et al. 2007).

In Italy, the species is known in all regions (Agnelli et al. 2004, Lanza 2012) with declining populations. Additionally, it is listed as endangered by the Italian Red List (Rondinini et al. 2013), and its conservation status is even now considered inadequate (Genovesi et al. 2014).

Careful monitoring and population data collection, with the aim of tracking population change, are necessary for species conservation. Moreover, these data are essential for effective conservation and management decisions (e.g. Spellerberg 1991, Battersby & Greenwood 2004, Pereira & Cooper 2006). Collecting accurate data on bats abundance and its regional variation is rather difficult (Hayes et al. 2009). In the temperate zone, long-term population variations are usually estimated by counting hibernating bats in their underground roosts (Horáček 2010, Uhrin et al. 2010, Ingersoll et al. 2013). This type of monitoring is the most frequent and widespread source of bats data in Europe and has been used for the definition of a prototype indicator of trends in European bats populations (Van der Meij et al. 2015).

On the contrary, the population trends based on the monitoring of breeding colonies are scarcer, despite summer roost colony counts often being a preferred monitoring method (Battersby 2010). The few available examples concern *Rhinolophus hipposideros* in Great Britain and Ireland (Warren & Witter 2002, Roche et al. 2012), *Rhinolophus ferrumequinum*, *Rhinolophus hipposideros*, *Myotis emarginatus* and *Myotis myotis* in Bretagne-France (Baudouin 2013) or a few other bats species in Great Britain (Barlow et al. 2015). Overall, systematic bat monitoring in summer roosts is a relatively new approach, and therefore

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long-term population variations are not available so far (Bartonička & Gaisler 2010).

The aim of this study was to analyse long-term population changes of a reproductive population of *Rhinolophus hipposideros*. We monitored 33 maternity roost sites in north-western Italy across a 19-year period, and analysed the data through a TRIM statistic to calculate a robust estimation of population trend.

Material and Methods

Thirty three maternity roosts were visited in the years 2000 to 2018, in an area of north-western Italy (Fig. 1) between the Piedmont (13 roosts) and Liguria regions (20 roosts).

Standardized counts were made through the detection of only adult females, by counting emerging bats with an ultrasonic detector or by photographic methods or direct counts inside the roost, based on the characteristics of the roost and its accessibility, as recommended by Battersby (2010). For each roost, only one inspection per year between late June and the first ten days of July was performed using the same counting methodology each year to minimize disturbances (Fig. 2).

In order to analyse population trends, we used the TRIM-software (trends and indices for monitoring data; Pannekoek & Van Strien 2001, Gregory et al. 2005) through package "Rtrim" v 2.0.6 that allows implementing TRIM within the R statistical environment (Bogaart et al. 2016). TRIM is a widely used freeware program with an efficient implementation of log-linear Poisson regression models to analyse time series of count data (Gregory et al. 2005), and is also used for analysing trends in hibernating bats (Uhrin et al. 2010, Van der Meij et al. 2015). TRIM was developed for the analysis of time-series of counts with missing values from individual sites (Pannekoek & van Strien 2001). In our study, we have a few missing counts in some colonies. Two roost were counted only one year, four for two years, five for three years, four for four years, three for five years, two for six years, four for seven years, three for eight years, two for nine years and four for more than ten years, one of which for nineteen.

The presence of missing values for some sites prevents the use of a model that takes the "site" and "year of count" parameter into account (model 3 time effect), since the time effects model needs each site and each year to have at least one non-zero count in TRIM. For that reason, we performed a linear trends models (model 2) analysis by using sites with at least three counts in the selected time period. The different

environmental and climatic characteristics of the two regions were considered as covariates for the analysis. The Wald statistic was performed to test the slope parameter significance.

Mean yearly change rate estimates and confidence intervals were used to classify the trends following the criteria indicated by Soldaat et al. (2007).
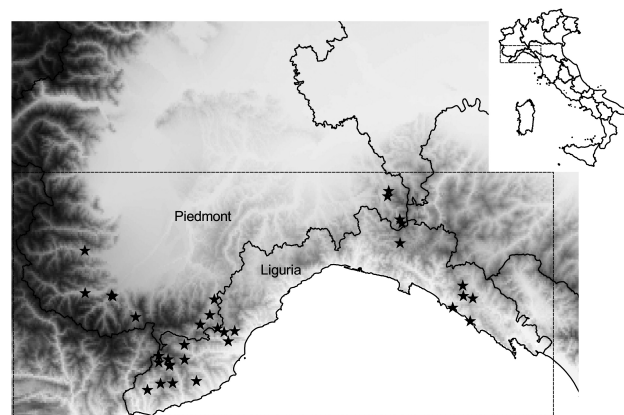

Fig. 1. Maps of study area (dashed rectangle) and maternity roost monitored (stars).
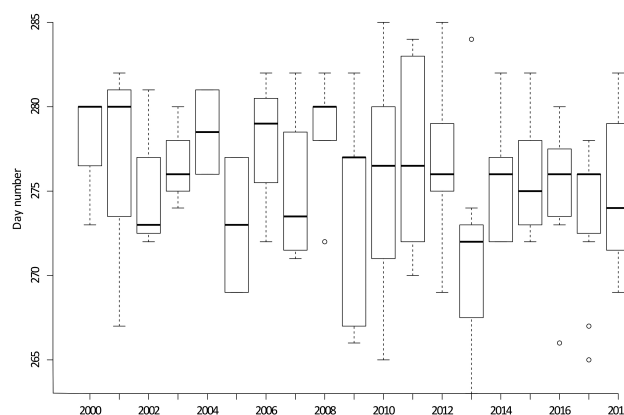

Fig. 2. Boxplot of Julian Date (day number: 1st January = day 1) for yearly maternity roost counts from 2000-2018 (June-July).
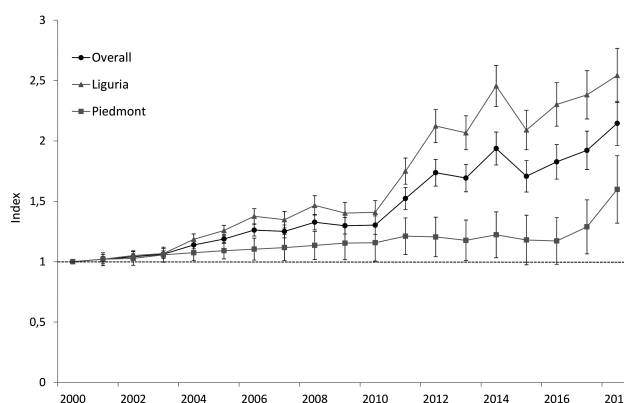

Fig. 3. Trend of summer population (with SE bars) of *Rhinolophus hipposideros* females in the study area.

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Table 1. Number of roosts and number of *Rhinolophus hipposideros* breeding females counted per year.

Year	Liguria		Piedmont		Total	
	No. roost	No. female	No. roost	No. female	No. roost	No. female
2000	2	70	1	9	3	79
2001	2	63	1	10	3	73
2002	2	58	1	8	3	66
2003	2	48	1	11	3	59
2004	1	54	1	11	2	65
2005	1	36	1	11	2	47
2006	2	140	1	10	3	150
2007	4	180	1	9	5	189
2008	4	214	1	9	5	223
2009	7	269	3	58	10	327
2010	16	566	4	66	20	632
2011	2	137	8	152	10	289
2012	5	294	7	131	12	425
2013	5	216	7	130	12	346
2014	10	496	4	78	14	574
2015	10	522	4	56	14	578
2016	1	105	7	98	8	203
2017	2	136	8	198	10	334
2018	4	189	11	287	15	476
		3793		1342		5135

Results

Throughout the 19-years-long monitoring period, a total of 5135 *Rhinolophus hipposideros* were counted in maternity roosts (Table 1), with a mean of 273 (SD = 198.1) per year (mean Piedmont = 73, SD = 85.9; mean Liguria = 200, SD = 164.6). The differences between the two regions were significant (Wilcoxon test $Z = 167$, $p = 0.0023$).

The population analysis model shows a significant overall gradient of 1.047 (Wald test = 24.89, $p < 0.001$), which corresponds to an average annual increase of 4.7 % over the period considered (Table 2, Fig. 3). The Wald test for significance of covariates does not show a general significant effect on slopes (Wald test = 14.39, $p = 0.703$). Only after 2010 is there a significant change in the slopes of the two regions

(Wald test = 66.18, $p < 0.001$) with a significant moderate increase in Liguria (slope = 1.056, SE = 0.013) corresponding to a 5.6 % change per year and a stable trend in Piedmont (slope = 1.017, SE = 0.015) with an average increase of 1.7 % per year.

Discussion and conclusion

The analysis suggests that in the study area, the reproductive population of *Rhinolophus hipposideros* monitored in the maternity roost has increased throughout the period 2000-2018, with large increases after 2010 and different trends in the two regions considered. The results agree with the recently observed trends in other European countries for this species. The annual increase observed in this study is similar to that detected in the U.K. where a growth of 65.5 % was detected from 1999 to 2012 (Barlow et al. 2015) with an average annual increase of 5 % in the short term (Warren & Witter 2002). Similar increases, though more limited, have been observed in Ireland (Roche et al. 2012) and in other European countries (e.g. Slovakia, Poland) where positive trends have been detected (Furmankiewicz et al. 2007, Chytil & Gaisler 2012). In some regions of France close to our study area, there have been positive trends (e.g. Rhone-Alpes), even though they are not precisely quantified (Groupe Chiroptères de la LPO Rhone-Alpes 2014), or even small decreases (e.g. Provence; Drousie & Cosson 2016).

The positive trends observed in the reproductive period in some populations are confirmed by the long-term trends of hibernating bats observed in various European countries (Van der Meij et al. 2015). *Rhinolophus hipposideros* has shown an average annual increase of 6 % hibernants in some European countries, with variations between 4 % and 16 % (Van der Meij et al. 2015). Similar trends were also found locally in Austria (Spitzenberger & Engelberger 2013), Czech Republic (Chytil & Gaisler 2012), and in some French regions (Groupe Chiroptères de la LPO Rhone-Alpes 2014), although in some sites in Austria, a subsequent decrease has taken place (Spitzenberger & Engelberger 2013).

The trends we detected in the Piedmont and Liguria regions are the first quantitative information about the

Table 2. TRIM: parameters calculated by the linear model.

Region	Wald-test	*P*	Slope	SE	Trend
Overall	24.89	< 0.001	1.047	0.009	Moderate increase
Piedmont	1.15	0.286	1.017	0.015	Stable
Liguria	19.55	< 0.001	1.056	0.013	Moderate increase

species increase, given that it was formerly assessed as "endangered" in Italy (Rondinini et al. 2013) and considered "in decline" (Agnelli et al. 2013), despite the lack of long-term monitoring. However, some caution is needed in interpreting these results, because of the limited number of monitored maternity roosts related to only two geographical areas of north-western Italy. Moreover, the lack of knowledge about other demographic parameters makes it difficult to explain the different trends. This fact could be explained by different environmental characteristics, the forest size and preservation, the habitats normally used for foraging activity (Bontadina et al. 2002) and, last, by consequent diet changes (Bono & Toffoli 2016), as well as likely different reproductive success. In fact, the Liguria colonies are mainly found in forested or non-anthropized areas, while in Piedmont we observe greater urbanization (Bono & Toffoli 2016).

Despite this encouraging positive trend that corresponds to the observations in other European areas, in our study area there are threats that should not be underestimated. Some maternity roosts are indeed in a precarious conservation status and are subject to anthropogenic disturbance due to renovations of the bat-inhabited buildings or because of their deterioration due to the state of neglect. Building deterioration can lead to roost size reduction or its desertion, as observed in some monitored sites in Liguria. Environmental transformations due to urbanization, more evident in Piedmont, habitat fragmentation, and foraging area reduction can jeopardize the conservation status of this species (Dietz & Kiefer 2016).

In conclusion, for a correct conservation of the horseshoe bat, it would be necessary to continue regular monitoring in a large number of maternity roosts to standardise techniques and dates of counting as much as possible. Furthermore, the acquisition of other demographic parameters is needed in order to even better evaluate reproductive success.

Acknowledgements

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