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Consistent demographic trends in Savi's pine vole between two distant areas in central Italy

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Key words: small mammals, *Microtus savii*, live trapping, orchards, mark-recapture, population structure.

Abstract

The Savi's pine vole, *Microtus savii* is an Italian species living in grasslands, both natural and anthropogenic (i.e. agroecosystems) where it is generally considered a pest because it may damage crops and orchards. As for most rodent pests, the extent of the damage might depend on population density and temporal food availability (e.g. Brown et al. 2007, Jacob & Tkadlek 2010). However, data on demographic parameters are not available, making ecologically-based management strategies difficult to plan. Therefore, we conducted a study on Savi's pine vole demography for one year in two study areas in central Italy using capture-mark-recapture method. Density values ranged from 3 to 32 ind./ha, the highest population densities occurred in October, while the lowest occurred in February-April in both study areas. Turnover rates of both populations were very high, with time of

residency usually no longer than two months. Juveniles' survival was constant in both study areas, but it differed between the two populations regarding adults. The two populations showed similar demographic trends along the year with relatively small intra-annual fluctuations. The breeding season covered the whole year in both areas. The results here presented contribute to add meaningful information about this poorly known species, and could be useful to plan population control strategies for this rodent in agroecosystems.

Introduction

Microtine rodents are a large subfamily of the *Cricetidae* family, distributed across the whole Holarctic region (e.g. Carleton & Musser 1984). They inhabit a wide range of habitats including forests, meadows, prairies, riparian zones and agricultural fields (Nowak 1999, Capizzi & Santini 2007). The numbers of microtine rodents show large variations among species and even among populations of a single species. Populations may undergo seasonal variations and multi-annual cycles (e.g. Tkadlec & Stenseth 2001, Lambin et al. 2006, Jacob & Tkadlec 2010, Korpela et al. 2013). Rodents can cause considerable damage to agricultural crops and forest trees, the more extensive during population peaks. Crop losses can exceed several million euros in Europe per year (e.g. Jacob & Tkadlec 2010). However, among the many microtine European species, not all are equally well studied, and effective management practices are feasible only when there is a thorough understanding of the ecology and behaviour of the species (e.g. Singleton et al. 1999, Brown et al. 2006, Clapperton 2006).

One of the least studied European species is the Savi's pine vole (*Microtus savii*), the most widespread vole species of Italy (Ranchelli et al. 2016). The habitat of Savi's pine voles mainly consists in grasslands, ecotonal areas, fallow fields, banks of ditches and canals, as well as agricultural crops and orchards (Cagnin & Grasso 1999, Capizzi & Santini 2007). Savi's pine voles live in a system of underground burrows, and feed on annual and perennial herbaceous plants, both wild and cultivated (Caroli 1992, Capizzi & Santini 2007). During late autumn and winter, they may cause extensive debarking of tree roots and stems, leading ultimately to plant death, being therefore considered as a pest species (Capizzi et al. 2014).

To date, few studies have investigated the biology of the Savi's pine vole, and no information is available regarding demographic structure (as reviewed by Ranchelli et al. 2016). Only anecdotal observations (Contoli 2008) have suggested that Savi's pine vole populations densities may exhibit both multiannual and seasonal fluctuations, although extreme cyclical population outbreaks are not reported as it happens for common voles (*Microtus arvalis*) (Jacob & Tkadlec 2010). In allowing for the possibility to fill this gap of information, we followed two populations of *M. savii* focusing on

population density, survival rate, turnover, time of residency in the population and sex ratio. The aim of our study was to improve for the first time knowledge on demography of this endemic species in agricultural landscape. Our data should be useful for planning and developing efficient control strategies for this species known as a pest (e.g. Jacob 2013, Ranchelli et al. 2016).

Materials and methods

Study area

This study was carried out in two peach orchards of 2 ha each. These plots are located in two regions of central Italy, Emilia-Romagna and Tuscany, where climatic conditions are similar.

The study area in Emilia Romagna is located near the town of Imola (44°21' N, 11°42' E), in a highly-fragmented, predominantly rural area, with average annual rainfalls of 750 mm and mean annual temperatures ranging between +2.6°C and +23.7°C. The landscape of two study areas is similar, characterized by plain topography and formed by agricultural plots separated by ditches and secondary roads. Savi's pine voles here coexist with common voles (*Microtus arvalis*), with the latter attaining very low densities. The study area in Tuscany, about 125 km away from the previous one, is located near the town of Foiano della Chiana (43°15' N, 11°48' E), hereafter Foiano, an area relatively far away from urban settlements, with average annual rainfalls of 700 mm and mean annual temperatures between +5.8°C and +23.0°C. The only vole species to be found in this area is the Savi's pine vole. Both areas consist of peach orchards, similarly managed, with trees ageing from 5 to 15 years, with a between-trees distance ranging from 1.5 to 3 meters (depending on age), a between-rows distance of 4.5 meters, and permanent grass-cover among rows. Both areas are treated with fungicides (in summer and autumn), herbicides (in summer) and insecticides (in summer only in Foiano), but not rodenticides.

Trapping and handling

This study was performed by the capture-mark-recapture method (Nichols & Pollock 1983). Sampling took place monthly, from July 2014 to June 2015 in Imola, and from August 2014 to July 2015 in Foiano. Each sampling session lasted 8 days, with traps kept active continuously for 6 trapping days, for a total of 144 hours of sampling, which remained constant throughout the year. Traps were checked every 8 hours in summer, and every 4-5 hours in winter, to reduce trapping mortality. The two sampling areas remained 2 hectares in size over the 1-year study. Multiple-capture live-traps (Ugglan Special Traps n. 2, Grahnab AB, Hillerstorp, Sweden) were used, whose number (N = 183) remained constant both through sessions and study areas. Traps were baited with apples and provided with cotton for thermoregulation and lowering of stress response.

A preliminary study showed that Savi's pine voles do not enter traps scattered in the field, and the

only way to effectively trap them is to place the traps horizontally, directly inside the first section of the tunnel, so that no light passes through (Dell'Agnello et al. 2017). For this reason, it was crucial to pinpoint 'active' holes, i.e. holes belonging to currently-used tunnels. To do this, on the first day of every sampling session, the study area was walked through in all its width, and all tunnel holes were closed (Bertolino et al. 2015). After 24 hours, re-opened (active) burrow entrances were counted and traps were placed only in these positions, to optimize catching success.

Captured animals were marked using a syringe-injected, 1.4 mm x 9 mm ISO FDX-B glass transponder (Planet ID GmbH, Essen, Germany). Animals weighing less than 10 grams were marked by fur clipping, and with PIT tags when recaptured once over this weight threshold. Animals were assessed for body mass using a handy mechanical spring scale, sex, age and breeding condition. Males with scrotal testes and females with apparent nipples, open vagina, vaginal plug, or evident pregnancy were considered as reproductively active individuals. We used body mass (≤ 15 g juveniles, >15 g adults) to assess age to distinguish juveniles from reproductively competent adults, but the distinction was confirmed by external appearance and breeding condition. Data were recorded on the transponder reader at every capture.

Data analysis

Data on population abundance and demographic parameters were analysed using the program MARK version 8.0 (White & Burnham 1999). We used an information-theoretic approach to select models that were most informative using the Akaike Information Criterion corrected for small sample size (AICc) (Pradel 1996). Candidate models were ranked based on the AICc score and we used delta AICc (Δ AICc) and the Akaike weights (w_i) to select models with the best support. A Δ AICc < 2 suggests substantial evidence for the model. The Akaike weights indicate the probability that the model is the best among the whole set of candidate models.

We used a Robust Design approach to estimate the population densities, and a Multistate Recapture Model to evaluate survival. The Pollock's Robust Design can be used when the trapping scheme is composed by primary periods between which the population is open to immigration and emigration, typically between trapping sessions, and secondary periods that are close together temporally, i.e. within a trapping period of few days. In our cases, the population was considered closed during the 6-days monthly sessions, while it was considered open from one month to the other. The Robust Design estimate the probability of first capture (p), the probability of recapture (c) and the population size (N); it allows also to estimate temporary emigration factors here not considered. The Multistate Recapture Model was used to evaluate the survival (S) of juveniles, and adult males and females between monthly periods.

Turnover, defined as the rate of renewal of the population, was calculated using this formula: $[(N_r+N_l)/N_{rd}] \times 100$ (Bertolino et al. 2001), where N_r is the number of recruits (animals caught during session t that were not present before); N_l is the number of losses (animals trapped during session $t - 1$ and not caught in session t or after); N_{rd} is the number of residents (animals present in session $t - 1$ that were recaptured at time t or after). Time of residency was defined as the time during which a vole can be considered present in the population (Briner et al. 2007). It was computed as the time passed between the first and the last capture event, plus two weeks (half of inter-session time). Only adult individuals captured at least twice, in different sessions, were considered for this analysis. A χ^2 test was used to compare time of residency of males and females, and between study areas. Analysis of variance using a GLM approach was used to test differences in body mass of adult voles pooling data in 4 periods of 3 months. We added 'study area' as random factor to account for variability differences due to site. Post-hoc comparisons were carried out using the LSD test (Sokal & Rohlf 1995). All tests were performed using IBM SPSS 22 (IBM®, Chicago, IL) and R Core Team (version 2.15.3).

Results

Population densities ranged from 8 ind./ha to 32 ind./ha in Imola and from 3 ind./ha to 30.5 ind./ha in Foiano. Density values followed a similar trend in the two areas, with an increase from July to October, a slow decrease till March-April followed by a rapid increase (Fig. 1).

The juvenile/adult ratio, showed an increase in the percentage of juveniles in both populations in the late spring, with a peak in July, where in Imola juveniles made up 54% of all captures (Fig. 2). Even the number of reproductively-active individuals varied over time for both populations, with maximum values in late winter and spring and minimum values in summer (Fig. 3).

The best supported multistate recapture model for Imola indicated a constant survival probability in juveniles (0.56 ± 0.14), and a temporal effect in adults (Fig. 4), with a decreasing trend in survival from summer to following spring. In Foiano a first multistate recapture model indicated a constant survival probability both in juveniles (mean \pm SD 0.39 ± 0.11) and adults (0.49 ± 0.04) ($AIC_c=350$, $w_i=0.63$). There was, however, a substantial evidence for the support of a second model as well, with the same constant survival probability in juveniles (0.39 ± 0.11), and a sex-effect in adults with a slightly higher survival in males (0.51 ± 0.06) compared to females (0.47 ± 0.06) ($\Delta AIC_c=1.88$, $w_i=0.24$) (Table 1).

Turnover rates (Fig. 5) of both populations were conspicuously high: from 60% to 900% in Imola, from 30% to 500% in Foiano; in nearly all capture session, the number of recruits and losses (i.e. turnover rate) was more than twice the number of residents (mean turnover rate of 230 ± 245 in Imola; 246 ± 144 in Foiano). Time of residency, consequently, was quite short in both study areas, being on

average 88 ± 7.9 days in Imola, 73 ± 5.4 days in Foiano. The average time of residency was not significantly different neither between males and females (Imola: $\chi^2=1.8$; $df=1$; $p=0.17$. Foiano: $\chi^2=0.6$; $df=1$; $p=0.43$) nor between study areas ($\chi^2=1.5$; $df=1$; $p=0.22$). No statistical differences were observed in the sex ratios among seasons (Foiano: $\chi^2=0.2$; $df=3$; $p=0.98$. Imola $\chi^2=2.9$; $df=3$; $p=0.41$) and study areas ($\chi^2=0.6$; $df=1$; $p=0.43$). Differences in body mass were investigated with sex and season as fixed factors, and study area as random factor. Test of between-subjects' effects revealed the presence of statistically significant differences for sex ($F_{1,3,219}=438.4$, $p < 0.05$; marginal mean, male= 21.53 female= 21.04), but not for study area ($p=0.27$) and season ($p=0.38$). The analysis did not reveal any two- (at least $p=0.31$) or three-way interaction ($p=0.18$).

Discussion

The present study is the first one in which individuals of two different populations of *M. savii* have been individually and regularly (monthly) trapped. These populations investigated in two different areas showed consistent population patterns. Therefore, our study provided the first significant results until now on the demographic parameters of this poorly known species.

Density values recorded for Savi's pine vole in our study areas (3-32 ind./ha) were much lower than those reported for other species of the genus *Microtus* in Europe, e.g. *Microtus arvalis* (Briner et al. 2007) and *Microtus agrestis* (Burthe et al. 2010). Density values of other non-cyclic *Microtus* species (*M. lusitanicus* and *M. duodecimsotatus*) are much higher than our recorded values too (Santos 2009). These values appear rather low even when compared to previous studies of the same species. Salvioni (1995) reported a minimum of 50 ind./ha and a maximum of 100 ind./ha in Switzerland, while Contoli (2008) reported density values ranging from 10 to 100 ind./ha, which could rise to 1000 ind./ha. None of these studies, however, was conducted in orchards, so there is no real basis for comparison for this type of environment. In our study, densities in both areas did not exceed 30-32 ind./ha, attaining minimal values in winter, rising in spring, and reaching peak values in autumn. Density peaks in late spring and mid-autumn occur in the months in which most births are concentrated when juveniles enter the population. Monthly fluctuations were rather small, compared to those reported for other European species (Korpimäki et al. 2004; Jacob & Tkadlek 2010). Meteorological data of monthly rainfall as well as minimum and maximum temperatures of the two study areas are given in supplementary material Table S1.

Our data regarding reproductively-active individuals show how the breeding period of this species extends throughout the year. This continuous breeding probably is due to relatively mild temperatures and temperatures and food availability all year around (Miñarro et al. 2017). Salvioni (1995) indicated a breeding season extending from March to November, with maximal reproductive activity in spring

and falls in August. Conversely, our studied populations showed no interruption of the reproduction, with a peak of reproductively-active individuals between the end of winter and spring. This new result for our species is concordant with the one obtained for another microtine pest species, the Lusitanian pine vole, *Microtus lusitanicus* that inhabits apple orchards and breeds along the whole year (Miñarro et al. 2017). The same outcome has been reported in the montane water vole, *Arvicola scherman* (Somoano et al. 2017). The juvenile/adult ratio reaches the maximum values in June and July. This can be explained considering that the average gestation period ranges from 22 to 24 days (Caroli et al. 2000), and new-borns reach independence around 24–25 days of age (Santini 1983). The presence of new-borns during the winter month could depend on the availability of food resources, which in our study areas remained relatively abundant throughout the year. Neither population appeared completely stable, though, because they both exhibited very high turnover rates and quite a short time of residency. In general, survival rates were much lower than those of other fossorial microtine species of Mediterranean Europe (e.g. *Microtus duodecimcostatus*, Paradis & Guédon 1993). Populations were totally renewed in a very short time, and this cannot be attributed only to the normal generational renewal (see survival rates). One possible explanation may be a high predatory pressure, since this species is preyed on by raptors as the kestrel (*Falco tinnunculus*) and the barn owl (*Tyto alba*), but also of Tawny owl (*Strix aluco*) and Long-eared owl (*Asio otus*) (Capizzi & Luiselli 1998) both present in the study areas. Moreover, Savi's pine voles are an important part of the diet of terrestrial predators like the red fox (*Vulpes vulpes*) and the weasel (*Mustela nivalis*) (Ranchelli et al. 2016). Population models indicate that change in survival are more important than reproductive output in determining voles' population cycles (Norrdahl & Korpimäki 2002, Korpimäki et al. 2004). For instance, early studies in the seventies (e.g. Krebs & Myers 1974) showed that juvenile survival is one of the major factors in determining the population growth in some American *Microtus* species. On one hand, the abundance of voles in each year can be dependent on the current annual growth rate, but can be influenced also by the density of the former year, in which population density and population growth rate show a negative feedback (Reed & Slade 2008, Barraquand et al. 2014). On the other hand, several extrinsic factors, such as food availability and quality, pathogens and parasites can affect their mortality and fitness (e.g. Cavanagh et al. 2004). Since our results show that juvenile survival did not vary between seasons in both study areas, they may partly explain the absence of population cycles in Savi's pine voles. However, a sampling conducted over a longer time should corroborate these findings, confirming (or not) the stability in the juvenile survival in both populations.

Density values reported here were unexpectedly low compared to other species' – both cyclic and non-cyclic – reported values. However, due to the temporal limit of the study we could not say if

these were the bottoms of synchronized cycles, or the mean values in these orchards. Our data on turnover and time of residency indicate a high renewal rate in both populations. Ascertaining if Savi's pine vole represent a threat for these kinds of crops even at such low densities through damage assessment would certainly be important. However, only a more specific study focused on space use, and dispersal during the year, could help to improve the understanding of Savi's pine vole's population dynamics.

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Conflict of Interest: The authors declare that they have no conflict of interest.

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Table 1 - Outputs of the first Multistate Recapture Models for the two study areas of Foiano and Imola. Juv = juveniles; Ad = adults.

Model	AICc	Δ AICc	AICc weight	Model likelihood	No. Parameters	Deviance
Foiano						
Survival Juv (.) Survival Ad (.) p Juv (.) p Ad (.)	350.675	0.000	0.626	1.000	6	196.820
Survival Juv (.) Survival Ad (sex) p Juv (.) p Ad (.)....	352.562	1.887	0.244	0.389	7	196.548
Survival Juv (.) Survival Ad (t) p Juv (.) p Ad (.)....	353.817	3.142	0.130	0.208	16	177.229
Survival Juv (.) Survival Ad (sex*t) p Juv (.) p Ad (.)	355.016	4.341	0.079	0.173	17	175.841
Imola						
Survival Juv (.) Survival Ad (t) p Juv (sex) p Ad (.)	627.578	0.000	0.350	1.000	16	305.153
Survival Juv (.) Survival Ad (t) p Juv (.) p Ad (.)	628.199	0.621	0.257	0.733	15	307.774
Survival Juv (.) Survival Ad (t) p Juv (sex) p Ad (sex)	629.326	1.748	0.146	0.417	17	304.901
Survival Juv (.) Survival Ad (t) p Juv (.) p Ad (sex)	629.983	2.405	0.105	0.300	16	307.558
Survival Juv (.) Survival Ad (t) p Juv (sex) p Ad (t)	630.973	3.395	0.064	0.183	21	298.546
Survival Juv (.) Survival Ad (t) p Juv (.) p Ad (t)	631.663	4.085	0.045	0.130	20	301.236

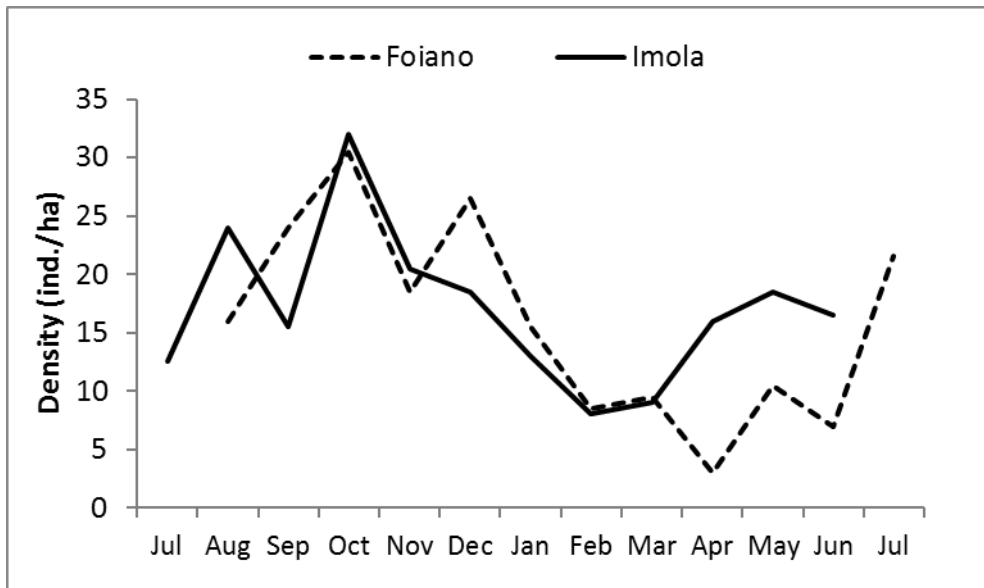


Fig. 1 – Population densities trends of *Microtus savii* during the sampling period (Imola: July 2014–June 2015; Foiano: August 2014–July 2015).

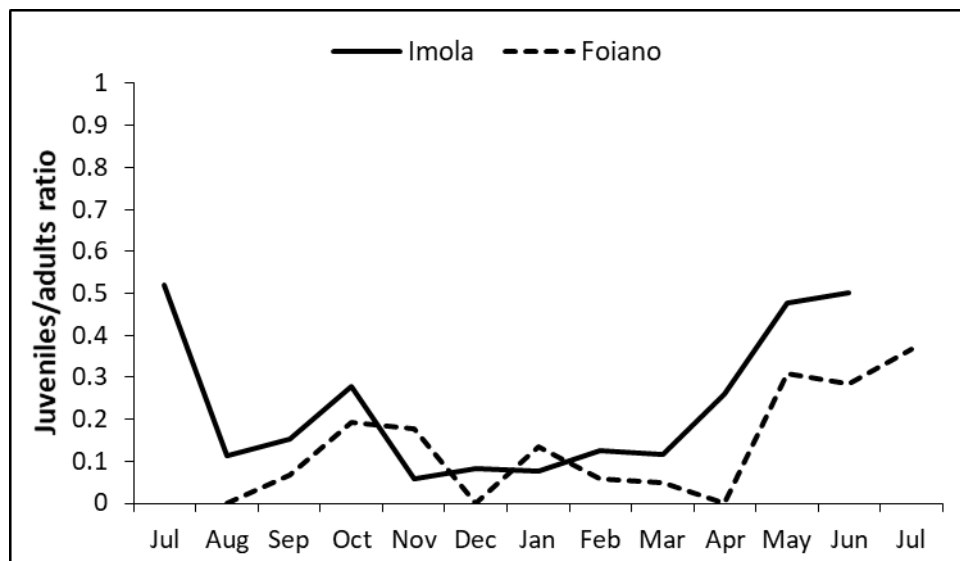


Fig. 2 – Monthly juveniles/adults ratio of *Microtus savii* in the study areas.

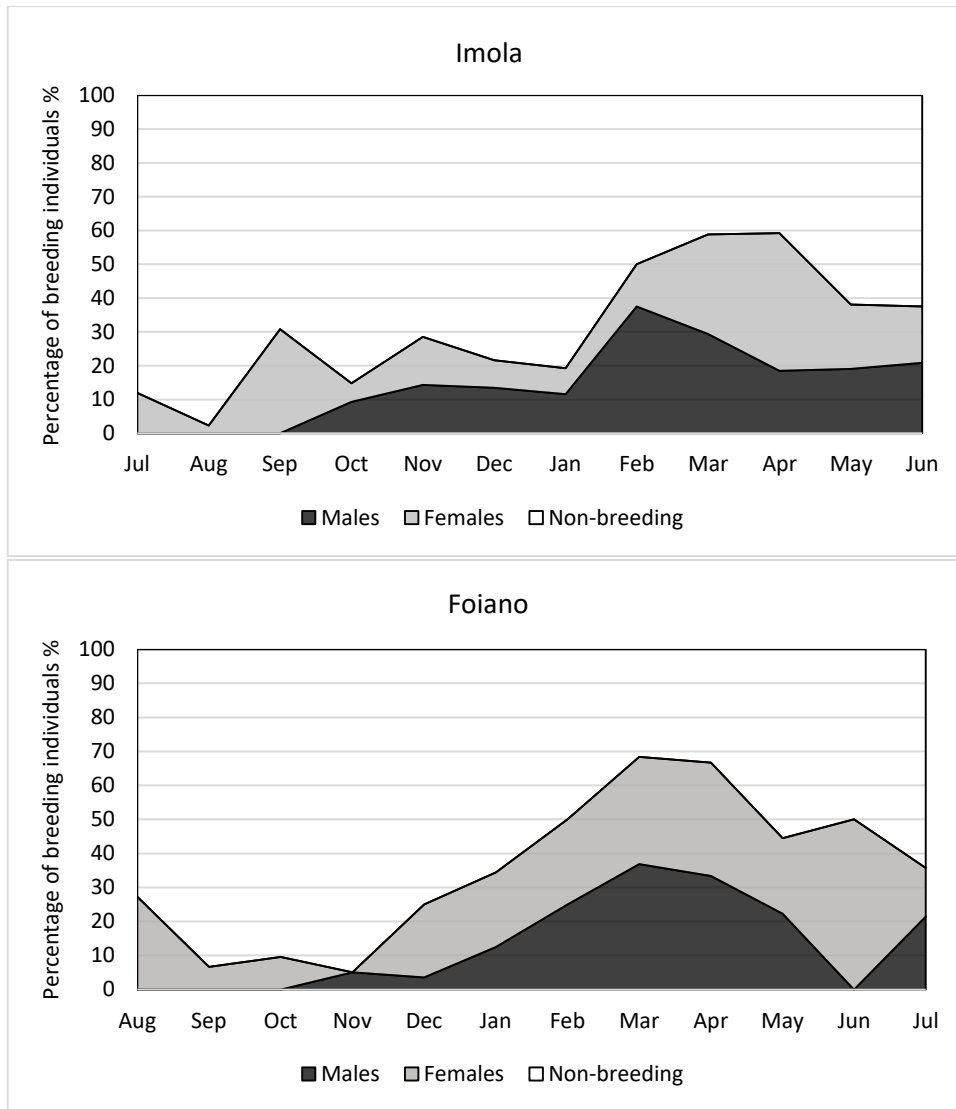


Fig. 3 – Proportion of reproductively – active males and females.

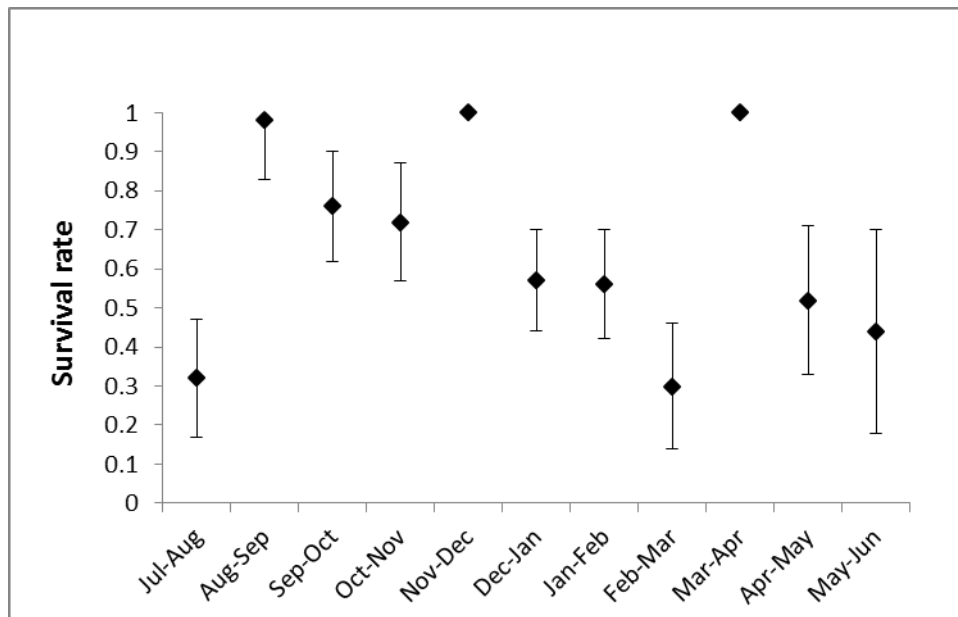


Fig. 4 – Adults survival rate in Imola. Plot showing means and dispersion measures (standard errors) of *M. savii* survival rate throughout the study period.

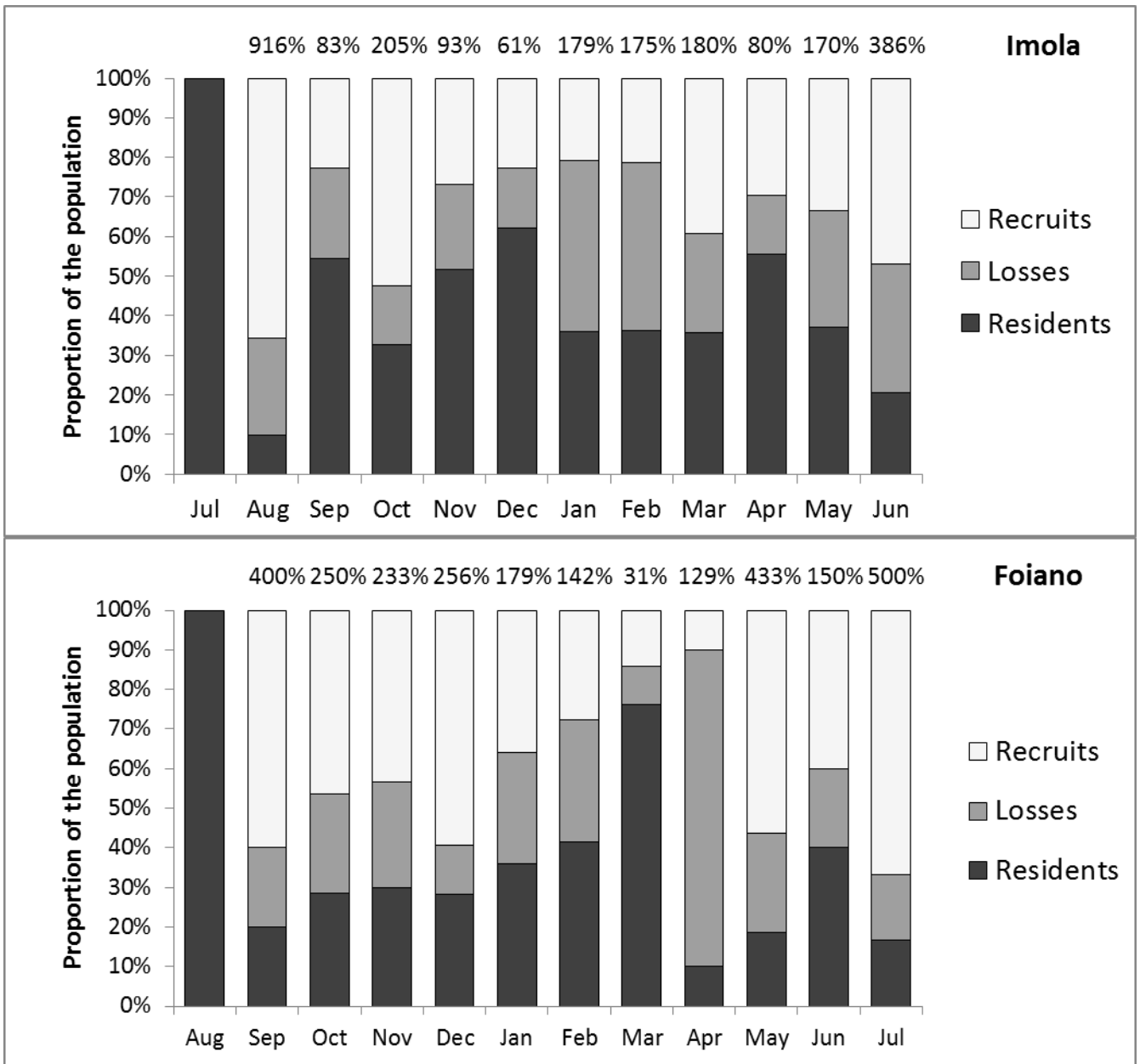


Fig. 5 – Turnover rates in Imola and Foiano.