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Rodent eradication on Molara Island and surrounding islets (NE Sardinia): from success to the riddle of reinvasion

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The eradication of rodents on islands, aimed at the conservation of seabird populations and other components of insular ecosystems, is becoming a common management practice. To restore island ecosystems, rodents (mainly rats Rattus spp. *and house mouse* Mus musculus*) are removed according to standardized methods, including the helicopter delivery of poisoned baits, which has allowed access to larger islands (up to more than 12,000 ha) or difficult to access islands (Howald et al 2007, Veitch et al., 2011). In recent years eradication projects have been carried out in the Mediterranean Sea (Genovesi & Carnevali, 2011), on small to medium sized islands: the largest of these where success has been confirmed are Lavezzi (73 ha, Pascal et al. 2008), Zannone (103 ha, Francescato et al. 2010) and Giannutri (240 ha, Sposimo et al. 2008), all treated by networks of bait stations placed on the ground.*
High predation rates by black rats Rattus rattus *on the largest population of Yelkouan shearwater* Puffinus yelkouan *(Baccetti et al. 2009; Zenatello et al. in press.) (Fig. 1), suggested the urgent adoption of conservation measures in the Tavolara archipelago (NE Sardinia), within the Tavolara – Punta Coda Cavallo Marine Protected Area. An action plan for an overall decrease of the rat impact was produced and operational strategies were evaluated for all different islands and islets of this area.*

Fig. 1. Yelkouan shearwater *Puffinus yelkouan*. Photo: Massimo Putzu

Here we report on the first actions that were put into practice, namely: black rat eradication from Molara island (360 ha), achieved by aerial distribution (cf. Veitch 2002) in October 2008, and ground-based black rat eradication on three islets.

Study area, materials and methods

Study area

Tavolara's group (Fig. 2) is composed of 2 main islands, Tavolara and Molara (600 e 340 ha respectively, 1900 and 1600 m from the nearest point of the Sardinia's coast; Fig. 3-4) and 4 islets, with a surface area between 2.2 and 13.6 ha, set in an intermediate position between the coast and the main islands.

The only wild mammals inhabiting these islands were the black rat *Rattus rattus* (absent only from two islets,) and *Mus musculus* (absent only from Molara). Feral cats are present on Tavolara, cows on Molara and goats are present on both.

Fig. 2.Study area

Fig. 3. Molara Island from the top of Tavolara. Photo: Massimo Putzu

Trappings

In March and September 2008 we performed two four night trapping sessions (March: 19-22; September: 2-5) on Molara, in order to estimate the relative abundance of rats.

Fig. 4. Tavolara from Molara. Photo: Massimo Putzu

Materials and methods

A spreader bucket for the aerial broadcast of pellet baits has been purchased from HeliOtago, New Zealand and helicopters (Eurocopter Ecureuil AS 350 - B2) have been hired for the operation (Fig. 5). The pilot had no previous experiences of similar works.

Bait was chosen according to the results of palatability and longevity tests. The selected product was Brocum® (with 0.005% brodifacoum as the active ingredient). The formulation was 2-gram cereal pellets. Distribution trials for instrumental calibration and pilot training have been conducted using non-toxic baits. Trials showed that, from a height of 50 m, in absence of wind, pellets were distributed on a radius of 45 m, i.e. on 90 m-wide transects.

Livestock protection

To avoid poisoning of free-ranging livestock, two enclosures were built and c. 50% of the goats and 80% of cattle were herded in (total enclosures area: 1 ha).

Rat eradication on Molara

A transect map was made with GIS software and transferred on the helicopter's GPS. Distribution transects were spaced 50 m from each other, for a total transects length of 53 km (Figure 2).

In October 2008 two aerial distributions of bait were performed, spreading 12.3 kg of bait/ha on 1st October and 11.6 kg/ha on 21st October. The pilot's assistant manually switched the spread on and off. The two enclosures were excluded from aerial delivery. In these areas bait was placed inside tamper-resistant bait stations, and so was a buffer area surrounding the enclosures.

A 20 m wide belt running all along the sea coast, larger in two areas (Fig. 6), was baited by hand.

Post-eradication monitoring

To assess the success of the rat eradication we placed and monitored 29 monitoring stations, containing gnawing sticks, toxic and non-toxic wax blocks; six of them being located at most likely landing places.

Rat and mouse eradication/control on other islands.

Ground-based rodent eradication was performed on the three islets in winter 2009-2010. To determine eradication units for future operations (also on Tavolara) and evaluate the risk of rat reinvasion of Molara, rat specimens were trapped and collected for genetic analyses, in order to test possible genetic flow between rat populations in the archipelago and on the adjacent Sardinian coast. Genetic analysis was performed by genotyping 8 microsatellite loci (see Abdelkrim et al., 2005b; 2009) in four black rat populations (Tavolara Is., N = 30; Molara Is., N = 30; Piana Is., N = 30, Sardinian mainland - Capo Coda Cavallo, N = 24). DNA was isolated from rat tail tissues using the Puregene Kit (Gentra System), re-suspended in TE buffer and then preserved at -20°C. Each individual was genotyped for 8 microsatellite loci already used on *Rattus rattus* (see Abdelkrim et al., 2005b; 2009).

Results

Molara rat eradication

The relative abundance of rats on Molara was higher in late winter (0.75 ind/trap/night in March) and much lower in late summer (0.38 ind/trap/night in September), suggesting that summer was a critical period for rat survival. This evidence allowed us to identify the latter season as the best period to carry out the rat eradication on Molara.

A total of 7.4 tons of rodenticide bait was delivered on the island. Several technical problems occurred during the operation. The most serious of which was the malfunctioning of the spinner engine on both delivery sessions, so most transects were covered by vertical dropping (see Micol e Juventin 2002 for a similar episode). Pellet distribution on the ground was found to be in 10 m (wide stripes, alternating to c.40 m) wide empty stripes.
The comparison of the helicopter tracks recorded by GPS and the original planned transects showed marked discrepancies (up to 35 m in some cases). Nevertheless, in the first delivery only six areas, all smaller than 1 ha, were at distances greater than 40 m from the nearest treated point.
Considering both deliveries together, no areas existed with a distance of more than 30 m from the nearest treated point.
Starting two days after the first delivery, several rain showers were recorded, which steadily degraded most fallen pellets; after 21 days, on the date of the second delivery, most previous pellets already seemed considerably degraded.
On the day following the second delivery, a heavy and un-forecast rainfall (33 mm recorded by the nearest weather station) almost completely degraded the newest pellets. If any rat had survived the first delivery, therefore, pellets from the second one were available to them in adequate conditions only for a single night.
Carried out in a season when very few gulls (Laridae) are locally present, poisoning affected few non target species. Corpses of two barn owls (*Tyto alba*) and two ravens (*Corvus corax*) were found, both presumably dead after secondary poisoning.
Livestock that could not be herded into the enclosures was affected for an estimated one third of the goats; while none of the 6-7 free ranging cows died nor showed any problems.
Yelkouan shearwater reproductive success greatly increased after rat eradication on Molara, from total failure at the previous rat eradication reaching values of 0.6-0.8 fledglings per pair in 2009 and 2010.
After 21 months of an apparent absence of rats, signs of their presence were discovered in July 2010 along 1 km of the Molara coast, facing the Sardinian mainland, 1600 m far.

These signs of rats followed the appearance of several domestic rabbits (quickly captured and removed) in the previous winter. The responsibles of the introduction of rabbits to Molara are unknown.

Rats eradication from islets

From all three islets black rats were easily eradicated, but success was short lived.
After 6 months they were found again on the islet nearest to the mainland (Proratora, 4.6 ha, 170 m offshore), and also after two years at Cavalli (2,2 ha, 300 m offshore), although not yet on adjacent Piana (13,6 ha, 660 m far from land and 530 m from Cavalli).

Genetic analyses

All the analyzed loci are polymorphic, with a mean number of alleles equal to 10.
The highest values of genetic variability have been recorded for the Capo Coda Cavallo population, while the lowest one is for the population of Piana (Tab. 1). Molara and Tavolara populations have similar value of allelic richness, number of alleles and heterozygosity values (Tab. 1). Piana and Tavolara populations show evidence of heterozygosity excess.

Table 1. Mean allelic richness per locus (Na), observed heterozygosity (Ho), expected unbiased heterozygosity (He) and the P- value of departure from the Hardy-Weinberg equilibrium (P).

Population	Na	Ho	He	P
Piana	2.36	0.352	0.355	P < 0.001
Tavolara	4.22	0.510	0.554	P < 0.001
Molara	4.42	0.488	0.545	P = 0.28
Capo Coda Cavallo	6.67	0.715	0.724	P = 0.03

Fig. 5. Helicopter and bucket. Photo: Nicola Baccetti

All four populations are characterized by a high number of private alleles, a clear evidence of inter-population differentiation. Statistically, this is confirmed by AMOVA that shows low levels of gene flow among our populations (Fst = 0.33, P < 0.001), as well as by SAMOVA showing that the number of population groups that maximised the distribution of genetic variation was k = 4. Moreover, the cluster analysis conducted with the program STRUCTURE recorded the presence of four groups of populations (K = 4), each corresponding to one of the four analysed populations.
Finally, STRUCTURE indicates that the genotypes of two specimens of *R. rattus* collected on Molara during the post-eradication monitoring cluster within the mainland population (Fig. 7).

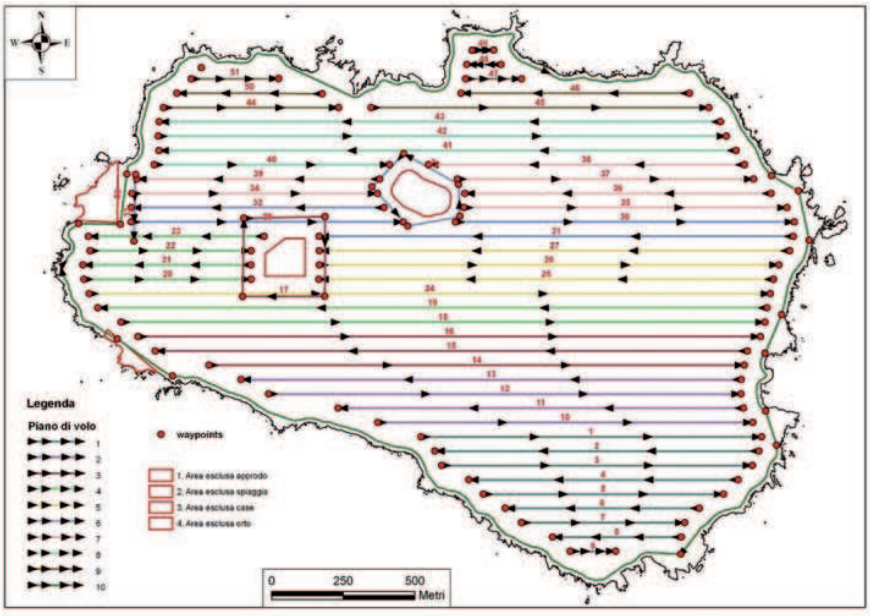

Fig. 6. Flight plan

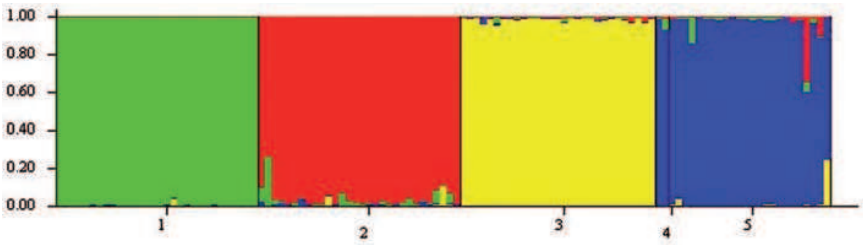

Fig. 7. Cluster analysis: the run with the highest posterior probability corresponds to K = 4. Black vertical bars delineate predefined populations: Group 1, Piana in green; Group 2, Tavolara, in red; Group 3 Molara pre-eradication, in yellow; Group 4, two Molara individuals collected after the eradication, in blue; Group 5, Capo Coda Cavallo, also in blue.

Conclusions

Aerial bait delivery proved an effective way to eradicate rats on Mediterranean mid-sized islands, where a ground-based action would not be possible for technical and/or economic reasons. The Molara operation represented the first case in the Mediterranean and Europe of using a helicopter and bucket. Problems with instruments and the lack of a trained pilot made sticking to strict protocol (Cromartry et al. 2002, McClelland 2011) impossible. The problems with instruments were at least partially due to inexperienced staff in their utilization (experiences gained on Molara have helped two eradications realized after with the same bucket, on Sa Dragonera, J. Mayol *et al*. this volume., and Montecristo, P. Sposimo *et al*. unpublished., where all instruments worked without noticeable problems). On the other hand, the failure of the spinner engine had also happened during the (succesful) eradication of an island larger than Molara (Saint-Paul Island, 800 ha, Micol e Juventin 2002), and prompted us not to suspend the flight.

The four populations of *R. rattus* collected in the Marine Protected Area Tavolara Punta Coda Cavallo resulted to be genetically differentiated based on summary statistics, as well as on cluster analysis. Thus, four independent eradication units with extremely reduced or absent gene flows existed within the Marine Protected Area. The two specimens collected during post-eradication monitoring on Molara Island clearly clustered with the Sardinian mainland population and not with the pre-eradication Molara population. The extinction of the private alleles that were present in all rats previously examined strongly suggests that: 1) a new population is present; 2) the combined effects of inexperienced staff, heavy rains and engine malfunctioning had not affected the success of the eradication efforts; 3) the potential source for rat recolonization is the Sardinia mainland population. Assessing whether recolonization occurred spontaneously (which would entail the largest sea crossing recorded for the species, 500 m being the maximum known, e.g. Russell et al., 2005; Russell and Clout, 2005) or following intentional introduction is unfortunately impossible to know. Unintentional transportation is also possible, but we judge this as the most unlikely option. The concurrent – and definitely intentional - introduction of rabbits represents an additional evidence of the intentional introduction option. However, the rapid reinvasion of the islet nearest to the mainland (130 m), followed by that

of the second nearest, shows that rat recolonization by swimming is a frequent phenomenon and confirms that a careful evaluation of the risks of recolonization is a fundamental measure before a rat eradication project is carried out (see Capizzi et al., 2010).

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