LIFE ilhas barreira

REPORT

Action A3 – Gathering baseline information of introduced mammal species

Lisboa | August | 2022

Gathering baseline information of introduced mammal species.
Report of the Action 3 of the project LIFE Ilhas Barreira

SPEA

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Reference
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Summary

The presence of predatory mammals on seabird breeding areas are a threat to the survival of eggs and chicks, and can drive birds to the abandonment of colonies. We collected data on presence, abundance, sesonal fluctuations and distribution of rodents and cats on the Barrier Islands of Ria Formosa, where two seabird species of special concern breed, the Audouin's gull (*Larus audouinii*) and the Little tern (*Sternula albifrons*).
On Barreta/Deserta island three species of rodents were identified, the native Algerian mouse (*Mus spretus)*, and the invasives Brown rat (*Rattus norvegicus*) and Black rat (*Rattus rattus*). Their presence was also registered on Culatra Island and in Ancão peninsula. The Algerian mouse is highly abundant on Barreta Island, mainly after the reproduction peak period in August-September. Despite the high density values recorded, their diet does not pose a threat to the breeding birds present. On the other hand, rats (*Rattus* spp.) are less abundant but have a wide distribution on Barreta Island. Control measures to reduce the rat population can be placed in areas where their presence has been confirmed and in potential entry zones.
The presence of cats was registered in all Barrier Islands, with the exception of Cabanas Island. The Ancão peninsula registered the highest cat abundance followed by Tavira Island. On Barreta Island we were able to individually identify 7 adult cats. Their distribution was mainly centered between the restaurant and the boardwalk, with the exception of one cat that centered its activity on the lagoon in the western part of the island.
The control of rodents and cats on Barreta will improve the breeding conditions of seabirds and land birds that breed on the island, as well as the improvement of native vegetation, other native species, and a sensitive habitat.

Resumo

A presença de mamíferos predadores em áreas de reprodução de aves marinhas é uma ameaça à sobrevivência de ovos e crias, podendo levar ao abandono das colónias. Recolhemos dados sobre a presença, abundância, flutuações sazonais e distribuição de roedores e gatos nas Ilhas Barreira da Ria Formosa, onde nidificam duas espécies de aves marinhas de especial preocupação, a gaivota-de-audouin (*Larus audouinii*) e a chilreta (*Sternula albifrons*).
Na ilha da Barreta/Deserta foram identificadas três espécies de roedores, o nativo ratinho-das-hortas (*Mus spretus),* e as espécies invasoras ratazana castanha (*Rattus norvegicus*) e o rato-preto (*Rattus rattus*). A sua presença foi também registada na ilha da Culatra e na península do Ancão. O ratinho-das-hortas é bastante abundante na ilha da Barreta, principalmente após o período de pico de reprodução em agosto-setembro. Apesar dos altos valores de densidade registados, a sua dieta não representa uma ameaça para as aves reprodutoras presentes. Por outro lado, as ratazanas (*Rattus* spp.) são menos abundantes, mas têm uma ampla distribuição na ilha da Barreta. Medidas de controle para reduzir a população podem ser colocadas em áreas onde sua presença foi confirmada e em potenciais zonas de entrada.
A presença de gatos foi registrada em todas as Ilhas Barreira, com exceção de Cabanas. A península do Ancão registou a maior abundância de gatos seguida da ilha de Tavira. Na ilha da Barreta conseguimos identificar individualmente 7 gatos adultos. A sua distribuição centrou-se principalmente entre o restaurante e o passeio marítimo, com exceção de um gato que centrou a sua atividade na lagoa na parte ocidental da ilha.
O controlo de roedores e gatos na ilha da Barreta irá melhorar as condições de reprodução das aves marinhas e terrestres que se reproduzem na ilha, bem como a melhoria da vegetação autóctone, de outras espécies nativas, e de um habitat sensível.

1 | Introduction

Mammals can pose serious threats to insular ecosystems (Courchamp *et al.*, 2003). Species such as rats, cats, goats, rabbits and pigs, were, in most cases, introduced by humans to islands, or their colonization was facilitated by migration and human activities (Mack *et al.*, 2000; Courchamp *et al.*, 2003). The presence of introduced mammals can cause damage to local fauna and flora (Courchamp *et al.*, 2003), and even cause the extinction of vulnerable native species through predation, competition, disease transmition, grazing, and habitat alteration (Mack *et al.*, 2000; Harris, 2009).

Impacts of rodents extend to a wide variety of animals, from other native rodent species (Harris, 2009) to reptlies (Case and Bolger, 1991), hatchling sea turtles (Caut *et al.*, 2008), seabirds (Jones *et al.*, 2008), arthropods (Cole *et al.*, 2000), and land snails (St Clair, 2011). Also, their intense predation upon seeds seeds and fruits may represent a threat to endemic plant species, and promote the dispersion of invasive plants (Meyer and Butaud, 2009; Grant-Hoffman and Barboza, 2010).

Feral cats are also responsible for at least 14% of global bird, mammal, and reptile extinctions on islands (Medina *et al.*, 2011), leading to several erradication campaigns to promote native species recovery (Nogales *et al.*, 2004). As an opportunistic predator, cat diet includes a wide variety of animals, with primary prey often being based on abundance (Nogales *et al.*, 2004).

To seabirds, mammals can cause rapid declines in numbers and range, due to their vulnerability to introduced predators (Towns *et al.*, 2011). Cases of extinction due to predation are rare, as seabirds often breed in more than one island (Nogales *et al.*, 2004), although several local extripations occurred (Towns *et al.*, 2011).

On the sandy Barrier Islands of Ria Formosa, in Algarve, two seabird species of special concern breed, the Audouin's gull (*Larus audouinii*) and the Little tern (*Sternula albifrons*). The presence of predator mammals on their breeding areas are a threat to these populations, as they predate eggs, chicks, and adults, and drive birds to the abandonment of breeding colonies (Oro *et al.*, 1999; Medeiros *et al.*, 2012). Events of predation of Audouin's gulls' chicks by cats have already been registered on Barreta/Deserta Island, and recent records also point to predation of Little tern eggs and chicks by cats and rats on Ancão peninsula.

Despite rodents and cats were known to be present on Barreta Island (where the largest colony in the country of Audouin's gull is located), little was known about which species have been present and their current abundance and distribution on this island, or on the neighbouring islands.

Under the Action A3 of the Life Ilhas Barreira (LIFE18 NAT/PT/000927), we collected data on abundance, seasonal fluctuations on population size, and distribution of rodents and cats at Barreta Island and neighbouring islands. Gathering this information will be helpful to implement the follow actions foreseen under the project, namely the control of invasive rodents and the removal of cats from Barreta Island, as foreseen in the Action C3 - Control of invasive mammals (rodents and cats). These actions will reduce the predation on breeding seabirds, landbirds, and other native fauna, as well as threatened flora.

2 | METHODOLOGY

2.1 Study area

Ria Formosa is a complex coastal lagoon system, located in Algarve (southern Portugal), and enclosed by 5 barrier Islands (Barreta/Deserta, Culatra, Armona, Tavira, and Cabanas) and two peninsulas (Ancão and Cacela), which extend for over 60 km (Fig. 1).
The Barrier Islands are a unique system that support priority habitats such as fixed dunes “grey dunes”, essential for the stabilization of dune and protection of the shoreline, the presence of interesting floristic species with 14 Iberian endemisms, and priory bird species.
Ria Formosa is home to 3 breeding seabird species: the Audouin’s gull, has a global conservation status of Vulnerable, with the only breeding ground in Portugal located on Barreta Island; the Little tern, with the most important portuguese breeding population located inRia Formosa; and the Yellow-legged gull (*Larus michahellis*). The Barrier Islands also hold several other breeding birds, native reptiles and arthropods, and the surrounding sea grounds have significant ichthyofauna richness, playing an important role as a fish nursery.
By its natural values, Ria Formosa is classified as special preotected area (SPA PTZPE0017), a Natural Park (designated under national legislation), and a Site of Community importance (SCI PTCON0013).
Main biodiversity threats are related to huge touristic pressure, especially during the summer months, bycatch and mortality in fishing gears, and the presence of invasive alien mammals (rats and cats) on the islands.

Figure 1 | Location of Ria Formosa SPA and the Barrier Islands on the Algarve coast.

2.2 Rodents

Information of the rodent species that are present on the Barrier Islands is scarse. It is imperative to increase knowledge about these populations in order to assess the threats they may pose to the nesting seabirds and other native fauna on Barreta Island, especially if we are facing invasive species. Several methods were perfomed in order to access species diversity, population fluctuations, and distribution maps.

2.2.1 Capture-recapture on Barreta Island

Capture-recapture methods were used to access species composition and to estimate rodents' densities and abundance fluctuations over the year. Two grids of 5×4 traps, spaced 25 m, were set on the eastern part of Barreta Island, with one grid (grid A) located near the pier and the restaurant, and the other grid (grid B) located in a Yellow-legged and Audouin's gull colonies (Fig. 2). In each grid, 20 Sherman® traps (XLF15 model, 10×10×38cm) were placed to capture bigger (e.g. *Rattus* sp.) and smaller (e.g. *Mus* sp.) rodent species. At the middle points of each Sherman® trap, a Pest-stop® trap (43 traps in total) was placed in order to cover the minimal range of smaller species, resulting in a final grid of 9x7 traps spaced 12.5m apart (Fig. 3). Logistical aspects (as the proximity from the only harbour and from the team settlement place) were also took into consideration on the choice of the grid locations.
Both grids were sampled once a month during 4 nights, over a one-year period from January 2020 to February 2021, unless stated otherwise (Table 1). Changes to the sampling periods were due to adverse weather events and COVID-19 circulation restrictions.
All traps were baited with seeds and/or peanut butter, and were visited early in the morning.

Figure 2 | Location of the two grids of traps to capture rodents on the eastern part of Barreta Island. Each grid had 63 traps located at the centroid of each cell.

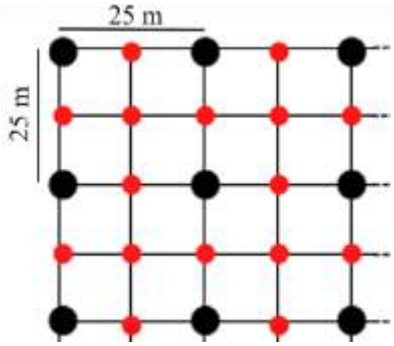

Figure 3 | Scheme of the trap grids. Black and red circles indicate the position of Sherman® trap and Pest-stop® taps, respectively.

Each captured rodent was marked with a uniquely numbered earring on the posterior part of the ear, and if not damaged, an ear tissue sample was also collected for DNA analysis. Data on the morphologic characteristics were also collected: sex, age (coded as adult or juvenile, based on body size and sexual maturation - testes in the scrotal zone or perforated vaginas were considered active adult individuals), reproductive status, weight, ear length, hind paw length and tail length. Species identification was carried out by morphological characteristics and confirmed by genetic analysis of 89 tissue samples. The taxonomic identification of the species was
based on the sequence of the d-loop, a fragment of mitochondrial DNA. Results can be found on Annex A.

Monthly relative abundance was calculated for each grid by calculating the Trap Success Index (TSI) (Mills *et al.*, 1991) given by:

$$TSI = \frac{\text{nº of rodents captured}}{\text{total nº traps set} - [\,0.5 \times (\text{sprung traps} + \text{nontarget captures})]}$$

The results were expressed in number of captures per 100 trapping nights.

From the calculated TSI, distribution maps of the captured rodent species were prepared using the geographic information system software QGIS (QGIS.org, 2022).

Table I | Periods of capture-recapture sampling in Barreta Island, from January 2020 to February 2021.

Month	Grid	Period
January	A	21/01/2020 to 24/01/2020
February	A / B	06/02/2020 to 09/02/2020
March	A / B	17/03/2020
May	A / B	08/05/2020 to 11/05/2020
June	A / B	16/06/2020 to 19/06/2020
July	A / B	10/07/2020 to 13/07/2020
September	A / B	12/09/2020 to 15/09/2020
October	A / B	09/10/2020 to 12/10/2020
November	A / B	27/11/2020 and 28/11/2020
December	A / B	15/12/2020 and 17/12/2020 to 18/12/2020
February	A / B	19/02/2021 to 20/02/2021 and 22/02/2021

Also, density (ind/ha) of species captured was estimated for each grid by modelation of capture data using the package “secr” in R version 3.6.3 (R Core Team, 2020). Several models were performed evaluating different detection functions (halfnormal, exponential, and hazard rate) following a Poison distribution. The best fitting model for each grid was selected based on the lowest AIC value.

2.2.2 Wax-blocks

To identify rodent species that may be too shy to enter the traps, a wax-block (a mix of peanut butter and paraffin) was set near each trap during the traping periods, so they could be identified based on the bite marks. Wax-blocks were set on the first day of capture, and replaced each day if they had been bitten/consumed.
To map the distribution of rodents that bit the wax-blocks, an index was calculated for each cell of both grids, by the number of blocks biten by the total block placed multiplied by 100. The results were expressed as the number of blocks bitten by 100 blocks placed.

2.2.3 Extra sampling on Barreta and adjacent islands

To access rodent species that may disperse to Barreta island, extra sampling was carried out in the neighbour Culatra Island (in Farol) and Ancão peninsula, with 18 and 35 traps, respectively (Fig. 4). Also, as there was evidence of the presence of rats on Barreta Island, extra sampling was performed around the harbour, with 15 traps, and in the lagoon on the west end of the island, with 20 traps (Fig. 4). Two types of traps were used, Tomahawk® Live Traps (model 102, 16"x5"x5"), and Sherman® traps (XLF15 model, 10×10×38cm). Traps were set alternately, 25 m apart in a line. Each capturing session had the duration of 4 nights, and traps were baited with peanut butter and chorizo. The periods of trapping are presented on Table II.
Similarly, to the capture-recapture method used in the two grids on Barreta Island, each captured rodent was marked with a uniquely numbered earring, morphometrics were collected, as well as tissue samples for DNA analysis.
Distribution maps of each species were elaborated as the total number of captures per trap.

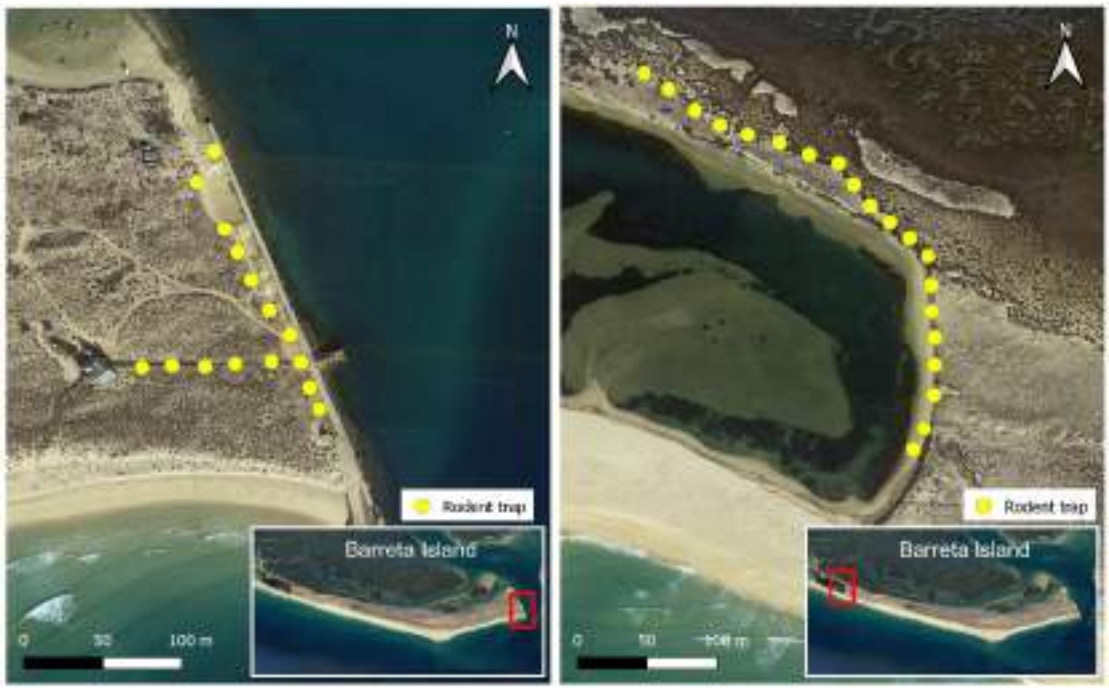

Figure 4 | Location of the extra trapping sessions on the Barreta island (harbour - top left; lagoon – top right), on Culatra (bottom left) and Ancão peninsula (bottom right).

Table II | Periods of extra capture-recapture sampling in Barreta Island, Ancão peninsula and Culatra island, from April to November 2021.

Month	Location	Period
April	Ancão Peninsula	18/04/2021 to 21/04/2021
May	Culatra Island	08/05/2021 to 11/05/2021
June	Barreta Island - Harbour	20/06/2021 to 23/06/2021
July	Barreta Island - Harbour	11/07/2021 to 14/07/2021
September	Barreta Island - Lagoon	17/07/2021 to 20/07/2021
October	Culatra Island	25/10/2021 to 28/10/2021
November	Ancão Peninsula	15/11/2021 to 18/11/2021

2.2.4 Occasional sightings of rats

Occasional sightings of rats (*Rattus* spp.) and their tracks, scats, and other signs, were also opportunistically collected during other activities developed under the project. Those sightings were used to fill any gaps on rat distribution on Barreta island.

2.3 Cats

2.3.1 Camera-traps

One grid of 22 camera-traps (PRIMOS Mug Shot Trial Camera Model 65064, 12MP) spaced 500*500 m was set on Barreta Island (Fig. 5). This grid covered near two thirds of the island in terms of length because the last third was a narrow sandy strip, which was assumed to have non-suitable habitat for cats. Cameras were installed 30 cm from a passageway used by cats, and oriented so as to make an angle of 23° with the trail (Fleming *et al.*, 2014), and set to take three photos with one second apart upon activation of the motion sensor. Cameras were active from January to February 2021, with memory cards and batteries replaced once a month by two team members.

All images were processed using Timelapse software. A collaboration with Microsoft was set in order to use their MegaDetector to help on image processing, as normally this methodology produce an enormous amount of data and empty images. Photos of cats were used to individually identify cats, estimate the total number present on the island, and to map their individual distribution.

For mapping cat distribution, cat photos were filtered in order to eliminate photos taken within 3 seconds of the previous recording.

Figure 5 | Location of the 22 camera traps set on the Barreta island, on a grid spaced 500*500 m.

2.3.2 Spotlighting

In order to count cats and to identify hot spots of cat activity, one transect, covering the same two thirds of Barreta Island sampled with camera traps (see section 2.3.1), was sampled every month from February 2020 to February 2021. This transect was 5.49 km long and was divided in four segments (Fig. 6). It was sampled by a team of two people at sunset or 30 min before the sunrise for three consecutive days, unless limited by the weather conditions. Sighting data were recorded in the WVS Data Collection App https://apkpure.com/wvs-data-collection-app/com.wvsdatacollection.android).

An abundance index was calculated by the mean number of cat sightings per kilometre. The resulting value was intended to be used in the future to evaluate the success of the feral cat removal operation (foreseen under Action C3) comparing abundance index values of before and after removal (Mitchell and Balogh, 2007).

Figure 6 | Transect with 5.49km length used during spotlighting surveys of cats on Barreta Island. The transect was divided in 4 segments.

2.3.3 Track counts

To monitor cat footprints, 56 stations 1*8 m placed 100m apart were marked on Barreta Island (Fig. 6). Stations were set in places with low vegetation and the sand was smoothed. The presence of cat tracks was registered during three consecutive days every month over one year (from February 2020 to February 2021) by two team members. A measure of 'imprintability' was taken in each station to account for variations in footprint detectability. The imprintability score was given by walking 10 steps along the station and record the value of each footprint on a scale from 0 to 3 (0 - no print visible; 1 - print barely visible; 2 – complete outline of print and some details of the sole visible; 3 - complete outline of print and all details of the sole visible) varying from 0 to 30. A station with a score of 0 to 5 – poor printing potential (1), 6 to 15 – reasonable (2), 16 to 25 – good (3), 26 to 30 – excellent (4). All stations with poor printing potential (1) were not used to calculate abundance indexes.

In order to compare cat abundance on Barreta Island with the other Barrier Islands, track counts were carried out twice a year in Culatra, Armona, Tavira and Cabanas islands and Ancão peninsula, following the periods present on Table III. Locations of track stations are represented through out Figure 7 to Figure 12.

Two abundance indexes were calculated: the Allen index, given by the number of stations with cat tracks per transect per day, and the Catling index, given by the percentage of station nights with tracks (Mitchell and Balogh, 2007).

Table III | Periods of track counts and number of track stations monitored in Barrier Islands.

Site	Nº of track stations	Period
Barreta Island	56	February 2020 to February 2021
Culatra Island	62	February 2021 and October 2021
Armona Island	67	February 2021 and October 2021
Tavira Island	114	March 2021 and November 2021
Cabanas Island	78	April 2021 and September 2021
Ancão peninsula	16	March 2021 and November 2021

Figure 7 | Location of track stations on Barreta Island.

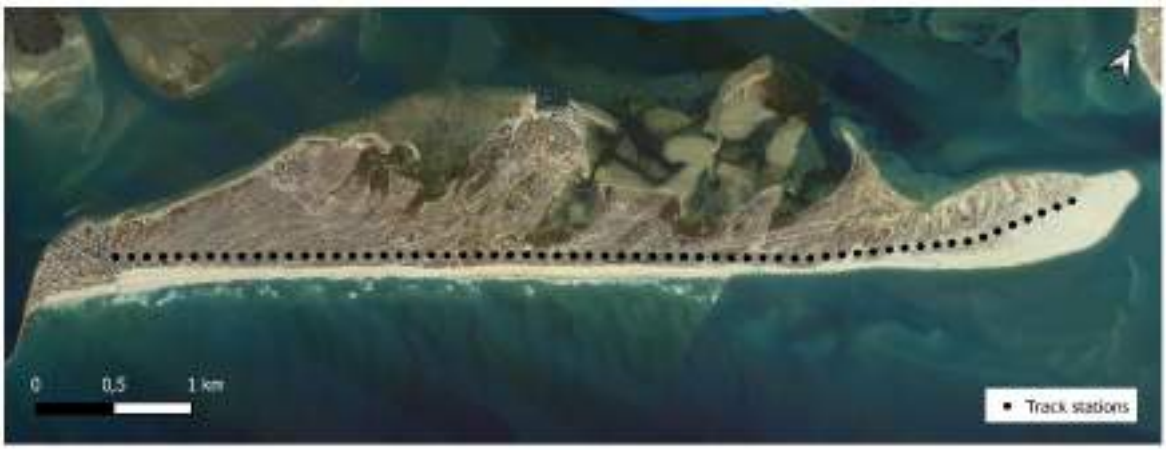

Figure 8 | Location of track stations on Culatra Island.

Figure 9 | Location of track stations on Armona Island.

Figure 10 | Location of track stations on Tavira Island.

Figure 11 | Location of track stations on Cabanas Island.

Figure 12 | Location of track stations on Ancão peninsula.

3 | Results

3.1 Rodents

3.1.1 Capture-Recapture

Only the Algerian mouse was captured during the trapping sessions carried out in the two grids installed on Barreta island. Morphological characteristics of the adult mice population is registerd on Table IV.

Table IV | Morphological characteristics of adult Algerian mice captured in Barreta island, in which N represent the number of individuals sampled, m the mean value, std the standart deviation, and Range the lower and upper limits.

	Females				Males			
	N	m	std	Range	N	m	std	Range
Weight (g)	27	13.18	2.90	(10 - 18)	35	12.11	2.70	(10 - 18)
Tail (mm)	24	65.73	4.63	(56 - 72)	31	65.33	6.20	(49 - 76)
Ear (mm)	25	13.36	1.70	(10 - 16)	36	13.18	1.93	(8 - 17)
Hind paw (mm)	27	15.57	0.91	(14 - 18)	36	15.78	0.88	(14- 19)
Head+body (mm)	1	67.5	-	-	2	66.25	0.78	(65 - 67)

On total, we captured 199 Algerian mice (from which 89 were recaptures), over January 2020 to February 2021. A greater number of captures was recorded in grid B, with 84 captures and 85 recaptures. Any rodent was captured from January to July 2020 in grid A. Monthly variation of captures is expressed on Fig. 13, and monthly TSI is represented on Fig. 14.

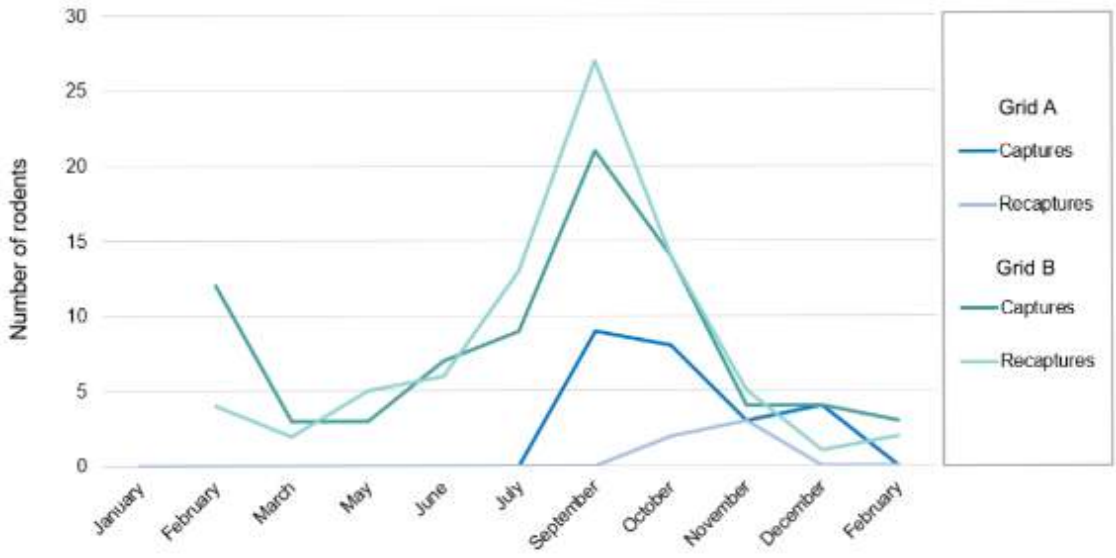

Figure 13 | Monthly number of Algerian mice captured and recaptured in grid A and grid B over January 2020 to February 2021.

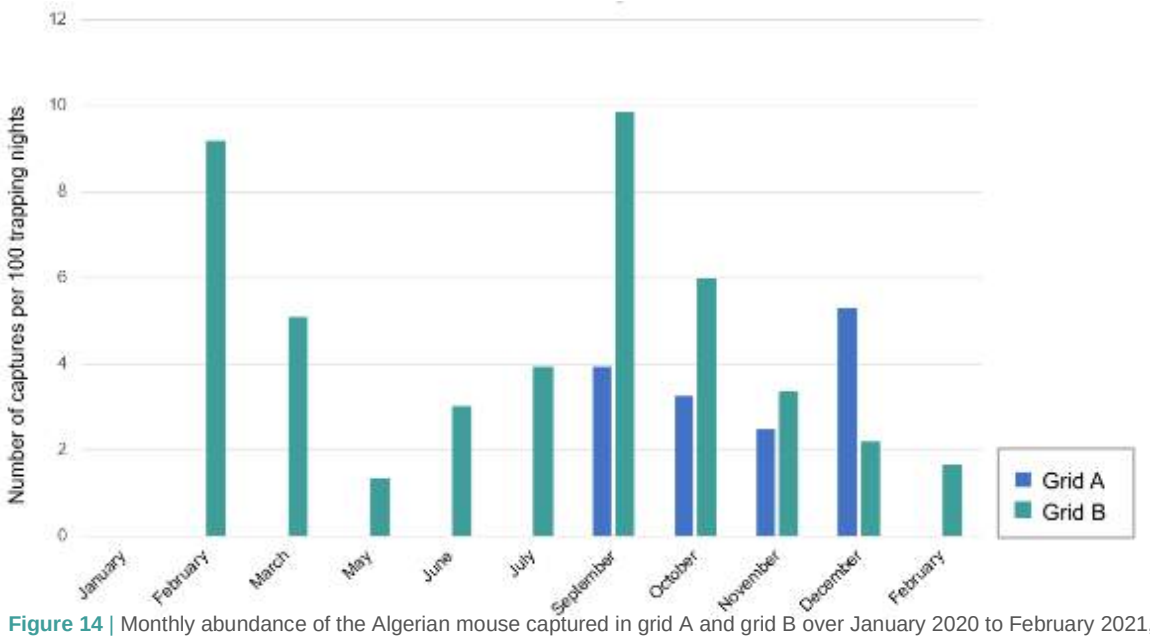

Figure 14 | Monthly abundance of the Algerian mouse captured in grid A and grid B over January 2020 to February 2021, expressed by the trap success index (TSI). Number of captures includes captures and recaptures.

The trap index success for each trap was calculated and used to map the distribution of the Algerian mouse within each grid (Fig.15).

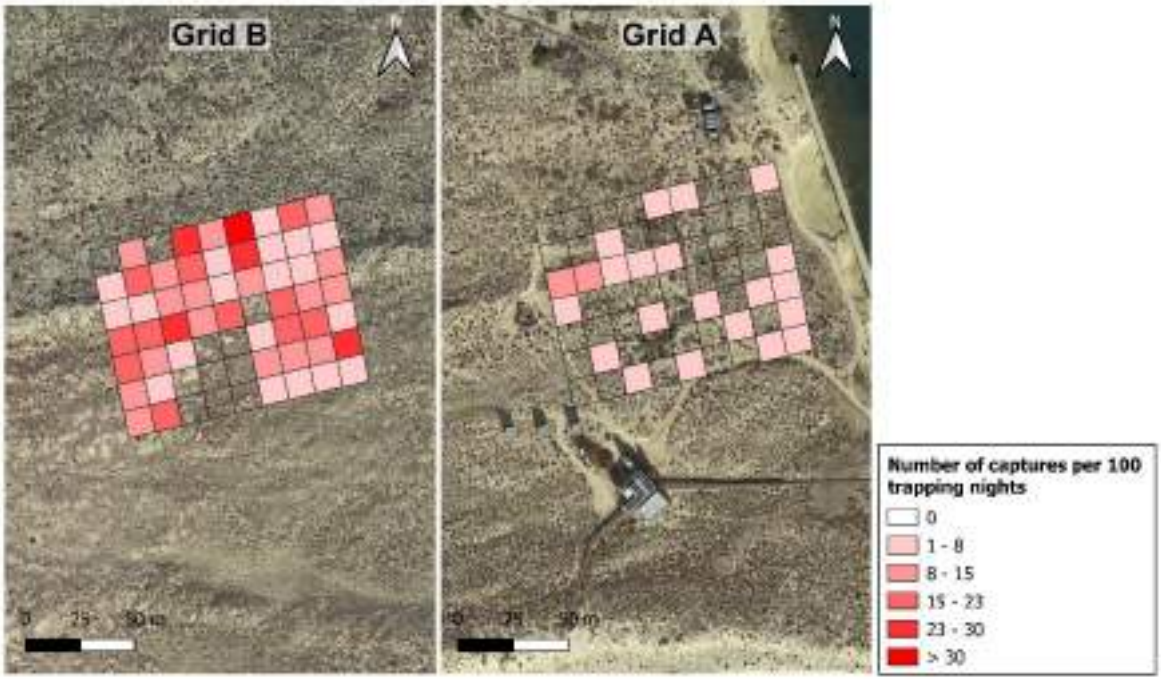

Figure 15 | Distribution maps of the Algerian mouse *Mus spretus* captured in the grid A (near the restaurant) and grid B (in the Yellow-legged and Audouinii's gull colonies) on Barreta Island over the period of capture-recapture.

Estimations of the Algerian mouse density on Barreta Island, calculated by modelation of capture data, are expresed on the Table V. For grid B the best fitting model is a model with an exponential detection function (formula: D~session, g0~1, sigma~1), in which density vary between capture sessions (one session = one sampling month), but within sessions the population is considered closed to gains and losses. The values of density for grid B are therefore a range between the lowest and highest estimated density in all sessions.

As grid A had fewer captures and recaptures, the same model did not explain the variance of data, which had a better fitting model with an halfnormal detection function (formula: D~1, g0~1, sigma~1), in which density remained constant throughout the capture period.

Table V | Density estimates of the Algerian mouse on Barreta Island, based on modelling capture–recapture data.

Grid	Density (ind/ha)	Std error	Detection function	Distribution	AIC
A	26.28	18.49	halfnormal	poisson	270.30
B	3.73 – 18.23	1.84 – 4.32	exponential	poisson	1387.11

3.1.2 Wax-blocks

Teeth marks from wax-blocks revealed the presence of rodents from the *Mus* genus (probably the Algerian mouse), and of rats *Rattus* spp. on both grids. Identification on a species level it is highly unlikely as teeth marks from rodents of the same genus are very similar. Distribution maps of rodents based on and index of the number of bitten wax-blocks are represented on Fig. 16 and Fig. 17.

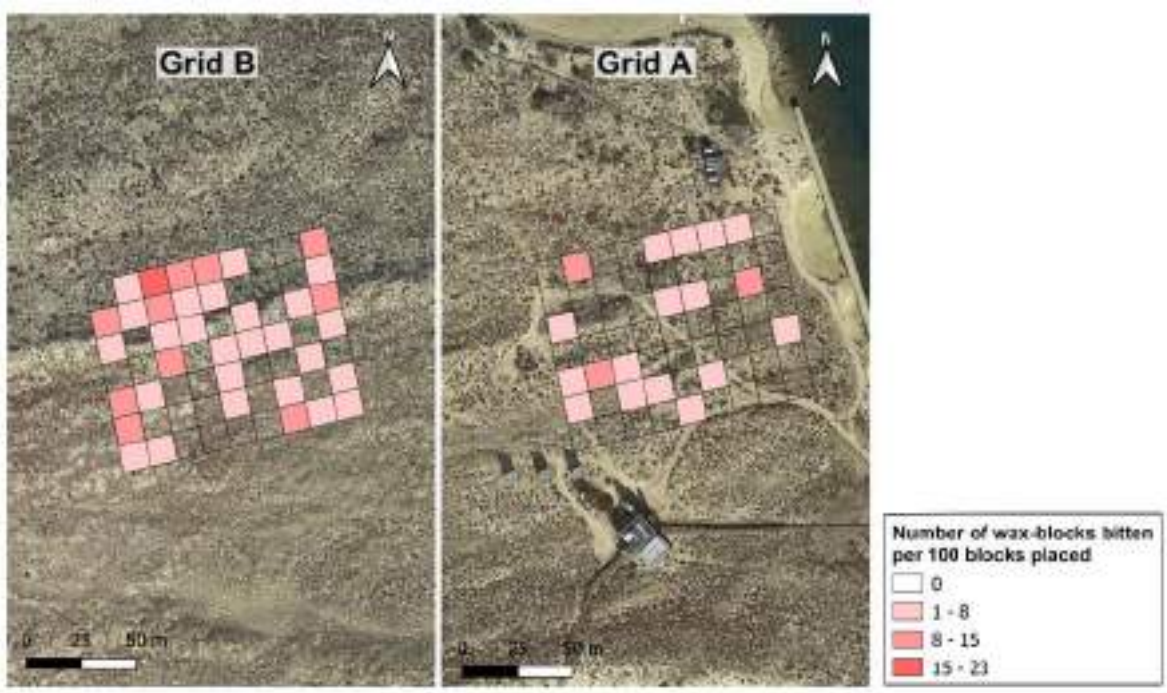

Figure 16 | Distribution maps of bait blocks bitten by the Algerian mouse *Mus spretus* placed in grid A (near the restaurant) and grid B (in Yellow-legged and Audouinii's gull colonies) on Barreta Island over the study period.

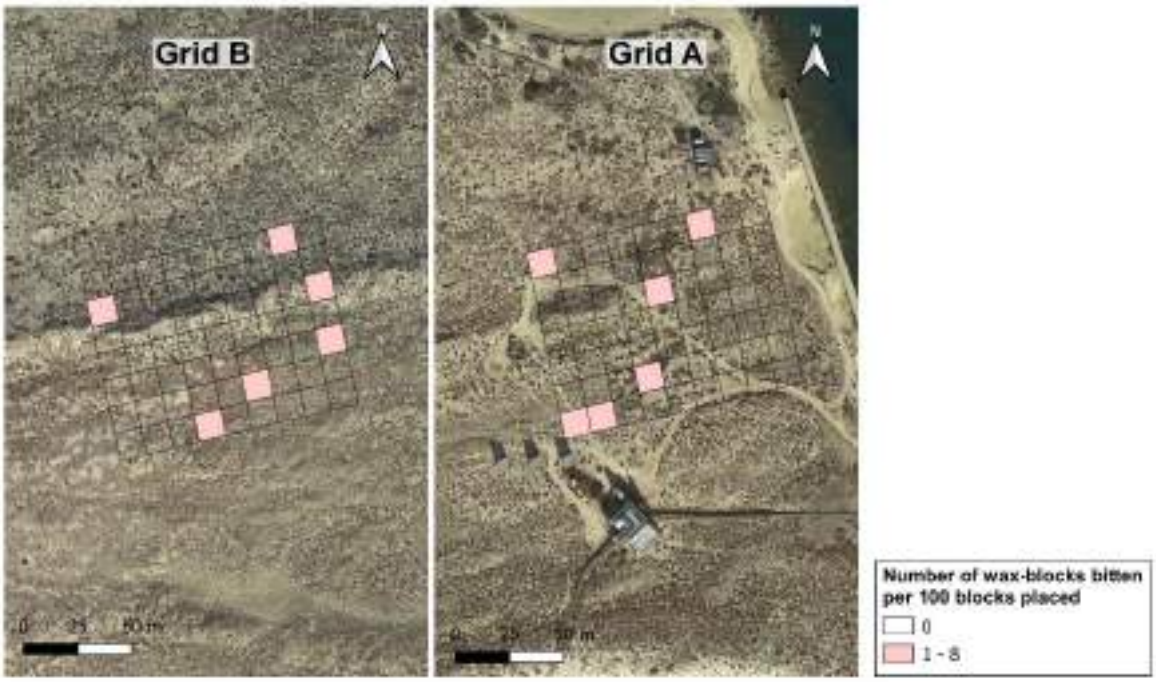

Figure 17 | Distribution maps of bait blocks bitten by rats *Rattus* spp. placed in grid A (near the restaurant) and grid B (in Yellow-legged and Audouinii's gull colonies) on Barreta Island over the studyperiod.

The first wax-block bitten by rats on grid B was registered in February 2020, whilst on grid A only in November 2020. Monthly distribution of rats based on wax-blocks is represented on Fig. 18.

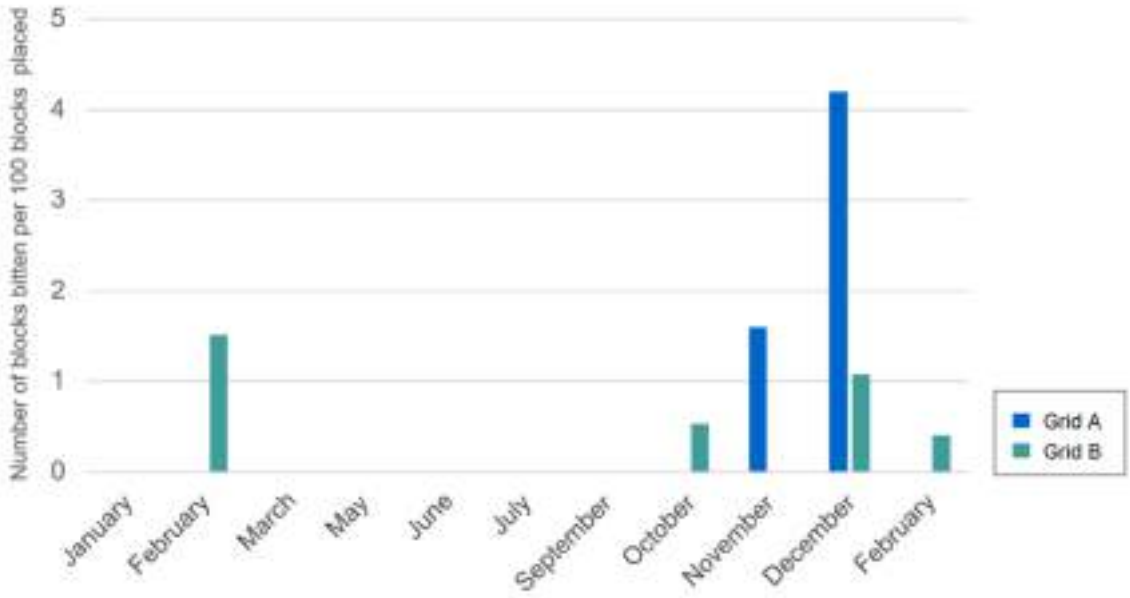

Figure 18 | Montlhy relative abundance of rats *Rattus* spp., in grid A (near the restaurant) and grid B (in Yellow-legged and Audouinii's gull colonies) on Barreta island over the study period, based on bitten wax-blocks.

While wax-blocks revealed to be useful for detecting the presence of rats on both grids, even though they weren't caught in the traps, the methodology should not be used for assessing rodent's abundance in grid B, as the majority of wax-blocks placed disappeared (Tab. VI). Lost wax-blocks are likely caused by gulls that steal the blocks to feed. Most blocks disappeared

during the period of higher density of gulls present on the island related with gull breeding season (March to July). Bird beak marks were also found during wax-blocks monitoring. The consumption of wax-blocks by gulls compromised the ability to detect the presence of rodents during those months in areas around gull colonies.

Table VI | Percentage of missing wax-blocks registered in grid A and grid B from January 2020 to February 2021.

	% of missing wax-blocks	
	Grid A	**Grid B**
January	0	-
February	0	67
March	3	99
May	1	56
June	4	22
July	0	12
September	0	>1
October	0	0
November	0	0
December	0	2
February	0	6

3.1.3 Extra sampling

Rodents captured on the extra sampling carried out on Barreta and Culatra islands and Ancão peninsula are presented on Table VII. The presence of rats *Rattus* spp. was registered in the lagoon of Barreta Island and on Culatra Island. No rats were captured on Ancão peninsula. The Algerian mouse, which was present on Barreta Island, was also registered on Ancão peninsula, and possibly on Culatra island.
Maps on the locations of rodent captures are represented on Figures 19, 20 and 21.

Table VII | Species and number of rodents captured on the extra sampling carried out in Deserta, Culatra and Ancão peninsula.

Island	Month	Species captured	Nº of captured rodents	Nº of traps
Ancão peninsula	April	*Mus* sp.	4	35
	November	*Mus spretus*	1	35
Culatra	May	*Mus* sp. *Rattus* sp.	1 1	18
	October	-	0	18
Barreta - Harbour	June	*Mus spretus*	1	15
	July	*Mus spretus*	3	15
Barreta - Lagoon	September	*Mus spretus* *Rattus norvegicus*	6 11	20

Figure 19 | Distribution maps of rats *Rattus* sp.(right) and mouse *Mus* sp. (left), captured on Culatra Island. Location of traps is represented by black dots. Yellow dots represent the locations were rodents were captured

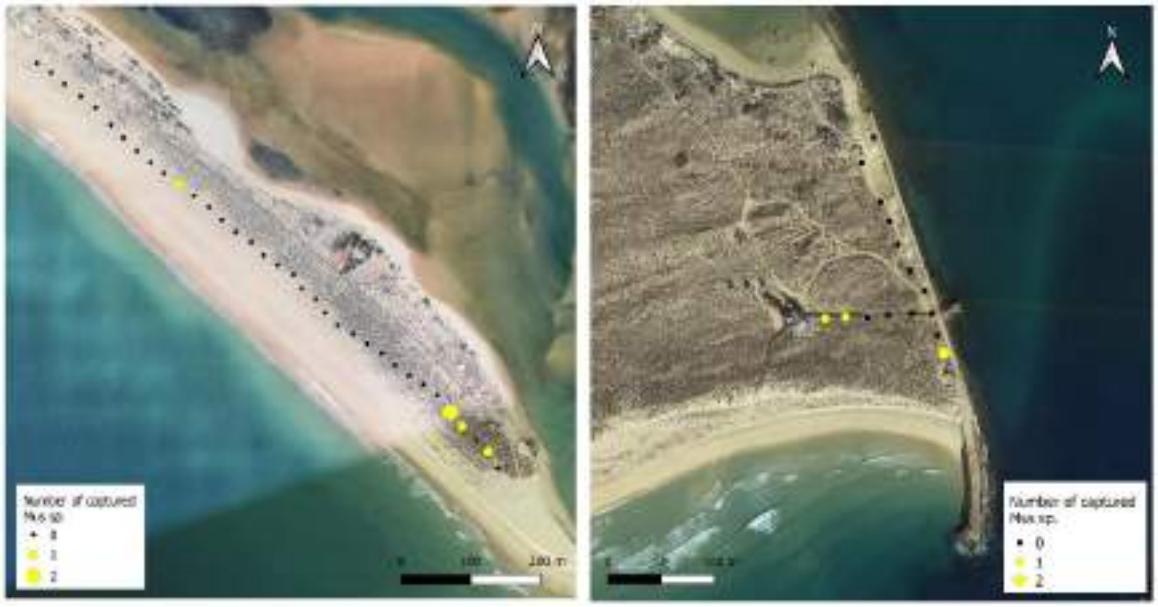

Figure 20 | Distribution maps of Algerian mouse *Mus spretus*, captured on Ancão peninsula (left) and in the harbour on Barreta Island (right). Location of traps is represented by black dots. Yellow dots represent the locations were rodents were captured

Figure 21 | Distribution maps of Algerian mouse *Mus spretus* (right), and brown rats *Rattus norvegicus* (left) captured around the lagoon on Barreta Island. Location of traps is represented by black dots. Yellow dots represent the locations were rodents were captured

3.1.4 Occasional sightings of rats

To complement the information about the presence of rats on Barreta island, locations of occasional sighthinhg, signs of presence (scats and footprints), as well as corpses were used to map the distribution of rats on the island (Fig. 22). Both the brown and the black rats were seen on Barreta Island. Rats seem to have a wide distribution across the island, but appear to

be more frequent in the north side, close to inshore waters, around the harbour and in the lagoon on the west end of the island.

Figure 22 | Distribution maps of occasional sightings of brown rats *Rattus norvegicus* and black rats *Rattus rattus* on Barreta Island from February 2020 to August 2021.

3.2 Cats

3.2.1 Camera-traps

The 22 camera-traps placed on Barreta Island produced 1,262,338 photos from January 2020 to February 2021. From those, 94% where “empty” images (Fig. 23), caused mainly by the wind moving the vegetation and triggering the sensor. Images of cats represented <1% of the total images. Besides cats, cameras also registered the presence of rodents (both the Algerian mouse and rats), domestic dogs, foxes, and humans crossing the restricted area of the island.

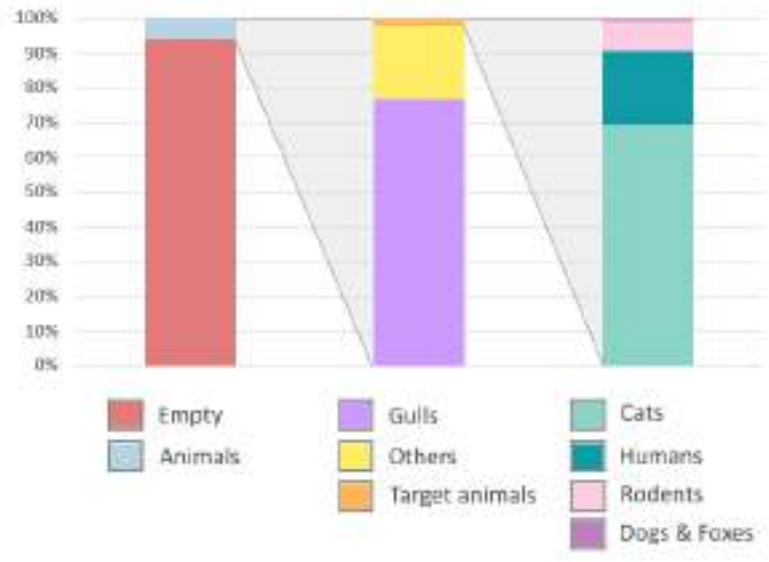

Figure 23 | Percentage of photos of each category registered by the 22 camera-traps installed on Barreta Island from January 2020 to February 2021.

From the cat images recorded by cameras, 7 cats were individually identified. Distribution map of cats, created from the frequency of photos taken by the cameras, is represented on figure 24. Cats seem to wander on the eastern part of the island, around the restaurant and the boardwalk, although their presence was also registered near the lagoon on the west end. Distribution maps of each cat can be found on Annex B.

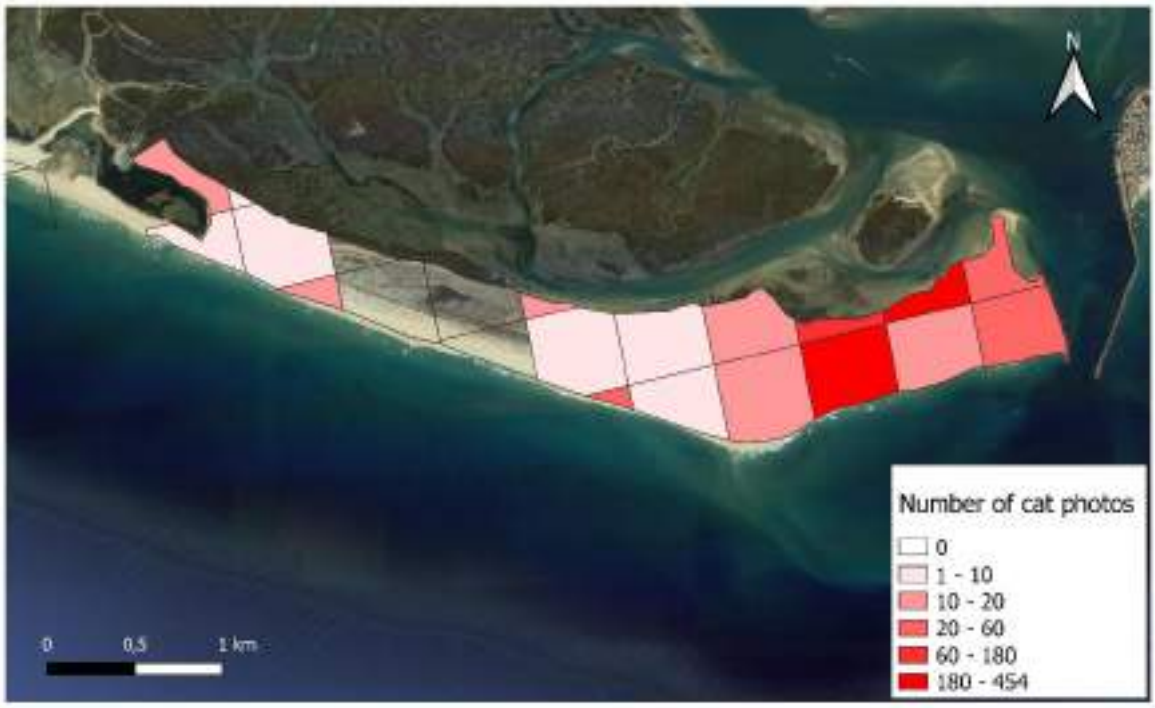

Figure 24 | Distribution map of feral cats photographed by 22 camera-traps installed inside each cell on Barreta Island, in a grid spaced 500*500 m apart, from January 2020 to February 2021.

3.2.2 Spotlighting

From January to December 2020,23 spothlighting surveys were performed to detect cats on Barreta Island. Rhere were recorded sixteen sightings of cats. Map of the location of sightings is in figure 25. Most of cat sightings occurred on segment 3 (with 9 sightings), followed by segment 4 (5 sightings), and segment 2 (2 sightings). On the segment 1, on the west part of the island, no sightings were registered during this period. Number of sightings varied by month, with the highest number of occurring in February (Table VIII). Mean abundance index was calculated at 0.13 cats/km (monthly abundances are present at Table VIII).

Figure 25 | Distribution map of feral cats spotted during spotlighting surveys on a transet (divided in 4 segments), covering Barreta Island.

Table VIII | Monthly variation of the number of cat sightings, and average cat abundance per km, registered during spotlighting survey in Barreta island.

Month	Nº of spotlighting days	Nº of cat sightings	Nº cats per km	Overall nº cats per km
January	2	1	0.09	
February	3	5	0.30	
May	2	1	0.09	
June	3	2	0.12	
July	3	1	0.06	0.13
September	3	1	0.06	
October	3	3	0.18	
November	1	1	0.18	
December	3	1	0.06	

3.2.3 Track counts

From February 2020 to February 2021, footprint stations were monitored for 26 days on Barreta Island. September and October were the months with the highest number of stations with cat tracks, while January and February 2021 the ones with the lowest number of footprints (Table IX). Footprints were registered all over the island, particularly in the lagoon on the west end of the island (Figure 26).

To compare cat abundance, the Catling and Allen index values were calculated for each month (Table X).

Table IX | Monthly variation of the number of cat footprints, registered during track surveys on Barreta Island. The number of operable stations represents the total number of stations after removing the stations with imprintability score of 1 (see the methods for more details).

Month	Nº of operable stations	Nº of nights	Nº of stations with imprintability score of 1	Nº of nights with cat tracks	Nº of stations with cat tracks
February	133	3	0	3	9
May	108	2	2	1	3
June	168	3	0	3	7
July	165	3	1	3	5
September	144	3	5	3	12
October	167	3	0	2	12
December	150	3	6	2	4
January	159	3	2	0	0
February	162	3	2	1	1

Figure 26 | Distribution map of feral cat footprints monitored in 56 track stations placed 100 m apart, on Barreta Island, from February 2020 to February 2021.

Table X | Monthly variation of cat footprints abundance, registered during track surveys on Barreta Island.

Month	Catling index value	Overall Catling index value	Allen index value	Overall Allen index value
February	100		0.08	
May	50		0.03	
June	100		0.04	
July	100		0.03	
September	100	69.23	0.08	0.04
October	66.67		0.07	
December	66.67		0.03	
January	0		0	
February	33.33		0.01	

Track countings on the other Barrier Islands (Culatra, Armona, Tavira and Cabanas islands and Ancão peninsula) were carried out twice during 2021, and the results are present on Table XI. Cabanas Island was the only one where no footprints were recorded. Maps of cat footprint distribution are represented through figures 27 to 31.
The Ancão peninsula registered the highest cat abundance followed by Tavira and Barreta islands (Table XII).

Table XI | Monthly variation of the number of cat footprints, registered during track surveys on Culatra, Armona, Tavira and Cabanas islands and Ancão peninsula during 2021.

Island	Month	Nº of operable stations	Nº of nights	Nº of stations with imprinta-bility score of 1	Nº of nights with cat tracks	Nº of stations with cat tracks
Culatra	February	183	3	0	0	0
	October	186	3	2	3	4
Armona	February	195	3	6	0	0
	October	201	3	0	2	7
Tavira	March	319	3	23	3	32
	November	318	3	9	3	12
Cabanas	April	231	3	0	0	0
	September	220	3	14	0	0
Ancão Peninsula	March	48	3	0	3	5
	November	48	3	0	3	4

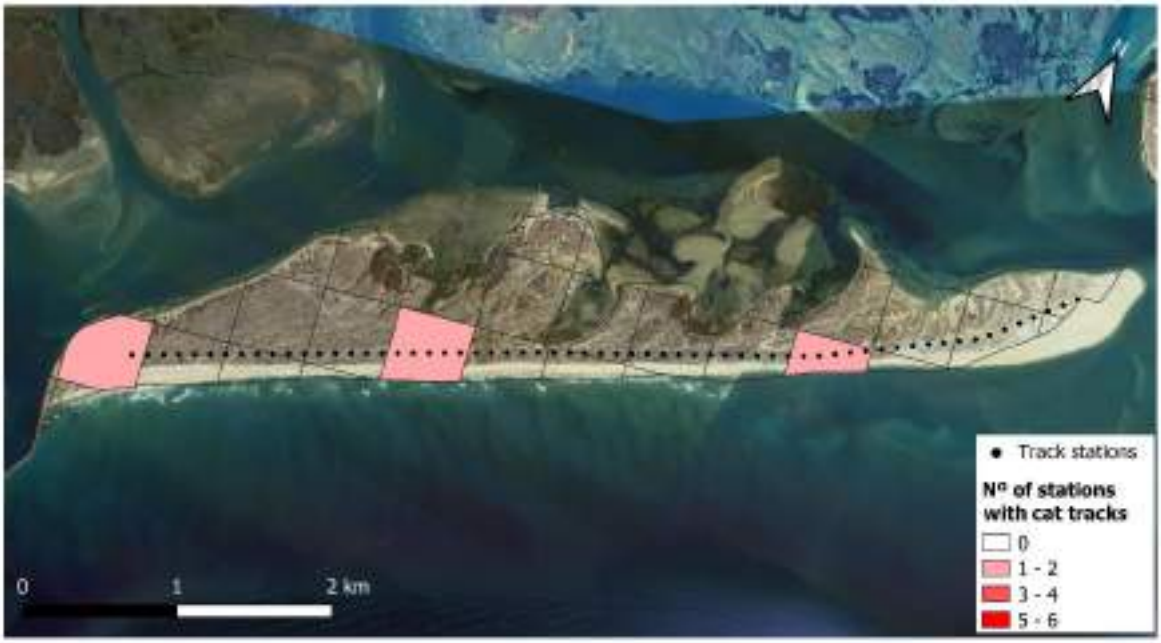

Figure 27 | Distribution map of feral cat footprints monitored in 62 track stations placed 100 m apart, on Culatra island, during three consecutive days in February and October 2021.

Figure 28 | Distribution map of feral cat footprints monitored in 67 track stations placed 100 m apart, on Armona island, during three consecutive days in February and October 2021.

Figure 29 | Distribution map of feral cat footprints monitored in 114 track stations placed 100 m apart, on Tavira island, during three consecutive days in March and November 2021.

Figure 30 | Distribution map of feral cat footprints monitored in 78 track stations placed 100 m apart, on Cabanas Island, during three consecutive days in April and September 2021.

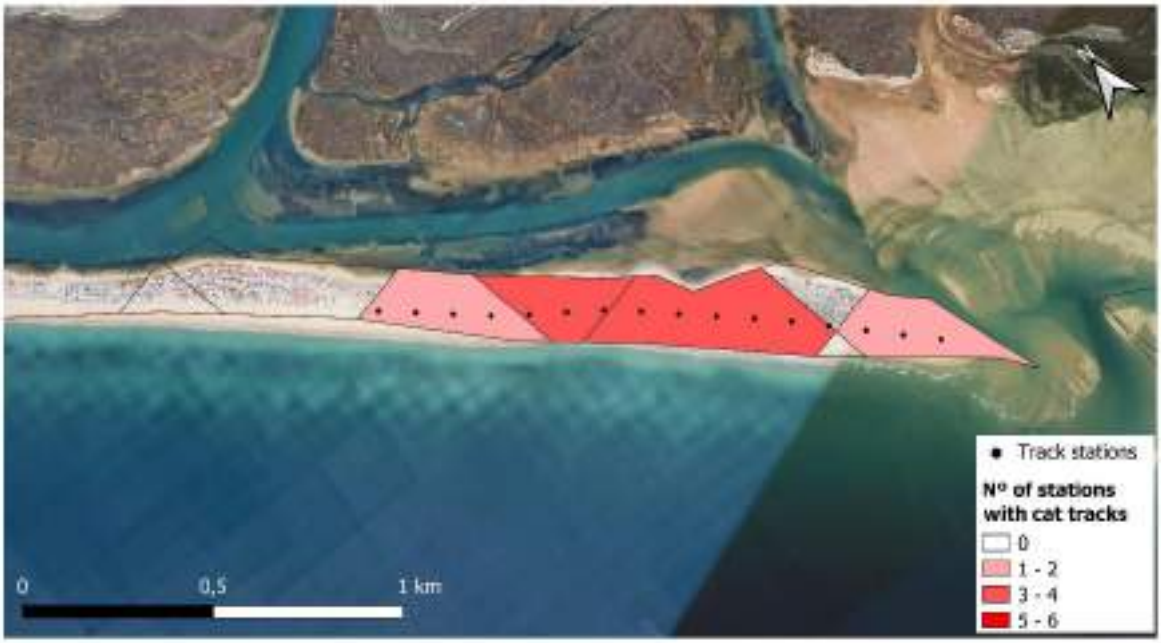

Figure 31 | Distribution map of feral cat footprints monitored in 16 track stations placed 100 m apart, on Ancão peninsula, during three consecutive days in March and November 2021.

Table XII | Monthly variation of the number of cat footprints, registered during track surveys in Culatra, Armona, Tavira, Cabanas, and Ancão peninsula during 2021.

Island	Overall Catling index value	Overall Allen index value
Barreta	69.23	0.04
Culatra	50	0.01
Armona	33	0.02
Tavira	100	0.08
Cabanas	0	0
Ancão Peninsula	100	0.09

3.4 Monitoring effort

Capture-recapture rodents on Barreta Island, from January 2020 to February 2021, was performed for 33 days, and lasted for a minimum of 99 hours and 47 minutes, with a two-person team. Extra samplings during 2021, were performed for 28 days, and lasted for a minimum of 26 hours and 18 minutes. These sums do not include the time needed to install the traps, nor the collection and cleaning.

Monitoring of camera traps on Barreta island, including replacing batteries and collecting SD cards, was done once a month, with a total of 11 days from January 2020 to February 2021, in 66 hours with a two-person team. Spotlighting transects for cat sighting were done for 23 days, with a total of 46 hours. Cat track counts on Barreta Island were done during 35 days, with a total of 123 hours; during 8 days with a total of 8 hours and 43 minutes in Ancão peninsula; during 8 days with a total of 29 hours and 16 minutes on Culatra Island; during 8 days with a total of 33 hours and 10 minutes on Armona Island; during 8 days with a total of 43 hours on Tavira Island; and during 8 days with a total of 26 hours and 40 minutes on Cabanas Island.

Mean monitoring time of mammals on Barreta Island is present on Table XIII, as well as the ranking of expertise, equipment, and start-cost nedded for each methodology used.

Table XIII | Ranking of monitoring techniques used to gather information of cats and rodents on Barreta Island.

	Mean duration	Labour	Expertise and training	Specialized equipment	Start-Cost
Cats					
Camara-traps	6h	High	Medium	High	High (need to purchase camera-traps)
Track counts	3.5h	Medium	Medium	Low	Low
Spotlighting	2h	Medium	Medium	Low	Medium (need to purchase gps, headlamps, and rangefinder)
Occasional sightings	-	Low	Low	Low	Low
Rodents					
Capture-Re-capture	20 min (per trap, if rodent trapped)	High	High (need to know how to set traps properly, identify and handle rodents)	High	High (need to purchase traps, measuring and tagging equipment)
Wax-blocks	1 min (per block)	Low	Medium (need to know how to identify rodents' teeth marks)	Low	Low
Occasional sightings	-	Low	Medium (need to know how to identify rodents' scats & footprints)	Low	Low

4 | Discussion

The present study allowed us to collect splicit information on distribution and abundance of the introduced mammals (rodents and cats) of Barrier Islands. In addition, it contributed to improve the knowledge on the native Algerian mouse local status. Using different monitoring techniques, a complete and comprehensive picture of those populations was obtain, both in terms of spatial and seasonal variations.

On Barreta Island three rodent species were identified in the course of the monitoring actions, the Algerian mouse, the brown rat, and the black rat. Recent sightings have also verified the presence of shrews (unidentified species) in a localized area of the island in the center west, called Lagoa Seca (36°58'03.6"N 7°54'39.4"W).

The Algerian mouse is a native rodent species distributed throughout Portugal, centre and south of Spain, the southern region of France, and in the African continent from Morocco to Algeria and Tunisia (Palomo *et al.*, 2009). With an average life expectancy of < 4 months and a longevity up to 15 months, the Algerian mouse is sexual inactive in winter (between November to January), with two peaks of maximum activity in April-May and August-September (Palomo *et al.*, 2009). The highest abundance of Algerian mice on Barreta Island was registered exactly in September, probably reflecting the emergence of new individuals after reproduction. The intensity and the long periods of reproduction explain the high abundance fluctuations recorded. Although our results point to a high density of mice on Barreta Island (fluctuating between 800 and 5600 from our density estimation data), the diet of this species is essentially made up of seeds, fruits, and insects (Palomo *et al.*, 2009), not likely posing a threat to the breeding birds present. The Algerian mouse is closely related to the house mouse (*Mus musculus*), a common predator of seabirds on islands (Croxall *et al.*, 2012). Although sympatric, the two species tend not to share habitat, with hybridization occurring only in laboratory conditions (Palomo *et al.*, 2009). With the DNA testing of rodents confirming the Algerian mouse identificarion (Annex A) is very unlikely that the house mouse is also present on Barreta Island.

Rats have contributed to the extinction of many species, including small mammals, birds, reptiles, invertebrates, and plants (Global Invasive Species Database, 2022). The black and the brown rat are among the species that cause more impact on seabirds (Jones *et al.*, 2008), through intense predation of eggs and chicks. The proximity of urban and rural areas favors the presence of both brown and black rats on the Barrier Islands, being seen occasionally swimming between islands and islets of Ria Formosa. The low number of rats captured on Barreta Island, together with the low number of blocks presenting rat bites, suggest a reduced abundance but high dispersion throughout the island. Also, the temporal patern shown by the wax-block monitoring, with a peack of abundance during the last months of the year in the area near the pier and closer to the inshore coast, might indicate rats are regularly entering on Barreta Island, coming from the surrounding grounds. In fact, local fishermen stated they used to spread rodenticide along the pier when abundance of rats was suspected to increase. Overall, Deserta Island is likely to have low suitable habitat to hold a high density of rats, even brown rats, which are less habitat quality demanding. This may justify the low densities recorded along the entire study area.

In order to minimize the threat caused by rats, auto-reset traps are foreseen to be placed in areas where their presence has been confirmed, in potential entry zones and near Audouin's gull colonies. Although a full eradication is always desirable, to keep Barreta Island free of rats would be extremely difficult given the high potential for recolonization, and without compromising the survival of the native Algerian mouse.

Evidence of the presence of cats was found in all islands except on Cabanas. Highest abundance of cat footprints was registered in Ancão peninsula, followed by Tavira and Barreta islands. Besides the presence of pet cats, the Ancão peninsula also holds a large population of stray cats that in recent

years was subject of a Trap-neuter-return program by Animais de Rua. A high density of cats in the eastern part of the peninsula is likely to pose a serious threat to the breeding colony of Little terns present.

Tavira Island is an uninhabited island with the highest density of cat footprints of all Barrier Islands. The presence of cats on the island can be facilitated by the restaurant and the camping area, and by the close proximity to the coast, with an easy acess by foot (through a bridge located in Barril beach). Little terns and Kentish plovers *Charadrius alexandrinus* nesting on the island might be at risk of predation.

Culatra and Armona islands have permanent resident population, with many domestic cats. The low abundance of cat footprints registered on these islands can be explained by the small home ranges of domestic cats, centered around their homes (Kays *et al.*, 2020). Footprint records far from urbanized areas may also point to the presence of feral cats.

Barreta Island use to hold a small resident populaton of fishermen and their families, in two residential areas, one near the harbour and the other in the center of the island. On the last decades people were realocated and houses demolished, leaving only one fisherman on the island to date. The presence of cats on the island probably started during human ocupancy, and continued with the offsprings of cats left behind. Fishermen also reported cases of people purposely abandoning cats on the island on the last years.

Based on images of camera-traps installed on the island, 7 adult cats were individually identified. Their distribution was mainly centered between the restaurant and the boardwalk, with one cat centering its activity in the lagoon on the west end of the island. This distribution was probably related to an easy access to food near the restaurant and the fisherman house near the harbour.

From the spotlighting and track surveys, cat sightings were more frequent in February, with the highest number of footprints recorded during September and October. A greater movement of cats during this period can reveal a difficulty in finding food, or be the period where female cats go into heat. The information gathered will facilitate the implementation of control measures, such as choosing the best places and periods for captures.

The control of rodents and cats on Barreta Island will improve the nesting conditions of seabirds and land birds that breed on the island, as well as the improvement of native vegetation, other native species, and a sensitive habitat.

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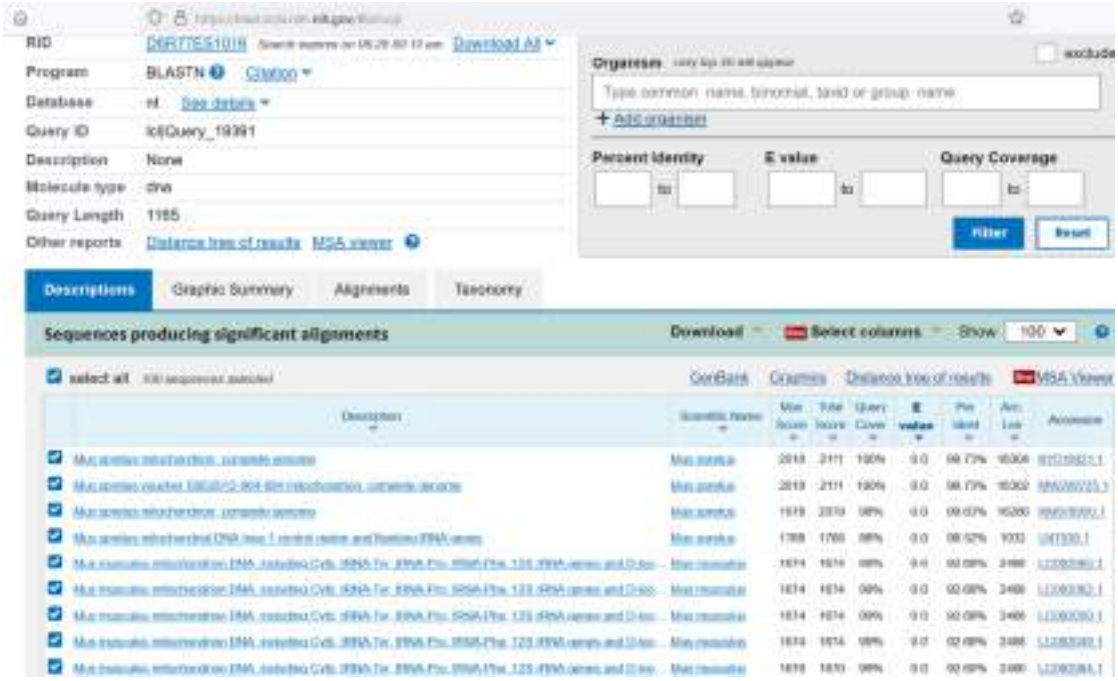
APPENDIX

A – Results of the genetic analysis of 89 tissue samples of Algerian mouse captured in Barreta island,

ID	Date	Site	DNA extraction date	Species
B36	06/02/2020	Barreta	13/04/2021	*Mus spretus*
B4	06/02/2020	Barreta	13/04/2021	*Mus spretus*
B58	06/02/2020	Barreta	13/04/2021	*Mus spretus*
B6	06/02/2020	Barreta	13/04/2021	*Mus spretus*
227	07/02/2020	Barreta	13/04/2021	*Mus spretus*
228	07/02/2020	Barreta	13/04/2021	*Mus spretus*
229	07/02/2020	Barreta	13/04/2021	*Mus spretus*
230	07/02/2020	Barreta	13/04/2021	*Mus spretus*
231	08/02/2020	Barreta	13/04/2021	*Mus spretus*
233	09/02/2020	Barreta	13/04/2021	*Mus spretus*
234	17/03/2020	Barreta	30/05/2021	*Mus spretus*
235	09/05/2020	Barreta	30/05/2021	*Mus spretus*
300	11/05/2020	Barreta	07/06/2021	*Mus spretus*
236	16/06/2020	Barreta	30/05/2021	*Mus spretus*
237	16/06/2020	Barreta	30/05/2021	*Mus spretus*
238	16/06/2020	Barreta	30/05/2021	*Mus spretus*
239	16/06/2020	Barreta	30/05/2021	*Mus spretus*
242	10/07/2020	Barreta	30/05/2021	*Mus spretus*
243	10/07/2020	Barreta	30/05/2021	*Mus spretus*
244	10/07/2020	Barreta	30/05/2021	*Mus spretus*
245	10/07/2020	Barreta	30/05/2021	*Mus spretus*
246	11/07/2020	Barreta	30/05/2021	*Mus spretus*
247	12/07/2020	Barreta	30/05/2021	*Mus spretus*
249	13/07/2020	Barreta	30/05/2021	*Mus spretus*
250	12/09/2020	Barreta	30/05/2021	*Mus spretus*
280	12/09/2020	Barreta	04/06/2021	*Mus spretus*
281	12/09/2020	Barreta	04/06/2021	*Mus spretus*
282	12/09/2020	Barreta	04/06/2021	*Mus spretus*
283	12/09/2020	Barreta	04/06/2021	*Mus spretus*
284	12/09/2020	Barreta	04/06/2021	*Mus spretus*
285	12/09/2020	Barreta	04/06/2021	*Mus spretus*
286	12/09/2020	Barreta	04/06/2021	*Mus spretus*
287	12/09/2020	Barreta	04/06/2021	*Mus spretus*
288	13/09/2020	Barreta	04/06/2021	*Mus spretus*
289	13/09/2020	Barreta	04/06/2021	*Mus spretus*
290	13/09/2020	Barreta	07/06/2021	*Mus spretus*
293	13/09/2020	Barreta	07/06/2021	*Mus spretus*
294	13/09/2020	Barreta	07/06/2021	*Mus spretus*
295	13/09/2020	Barreta	07/06/2021	*Mus spretus*
296	13/09/2020	Barreta	07/06/2021	*Mus spretus*
297	13/09/2020	Barreta	07/06/2021	*Mus spretus*

298	13/09/2020	Barreta	07/06/2021	*Mus spretus*
299	13/09/2020	Barreta	07/06/2021	*Mus spretus*
251	14/09/2020	Barreta	30/05/2021	*Mus spretus*
252	14/09/2020	Barreta	30/05/2021	*Mus spretus*
253	14/09/2020	Barreta	30/05/2021	*Mus spretus*
255	14/09/2020	Barreta	30/05/2021	*Mus spretus*
256	15/09/2020	Barreta	30/05/2021	*Mus spretus*
257	15/09/2020	Barreta	30/05/2021	*Mus spretus*
258	15/09/2020	Barreta	30/05/2021	*Mus spretus*
259	15/09/2020	Barreta	30/05/2021	*Mus spretus*
279	20/09/2020	Barreta	04/06/2021	*Mus spretus*
260	09/10/2020	Barreta	30/05/2021	*Mus spretus*
261	09/10/2020	Barreta	30/05/2021	*Mus spretus*
262	09/10/2020	Barreta	02/06/2021	*Mus spretus*
263	09/10/2020	Barreta	02/06/2021	*Mus spretus*
264	09/10/2020	Barreta	02/06/2021	*Mus spretus*
265	09/10/2020	Barreta	02/06/2021	*Mus spretus*
266	10/10/2020	Barreta	02/06/2021	did not work
267	10/10/2020	Barreta	02/06/2021	*Mus spretus*
268	10/10/2020	Barreta	02/06/2021	*Mus spretus*
269	10/10/2020	Barreta	02/06/2021	*Mus spretus*
270	10/10/2020	Barreta	02/06/2021	*Mus spretus*
272	11/10/2020	Barreta	02/06/2021	*Mus spretus*
274	11/10/2020	Barreta	02/06/2021	*Mus spretus*
275	11/10/2020	Barreta	02/06/2021	*Mus spretus*
278	12/10/2020	Barreta	04/06/2021	*Mus spretus*
301	12/10/2020	Barreta	07/06/2021	*Mus spretus*
302	12/10/2020	Barreta	07/06/2021	*Mus spretus*
303	27/11/2020	Barreta	12/04/2021	*Mus spretus*
304	27/11/2020	Barreta	12/04/2021	*Mus spretus*
305	27/11/2020	Barreta	12/04/2021	*Mus spretus*
306	27/11/2020	Barreta	12/04/2021	*Mus spretus*
307	27/11/2020	Barreta	12/04/2021	*Mus spretus*
308	28/11/2020	Barreta	12/04/2021	*Mus spretus*
309	28/11/2020	Barreta	12/04/2021	*Mus spretus*
310	15/12/2020	Barreta	12/04/2021	*Mus spretus*
325	18/12/2020	Barreta	12/04/2021	*Mus spretus*
327	18/12/2020	Barreta	12/04/2021	*Mus spretus*
384	18/12/2020	Barreta	12/04/2021	*Mus spretus*
385	18/12/2020	Barreta	12/04/2021	did not work
314	19/02/2021	Barreta	12/04/2021	*Mus spretus*
378	19/02/2021	Barreta	12/04/2021	*Mus spretus*
311	20/02/2021	Barreta	12/04/2021	did not work
386	20/02/2021	Barreta	12/04/2021	*Mus spretus*
312	22/02/2021	Barreta	12/04/2021	*Mus spretus*

316	22/02/2021	Barreta	12/04/2021	*Mus spretus*
331	22/02/2021	Barreta	12/04/2021	*Mus spretus*
332	22/02/2021	Barreta	12/04/2021	*Mus spretus*



B – Distribution maps of feral cats on Barreta Island, photographed by some of the 22 camera traps installed in a grid spaced 500*500 m apart, from January 2020 to February 2021.

- CAT1

- CAT2


N
Number photos CAT2
0
1 - 4
4 - 10
>10
0
0,5
1 km

CAT3


N
Number photos CAT3
0
1 - 4
4 - 10
>10
0
0,5
1 km

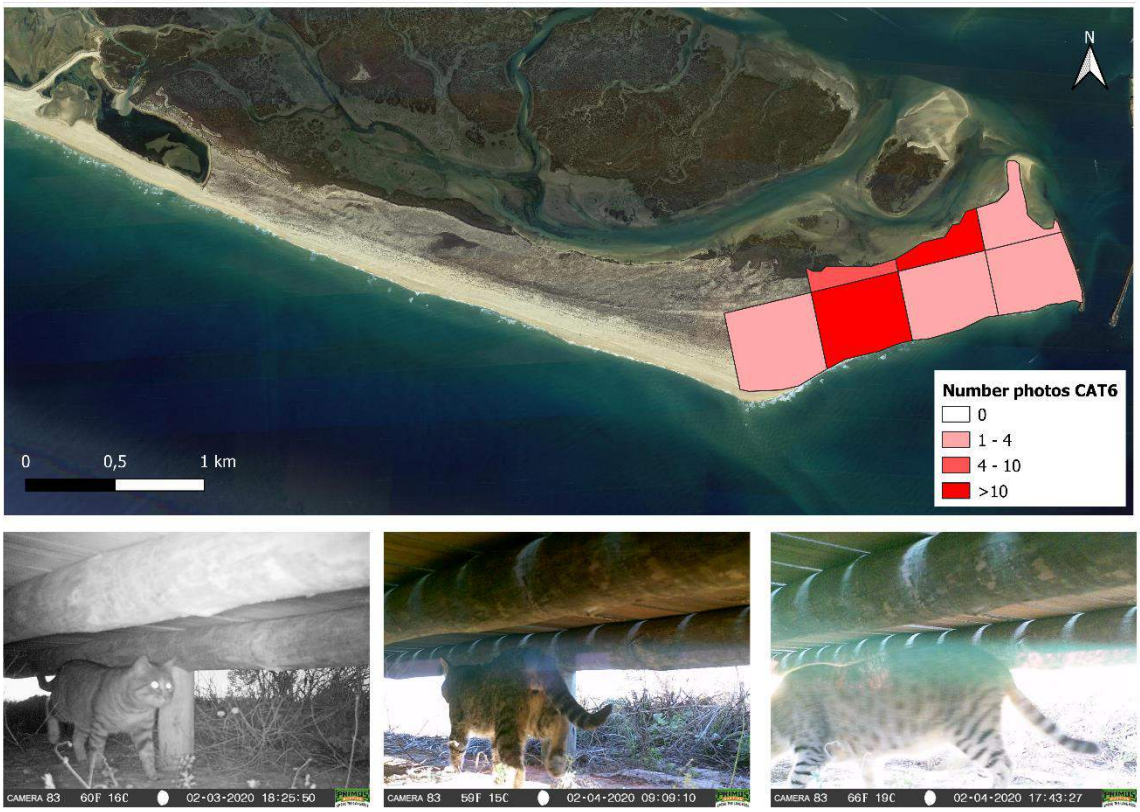
- CAT4


N
Number photos CAT4
0
1 - 4
4 - 10
>10
0
0,5
1 km

- CAT5


N
Number photos CAT5
0
1 - 4
4 - 10
>10
0
0,5
1 km

- CAT6


N
0
0,5
1 km
Number photos CAT6
0
1 - 4
4 - 10
>10

CAMERA 83 60F 16C 02-03-2020 18:25:50

CAMERA 83 59F 15C 02-04-2020 09:09:10

CAMERA 83 66F 19C 02-04-2020 17:43:27

- CAT7


N
0
0,5
1 km
Number photos CAT7
0
1 - 4
4 - 10
>10