CONSERVATION OF MEDITERRANEAN TORTOISES UNDER GLOBAL CHANGE

CONSERVACION DE LAS TORTUGAS MEDITERRANEAS ANTE EL CAMBIO GLOBAL

Eva Graciá, Roberto C. Rodríguez-Caro, Andrés Giménez

Asociación Herpetológica Española

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Eva Graciá, Roberto C. Rodríguez-Caro y Andrés Giménez llevan más de 20 años investigando distintos aspectos de las tortugas de tierra, en particular de la tortuga mora. Roberto y Eva realizaron sus tesis doctorales dirigidos por Andrés. Eva se ha especializado en genética y filogeografía, y Roberto en modelado y ecología de poblaciones. Los tres continúan trabajando en equipo.

Eva Graciá, Roberto C. Rodríguez-Caro, and Andrés Giménez have been studying various aspects of terrestrial tortoises for over 20 years, with a particular focus on the spur-thighed tortoise. Roberto and Eva completed their doctoral theses under the supervision of Andrés. Eva has specialized in genetics and phylogeography, while Roberto focuses on modeling and population ecology. The three continue to work as a team.

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A la memoria de Ramón Mascort
To the memory of Ramón Mascort

Bulgaria, 2017, cogiendo una *Emys*
Bulgaria, 2017, catching an *Emys*

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Retos de conservación para las tortugas de tierra mediterráneas

Los quelonios, que incluyen tortugas, galápagos y tortugas marinas, se encuentran entre los vertebrados más amenazados del planeta. Según el Turtle Taxonomy Working Group (2021), cerca del 50% de las 357 especies reconocidas en la actualidad están amenazadas, siendo clasificadas en la Lista Roja de la Unión Internacional para la Conservación de la Naturaleza (UICN) como En Peligro Crítico (CR), En Peligro (EN) o Vulnerable (VU). Las tortugas terrestres (familia Testudinidae), merecen especial atención ya que aproximadamente el 60% de sus 53 especies están amenazadas y 8 se han extinguido en tiempos recientes (Kehlmaier *et al*., 2019, 2023). Las principales amenazas para este grupo son la pérdida y degradación del hábitat, el expolio y tráfico de sus poblaciones, tanto para consumo como para el comercio de mascotas, el cambio climático o las enfermedades emergentes. Su limitada capacidad de dispersión y su madurez sexual tardía las hacen especialmente vulnerables a las presiones humanas (Stanford *et al*., 2020; Rodríguez-Caro *et al*., 2023).

Acompañando a tan alarmantes cifras, otros motivos hacen que el diagnóstico de la situación sea verdaderamente grave para las tortugas de tierra. Primero, que ya hemos sufrido una importante pérdida de diversidad por la extinción de las cinco especies de tortugas gigantes *Cylindraspis*, tras el descubrimiento de las Islas Mascareñas. Su extinción hace 150-250 años supuso un duro declive en términos de pérdida de biodiversidad para estas islas (Kehlmaier *et al*., 2019, 2023). Segundo, que el impacto humano incide especialmente sobre estrategias vitales únicas y la pérdida es mayor de lo que cabría esperar por azar, siendo la pérdida de hábitat el factor de amenaza más relevante. Esto hace que se encuentren particularmente afectadas tortugas con rasgos vitales extremos como los de la tortuga panqueque (*Malacochersus tornieri*) con gran longevidad, pero con un único huevo por puesta y una única puesta por año (Rodríguez-Caro *et al*., 2023).

El colapso de las poblaciones de tortugas puede tener un efecto importante en el funcionamiento de los ecosistemas por su papel como herbívoras, dispersoras de semillas, presas o como especies mineras de suelos (Falcón y Hansen, 2018). Este impacto negativo es global, pero especialmente acusado en islas, en donde adquieren protagonismo como especies clave. Sabemos de tortugas de tierra extintas en muchas islas oceánicas remotas y archipiélagos, incluidos las anteriormente mencionadas Mascareñas, las Bahamas, las Bermudas, las Islas Canarias, Cabo Verde, Fiyi, las Seychelles Graníticas y Vanuatu (Turtle Taxonomy Working Group, 2015). Esto ha propiciado proyectos de introducción de tortugas (*rewilding*) en algunas islas del oeste del océano Índico en donde las especies nativas se han extinguido recientemente (Griffiths *et al*., 2010; Falcón y Hansen, 2018). Además, la evaluación del estatus de conservación de la cuarta parte de las tortugas de tierra amenazadas se llevó a cabo por la UICN hace más de diez años, siendo su situación actual probablemente peor. Podríamos estar, por tanto, infravalorando sus necesidades de conservación.

Pese al estado de conservación tan precario de las tortugas de tierra, en el entorno Mediterráneo no existen estrategias comunes y consensuadas para la gestión y conservación de sus poblaciones. En 2019 el *Mediterranean workshop to develop tortoise conservation strategies* reunió en Alicante (España) a 61 participantes incluyendo científicos, miembros asociaciones conservacionistas, técnicos de centros de recuperación, miembros de UICN, autoridades CITES y gestores de medio ambiente en materia de conservación de Portugal, España, Marruecos, Italia, Serbia, Alemania y Turquía. A través de 31 ponencias se discutió sobre el estado de conservación de la tortuga mediterránea (*Testudo hermanni*) y de la tortuga mora (*T. graeca*) y se sugirieron estrategias para garantizar su conservación en la región Mediterránea. Las principales conclusiones del encuentro fueron publicadas en *Basic and Applied Herpetology* (Graciá *et al*., 2021), y se resumen en:

i) Las tortugas son especies silvestres y amenazadas, y debemos conservarlas "en" y "junto con" su hábitat natural. Sus principales amenazas son la pérdida de hábitat y el expolio de sus poblaciones, pero también debemos poner atención a los incendios, la transmisión de enfermedades, la introducción de especies exóticas y el cambio climático.

ii) Son necesarios mayores esfuerzos a largo plazo para diagnosticar y monitorear con precisión el estado de conservación de las poblaciones silvestres, y los flujos que existen entre animales cautivos y silvestres.

iii) El comercio de tortugas debería estar prohibido porque amenaza a la conservación de las poblaciones silvestres de tortugas. Las prácticas ilegales relacionadas con las tortugas deben ser perseguidas. Debemos considerar a las tortugas como animales silvestres, amenazadas y merecedores de protección. Podremos disfrutarlas mediante la observación responsable en su hábitat.

iv) Las tortugas pueden ser portadoras de patógenos y parásitos y su estado sanitario no puede ser evaluado por completo. Siempre debemos considerarlas como posibles vectores de enfermedades de tortugas y de zoonosis. Debemos acordar protocolos de bioseguridad e implementarlos cuando trabajemos con estas especies.

v) Los científicos participantes en este encuentro planeamos constituir una red científica para la conservación de las poblaciones de tortugas de tierra mediterráneas. Necesitaremos el apoyo de administraciones públicas.

Este libro publica algunas de las ponencias de aquella reunión. Los temas tratados versaron en torno a la conservación de poblaciones silvestres, el tráfico de especies, la gestión de las tortugas mantenidas en cautividad y la potencial emergencia de enfermedades y parásitos. Este libro también se estructura así.

Han transcurrido varios años desde aquella reunión, pero los temas siguen siendo de actualidad. Tenemos entonces la esperanza de que este libro sirva para mantener la esencia de aquella reunión y promover la creación de una red que pretenda la conservación de las tortugas de tierra del entorno Mediterráneo, incluyendo en el futuro muchas más poblaciones y países. También a las otras tres especies del género *Testudo*: *T. kleinmanni*, *T. marginata* y *T. horsfieldii*.

Conservation challenges for mediterranean tortoises

Chelonians, which include tortoises, freshwater turtles, and sea turtles, are among the most threatened vertebrates on the planet. According to the Turtle Taxonomy Working Group (2021), nearly 50% of the 357 currently recognized species are threatened, classified on the International Union for Conservation of Nature (IUCN) Red List as Critically Endangered (CR), Endangered (EN), or Vulnerable (VU). Tortoises (family Testudinidae) deserve particular attention, as approximately 60% of their 53 species are threatened and 8 have gone extinct in recent times (Kehlmaier *et al*., 2019, 2023). The main threats to this group include habitat loss and degradation, poaching and trafficking for consumption or the pet trade, climate change, and emerging diseases. Their limited dispersal ability and delayed sexual maturity make them especially vulnerable to human pressures (Stanford *et al*., 2020; Rodríguez-Caro *et al*., 2023).

Alongside such alarming figures, other factors make the conservation outlook for tortoises truly dire. First, a significant loss of diversity has already occurred due to the extinction of the five species of giant tortoises in the genus *Cylindraspis* following the discovery of the Mascarene Islands. Their extinction 150–250 years ago represented a severe decline in biodiversity for these islands (Kehlmaier *et al*., 2019, 2023). Second, human impacts disproportionately affect unique life-history strategies, and the resulting loss is greater than would be expected by chance, with habitat loss being the most critical threat. This means that tortoises with extreme traits, such as the pancake tortoise (*Malacochersus tornieri*), which is long-lived but lays only a single egg once a year, are particularly affected (Rodríguez-Caro *et al*., 2023).

The collapse of tortoise populations can significantly impact ecosystem functioning due to their roles as herbivores, seed dispersers, prey, or even soil engineers (Falcón & Hansen, 2018). This negative impact is global, but especially severe on islands, where tortoises often play key ecological roles. Numerous land tortoise species have gone extinct on remote oceanic islands and archipelagos, including the aforementioned Mascarene Islands, as well as the Bahamas, Bermuda, the Canary Islands, Cape Verde, Fiji, the granitic Seychelles, and Vanuatu (Turtle Taxonomy Working Group, 2015). These losses have led to rewilding projects involving tortoise introductions on several islands in the western Indian Ocean, where native species have recently gone extinct (Griffiths *et al*., 2010; Falcón & Hansen, 2018). Furthermore, the conservation status of a quarter of the threatened tortoises was last assessed by the IUCN over a decade ago, and their current situation is likely worse. As a result, we may be underestimating their conservation needs.

Despite the precarious conservation status of tortoises, there are no common or coordinated strategies for the management and conservation of their populations in the Mediterranean region. In 2019, the Mediterranean Workshop to Develop Tortoise Conservation Strategies brought together 61 participants in Alicante (Spain), including scientists, members of conservation organizations, wildlife rehabilitation technicians, IUCN members, CITES authorities, and environmental managers from Portugal, Spain, Morocco, Italy, Serbia, Germany, and Turkey. Through 31 presentations, the conservation status of the Mediterranean tortoise (*Testudo hermanni*) and the spur-thighed tortoise (*T. graeca*) was discussed, and strategies were proposed to ensure their conservation in the Mediterranean region. The main conclusions of the meeting were published in Basic and Applied Herpetology (Graciá *et al*., 2021), and can be summarized as follows:

i) Mediterranean tortoises are wild endangered species that must be protected "in", and "together with", their natural habitat. Their main threats are habitat loss and fragmentation and pet trade, but we should also pay attention to wildfires, spread of diseases, the introduction of exotic species or lineages and climate change.
ii) Long-term efforts and stablished protocols are needed to accurately diagnose and monitor the conservation status of wild populations, as well as flows among captive and wild animals.
iii) Tortoise trade should be banned because it threatens the conservation of wild populations of tortoises. Illegal practices related to tortoises should be decidedly persecuted. People should consider tortoises as wild animals that are endangered and deserving of protection. It is possible to enjoy them by responsibly observing them in their habitat.
iv) Tortoises may carry pathogens or parasites and their sanitary status cannot be fully assessed. They should always be considered potential vectors of tortoise pathogens and of zoonotic agents. Hygiene protocols should be implemented when managing them.

v) The researchers attending this workshop aimed to develop a scientific network for the longterm monitoring of *T. graeca* and *T. hermanni* populations in the Mediterranean Region. They will need the support from public administrations.

This book includes some of presentations from that meeting. The topics addressed focused on the conservation of wild populations, the illegal wildlife trade, the management of captive tortoises, and the potential emergence of diseases and parasites. The book is organized around these same themes.

Several years have passed since that meeting, but the issues remain just as relevant today. We therefore hope that this book helps preserve the spirit of that gathering and encourages the development of a collaborative network for the conservation of Mediterranean tortoises—one that will eventually include more populations and countries, as well as the other three species of the genus *Testudo*: *T. kleinmanni*, *T. marginata*, and *T. horsfieldii*.

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Conservación de poblaciones silvestres

La conservación de las poblaciones silvestres de tortugas de tierra enfrenta desafíos que requieren de enfoques integrales. Para evaluar el estado de conservación de sus poblaciones es necesario comprender tanto las distribuciones actuales como históricas, así como cambios en la abundancia poblacional, lo que permite identificar tendencias. Pero las tortugas terrestres son especies longevas, por lo que los cambios en la abundancia solo se pueden observar en periodos largos de tiempo. Los estudios científicos que soporten las evaluaciones de sus estados de conservación deben entonces considerar enfoques a largo plazo. El seguimiento de las poblaciones es imprescindible, tanto en el diseño de estrategias de conservación adecuadas, como para evaluar la efectividad de las acciones de conservación que se lleven a cabo. Mediante el seguimiento a largo plazo es posible detectar amenazas emergentes y adaptar las estrategias de conservación a las verdaderas necesidades de estas especies vulnerables.

El principal factor de amenaza de las tortugas es la pérdida y fragmentación del hábitat, principalmente por cambios de uso del suelo, como sucede por la expansión de áreas urbanas y agrícolas. También es clave considerar la dimensión social de la especie como mascota, lo que provoca el expolio de individuos en el medio silvestre. Esta consideración también deriva en la introducción o translocación accidental o intencionada de tortugas, un fenómeno cada vez más frecuente que puede implicar exogamia genética y la emergencia de enfermedades.

Otros factores de amenaza importantes son los incendios forestales, que impactan directamente sobre las poblaciones pero también modifican la calidad del hábitat.

A todo esto se suma la realidad del cambio climático, que afecta tanto a la disponibilidad de hábitats adecuados, como a la capacidad de las tortugas de desarrollarse en ellos. El diseño de redes de áreas protegidas debería tener en cuenta las proyecciones de la distribución de las especies en escenarios previstos de cambio climático, garantizando la conectividad de las poblaciones silvestres para asegurar su conservación y la de sus hábitats en las próximas décadas. Para esto es necesario caracterizar los hábitats de tortugas y entender los factores clave para la viabilidad de las poblaciones, incluyendo factores climáticos, pero también otros como la vegetación o la litología.

Conservation of wild populations

The conservation of wild tortoise populations faces challenges that require integrative approaches. To assess their conservation status, it is necessary to understand both current and historical distributions, as well as changes in population abundance, which help identify trends. However, tortoises are long-lived species, so changes in abundance can only be observed over extended periods. Therefore, scientific studies suporting the assessment of their conservation status must adopt long-term approaches. Monitoring populations is essential for designing appropriate conservation strategies and for evaluating the effectiveness of conservation actions. Long-term monitoring also enables the detection of emerging threats and the adaptation of conservation strategies to meet the actual needs of these vulnerable species.

The main threat to tortoises is habitat loss and fragmentation, mainly due to land use changes, such as the expansion of urban and agricultural areas. It is also crucial to consider the species' social dimension as a pet, which leads to the poaching of individuals from the wild. This aspect also results in the accidental or intentional introduction or translocation of tortoises, an increasingly common phenomenon that can lead to genetic outbreeding and the emergence of diseases. Other significant threats include wildfires, which directly impact populations but also alter habitat quality.

Adding to this is the reality of climate change, which affects both the availability of suitable habitats and the ability of tortoises to thrive in them. The design of protected area networks should consider species distribution projections under predicted climate change scenarios, ensuring the connectivity of wild populations to safeguard them and their habitats in the coming decades. This requires characterizing tortoise habitats and understanding the key factors for population viability, including climatic factors, but also others like vegetation and lithology.

Pujante-Expósito, N., Fahd, S., Felber, F.J., Rodríguez-Caro, R.C., Graciá, E., Semaha, M.J., & Giménez, A. 2025. Use of local ecological knowledge as a source of information on the distribution of *Testudo graeca* in North Africa. *In*: Graciá, E., Rodríguez-Caro, R.C., & Giménez, A. (eds.). *Conservation of Mediterranean tortoises under global change*. Madrid. Asociación Herpetológica Española.

Use of local ecological knowledge as a source of information on the distribution of *Testudo graeca* in North Africa

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Abstract

The spur-thighed tortoise (*Testudo graeca*) has three lineages in Morocco (*T. g. graeca*, *T. g. marokkensis*, *T. g. whitei*). Ecological modelling exercises and demographic reconstruction from genetic information suggest different spatial dynamics following the Last Glacial Maximum: *T. g. whitei* expanded from several refugia, *T. g. marokkensis* contracted and *T. g. graeca* shifted to the north. In this work, our aim is to use local ecological knowledge (LEK) as a source of information to enhance our understanding of the distribution of species in Morocco. We conducted several campaigns by interviewing local shepherds in the study area in 2016 and 2018. These campaigns allowed the number of occurrence records of the species to rise from 129 to 323. Thanks to interviews, we obtained 176 records of absence of the species. LEK emerges as a valuable source of high-quality information about the distribution of specific biological species thanks to the insights shared by local inhabitants.

Resumen

La tortuga mora (*Testudo graeca*) presenta tres linajes en Marruecos (*T. g. graeca*, *T. g. marokkensis* y *T. g. whitei*). Los ejercicios de modelado ecológico y de reconstrucción demográfica a partir de información genética sugieren dinámicas espaciales diferentes tras el Último Máximo Glacial: *T. g. whitei* se expandió desde varios refugios, *T. g. marokkensis* se contrajo y *T. g. graeca* se desplazó hacia el norte. En este trabajo, nuestro objetivo es utilizar el Conocimiento Ecológico Local como fuente de información para mejorar nuestra comprensión sobre la distribución de la especie en Marruecos. Realizamos varias campañas entrevistando a pastores locales en el área de estudio durante 2016 y 2018. Estas campañas han dado como resultado un aumento en el número de registros de presencia de la especie de 129 a 323. Además, gracias a las entrevistas hemos obtenido 176 registros de ausencia. El Conocimiento Ecológico Local emerge como una valiosa fuente de información de alta calidad relativa a la distribución de determinadas especies biológicas, gracias a las percepciones de los habitantes locales.

Keywords: spur-thighed tortoise, species distribution, Marocco.
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1. Introduction

The spur-thighed tortoise *Testudo graeca* (L., 1758), is a medium-sized terrestrial tortoise and one of the most charismatic species of circum-Mediterranean fauna (Anadón *et al*., 2012). In the western Mediterranean, the core *T. graeca* range lies in North Africa, with small isolated populations in the south-eastern part of the Iberian Peninsula and on some Mediterranean islands (Anadón *et al*., 2012). In Morocco, we found three *Testudo graeca* subspecies with different spatial dynamics following the Last Glacial Maximum: *T. g. whitei* expanded from several refugia, *T. g. marokkensis* contracted and *T. g. graeca* shifted to the north (Anadón *et al*., 2015).

The species is threatened throughout its range and is classified as Vulnerable according to the International Union for Conservation of Nature (IUCN) (IUCN Red List of Threatened Species, 2023). The main threats are habitat loss and fragmentation and poaching (Giménez *et al*., 2004; Pérez *et al*., 2004).

Sampling tortoise populations to obtain distribution and abundance estimates is costly given their ecology and poor detectability (Freilich *et al*., 2000; Pérez *et al*., 2002). Detection rates are directly related to interannual variability in activity patterns, which depend mainly on interannual climatic variations (Freilich *et al*., 2000). Finally, their populations have a very wide range of densities, with perfectly viable very low-density populations (Averill-Murray & Averill-Murray, 2005).

The distribution area of the *T. graeca* in North Africa remains poorly understood, with lack of comprehensive ecological and geographic descriptions across its entire range (Anadón *et al*., 2012). Works like those of Anadón *et al*. (2015) and Graciá *et al*. Z(2017) have defined the ecological and geographic limits of this species, but there are still areas of the region for which information is lacking.

Local ecological knowledge (LEK), defined by Olson and Folke in 2001 as "knowledge that a specific group of people have about their local ecosystems", has been proposed as a tool capable of producing relevant biological information for conservation efforts (Huntington, 2000; Folke, 2004). Previous works have used this tool to study the distribution of tortoises as an initial exploratory method (e.g., Lambert, 1983; Smith *et al*., 1999). LEK has also been employed to describe and model the abundance and distribution of *T. graeca* in Murcia and Almería, Spain (e.g., Anadón *et a*l., 2009, 2010).

The objective of this work is to increase existing knowledge on the distribution of *T. graeca* in Morocco using LEK as a source of information.

2. Materials and Methods

Study area

Our study area is located in North Africa. It specifically occupies the entire surface of Morocco, from 27.5ºN latitude northwards, except for Saharan areas. We used a grid that covers the entire surface of the study area, which was divided into grids sized 20 x 20 km.

Interviews

The local inhabitants selected to explore LEK were shepherds. The criteria of a previous work were followed given their proven suitability to collect data about the fauna of a given locality (Anadon *et al*., 2010).

Interviews were conducted during nine field campaigns between 2016 and 2018, which totalled 37 effective days. For each campaign, we planned a daily itinerary covering 200 km. We conducted one interview or two in each 20 x 20 km grid, as long as there were no major habitat changes, in which case the number of interviews to be conducted was increased. Shepherds were located in the field while performing their routine activity of caring for their livestock.

Interviews were conducted in French, Arabic and Amazigh. These interviews were semi-structured and began by asking general questions until the most detailed and interesting issues were mentioned, such as signs of the presence of the species and the interviewee's activity. Questionnaires were made up of three clearly differentiated parts: (i) general information about the interview; (ii) general information about the interviewee and his/her activity in the area; (iii) information of interest about tortoises.

Data processing

The data collected during the field campaigns were systematised in a database. In particular, the spatial data were implemented into a geographic information system using the QGIS software.

Before conducting the comparative spatial analysis of the data acquired by interviews and previously available information, we filtered the information provided by shepherds. We firstly considered the absence of the species to be valid when the interviewee had grazed the area for at least 10 years. It should be noted that the data on the presence of the species did not require a minimum grazing period in the area. Secondly, we found that some grids had more than one observation of presence, but they were counted as one because only the absence and presence of the species were studied. Lastly, if one shepherd reported seeing the species in a grid, but another did not, we still considered the occurrence citation to be valid.

3. Results

Interviews were conducted throughout the study area, and covered an average daily distance of 227 km. Interviews were held with 288 people, of whom 93% were men and 7% were women. Of these, 90% were shepherds and 10% belonged to other groups, such as hunters or farmers. In all, the interviews provided us with 370 records, of which 52% were of presence and 48% of absence. By adding the 194 new records of presence of the species to the 129 previously available records, we obtained 323 positive records and 176 new records of absence (Figure 1).

Figure 1: Map of the new records of absence (black) and the presence (orange) of *T. graeca* from the interviews. Green represents the previous citations of the presence of the species. The coloured polygons indicate the distribution area of the subspecies: *T. g. graeca* (blue), *T. g. marokkensis* (orange) and *T. g. whitei* (red) according to previous works (Anadón *et al*., 2015; Graciá *et al*., 2017).

4. Discussion

By applying LEK as a data collection tool and conducting 288 interviews on 37 field days, we increased the records of the presence of the *T. graeca* species by 40%, which extends our knowledge of its distribution area.

In addition, the use of LEK allowed us to obtain new data about the absence of the species. Such information is normally more costly to obtain by conventional and large-scale sampling methods. This information is significant for refining more accurate niche models (Brotons *et al*., 2004).

The reliability of the distribution data obtained by this methodology is variable depending on the taxa characteristics and the interviewed population (Chalmers & Fabricius, 2007). However thanks to previous studies, traditional shepherds have proven to be a source of rigorous information about the distribution of animals and plants. Nonetheless, numbers of shepherds are expected to lower in Morocco, just as they have decreased in Spain (Anadón *et al*., 2010; Hortalá *et al*., 2015).

In view of the obtained results, we can affirm that LEK from shepherds is a high-quality and low-cost source of information on the distribution of *T. graeca*, as described in previous studies (Anadón *et al*., 2009, 2010). Therefore, LEK can be especially useful when sampling methods are expensive or difficult to apply (Anadón *et al*., 2010), and the information that derives from it is extremely important for developing conservation measures for the species.

Interviews covered a significant portion of Morocco, but there are still some areas in this country without records where more fieldwork can be carried out to complete available information. It would also be valuable to apply this approach to other easily identifiable faunal groups, including mammals, reptiles and birds.

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What is long-term monitoring for? The example of survival rates of reintroduced Hermann's tortoises

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ABSTRACT

For long-lived species as tortoises short-term monitoring programs (e.g. 3-5 years) might not allow estimating the variability of demographic parameters. This is because short-term studies can be carried out in good times, obtaining high parameters estimations, as well as in bad times, with low parameters estimations. For example, survival rates (S) of reintroduced adult Hermann's tortoises at the Ebro Delta Natural Park may even remain constant until periods of 11 years and be highest (i.e. S = 1.0), whereas the mortality events may focus in short periods. Thus, a minimum duration over 10 years is recommended in reintroduction programs, as well as in monitoring native populations, to estimate survival rates variability, in the aim to assess these actions of conservation more efficiently.

RESUMEN

Para especies longevas, tales como las tortugas, es posible que los seguimientos a corto plazo (e.g. 3-5 años) no permitan estimar la variabilidad de los parámetros demográficos. Esto se debe a que los estudios a corto plazo pueden realizarse en períodos favorables, obteniéndose altos valores en los parámetros estimados, pero también en períodos desfavorables, estimándose entonces valores inferiores. Así por ejemplo, se ha observado que las tasas de supervivencia (S) de los adultos reintroducidos de tortuga mediterránea en el Parc Natural del Delta de l'Ebre se mantienen con valores máximos (S = 1.0) en períodos de hasta 11 años consecutivos, mientras que la mayor mortalidad se concentra en pocos años. Por ello, se recomienda que tanto en los programas de reintroducción como en los seguimientos de poblaciones nativas la duración de los seguimientos sea superior a los 10 años, de manera que se pueda estimar la variabilidad de las tasas de supervivencia y evaluar eficientemente estas acciones de conservación.

Keywords: demographic parameters, monitoring, Delta de l'Ebre, *Testudo hermanni*.
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1. INTRODUCTION

Survival rate is a key parameter in demographic studies, but their estimation for wildlife under field conditions is in most cases a challenge (Lebreton *et al*., 1992). One of the most common approaches to estimate survival probabilities is by maximum likelihood parameter estimation using capture-markrecapture (CMR) method, which accounts for imperfect detection of individuals (Lebreton *et al*., 1992). However, for long lived species, as chelonians, it is a time-consuming methodology due to the fact that several years of monitoring are needed to obtain representative data.

The Hermann's tortoise *Testudo hermanni* population at the Ebro Delta Natural Park has one of the most long-term monitoring programs for a reintroduced species of chelonians. Started in 1987, the annual monitoring using CMR methodology has enabled to analyze survival using different parameterization models: for reintroduced tortoises, combining information of recapture and recoveries (period 1987-2004; Bertolero *et al*., 2007); for wild born tortoises, descendants of reintroduced tortoises (period 1988-2009; Fernández-Chacón *et al*., 2011); and for reintroduced and wild born, all tortoises together (period 1987-2015; Bertolero *et al*., 2018). The opportunity to make available this long-term data is to estimate a more accurate survival rate that integrates year variation (whatever the cause).

The objective of the present work is to show that in small populations, as obtained in reintroduction programs, it is necessary to carry out long-term studies to estimate unbiased survival rates using a CMR approach.

2. Materials and Methods

This study was carried out on the Hermann's tortoise population reintroduced at the Ebro Delta Natural Park in 1987. This population inhabits a patchy habitat of dunes with vegetation, where tortoises have spread in an area of 55 ha. For a description of the reintroduction project and the habitat, see Bertolero *et al*. (2007, 2018). This population has been monitored since 1988, but most intensively from 1991 to 2019 (1988-1990: mean ± SD = 5.3 ± 5.1 days/year, range: 1-11 days/year; 1991-2019: mean ± SD = 39.1 ± 16.4 days/year, range: 16-86 days/year).

For the present study I focused in the two batches of released tortoises at one of the dunes (44 adult tortoises released in 1987-1988 and 22 adults and subadults released in 1997-1998 "the youngest tortoises were two males and one female, with a minim age of 8 years old"). All released tortoises were marked individually with notches at the carapace. As each batch of tortoises was released in two consecutive years, survival was analyzed in the period 1989-2019 for the first batch (G1) and in the period 1999-2019 for the second one (G2). Due to the particularities of this population where released tortoises showed high annual recapture probabilities in spring most of the years since 1991 (> 60%; Bertolero *et al*., 2007, 2018), recoveries probabilities of released tortoises were also high (probability of finding a dead tortoise, mean ± SE = 0.533 ± 0.074; Bertolero *et al*., 2007), and population cannot migrate (inhabit a protected peninsula), survival rates were analyzed as a known-fate model (i.e. model where the probability of detection is assumed equal to 1; Cooch & White, 2020).

Annual survival rate (S) was estimated directly as the ratio of number of tortoises found in year *j+1* to the number of tortoises found in the year *j*, independently for each batch of tortoises. If a tortoise was not found in the year *j*, but was found in the year *j+1*, it was considered as missed and included as alive in the year *j* (no alive tortoise was found in the year *j+1* and missed in the years *j-1* and *j*; then, released tortoises not found in a time span of two years had never been subsequently recaptured alive). Survival rate for each period of years considered was estimated as the geometric mean of the annuals survivals rates (e.g. for the four years period 1989 to 1992: S1989-1992 = $([n1990/n1989]*[n1991/n1990]*[n1992/n1991])^{\wedge(1/3)} = (n1992/n1989)^{\wedge(1/3)}$, where *n* was the number of tortoises found each year). Permutation test was used to compare mean survival rate between both batches of tortoises with 9999 permutations. For this, the geometric mean was estimated using the function Gmean of package DescTools (Signorell *et al*., 2020). In the same way, the 95% confidence interval was calculated with the same function, with 9999 bootstrap samples and the percentile limits calculated by the accelerated bias-corrected percentile method (Efron [1987] in Manly, 2001). The package survminer (Kassambara *et al*., 2019) was used to make the survival plot only for graphical representation. All analyses were carried out in R (R Development Core Team, 2018).

3. Results

Observed annual survival rates ranged from 0.828 to 1.0 for the G1 and from 0.636 to 1.0 for the G2. Some periods of years concentrated most mortality (e.g. S1992-1995 = 0.866 for the G1 or S1999-2001 = 0.661 years for the G2), while for other periods mortality was nil (Figure 1). Additionally, annual survival rates were highest (1.0) for several consecutives years in both batches of tortoises (until 11 years for the G1 and until 7 years for the G2; Figure 1). Long-term mean survival rate was similar for both batches of released tortoises (permutation test $p = 0.35$; G1 period 1989-2019: mean = 0.9687, bootstrapped IC 95%: 0.9488-0.9814; G2 period 1999-2019: mean = 0.9435, bootstrapped IC 95%: 0.8700-0.9819).

4. Discusion

The method used in this work to estimate the annual survival rates is very simple and does not account for imperfect detection of tortoises, neither incorporates covariables that can be relevant to explain some sources of mortality, as in previous works on this population where, among other factors, the presence of a predator and the effect of release cost were considered (Bertolero *et al*., 2007; 2018). Despite this, these estimated long-term survival rates were similar to those estimated previously by a more powerful Cormack-Jolly-Seber constrained model (mean ± SE = 0.978 ± 0.004; Bertolero *et al*., 2018). However, it is important to remark that this simple ratio method is not recommended to estimate survival rate in other chelonian monitoring programs, without a previous and careful assessment of their suitability.

The purpose of the used simple ratio method was only to obtain raw annual survival rates that vary across years (likewise a time dependent model in the CMR framework) to show

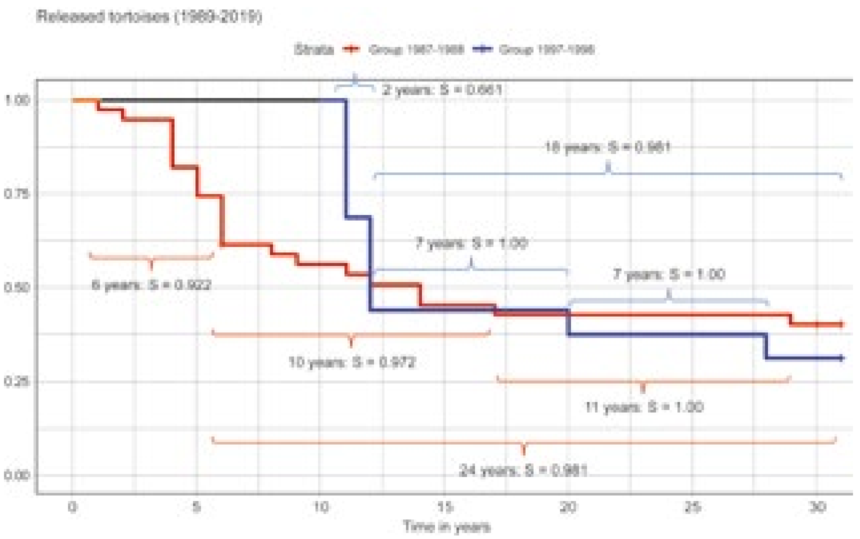

Figure 1: Survival curves for the both batches of released tortoises at the Ebro Delta Natural Park. Note that both curves cannot be compared directly in this plot, since the first part of the curve of the batch G2, from year 0 to year 10 (black line), is a representation artifact to allow both curves to coincide in years.

that, in some situations, short-term studies cannot represent long-term survival rates. This is because, by stochastic process, short-term to medium-term studies (approx. ≤ 10 years) can be carried out in good times, obtaining high parameters estimations, but also in bad times, obtaining low parameters estimations. Lebreton *et al*. (1992) pointed that 4 or 5 capture occasions can be a starting point, but they recommended from 11 to 21 capture occasions to understand the survival dynamics. Thus, for long-lived chelonians study periods with a minimum of 11 years may be required to obtain unbiased survival rates, at least when populations are small, as the reintroduction projects, but also when monitoring native populations. For many chelonian conservation projects that include reintroduction actions, these long-term monitoring programs may seem excessively long with the available resources. Thus, before starting, managers and wildlife agencies should keep in mind that these are, in fact, long-term programs with delayed results.

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Bringing science to the people: Citizen science for the long-term monitoring of *Testudo graeca*

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Abstract

Long-term monitoring programs are essential tools for the conservation of endangered, long-lived species. Since 1999, Miguel Hernández University (Spain) has monitored spur-thighed tortoise (*Testudo graeca*) locations in southeastern Spain. More than 150 locations have been studied, with over 6500 individual captures, shedding light on various aspects of the species' biology, genetics, ecology, and conservation status. Since 2015, Miguel Hernández University and the Spanish Herpetological Association have coordinated the *Testudo* Project, initially supported by Fundación Biodiversidad (Spain), in collaboration with local naturalist associations. This project is a volunteer-based citizen science program whose main goals are to provide data for assessing *T. graeca* population and to raise awareness about the importance of biodiversity in semi-arid ecosystems. More than 700 volunteers have participated in the *Testudo* Project. The results, together with the involvement of academia, government bodies, and local associations, have driven the implementation of key conservation actions aimed at preserving *T. graeca* population in southeastern Spain.

Resumen

Los programas de seguimiento a largo plazo son una herramienta esencial para la conservación de especies de vida larga amenazadas. Desde 1999, la Universidad Miguel Hernández (España) viene realizando el seguimiento de la tortuga mora (*Testudo graeca*) en el sureste ibérico. Se han estudiado más de 150 localidades, con más de 6500 registros, que han revelado cuestiones sobre la biología, la genética, la ecología y el estado de conservación de la especie. Desde 2015, la Universidad Miguel Hernández y la Asociación Herpetológica Española coordinan el Proyecto *Testudo*, impulsado por la Fundación Biodiversidad en colaboración con asociaciones naturalistas locales. Este proyecto es un programa de voluntariado basado en acciones de ciencia ciudadana cuyos objetivos principales son proporcionar datos para la evaluación de la población de *T. graeca* y concienciar sobre la importancia de la biodiversidad en los ecosistemas semiáridos. Los resultados del Proyecto *Testudo*, junto con la implicación del mundo académico, las administraciones y las asociaciones locales, han promovido la puesta en marcha de importantes acciones de conservación para la población de *T. graeca* en el sureste ibérico.

Keywords: conservation, ectotherms, long-lived species, tortoises, volunteering.
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1. Introduction

Long-term monitoring (LTM) programs are an essential tool in conservation biology, providing valuable information on population trends, species interactions, predation rates, population size, and life-history traits such as reproduction, growth, and survival (Antworth *et al*., 2006; Iverson *et al*., 2006; Moore *et al*., 2007).

LTM studies are particularly important for long-lived species with slow population dynamics, as their long generation times can delay the detection of negative effects caused by disturbances (Moore *et al*., 2007; Fisher *et al*., 2020). Some long-lived reptiles have been monitored for decades, including the New Zealand tuatara (*Sphenodon punctatus*) (>50 years) (Moore *et al*., 2007), the Coachella Valley fringe-toed lizard (*Uma inornata*) in southern California (>30 years) (Fisher *et al.*, 2020), loggerhead and leatherback sea turtles (*Caretta caretta* and *Dermochelys coriacea*) in Florida (>20 years) (Antworth *et al*., 2006), and the Allen Cays rock iguana (*Cyclura cychlura inornata*) in the Bahamas (>20 years) (Iverson *et al*., 2006), among others.

Citizen science is an interesting tool for collecting large volumes of data over the long periods required by LTM programs, and it represents a significant advance in biodiversity research (Bonney *et al*., 2009). In addition, citizen science promotes environmental education by engaging people with species' ecology and conservation challenges (Bonney *et al*., 2009; Martín-Forés *et al*., 2013).

Here, we present the combination of an LTM program and a citizen science program focused on the spur-thighed tortoise (*Testudo graeca*) in southeastern Spain. This long-lived chelonian is considered an umbrella species in the western Mediterranean. It is listed as "Vulnerable" by the IUCN, the Spanish National Catalogue of Threatened Species (RD 139/2011), and the Catalogue of Threatened Species of Wild Fauna of the Murcia Region (Law 7/1995), and as "Endangered" by the Andalusian Catalogue of Threatened Species (Law 8/2003). It is also included in Annexes II and IV of the EU Habitats Directive (Council Directive 92/43/EEC).

The LTM program has yielded substantial insights into the biology, ecology, and conservation status of the species. At the same time, the citizen science initiative, driven by volunteers, has not only contributed a large volume of data but has also played a key role in raising public awareness. Together, both efforts underscore the value of integrating long-term research with citizen participation, not only to improve our understanding of *T. graeca*, but also to promote broader environmental education and engagement.

2. Long-term monitoring and citizen science: valuable tools for *Testudo graeca* conservation

LTM are necessary to document and understand environmental influences over the lifetime of individuals and are also crucial to determine the effectiveness of management and conservation policies (Yoccoz *et al*., 2001). Since 1999, the Ecology Area of the Miguel Hernández University of Elche (UMH, Spain) has studied *T. graeca* populations in southeastern Spain. More than 150 locations have been monitored in the regions of Murcia and Almería (Figure 1), providing more than 6500 records. This information has been useful to assess the conservation status of *T. graeca.*

In 2015, the UMH and the Asociación Herpetológica Española (AHE), in collaboration with local naturalist associations (Ecologistas en Acción, Serbal, ACUDE, and AHEMUR), and thanks to initial funding from the Fundación Biodiversidad (supported by the Ministry of Agriculture, Food and Environment of Spain), launched the *Testudo* Project. This project established the long-term monitoring of several tortoise locations in southeastern Spain. The *Testudo* Project is based on a volunteer program whose general objectives are to provide a population assessment to support the conservation of the species, promote tortoise observation in the wild, and raise awareness about the importance of biodiversity in semi-arid ecosystems.

Figure 1: Study site locations within the distribution range of *Testudo graeca* in southeastern Spain.

3. Long-term monitoring: gathered data and knowledge

The data collected through long-term monitoring and the scientific and technical projects developed by the UMH have been key to studying crucial aspects of *T. graeca* conservation, including ecology, activity patterns, genetics, demography, and distribution (Table 1). Moreover, the extensive monitoring data generated by the program has supported the development legal protection lists (e.g., Spanish National Catalogue of Threatened Species, RD 139/2011), the design of conservation strategies for *T. graeca* populations in the Murcia and Almería regions, as well as participation in environmental impact assessment consultations.

Basic knowledge of the species' ecology has been developed to understand patterns of size and growth (Rodríguez-Caro *et al*., 2015; Semaha *et al*., 2024). In the southeastern Iberian population, individuals exhibit significantly smaller body sizes, with a clear south-to-north gradient—where the smallest recorded male sizes for the species have been described (Semaha *et al*., 2024). Studies on reproduction have shown that females lay two clutches during the spring, each consisting of an average of three eggs (Rodríguez-Caro *et al*., 2021). Additionally, the species' ability to store sperm for at least four years has been a key factor in its reproductive success, particularly under conditions of low population density (Jiménez-Franco *et al*., 2020).

Table 1: Some of the research topics addressed by the Ecology Department at the Miguel Hernández University of Elche, made possible thanks to the long-term monitoring of *Testudo graeca* in southeastern Spain.

Area	Topic	Reference(s)
Ecology	Movement patterns	Pérez *et al.*, 2002, Montesinos *et al.*, 2007; Anadón *et al.*, 2012; Graciá *et al.*, 2023
	Body size and growth	Semaha *et al.*, 2024, 2025
Genetics and Evolution	Populations origin	Graciá *et al.*, 2013a,b, 2015, 2017
	Genetic structure	Graciá *et al.*, 2011, 2013a,b, 2017; Mira-Jover *et al.*, 2024a
	Fitness	Mira-Jover *et al.*, 2024b
Demography	Age estimation	Rodríguez-Caro *et al.*, 2015
	Distribution and abundance	Anadón *et al.*, 2006b; Ballestar *et al.*, 2007b; Anadón *et al.*, 2009, 2010
	Population dynamics	Rodríguez-Caro *et al.*, 2013, 2016
	Reproduction and life traits	Jiménez-Franco *et al.*, 2020; Rodríguez-Caro *et al.*, 2021
Habitat	Landscape studies	Anadón *et al.*, 2005, 2006a, 2007, 2012; Rodríguez-Caro *et al.*, 2017b; Graciá *et al.*, 2020a; Jiménez-Franco *et al.*, 2022, 2024
	Fire	Sanz-Aguilar *et al.*, 2011; Rodríguez-Caro *et al.*, 2013, 2019
Interspecific relationships	Predation	Ballestar *et al.*, 2007a; Gil-Sánchez *et al.*, 2022
	Parasitism	Chavarri *et al.*, 2012; Benitez-Malvido *et al.*, 2019; Gonzalvez *et al.*, 2022
Conservation	Threats	Pérez *et al.*, 2004, 2012; Giménez *et al.*, 2004, Graciá & Giménez, 2015; Graciá *et al.*, 2016, 2020b

Molecular research has helped to identify the main evolutionary traits of the southeastern Spanish population. This data was essential to develop the first chromosome-level genome assembly of the genus *Testudo* (Mira-Jover *et al*., 2024a). Genetic studies determined that this population originated from North Africa approximately 20 000 years ago and has undergone a northward expansion since then, starting in Vera's Basin (Almería) through the provinces of Almería and Murcia (Graciá *et al*., 2013a, 2017). This expansion resulted in a well-defined genetic pattern, being identified as one of the first empirical examples of genetic surfing (Graciá *et al*., 2013b, 2017). Such a process can affect the fitness and viability of the population (Mira-Jover *et al*., 2024b).

Monitoring individual movements has revealed inferences about the species' landscape use and activity patterns (Pérez *et al*., 2002). The species' distribution has been described in natural areas and traditional crops. Agricultural intensification, pine reforestation, and urbanization are the main drivers of landscape fragmentation (Anadón *et al*., 2006a, 2007; Rodríguez-Caro, 2017b), which affect population viability (Jiménez-Franco *et al*., 2022). Climatic conditions also play a key role in habitat loss, as low winter temperatures (Rodríguez-Caro *et al*., 2016) and decreased rainfall negatively affect population viability (Anadón *et al*., 2006b). Moreover, the increase of the recurrence of extreme events such as drouths may drive to population extinctions (Rodríguez-Caro *et al*., 2021)

Besides habitat loss, LTM has helped to identify and quantify other major threats, such as wildfires and captive breeding. Wildfires represent a significant pressure on tortoise population in southeastern Spain (Sanz-Aguilar *et al*., 2011; Rodríguez-Caro *et al*., 2013, 2019). Although tortoises can persist under low-frequency fire regimes, recurrent fires severely undermine population viability (Sanz-Aguilar *et al*., 2011). Beyond causing direct mortality, fire events also have sublethal effects, such as reducing individual growth rates (Rodríguez-Caro *et al*., 2013).

Captive keeping is also common in this region. Around twenty years ago, it was estimated that approximately 100 000 tortoises were being kept as pets in the province of Murcia (Pérez *et al*., 2004). Moreover, the illegal collection of wild individuals can cause local extinctions (Pérez *et al*., 2012), a threat further exacerbated by the release of captive tortoises, which introduces pathogens and parasites (Chavarri *et al*., 2012) and genetic and morphological differences (Graciá *et al*., 2013b; Semaha *et al*., 2025).

4. *Testudo* project: bringing science to the people

Volunteer programs play a crucial role in bringing biodiversity issues closer to society. Since 2015, more than 700 people have taken part in the *Testudo* project, and over 2000 tortoise individuals have been studied in the field.

The *Testudo* project illustrates the importance of fostering collaboration among academia, government institutions, and civil society to address biodiversity conservation. In coordination with local ecological associations, volunteers not only contribute to data collection but also learn about the species' biology, ecology, and habitat characteristics. Most participants report a change in their perception of the species, its threats, and its conservation status after their involvement in the program (Rodríguez-Caro *et al*., 2017a, 2022), highlighting the educational value of volunteer-based citizen science. Moreover, public awareness and opinion are key elements in the design and implementation of conservation plans, as they influence both political decisions and management strategies (Martín-Forés *et al*., 2013; Martín-López *et al*., 2011).
The monitoring data have enabled the annual tracking of several populations, revealing that some have remained stable over the study period, while others have shown increasing or decreasing trends (Figure 2). This is crucial for identifying the causes of population declines and for implementing management measures aimed at the conservation of these populations.

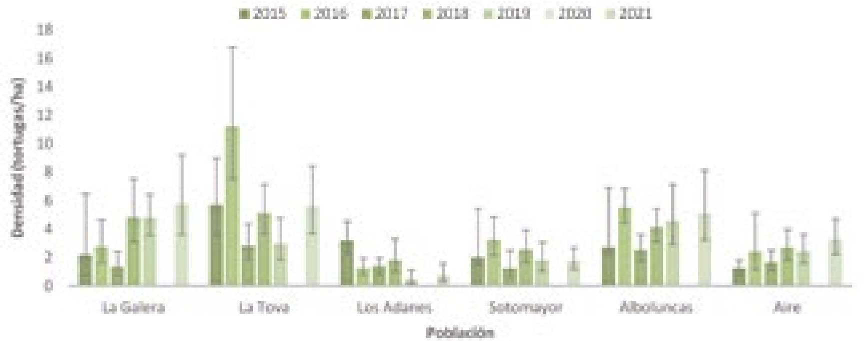

Figure 2: Population trends of six *Testudo graeca* locations monitored through the *Testudo* Project volunteer program. Estimated densities (individuals per hectare in 1 × 1 km cells) are shown for seven years (2015–2021), along with 95% confidence intervals. Figure adapted with modifications from Rodríguez-Caro *et al.* (2022).

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Mascort, R. Long-term monitoring of a population core of western Hermann's tortoise, *Testudo hermanni hermanni*, at Serra de l'Albera, in the north-eastern Iberian Peninsula. 2025. *In*: Graciá, E., Rodríguez-Caro, R.C., & Giménez, A. (eds.). *Conservation of Mediterranean tortoises under global change*. Madrid. Asociación Herpetológica Española.

Long-term monitoring of a population core of western Hermann's tortoise, *Testudo hermanni hermanni*, at Serra de l'Albera, in the north-eastern Iberian Peninsula

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Abstract

A long-term capture-recapture study of western Hermann's tortoise (*Testudo hermanni hermanni*) was carried out at Serra de l'Albera (NE Iberian Peninsula) during two five-year periods from 1996 to 2000 and from 2014 to 2018 covering a 23-year span. Different aspects of the species' biology and ecology were addressed in a 105-hectares study area. The tortoise population has remarkably declined in the last two decades, primarily due to various mammals predating adults, juveniles, hatchlings and a large number of nests. The population structure shifted towards old adults with minimal recruitment of juveniles.

Resumen

En la Sierra de l'Albera (noreste de la península ibérica) se ha realizado un estudio a largo plazo sobre la tortuga mediterránea occidental (*Testudo hermanni hermanni*) basado en el método de captura-recaptura durante dos períodos de cinco años, de 1996 a 2000 y de 2014 a 2018, comprendiendo en total un período de 23 años. Se abordaron diferentes aspectos de la biología y ecología de la especie en un área de estudio de 105 hectáreas. La población de tortugas sufrió una disminución notable en las dos últimas décadas, principalmente debido a la acción de diversos mamíferos depredadores que incidieron sobre adultos, juveniles, crías y en una gran cantidad de nidos. La estructura poblacional se desplazó hacia los ejemplares de mayor edad, con un reclutamiento muy bajo de juveniles.

Keywords: activity patterns, capture-recapture analyses, demography, field research, morphology, reproduction.
(†) Ramón Mascort passed away in 2025.

1. Introduction

The population of the western Hermann´s tortoise (*Testudo hermanni hermanni*) at Serra de l'Albera accrues distinctive morphological and genetic traits at its westernmost distributional area location (Bertolero *et al.,* 2011; Fritz *et al.,* 2006; Zenboudji *et al.,* 2016).

The population was first documented fairly recently (López Jurado *et al.,* 1979; Fèlix & Franquesa, 1980). Since then, some studies have been carried out at different sites following the capture-recapture method (Franch & Oller, 1989), telemetry with juveniles and adults (Casamitjana *et al.,* 2012; Mascort *et al.,* 1999) or a full census (Bertolero, 2017,2019). Fèlix *et al.* (1990) studied the impact of the massive forest fire in August 1986 on a core population, and its impact 4 years later (Fèlix, 1999), while a preliminary report of the present study was offered 26 years ago (Mascort, 1997). The objectives of this work are to describe the species' ecology and biology at Serra de L'Albera, and to assess its population dynamics and population structure.

2. Study area

The study area covered 105 hectares in the middle section of a west-facing valley in the buffer zone of the PNIN de l'Albera protected area (the Natural Place of National Interest of l'Albera). This area is an isolated, well-preserved plot where no tortoise reinforcement has been previously done. The altitude of the study area ranges from 120 to 250 m a.s.l. Most of the area is covered with sparse cork oak forest (*Quercus suber*), mixed with abandoned olive groves (*Olea europaea*) and isolated stone pines (*Pinus pinea*). A few downy oaks (*Quercus pubescens*), Mediterranean hackberries (*Celtis australis*) and Montpellier maples (*Acer monspessulanum*) grow on wetter spots. The extensive remaining scrubland area mainly comprises species like *Erica* ssp., *Calicotome spinosa* or *Spartium junceum*, while *Cistus* ssp., *Rosmarinus officinalis*, *Thymus vulgaris* and other small bushes grow on extensive sunny clearings.

3. Material and Methods

This study was carried out during two 5-year periods (from 1996 to 2000 and 2014 to 2018) from 1st March to 30th November each year. Monitoring was done during expected tortoise activity; i.e, daytime air temperatures between 18ºC and 30ºC. Line transects were done by the same person (RM). For all the tortoises, straight carapace length (SCL) was measured with a digital calliper. They were weighed, photographed, georeferenced (in 25 x 25 m plots) and marked in marginal scutes before being released at the same place. Only the animals rescued from sinks and wells were removed from the pit.

Density was estimated by taking into account the proportion of captured-recaptured animals for the last 10% records of all the animals per period.

To evaluate the predation effect, predated nests and related details were also recorded. Considering the difficulty of identifying egg-laying episodes, records of recently predated nests were used to described the nesting season, assuming that predators are able to smell eggs in nests for a few days after egg-laying (Nougarède, 1998; Swingland & Stubbs, 1985). Moreover, mesocarnivore scats were examined to investigate predation on tortoises.

4. Results and Discussion

During the 1996-2000 period, 567 hours were spent, which resulted in 562 records of 355 tortoises. During the 2014-2018 period, 1161 research hours produced 732 records of 364 tortoises. In all (1996-2018), 1728 hours were spent in the field and yielded 1294 records. The number of tortoises marked during the first period and recaptured during the second one was 85. Thirty tortoises were found dead (6 during the first period and 24 during the second one).

Activity

Activity started gradually in March and males began the season earlier. Females moved much more in May until the first half of July for the nesting season. Then males gradually became more active. In November, tortoises buried themselves (Figure 1). This seasonal pattern agrees with the pattern described by Bertolero (2002) in the River Ebro Delta.

In spring, tortoises showed unimodal activity in central daytime hours. On the first few days, tortoises spent more time basking and started feeding some days later. At the end of June, tortoises started bimodal activity (early in the morning and in the evening). In early September, they returned to the unimodal activity pattern. In October they still fed after midday.

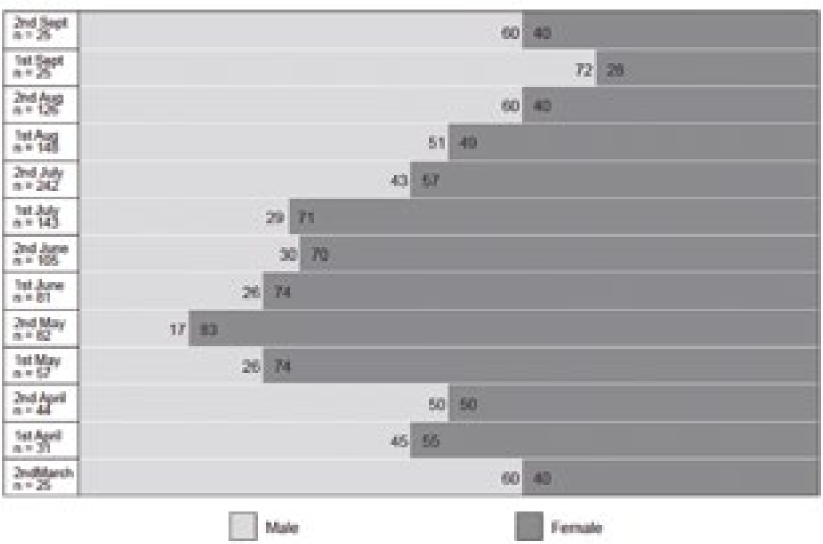

Figure 1: Seasonal activity patterns for males and females. Numbers refer to the percentage of active individuals found during that period.

Movements

Tortoises showed high site fidelity. Males can frequently cover short distances in one same plot, while females can take long-term trips. At the study site, tortoises were recorded to be occupying tiny areas or returning to their home areas after several years of wandering around (Table 1). This individual variation in movements, from total sedentary animals to others that keep going, has also been documented in south-eastern France (Stubbs & Swingland, 1985). Males' short-distance movements and females loop long-distance journeys agree with a previous study conducted in central Italy (Calzolai & Chelazzi, 1991).

Table 1: Tortoise movements of some tortoises during the study period (1996-2018). Initial and final SCL (mm) refers to the straight carapace length at first and last capture. Final distance is the straight-line distance between first and last locations.

Tortoise, sex, initial and final SCLmm	Location 1	Location 2 L1 to L2 (m)	Location 3 L2 to L3 (m)	Location 4 L3 to L4 (m)	Location 5 L5 to L6 (m)	Location 6 L6 to L7 (m)	Location 7 L7 to L8 (m)	Final distance	years lapse L-1 to L-7
7114 ♀	16/07/1999	01/08/2000	03/06/2014	01/07/2014	23/06/2018				
124-150 mm	0 m	70 m	40 m	35 m	45 m			80 m	19 years
1795 ♀	30/07/1996	09/08/1996	30/07/1998	06/07/1999	31/03/2015	26/03/2016	02/08/2016		
152-164 mm	0 m	180 m	160 m	140 m	230 m	220 m	100 m	200 m	20 years
1841 ♀	21/08/1996	26/05/1997	17/08/1998	16/07/1999	22/07/2000	01/07/2014	30/07/2016		
147-155 mm	0 m	15 m	70 m	25 m	20 m	140 m	140 m	55 m	20 years
1861 ♂	22/04/1997	17/08/1998	16/07/1999	02/09/1999	19/08/2000	14/04/2014	11/08/2015		
115-137 mm	0 m	80 m	80 m	15 m	10 m	15 m	10 m	20 m	18 years
1846 ♀	22/08/1996	29/08/2000	01/05/2014	02/08/2014	18/08/2014	10/07/2015			
75-138 mm	0 m	15 m	30 m	50 m	30 m	30 m		25 m	19 years
1917 ♀	22/07/1997	11/07/1999	30/07/2000	24/06/2014	11/06/2015	01/07/2015			
150 -166 mm	0 m	50 m	40 m	15 m	15 m	30 m		25 m	18 years
1925 ♀	25/04/1998	17/08/2000	16/05/2014	08/06/2015	14/06/2017	28/07/2018	06/07/2018		
136-159 mm	0 m	550 m	650 m	25 m	280 m	70 m	60 m	220 m	20 years
1942 ♂	30/07/1998	01/08/1999	17/07/2014	02/10/2015	24/08/2016				
118-130	0 m	50 m	30 m	50 m	50 m	06/05/2016	21/06/2018	70 m	17 years
1984 ♀	24/06/1999	10/03/2000	20/05/2000	14/08/2000	12/07/2014	50 m	40 m		
149-162 mm	0 m	15 m	10 m	15 m	5 m			10 m	19 years
1993 ♂	26/06/1999	17/08/2000	07/06/2014	06/05/2015	22/09/2017	10/03/2017			
94-142 mm	0 m	220 m	450 m	20 m	15 m	25 m		380 m	18 years
1994 ♂	06/07/1999	17/05/2014	17/07/2014	30/07/2014	26/03/2016	28/05/2018			
133-139 mm	0 m	50 m	110 m	140 m	70 m	30 m		140 m	19 years
7191 ♀	29/07/2000	15/04/2014	03/04/2015	13/04/2016	14/06/2017				
109-167 mm	0 m	280 m	15 m	50 m	60 m			5 m	18 years

On three times, small juveniles (< 68 mm SCL) were observed sharing the same area in pairs, although they remained at short distances from each other: for example, a 67 mm SCL individual was found approximately 60 cm from a 64 mm SCL one; a 56 mm SCL individual was about 50 cm from a 53 mm SCL one; and a 44 mm SCL individual was around 60 cm from a 39 mm SCL one.

Feeding

During this study, 105 feeding episodes were observed. In spring and early summer, tortoises ate the leaves and flowers of *Taraxacum officinale*, *Sonchus* spp., *Crepis* spp, *Trifolium campestre*, *Plantago lanceolata*, *Lobularia maritima* or *Sherardia arvensis*. As vegetation dried out, consumption of *Sedum* spp. leaves and flowers increased. In August, *Clematis flammula* leaves were the most widely consumed leaves. Moreover, some coprophagy events of cow and wild boar faeces were recorded, particularly in summer. A detailed list of the consumed food items at l'Albera can be found in Budó *et al.* (2009).

Reproduction

Mating

The smallest male and female observed displaying mating behaviour had straight carapace lengths of 113 mm and 132 mm, respectively. Mating happened at any time during the activity season, and intensified during the dry season from mid-July to the end of August (Figure 2). This result is similar to the mating season at the River Ebro Delta (Bertolero, 2002), but differs from that found in a wooded area in south-eastern France (spring and late summer; Swingland and Stubbs, 2009) or in Corsica (March-April and again in August-September; Nougarède, 1998). Immediately after the egg-lying season, males usually rambled through the dense vegetation covering dry creeks to search for a mate. As the mating season coincided with bimodal activity, mating behaviours happened either in the morning and/or the evening. The whole process was recorded for up to 80 minutes, but can probably last longer and might be limited only by the large amount of energy spent by males. Courtship rarely ends with true mating and was verified only 3 times. Courtship was also recorded in spring and autumn, especially on days after rain or with warm and wet weather. Male combat (two males facing one another) was rarely observed.

Nesting

Records of recently predated nests (n=265) were useful for establishing the nesting season. Depending on the year, nesting started between 15-25 May, although a few isolated nests were found up to 1 month before those dates. The last nests were probably excavated in the first week of July. The main nests' orientation was south, followed by nests on flat areas. The number of eggs per nest varied between one and six, with a mean value of three. The nests with one egg represented 5% (13 nests), 20% (54 nests) had two eggs, 37% (98 nests) contained three eggs; 32% (85 nests) had four eggs; 5% (13 nests) contained five eggs; 1% (2 nests) had six eggs.

A total of 127 eggs were measured. The mean egg size was 33.6 x 26.0 mm. The biggest recorded egg was 38.4 x 26.8 mm and the smallest egg was 30.2 x 24.4 mm.

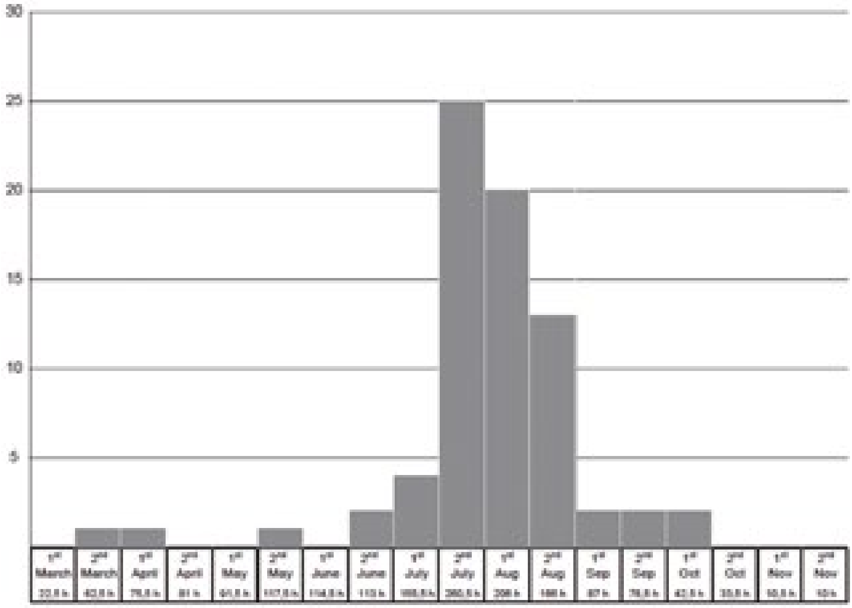

Figure 2: Mating activity recorded during the study. Bars represent the number of tortoises observed to be mating. The data after thee date are the total number of observation hours.

Hatching begins in Autumn (Nougarède, 1998). During this study, only two hatchlings were observed. On 2nd October 2000, a hatching was 32 mm SCL and weighed 9 g. The second one was observed on 24th October 2018, whose SCL was 38 mm and weight was 12 g.

Growth

The growth rate was estimated between recaptures 62-169 SCL (n=120). Growth was considerably uneven and depended on each tortoise. This fact prevented us from presenting a reliable year-by-year growing chart. Males' growth rate lowered when they reached 120 mm SCL (about 10 years old), which corresponds to sexual maturity. Females grew faster and were able to reach 130 mm SCL at about the same age.

When aged about 20 to 25 years old, tortoises, especially females, showed undetermined growth. Some females that measured over 160 mm SCL during the 1996-2000 period were found 15 to 22 years later to be 2 to 7 mm bigger. These animals were 40 to 45 years old in 2018. The SCL of the biggest female was 184 mm, and she weighed 1190 g. The biggest male's SCL was 154 mm and he weighed 660 g.

The growth rate recorded at the study site was much slower than at the River Ebro Delta. This was probably due to the harsher climate and environment conditions (Bertolero, 2002).

Population trends

During the first period, recaptures represented 41.7% of the last 10% found animals. At the end of the second period, 86.3% of the last 10% animals were recaptures. The number of tortoises estimated for the 1996-2000 period was 851, with density of about 8.1 tortoises/hectare. For the 2014-2018 period, the estimated abundance decreased to 480 tortoises, with density of around 4.6 tortoises/hectare. The rate of tortoises found per hour dropped from 1.00 (1996-2000) to 0.65 (2014-2018).

Population structure

Juveniles were well represented during the 1996-2000 period across all the size classes, and ranged from 30 to 100 mm SCL. However, juveniles were poorly represented during the 2014-2018 period, and the old adult class was overrepresented. This population pattern showed a low recruitment rate (Figure 3).
The sex ratio changed from 1:1.1 in 2000 to 1:2 in 2018, and was biased towards females. This pattern can be a consequence of climate warming because hotter incubation temperatures produce mainly females.

Predation

The beech marten (*Martes foina*) was the main predator of tortoises. It predated or partially damaged adults, and consumed eggs. In one beech marten territory (15 hectares), nine tortoises were found predated in 3 years (1 in 2015, 5 in 2016 and 3 in 2016) and 12 tortoises were found with one missing limb or two, usually hind limbs. Other predators could be wild boars (*Sus scrofa*), domestic dogs (*Canis familiaris*), badgers (*Meles meles*), foxes (*Vulpes vulpes*), weasels (*Mustela nivalis*) and brown rats (*Rattus norvegicus*).

5. Conclusions

This study provides key information about the activity, movement, feeding, reproduction, growth, population dynamics and population structure, and about the predatory impact, on the threatened species *Testudo hermanni*. The decline in population density and the change in population structure (with few recruitments and the high proportion of elderly individuals) threaten the population and suggest local extinctions in a medium term. This decline can be attributed mainly to predator pressure, which is increasing the consumption of tortoises, caused by the scarcity of

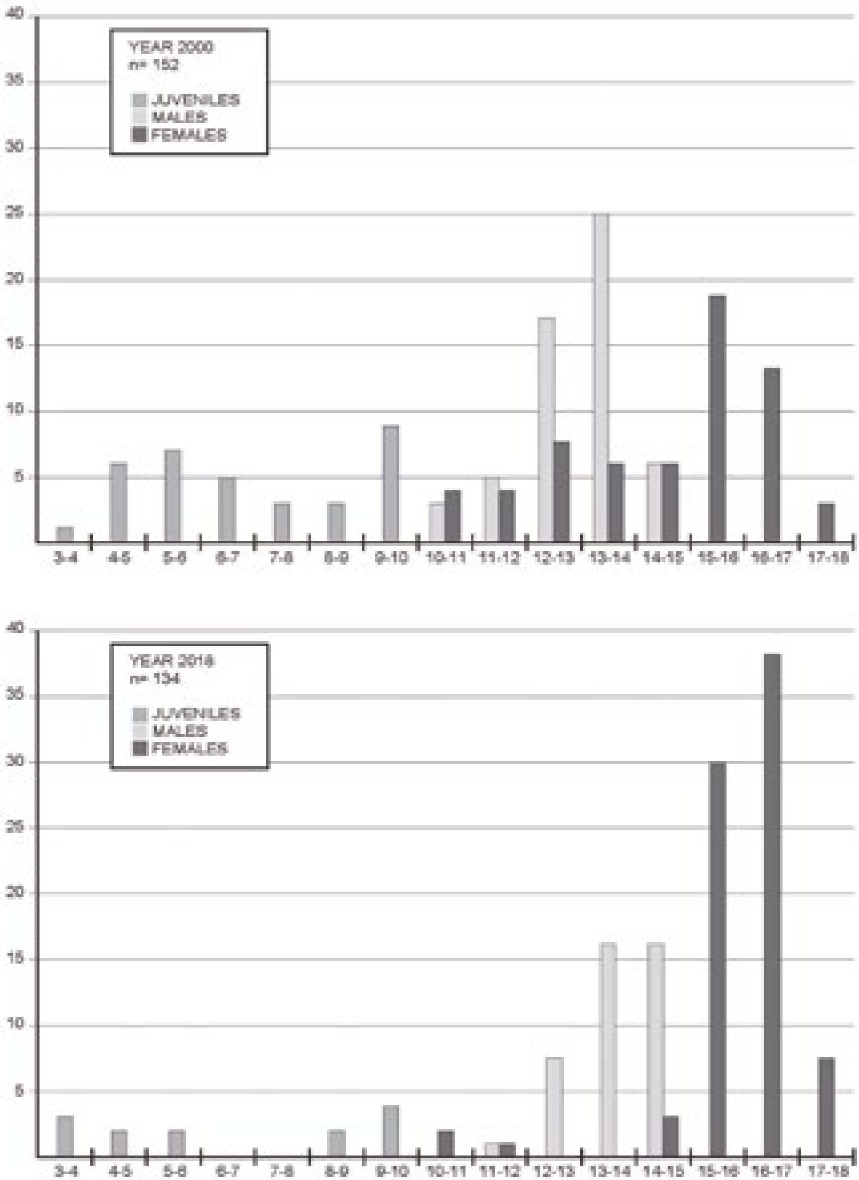

Figure 3: Up) Size classes in 2000. Down) Size classes in 2018

primary prey and also by the disappearance of old-time trappers. Furthermore, other threats like forest fires, emerging diseases and climate change (altering the sex ratio) endanger population persistence. So it is necessary to define and provide conservation actions to recover and preserve the *T. hermanni* population at Serra de l'Albera.

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Habitats of *Testudo hermanni* in Italy: an overview from a conservation perspective

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Abstract

Three *Testudo* species are present in Italy, of which the autochthonous *T. hermanni hermanni* is at most risk in this country. Like the rest of its range in the Mediterranean Region, this species is threatened mainly by illegal collection, habitat loss and alteration following the rapid expansion of intensive agriculture, the abandonment of traditional agricultural practices, urbanisation and fire. This study presents an overview of the main vegetation features and land uses of the sites inhabited by *T. hermanni*, which derives from field research conducted throughout the Italian species range. It particularly focuses on the presence of common elements among sites that differ in geographical location and land-use pattern. All the sites are characterised by: i) habitats ascribable to the ecological series having evergreen (more rarely deciduous) oak forests as climax vegetation; ii) habitat mosaic, including open areas (rich in Asteraceae and Fabaceae) and bushy vegetation, or the equivalent contact zones. The results are discussed within the framework of conservation strategies, including habitat management and relocation actions.

Resumen

Tres especies de *Testudo* están presentes en Italia, de las cuales la autóctona *T. hermanni hermanni* es la que corre mayor riesgo en este país. Al igual que el resto de su área de distribución en la región mediterránea, esta especie se ve amenazada principalmente por la recolección ilegal, la pérdida y alteración del hábitat tras la rápida expansión de la agricultura intensiva, el abandono de las prácticas agrícolas tradicionales, la urbanización y los incendios. Este estudio presenta una visión general de las principales características de la vegetación y los usos del suelo de los sitios habitados por *T. hermanni*, derivada de la investigación de campo realizada en todo el área de distribución de la especie italiana. Se centra particularmente en la presencia de elementos comunes entre sitios que difieren en ubicación geográfica y patrón de uso del suelo. Todos los sitios se caracterizan por: i) hábitats atribuibles a la serie ecológica que tiene bosques de robles perennifolios (más raramente caducifolios) como vegetación clímax; ii) mosaico de hábitats, que incluye áreas abiertas (ricas en Asteraceae y Fabaceae) y vegetación arbustiva, o las zonas de contacto equivalentes. Los resultados se discuten en el marco de las estrategias de conservación, incluyendo la gestión del hábitat y las acciones de reubicación.

Keywords: conservation, habitat management, Hermann's Tortoise, land-use cover, relocation.
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1. Introduction

Testudo populations are seriously threatened throughout the Mediterranean Region by illegal collection, habitat loss and alteration, mainly due to the rapid expansion of intensive agriculture, abandonment of traditional agricultural practices, urbanisation and fire (i.e., Hailey, 2000; Pérez *et al.*, 2004; Bertolero *et al.*, 2011). In Italy, three species occur: *T. graeca* Linnaeus, 1758; *T. marginata* Schoepff, 1792, naturalised and of ancient introduction; and *T. hermanni* Gmelin, 1789, which is the species at most risk in this country. This species is classified by IUCN as Near Threatened in the global assessment (van Dijk *et al.*, 2004), and as Endangered in the Italian assessment (Rondinini *et al.*, 2013).

Illegal collection and trade are still dramatically commonplace, as is the illicit release to nature of "pets", a possible cause of disease dissemination and genetic pollution. This entails reduced natural populations and the demanding problem of managing confiscated individuals. Relocation is a possible solution for the autochthonous subsp. *T. h. hermanni* (hereafter *T. hermanni*), but only after the genetic and health assessments of individuals and the correct selection of release areas (MATTM, 2019). Despite being present throughout the Peninsula, in Sardinia, Sicily and in several minor islands, *T. hermanni* distribution is highly fragmented within most of its range (Corti *et al.*, 2014). Populations often occupy residual patches of natural and seminatural vegetation, surrounded by unsuitable land uses. Hence they are at particular risk due to isolation.

Conservation actions, including habitat management, may be crucial to preserve suitable ecological conditions in these areas. As long-lived species with limited dispersal abilities, tortoises are very vulnerable to habitat loss and fragmentation (Hailey, 1989; Pough, 2004). Gaining knowledge on the habitats frequented by *T. hermanni* is necessary to plan effective conservation measures and correct relocation. This study provides a qualitative description of the main vegetation features and land uses at the sites inhabited by *Testudo hermanni* in Italy to: i) account for the variability of the habitats occupied by this species throughout its national range; ii) find key features that may guide the choice of release areas in relocation programmes; iii) provide habitat management indications. Data were collected as part of a project financed by

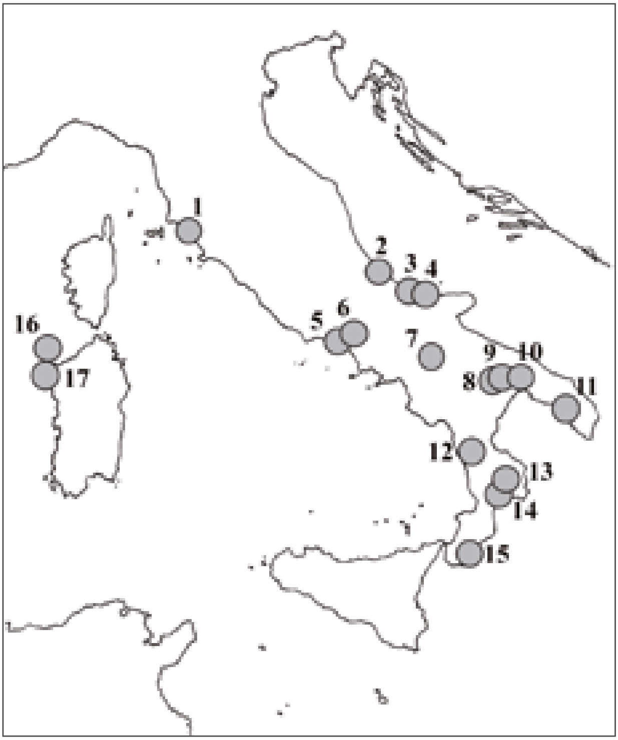

Figure 1: Distribution of the study sites in the Italian Peninsula and in Sardinia.

the "Ministero dell'Ambiente e della Tutela del Territorio e del Mare" (now Ministero della Transizione Ecologica) to compile guidelines for the relocation of confiscated *Testudo* individuals (MATTM, 2019).

2. Materials and methods

During the 2014-2016 period, 17 sites were surveyed throughout the Peninsula and Sardinia, where natural populations or recent sightings of *Testudo hermanni* were recorded (Figure 1). Each site was delimited based on natural or anthropogenic boundaries (i.e., water bodies, roads, intensive fields) and covered a minimum surface of 2 km^2 and, thus, covered average *T. hermanni* daily movements (Chelazzi & Francisci, 1979; Stubbs & Swingland, 1985). For each site, the following were identified: i) the main vegetation features, relative ecoregion and potential natural vegetation, as defined by Blasi *et al.*, (2014); ii) land-use composition by overlaying a 500 × 500 m grid to the satellite images of the study sites and assigning to each mesh the prevailing (> 50%) Corine Land Cover (CLC) class; iii) the 92/43/EEC habitats (Biondi *et al.*, 2010). At nine sites (Table 1), floristic spring surveys were performed (not herein reported in depth; see Corti *et al.*, 2016) following the Braun-Blanquet (1951) methodology in sampling areas of 100 m^2 in forests and of 25 m^2 in open habitats.

Table 1: Study sites (with a brief description) are listed under the corresponding ecoregions (D = Division; P = Province; S = Section; PNV = potential natural vegetation, from Blasi *et al*., 2014). Floristic surveys were performed in sites marked with *.

Site	Italian Ecoregions and site description
Mediterranean D., Thyrrhenian P., Northern and central Tyrrhenian S. PNV: deciduous oak forest with *Quercus cerris* (29%), *Q. frainetto* (12%) *Q. virgiliana* (12%), prevailing inland; evergreen oak forest with *Q. ilex* (14%) confined to the coastal strip and to south-facing carbonate slopes; widespread hygrophilous vegetation along the riparian zones (19%).	
1	Contact zone between oak (*Q. ilex*) and sclerophyllous forests and anthropogenic habitats, located near the coast; after-blaze and after-fire garigue (*Cistus* spp., *Ampelodesmos mauritanicus*) and ecotonal boundaries with bramble bushes adjoining fields. 9 km^2; 50-350 m a.s.l.
Mediterranean D., Adriatic P., Central Adriatic S. PNV: deciduous oak forests with *Quercus virgiliana* (66%), *Q. cerris* or *Q. pubescens*; widespread potential for hygrophilous vegetation of the riparian zone (21%).	
2	Mosaic of residual sub-Mediterranean forest (*Q. pubescens*) and cultivated lands (arable fields, olive groves and orchards) admixed with frequent minor landscape elements (reeds, hedges, dismantled quarries). 9.5 km^2; 60-110 m a.s.l.
Mediterranean D., Adriatic P., Southern Adriatic S. PNV: evergreen oak forests with *Quercus ilex* (35%), and other evergreen physiognomies, such as *Q. calliprinos* (2%) and *Pinus halepensis* (3%); uncertain setting of PNV types (44%) due to the high level of anthropisation, but certainly including semi-evergreen, semi-deciduous and deciduous oak forests (*Q. trojana*, *Q. ithaburensis* subsp. *macrolepis*, *Q. virgiliana*, *Q. dalechampii*, *Q. pubescens*, *Q. cerris* and *Q. frainetto*).	
3*	Residual sand dune vegetation, surrounded by roads and cultivations. 5 km^2; 0-10 m a.s.l.
4	Sand dune vegetation with junipers, garigue dominated by Rosmarinus officinalis, sparse *Pinus pinea* reforestations and *P. halepensis* along the coast; residual sclerophyllous maquis and oak forests inland. 21.5 km^2; 0-10 m a.s.l.
8	Not intensively cultivated area next to natural clay gullies; sparse bushy vegetation, due to frequent clearcutting, grazing and fires, and naturally drifting to oak forest, surrounding the arable fields. 8 km^2; 330-340 m a.s.l.
9*	*Pinus pinaster* re-forestation with sparse underwood vegetation made up of *Pistacia lentiscus* and bushes on a calcareous substratum. 7.5 km^2; 100-340 m a.s.l.
10*	Sub-Mediterranean deciduous forests alternated with pastures, cultivated lands and bushy transition vegetation on a compact calcareous substratum. 8 km^2; 320-460 m a.s.l.
11*	Residual coastal vegetation on a compact calcareous substratum with karst phenomena. Scarce halophilic vegetation (*Limonium*) along the coastal strip; glasswort habitats, pastures and sclerophyllous bushes inland. 4 km^2; 0-50 m a.s.l.
Temperate D., Apennine P., Central Apennine S. PNV: *Quercus cerris* (29%), *Ostrya carpinifolia* (25%), *Fagus sylvatica* (19%), *Q. virgiliana*/*Q. pubescens* (13%) forests.	
7*	Heterogeneous habitat matrix in which the original broad-leaved forests, still present in quite large surfaces, have been converted into pastures, cereal fields, sporadic olive groves and conifer reforestations. 16 km^2; 450-750 m a.s.l.

Mediterranean D., Thyrrhenian P., Southern Tyrrhenian S. PNV: thermophilous mixed evergreen and deciduous *Quercus virgiliana* forests (31%). *Q. ilex* evergreen forests (6%) and Mediterranean maquis in the area including sites 13, 14; potential for *Q. cerris* (25%), *Q. frainetto* (7%) and *Fagus sylvatica* (12%) deciduous forests at upper elevations.

5*	Coastal area with typical sand dune vegetation dominated by *Pistacia lentiscus* and pine forests (*Pinus pinea*). 2.5 km^2; 0-10 m a.s.l
6*	Limestone formation with arid rocky slopes, mainly devoted to pastures, characterised by sparse garigue and therophytic grasslands; small valleys covered by dense residual deciduous forests; fields rich in herbaceous and bushy boundaries at the slopes' base. 7.5 km^2; 160-300 m a.s.l.
12*	Traditionally cultivated areas (arable fields but also vineyards and olive groves) interspersed with rows of trees and bushes, used as property boundaries, and very small oak woodlots; scarce use of chemicals indicated by the abundance of archaeophytes. 16 km^2; 50-290 m a.s.l.
13	Slopes covered by abandoned vineyards, *Eucalyptus* reforestations and a mosaic of herbaceous and bushy vegetation of anthropogenic origin (pastures, fires) substituting the original oak forests. 8 km^2; 500-550 m a.s.l.
14	Abandoned olive groves and orchards, actually used as pastures and subject to re-colonization processes by the surrounding oak forests. 8 km^2; 170-470 m a.s.l.
15*	Vast area along the slopes of Aspromonte, from the riverbed up to mountain altitudes, with *Q. frainetto* forests and clearances mainly due to pastures and fires. 7.5 km^2; 160-780 m a.s.l.

Mediterranean D., Thyrrhenian P., Sardinian S. PNV: evergreen oak forests, with *Quercus suber* (36%) on clastic and igneous sub-plain sediments and *Q. ilex* (35%) on steep slopes or rocky substrata; coastal strip with varied Mediterranean communities (9%) with *Juniperus phoenicea* subsp. *turbinata*, *Olea europaea* var. *sylvestris*, *Q. calliprinos*, *Pinus halepensis* and *P. pinaster*.

16	Prevalence of garigue, made up of sclerophyllous vegetation or *Euphorbia arborea*, *Astragalus terraccianoi*, *Centaurea horrida*, alternated with meadows and maquis. 37 km^2; 0-300 m a.s.l.
17	Original oak forest (*Q. ilex*) substituted by a mosaic of Mediterranean maquis, open grassland and small garigue patches within a cultivated pine forest (*Pinus pinea*). 8.5 km^2; 0-50 m a.s.l.

3. Results

Site description and ecoregions' vegetation features are reported in Table 1. Ten CLC classes and 17 92/43/EEC Directive habitats were identified at the 17 study sites (Figure 2). Agricultural areas, present at all the sites, covered from 19% to 94% of the site surfaces (excluding site 16, with 9% of land cover). Broad-leaved forests characterised 14 sites, and covered 7-78% of their surfaces. Shrub and herbaceous vegetation associations were present at 14 sites, with 22-91% of surface cover (excluding site 12 with 3%). See Figure 2 for the land-use proportions at each site. The following were identified: 675 vascular plant species (listed in Corti *et al.*, 2016): 392 (58%) observed at only one site; 7 at 7 sites (*Anagallis arvensis* L., *Asparagus acutifolius* L., *Dactylis glomerata* L., *Dasypyrum villosum* (L.) *P. Candargy*, *Hypochoeris achyrophorus* L., *Sherardia arvensis* L., *Trifolium campestre* Schreb.); *Raphanus raphanistrum* L. and *Silene gallica* L. at 8 sites; only *Briza maxima* L. at all the sites. Of the 354 identified genera, 133 (38%) occurred at one site; 14 at 7 sites (*Allium*, *Anagallis*, *Avena*, *Convolvulus*, *Dactylis*, *Dasypyrum*, *Daucus*, *Euphorbia*, *Lathyrus*, *Lolium*, *Olea*, *Papaver*, *Plantago*, *Sherardia*); 12 at 8 sites (*Asparagus*, *Bromus*, *Cistus*, *Crepis*, *Echium*, *Hypochoeris*,

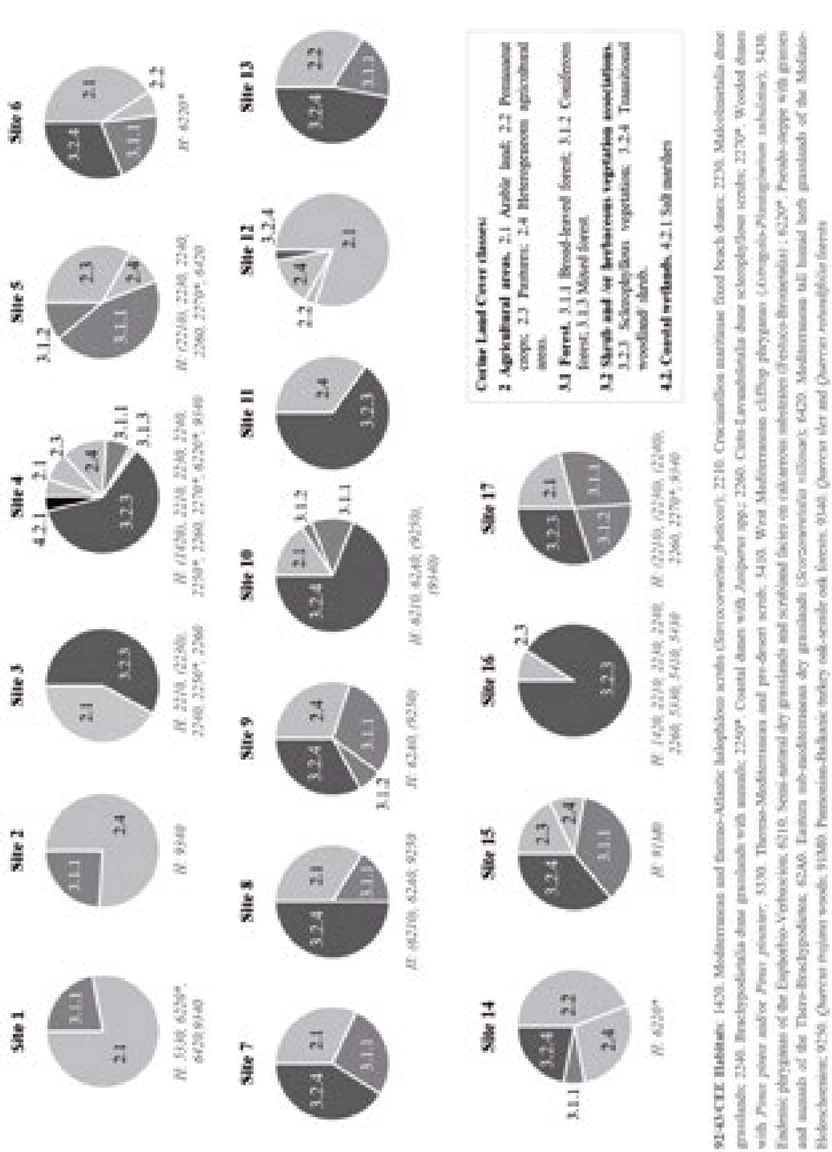

Figure 2: Percentages of surface cover of Corine Land Cover classes (pie charts) in 17 study sites in the Italian Peninsula and Sardinia and list of the 92/43/CEE Habitats (H) identified by direct observation or bibliographic reports (in brackets).

Medicago, *Quercus*, *Raphanus*, *Silene*, *Vicia*, *Vulpia*); *Briza* and *Trifolium* at all the sites. The families with higher species diversity were Fabaceae (13.9% of the identified plants), Asteraceae (11.9%) and Poaceae (11.3%).

4. Discussion

Surveying numerous areas where the endangered tortoise *Testudo hermanni* occurs throughout Italy resulted in a wide variety of environments. However, some common elements appear among sites, with significant implications for conservation strategies. Indeed the macroscopic differences observed among sites are due mainly to the different land uses and surrounding anthropic activities that have occurred over time rather than to primary ecological factors. All the sites are characterised by phytocoenoses that naturally evolve in Holm oak (*Quercus ilex*) forests or, less frequently, in deciduous oak forests, as observed in other countries (Celse *et al.*, 2014). The description of ecoregion potential natural vegetation confirms such evidence on a higher spatial scale. Based on aspects like biogeography, climate and physical land features (Blasi *et al.*, 2014), the ecoregion classification can be a useful interpretation key of the actual vegetation patterns in the areas characterised by long-lasting human exploitation and secondary successional processes, like most of those inhabited by *Testudo*. Accordingly, Stubbs (1989) suggests that *T. hermanni*'s preferred habitat, frequently identified as "arid, scrubby hillsides and farmland", is originally the holm oak forest, which have been profoundly modified by human activities over time. The assessment of the land-use cover percentages highlights that the copresence of open and close habitats is a further common element among the areas inhabited by *T. hermanni*. A mosaic of phytocoenoses and relative contact zones or clearances with herbaceous and sparse bush vegetation, characterise almost all the sites (pers. obs.). This environmental heterogeneity ensures the suitable ecological conditions for thermoregulation, refuging, egg laying and foraging (Rugiero and Luiselli, 2006; Filippi *et al.*, 2010; Celse *et al.*, 2014). *T. hermanni* is mainly herbivorous and feeds mostly on herbaceous annual species (Cheylan *et al.*, 2010). Given the different location and land-use pattern characterising the surveyed areas, it is not surprisingly that sites share a low portion of plant species and genera. However, the most frequent herbaceous species belong to the families most actively selected by *T. hermanni* (Asteraceae, Fabaceae and secondly Poaceae; Cheylan *et al.*, 2010), and that likely represent the basic elements of its diet. These herbaceous plants commonly occur in plain and hilly natural and seminatural open areas in Italy, and "food vicariance" phenomena probably arise within the genera of the same family. Similarly for scrub vegetation and bushes, it is possible to indicate the preferred taxa within specific habitats or defined areas (Del Vecchio *et al.*, 2011), but different species may perform the same ecological function in distinct environments. Agricultural lands of varying extensions are present at all the surveyed sites, which proves the strong impact of human activities on terrestrial tortoises' habitat. Actually, all the sampled populations are undergoing an alteration and habitat loss process. However, in the past, non-intensive activities like traditional agriculture and grazing may have favoured habitat complexity and the creation of open areas suitable for tortoises, but, nowadays, intensive agriculture, which entails the creation of huge cultivated surfaces and the intensive use of chemicals and machineries, is currently considered one of the main threats for *T. hermanni* (Cheylan *et al.*, 2010). If suitable conditions are preserved, tortoise populations may survive even in small areas (Hailey & Willemsen, 2003), where prevention from habitat loss due to land-use change is the primary conservation need. Nevertheless, considering that the phytocoenoses typical of the areas inhabited by *T. hermanni* naturally evolve to evergreen or deciduous oak forests, habitat management may become an equally key action in residual areas of natural vegetation or at sites where traditional farming and grazing have been abandoned. Indeed forest overgrowth may negatively affect populations on a local scale (Coututier *et al.*, 2014; Luiselli *et al.*, 2014), or may at least make surfaces wider (often not available), which are necessary for the survival of viable populations. In fact in more intact or uniform environments, where phytocoenoses display less discontinuous extensions, tortoises tend to concentrate their activities along ecotonal zones (e.g., in coastal pine-forests, Del Vecchio *et al.*, 2011) thus exploiting minor portions of sites. Management interventions are, thus, needed to maintain open areas and a sufficient degree of habitat complexity (Celse *et al.*, 2014). By way of conclusion, of the main environmental requirements for release areas in relocation programmes, we suggest the presence of: i) habitats ascribable to the ecological series having evergreen (or deciduous) oak forests as climax vegetation; ii) habitat mosaic, including about 25% of sparse bushy vegetation or the equivalent contact zones and open areas rich in Asteraceae and Fabaceae. Relocation areas should also: iii) be included within the species range, but far from sites inhabited by wild *Testudo* populations; iv) lack sources of (anthropogenic) threats (MATTM, 2019). An effort towards the census and conservation of the areas that present such features could contribute to *T. hermanni* conservation. We recommend habitat managing as a possible conservation tool to be evaluated on a site-specific basis.

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Effects of land-use changes on terrestrial tortoises: a spatially explicit individual-based model approach

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Abstract

Mediterranean ecosystems are undergoing habitat loss and fragmentation caused by human activities. These factors of change have become more noticeable in recent decades through both intensification processes and rural abandonment. They represent one of the main threats to biodiversity on a global scale. In this study, we analyse the main impacts of land-use cover change on the populations of the spur-thighed tortoise (*Testudo graeca*), a sensitive species to land-use changes that has limited movement capacity. To do this, we applied a spatially explicit individual-based model (STEPLAND) that simulates the movement and demographic processes of individuals in space over time. The results of this study show the effects on population density of different landscape scenarios of change: i.e, abandonment of traditional non-irrigated crops and intensification (intensive agriculture and urban infrastructure); scenarios with/without roads. These results are particularly useful for establishing conservation strategies for species with limited mobility based on landscape connectivity.

Resumen

Los ecosistemas Mediterráneos están sufriendo una pérdida de hábitat y fragmentación causada por actividades humanas. Estos factores de cambio se han acentuado en las últimas décadas, tanto a través de procesos de intensificación como de abandono rural, y representan una de las principales amenazas a la biodiversidad a escala global. En este estudio analizamos los principales impactos de los cambios en el uso del suelo sobre las poblaciones de tortuga mora (*Testudo graeca*), una especie especialmente sensible que posee baja capacidad de movimiento. Para ello, se aplica un modelo espacialmente explícito basado en el individuo (STEPLAND), que simula los procesos de movimiento y demográficos de los individuos en el espacio a lo largo del tiempo. Los resultados de este estudio muestran los efectos en la densidad poblacional de diferentes escenarios paisajísticos de cambio, como el abandono de cultivos tradicionales de secano y la intensificación (agricultura intensiva e infraestructuras urbanas), así como escenarios sin/con carreteras. Dichos resultados son de especial utilidad para establecer estrategias de conservación de especies de movilidad reducida basadas en la conectividad paisajística.

Keywords: habitat fragmentation, habitat loss, landscape connectivity, Mediterranean ecosystem, *Testudo graeca*.
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1. Introduction

Land-use cover change is one of the main drivers of global change that affect ecosystem structure and functioning (Foley *et al.*, 2005). Different types of land-use changes and linear infrastructures generate habitat loss and fragmentation, which directly threaten biodiversity by causing species losses. For this reason, these effects of land-use cover changes are particularly important in conservation ecology and biology (Sala *et al.*, 2000). Moreover, understanding the effects of dynamic landscapes that undergo habitat loss and fragmentation by roads are very interesting for animal species population viability (Arce-Peña *et al.*, 2019; van der Ree *et al.*, 2009), and may be crucial for ecosystem management and conservation (Carta *et al.*, 2018).

Landscapes in different parts of the world may be subject to land-use changes, which generate landscapes in transition gradient from natural to anthropic ecosystems, or in the opposite direction (Foley *et al.*, 2005). They may cause polarisation of landscapes, especially in typical mosaic structures of cultural and historical Mediterranean landscapes. On the one hand, rural land abandonment has increased the proportion of shrubland areas (Esteve-Selma *et al.*, 2003) through passive habitat rewilding (Pereira & Navarro, 2015). On the other hand, anthropisation processes have increased the proportion of intensive agriculture, urban areas and linear infrastructures (Laurance *et al.*, 2014). These contrasting types of land-use transitions have been observed worldwide (Tzanopoulos & Vogiatzakis, 2011; Carta *et al.*, 2018), but very little is known about their effects on local- and regional-scale biodiversity because of the difficulty of long-term species monitoring (Lindenmayer *et al.*, 2012). These effects of dynamic landscapes on populations tend to appear in the long term (also known as "extinction debt"), especially for long-lived species (Jiménez-Franco *et al.*, 2022). In this context, simulation models are particularly relevant for studying dynamic landscapes effects on population viability seeing that empirical analyses would be very costly in the long term (Graciá *et al.*, 2023).

The overall objective of this study is to evaluate the influence of different landscape evolution processes on the viability of the animal populations that inhabit Mediterranean mosaic landscapes to specifically quantify the effects of different types of land-use changes (agricultural abandonment and anthropisation process) and linear infrastructures (roads) on population density. We selected the spur-thighed tortoise (*Testudo graeca*) in south-eastern Spain as the study system and employed data on real land-cover changes in three reference years (1956, 1986 and 2010) in selected 5 km × 5 km landscapes. We used the spatially explicit individual-based model (IBM) STEPLAND, which synthesises data from almost two decades of intense field studies on the movement (Anadón *et al.*, 2012), habitat use and demography of the spur-thighed tortoise (Graciá *et al.*, 2020a; Jiménez-Franco *et al.*, 2020).

We hypothesised that various landscape evolution processes would affect population density differently, with positive effects of agricultural abandonment and negative effects of anthropisation proce^{s}ses due to habitat loss. We also hypothesised that linear infrastructures would decrease the population density of the study species due to landscape fragmentation. We hope that this modelling framework can be extrapolated to different contexts of successional changes in Mediterranean mosaic landscapes to predict the influence of these transitions on population demography, especially for limited dispersal ability species, and to establish management proposals for different animal populations.

2. Materials and Methods

Study system and landscape scenarios

We selected the spur-thighed tortoise *T. graeca* as a study system. It is an ideal model species because it inhabits transition biomes between Mediterranean regions and arid areas where dynamic land-use cover changes have occurred over time (De Aranzabal *et al.*, 2008), and anthropic threats are posed in its habitat due to habitat loss and fragmentation (Rodríguez-Caro *et al.*, 2017, Graciá *et al.*, 2020b). It is a long-life species whose courtships are observed through their breeding season in spring, in which males are very active while searching for females, and sperm storage is a key biological trait to avoid the mate-finding Allee effect with low densities (Jiménez-Franco *et al.*, 2020).

The study area of landscape evolution used for this study is located in a Mediterranean ecosystem in the Region of Murcia (SE Spain) that has hosted *T. graeca* populations during the historical period (Anadón *et al.*, 2006). As most Mediterranean countries, this area has undergone land-use transformations for socio-economic reasons (agricultural intensification since the 1980s, tourism and the construction of new linear infrastructures like highways; De Aranzabal *et al.*, 2008) by generating both anthropisation processes and agricultural abandonment with natural revegetation processes. Therefore, we selected real landscapes of 5 km × 5 km in the distribution area of *T. graeca*, which represent the main land-use transformations in Mediterranean ecosystems. The agricultural abandonment of traditional agricultural uses (involving crops, pastures and woody species, e.g. olives, almond trees, grapevines, etc.) has generated the "Los Mayorales" crops-scrub transition (Figure 1). This landscape evolution replaces traditional agricultural lands (crops habitat) in favour of an increase in Mediterranean scrub. We considered the anthropisation process (in the "Los Estrechos" study area) to be transitions towards artificial habitats (urban habitat) and agrarian intensification (intensive agriculture habitat) (Fig. 1). We highlighted two temporal events of change: a historical landscape of 1956, a land-use cover of 1987 (a period with changes in the agricultural system, plus social effects of rural abandonment) and land cover in 2010 (a period with changes in human intensification and new technologies for agricultural intensifications) (Figure 1). Moreover, the effects of linear infrastructures were evaluated as an independent land-use change for both landscape evolutions. Hence the presence and absence of linear infrastructures were contemplated for the different landscape scenarios for the three periods (1956, 1987 and 2010; Figure 1). Two types of roads were considered: highways, which are not permeable for tortoise movement; roads, which include other paved roads (see details of landscapes scenarios in Jiménez-Franco *et al.*, 2022). Note that the "Los Mayorales" landscape scenario has a pavement road, whereas "Los Estrechos" has both paved roads and a highway (Figure 1).

STEPLAND: a spatially explicit individual-based model to simulate spur-thighed populations' viability

The STEPLAND model integrates demographic processes with a previously developed IBM of the tortoise movement model by considering habitat structure (Anadón *et al.*, 2012; Graciá *et al.*, 2020a; Jiménez-Franco *et al.*, 2020). For model description purposes, we followed the Overview, Design concepts and Details protocol (ODD) proposed by Grimm *et al.* (2010). We describe the model with a full ODD version in Graciá *et al.* (2020a). The STEPLAND code, parameterisation and the main result files are available in the Figshare repository (Jiménez-Franco *et al.*, 2021). STEPLAND contains two types of entities: landscapes and tortoises. Landscapes are composed of a grid of 10 m × 10 m cells (see the details in the previous section). Tortoises have attributes sex, age and their location over time. We used initial population sizes of 250 individuals in a 5 x 5 km to represent low population densities of the species in natural Spanish populations (0.1 tortoises/ha; Anadón *et al.*, 2009). We performed an initial simulation for 50 years, where tortoises were randomly distributed across suitable habitats, proportionally to the area occupied by each type of land-use area in the landscape (for more details of simulations, see Jiménez-Franco *et al.*, 2022). It is important to consider that our STEPLAND model makes the movement of the study species different according to road type; although individuals can cross conventional roads, there is no permeability to movement on highways. However, we did not evaluate mortality due to road kills with our model.

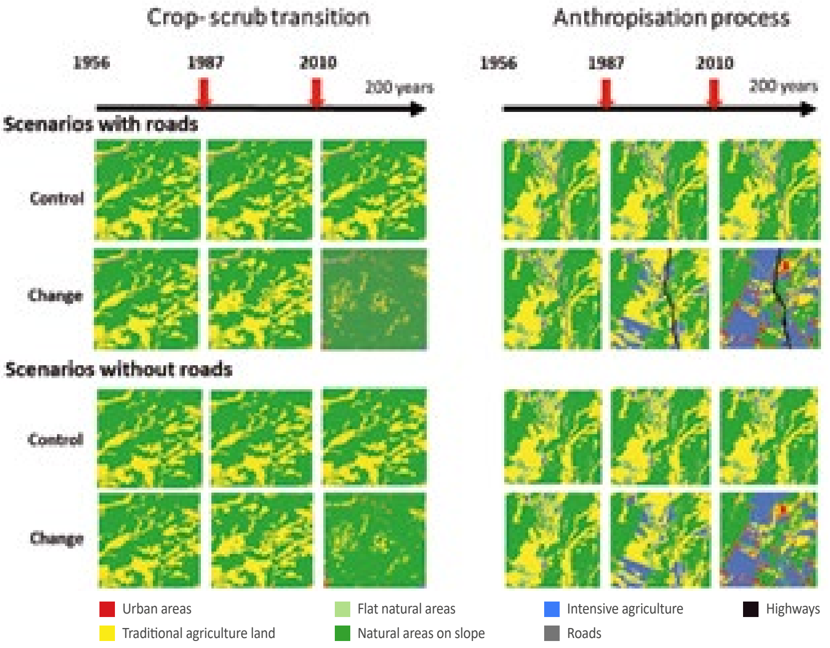

Figure 1: General overview of spatial and temporal landscape scenarios to study the effects of landscape evolution on *Testudo graeca* by using the individual-based model, STEPLAND. Black arrows show the temporal period from 1956 up to 200 years of simulation; red arrows depict the two landscape changes in 1987 and 2010, respectively. Landscape scenarios represent different transformation processes of land cover in SE Spain (5 x 5 km real areas): left: the "Los Mayorales" natural landscape scenario with agricultural abandonment to scrub; right: the "Los Estrechos" scenarios of natural landscape undergoing an anthropisation process of agricultural intensification and anthropogenic infrastructures. Each landscape evolution process shows lack of habitat transformation (without change scenarios as a control) and no linear infrastructures (without roads scenarios as a control). Habitat categories include different colours.

Design of simulation experiments and data analysis

We evaluated the effect of different land-use changes and linear infrastructures in two types of landscape evolutions (crop-scrub transition and anthropisation process) over 200 years (corresponding to approximately eight tortoise generations) in population density (ind/ha). To this end, we replicated 64 times each one of the eight types of landscape scenarios in a control/impact study design; that is, scenarios without and with land-use cover change over time (control *vs.* impact scenarios, respectively) and scenarios without and with roads (control *vs*. impact scenarios, respectively; Figure 1). Therefore, the total number of independent model simulations was 512 (2 landscape areas × 2 land-use cover change scenarios × 2 road effects × 64 replicates).

3. Results

The simulations in the two real landscape evolution processes (i.e., landscape scenarios "with change" and "with roads", Figure 1) showed that the population density of *T. graeca* had decreased in the "anthropisation process" landscape scenario. However, population density increased in the "crop-scrub transition" landscape scenario (Figure 2).

The effects of land-use cover changes (i.e., landscape scenarios without change and with change, control *vs*. impact, respectively, and considering linear infrastructures for both scenarios) showed similar effects on *T. graeca* population density for the study area of the crop-scrub transition. However, these effects on the *T. graeca* population density for the study area with the anthropisation process showed a moderate increase over time for the "control" scenario, with population density decreasing with time for the "impact" scenario (Figure 2; see the red lines with triangles and circles, respectively).

The effects of linear infrastructures (i.e., landscape scenarios "without" *vs*. "with" linear infrastructures; control *vs*. impact, respectively, and without considering land-use changes) on *T. graeca* population density were generally stronger in scenarios without roads than those with roads, especially in the study area with the anthropisation process (Figure 2; see the lines with triangles in blue and red, respectively). Therefore, the combined effects of land-use changes and the presence of linear infrastructures were the only simulation scenario that showed a clear decrease in population density during the simulation period, as well as a trend towards extinction (Figure 2).

4. Discussion

Our results showed that *T. graeca* population density changed over time in relation to the different simulation scenarios, especially in the study area of the anthropisation process. Considering the effects of land-use changes on population density, we did not find any differences in the study area of the crop-scrub transition. This could be due to the fact that *T. graeca* selects both a matrix of heterogeneous landscape between natural landscape and traditional agriculture areas, and homogeneous natural areas with scrub (Anadón *et al.*, 2006; Rodríguez-Caro *et al.*, 2017). Therefore, we highlight the fact that mosaic landscapes composed of agro-ecosystems with traditional agriculture should be conserved for the persistence of those species adapted to cultural landscapes (Fuller *et al.*, 2017). The results of the simulation models showed that loss of natural and traditional agricultural areas negatively influenced population density in the "Los Estrechos" study area. These results may be caused by the difficulty of breeding individuals having to find mates (Gascoigne *et al.*, 2009) due to landscape barriers by causing mate-finding Allee effects (Jiménez-Franco *et al.*, 2020). The results on population demography shown in this study were not produced immediately in time, but in the long term, when considering large temporal scales. These patterns on long term scales more commonly occur in long-lived species,

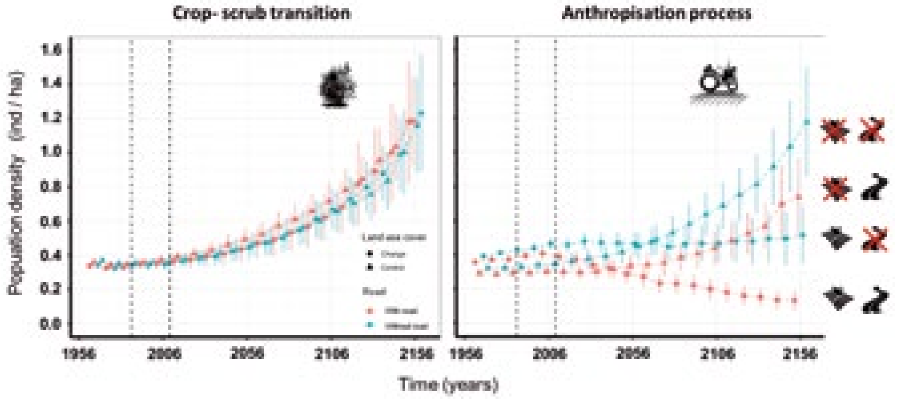

Figure 2: Mean population density (individuals per hectare, mean±confidence intervals) of *Testudo graeca* for different landscape evolution processes over 200 years (see Figure 1); left: crop-shrub transition; right: anthropisation process. The effects of land-use cover change are shown with symbols: triangles, control scenarios (without land-use cover change along simulation period); circles, impact scenarios (with land-use cover change during the simulation period), where the vertical dashed black lines indicate the two periods of land-use cover change for the "change" scenarios in 1987 and 2010, respectively. The effects of linear infrastructure (roads) appear in different colours: blue, control scenarios (landscapes without roads), where roads with reclassifying by adjacent land covers; red, change scenarios (landscapes with roads).

and suggest an important conservation issue in which past variation in land-use changes is largely a legacy of population dynamics that would have occurred 100-150 years later, and known as extinction debt (Lindborg & Eriksson, 2004; Jiménez-Franco *et al.*, 2022).

Moreover, the scenario with roads also showed negative effects on *T. graeca* population density in the "Los Estrechos" study area, where there is a highway with no permeable movement for individuals to generate mate-finding Allee effects (Jiménez-Franco *et al.*, 2020). It is worth considering that roads have a significant impact on wildlife worldwide (Polak *et al.*, 2014). Two ways to mitigate the impact of roads are to improve connectivity and to lower mortality through fences and wildlife crossings. Although our model does not evaluate road kills, it is important to note that the impact of road kills may be stronger on conventional roads than on highways. On conventional roads, the probability of individuals crossing is higher, whereas highways may be difficult to cross due to their structure and fences having been installed.

This modelling approach (IBM) is especially useful for management and conservation in order to better understand and predict the impacts of landscape evolution on populations when these land-use changes are based on real spatial and temporal dynamics. It allows to propose a proper land-use policy instead of restoring the impacts on biodiversity and ecosystem services caused by anthropisation processes (Bullock *et al.*, 2011), especially when endangered species live in association with land-use cover changes.

To conclude, our results show that both land-use changes and linear infrastructures (highways and roads) decrease *T. graeca* population density, with land-use changes having a stronger effect because they generate greater habitat loss (replacement of scrubland and crops with anthropic uses), while the effects of linear infrastructures decrease the landscape connectivity. However, the effects of linear infrastructures should be studied in more detail to identify loss of functional connectivity (e.g., at fragmented populations' genetic level), evaluate the impact of road kills on animal species, and assess mitigation measures to conserve animal populations, such us corridors and wildlife passages (Polak *et al.*, 2014).

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Effects of climate change on the potential distribution of *Testudo graeca* in southeastern Iberian Peninsula

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Abstract

The effects of climate change for the 2040-2070 time window on *Testudo graeca* distribution and abundance in the SE Iberian Peninsula were analysed using species distribution models (SDMs). This was donet by means of successive tests, where data quality progressively improved. The obtained results showed that the projected climate change effects reduced and the quality of data employed for SDMs improved. In the most elaborated models (*Testudo graeca* abundance using climate, topographical, lithological and land-use variables), climate modifications would severely alter this species' current area between 30% and 60%. These results are very different from those previously reported in more global studies. The sources of these possible discrepancies are discussed in data quality terms. The interest in acquiring data about local factors is emphasised because they significantly modulate the commonest bioclimatic models, especially in biogeographical ecotones. The use of these local factors will provide a more realistic approach to the response of current *Testudo graeca* populations, which is relevant especially for species whose dispersal is limited.

Resumen

Se han analizado mediante SDM los efectos del cambio climático, para la ventana temporal 2040-2070, en la distribución y abundancia de *Testudo graeca* en el sureste Ibérico. Dicho análisis se ha realizado mediante sucesivos ensayos donde se ha mejorado progresivamente la calidad de la información. Los resultados obtenidos muestran que los efectos del cambio climático proyectados se reducen en función de la calidad de la información usada para los SDM. En los modelos más elaborados (abundancia de *Testudo graeca* y variables climáticas, topográficas, litológicas y del uso del suelo) las modificaciones climáticas alterarían severamente entre el 30% y el 60% del área actual. Estos resultados son muy diferentes a los señalados en estudios previos más globales. Se discuten las fuentes de estas posibles discrepancias, en relación, a la calidad de la información utilizada, y se insiste en el interés de disponer de información sobre factores locales que modulan significativamente los modelos bioclimáticos más habituales, especialmente en ecotonos biogeográficos. El uso de estos factores locales permitirá disponer de una aproximación más realista sobre la respuesta de las poblaciones actuales de *Testudo graeca*, lo que resulta muy relevante especialmente en especies de dispersión limitada.

Keywords: distribution projection, bioclimatic models, *Testudo graeca whitei.*
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1. Introduction

To know a species' response to climate change is one of the greatest challenges for conservation policies to face. General models predict an altitudinal and latitudinal shift in most species, as well as a higher extinction risk for different Mediterranean taxa (Thuiller *et al.* 2005), especially in fragmented and restricted distribution populations. Exploring effects on species like the spur-thighed tortoise (*Testudo graeca*) is an urgent requirement, whose peninsular populations are located mainly in the semiarid landscapes of the SE Iberian Peninsula in Murcia and Almería (Anadón *et al.* 2006, 2007, 2010). Available studies indicate a wide typology of responses (Esteve-Selma *et al.* 2010, 2012, 2017, Esteve *et al.* 2015) from species with an expected significant expansion to those whose potential habitat shrinks, and have to move to a higher altitude and latitude, with local extinctions and others that might even disappear. To date however, the climate change impact on singular fauna species, such as the spur-thighed tortoise, has not yet been analysed in detail beyond a very suggestive general study (Araujo *et al.* 2011) that was carried out with certain methodological restrictions that its global goals imposed. Other related studies have determined the bioclimatic area of different *Testudo graeca* subspecies in the Mediterranean in relation to their evolution during the Holocene (Anadón *et al.* 2015).
Many modelling process factors can significantly affect the results. One key aspect is source data quality, which refers to both the species and physical factors that condition species distribution. Low-cost approaches (presence of data, models done with a few climate variables and low spatial resolution, among other issues) can provide results that are difficult to interpret, and can sometimes be of little use for biodiversity conservation policies. Some useful references in Araujo *et al.* (2011) match this debate.
In species with limited dispersal capacity, such as *Testudo graeca*, the main question to answer is not the possible occupation of new emerging areas of bioclimatic suitability associated with new climate conditions, which will be hard to colonise without assisted migration, but how current distribution areas will respond with populations directly affected by such changes. These current populations are those that most conservation strategies should be concerned about. Fine enough models run with the required precision provide a detailed understanding of these populations' possible behaviour, along with design intervention measures to guarantee their mid- and long-term conservation.

According to this logic, the objectives of this work were to: i) model *Testudo graeca* distribution and abundance in SE Spain; ii) explore the expected effects of climate change on *T. graeca graeca* distribution; iii) discuss the role of data quality on the type of results that can be obtained.

2. Methodology

Potential distribution models

Three GLM (Generalized Linear Model) models were developed for the distribution and abundance of the studied species in the southeast Iberian Peninsula on the 1 km^2 scale: two on its potential distribution and one on its abundance. Many climate variables (annual, seasonal and extreme temperatures) were employed to characterise the standard climate (1960-1990). Local factors were also used in two of these models, particularly topographical, lithological and land-use variables (these last two types of variables on two spatial scales, 1x1 km and 3x3 km, or at the landscape level), because they tend to improve the quality of species distribution models (Heikkinen *et al.* 2006, Luoto & Heikkinen 2008, Austin & Niel 2011), especially their geographical and/or environmental limits. To know more about the set of utilised variables, both presence-absence and abundance variables, and environmental variables, can be consulted in the articles of Anadón *et al.* (2006, 2010).

The distribution models were evaluated according to the ROC (Receiver Operating Characteristics) curve and the AUC (Area Under Curve) parameters, whose value varies between 0.5 (worst possible value) and 1 (best possible value). The ROC curve also identified the probability value that best defined species presence. As the presence probability on the 1 km^2 scale can be interpreted as a habitat suitability measure, it was used to identify different habitat quality categories.

The differential characteristics of the three models are as follows:

a) First distribution model: definition of the potential bioclimatic area. The considered reference area was the southeast Iberian Peninsula (Murcia and Almería provinces). The biological data of species presence-absence were obtained from 1,115 cell units at 1 km^2. The independent variables were only climate ones. The logistic GLMs were used.

b) Second distribution model: Role of local factors in the bioclimatic area. The same data as in the first model were used, but referred to the potential bioclimatic area defined by the first model and, apart from the previously obtained climate model, it included topographical, lithological and land-use variables. The logistic GLMs were also employed.

c) Abundance model in the bioclimatic area. It is based on the abundance estimates obtained from surveys held with shepherds, calibrated with density data from field surveys (for details, see Anadón *et al.* 2009, 2010). The GLM of the Negative Binomial family were used. The model refers to the potential bioclimatic area on the 1 km^2 scale with all the available climate, topographial, lithological and land-use variables.

Climate change projections

A regional adaptation of climate model MM5 was employed with several nested spatial domains, including a high-resolution domain (10 km^2) (Jerez *et al.* 2013). The expected effects were projected in scenarios B1 (less intensive in emissions, 549 ppm of CO_2 in 2100) and A2 (more intensive in emissions, 850 ppm of CO_2 in 2100). For both, the 2040-2070 time horizon was considered.

For the 2040-2070 period, an increase in the average temperature of 1.8-1.9ºC and a significant reduction in the number of frost days (26-30%) were expected in the southeast Iberian Peninsula. Rainfall forecasts point out a more complex spatial pattern and an average decrease of 18% in B1 (only 6% in A2).

The spatial resolution of climate scenarios at the 10 km^2 resolution was changed (downscaling) as variations from the standard climate (1960-1990) at the 1 km^2 resolution.

3. Results And Discussion

Bioclimatic area definition

T. graeca responds mainly to autumn rainfall and the number of frost days and, more secondarily, to summer temperature and winter rainfall. The factors included in the model adequately explained species distribution because they absorbed 65% of total deviance. The ROC curve and AUC parameters revealed the model's high predictive potential (AUC = 0.95). According to ROC, the distribution area would be defined by the 0.2 presence probability threshold.

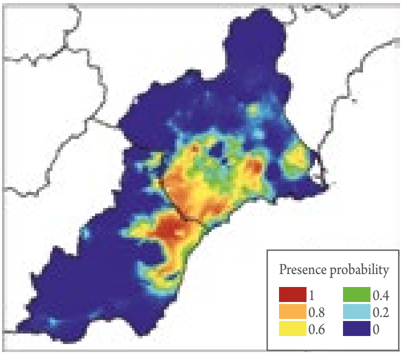

Figure 1: Potential (bioclimatic) distribution of *Testudo graeca* in the southeast Iberian Peninsula.

The potential bioclimatic distribution (Figure 1) was distributed over 5,279 1 km^2 grids, most of which were connected to one another. This area collected a total net habitat (i.e., the accumulated total habitat in these 5,279 1 km^2 grids considering each grid's presence probability, whose average value was 0.6) of around 3,195 km^2.

Role of local factors in the bioclimatic area

The habitat suitability model in the bioclimatic area included, apart from the previous climatic model, three local factors on the landscape level (3x3 km): preference for siliceous materials, rejection of irrigated-tree crops and preference for traditional dryland (rain-fed crops). The model explained 24% of deviance with an AUC of 0.81.

As seen in Figure 2, this new model redesigned suitable *T. graeca* areas by adjusting them to different physiographic elements, delimiting mountains and other optimal elevations for the species and the large valleys occupied by intensive agricultural activities, where probability fell below the threshold that defined habitat suitability (0.2). This area was reduced to 4,701 km^2, which was 11% less than the potential bioclimatic area. However, the net habitat slightly increased to 3,323 km^2 as the optimal (high probability) areas were better delineated and broader than in the bioclimatic model.

Abundance model in the bioclimatic area

T. graeca abundance in the bioclimatic area responded to the rejection of irrigated-tree crops, frost days, winter temperatures, autumn temperatures and preferences for traditional drylands. The explained deviance was 32.74%. This model (Figure 3) redefined habitat quality as a reduction in the main optimal reliefs for the species, with a certain topographic gradient and mosaic arrangement for wider density ranges

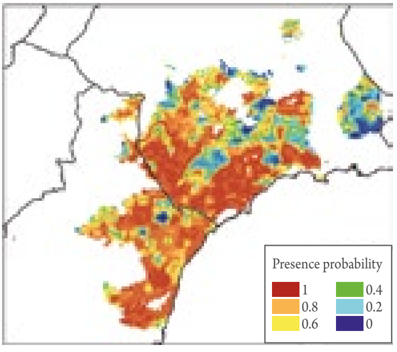

Figure 2: Final *T. graeca* distribution (including local factors) in the bioclimatic area under present climate conditions

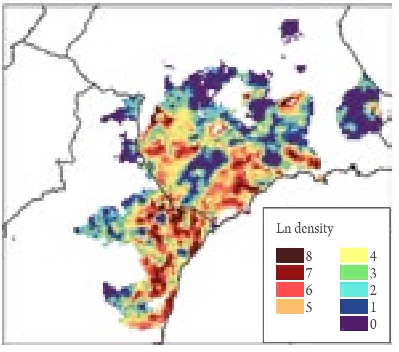

Figure 3: Abundance model in the bioclimatic area of *T. graeca* under present climate conditions.

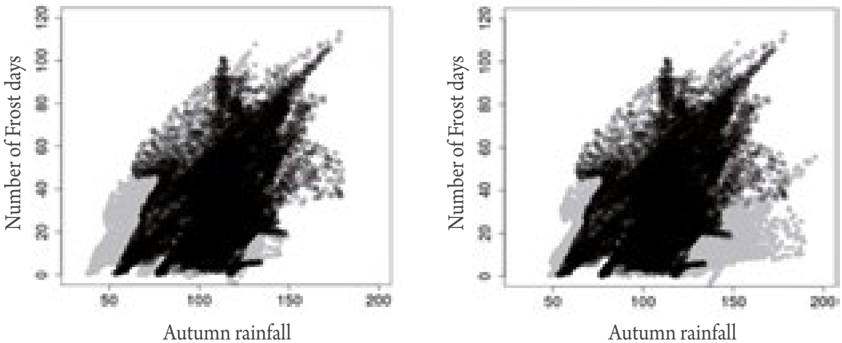

Figure 4: Degree of overlap of the present and future climate spaces according to the two climate variables with the heaviest weight in the *T. graeca* distribution model. Black points: present conditions. Grey points: new climate conditions in scenarios.

(levels 6 to 8 corresponding to densities from three to more than 14 specimens/ha; Anadón *et al.*, 2009).

Climate change scenarios

An important issue when projecting future climates is the possible existence of non-analogous climate conditions between present and future climates. This means that exploring the degree of overlap between both is necessary (Fitzpatrick & Hargrove 2009, Austin & Niel 2011) as the degree of confidence in projections is higher in areas with similar climate conditions (both climates overlap) and is lower in non-analogous ones (Austin & Niel 2011). The relevant climate space for *T. graeca* was characterised using the two main climatic variables of the species distribution model: autumn rainfall and frost days. Figure 4 provides the results about the degree of overlap of the present and future climate spaces in B1 and A2.

As Figure 4 shows, most of the future climate space would be analogous to the present one, but some areas (between 10% -B1- and 20% -A2- of the total) would represent novel combinations of autumn rainfall and number of frost days. In the areas with non-analogous climate conditions, the model projection results should be considered more cautiously.

Potential T. graeca *distribution and abundance with climate change*

First model. The bioclimatic area in scenario B1 showed a marked shift to the NW (Figure 5) from a coastal and pre-coastal distribution to the foothills of the Segura mountain ranges. This northward shift in latitude (including a rise in altitude) is a common result for many other species (Esteve *et al.* 2012, 2015). One relevant result was that this shift would give rise to a new spatial distribution with a low degree of

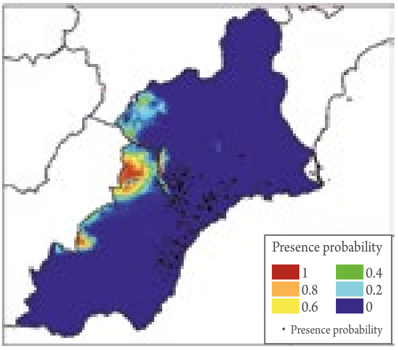

Figure 5: Potential (bioclimatic) *T. graeca* distribution in the B1 climatic scenario. Black dots denote current species presence.

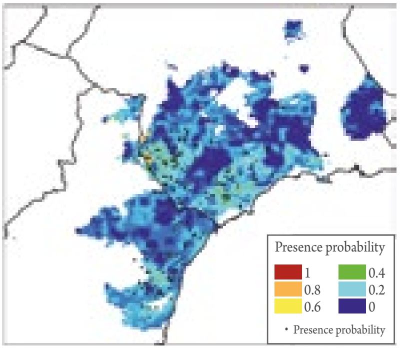

Figure 6: Final distribution model (including local factors) in the bioclimatic area of *T. graeca* in the B1 climate scenario.

overlap with the current habitat, which would mostly disappear. The overlap zone was located on the northern limit of the current distribution, which was relatively far from the current optimum. The present potential habitat would decrease by 98% in B1 and would totally disappear in A2 in both area and net habitat terms.

Second model. In this second model (Figure 6) which, together with the climate ones also included the physical and land-use variables, the reduction in distribution was 91% in area and 87% in habitat quality. The reduction pattern was latitudinal, and habitat suitability was poor (around 0.2-0.4 presence probability) in the main current distribution areas.

Third model. With the abundance model, the shift to a northern latitude was evidenced (Figure 7), although the current area proved much more resistant by maintaining good areas for the species in its main present areas, especially inland, northwardly

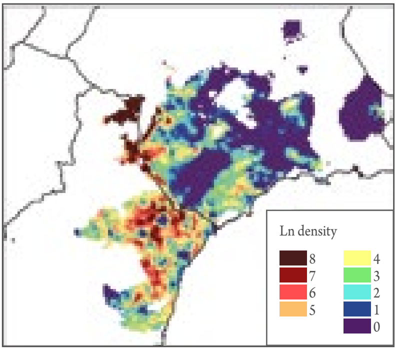

Figure 7: Abundance model in the bioclimatic area of *T. graeca* in the B1 climatic scenario.

and in the Almería populations. The reductions in both distribution area and population estimates varied between 30% (B1) and 60% (A2).

The works of Araujo *et al.* (2011) for spur-thighed tortoises in the Iberian Peninsula establish a potential bioclimatic area covering more than 27,000 km^2, of which half ranges from Murcia to Almería. These values are, according to Araujo *et al.* (2011), higher by an approximate factor of 2.5 than those obtained for these provinces in our work (< 5,300 km^2). These differences can be explained by: the strict use of presence data (instead of presence-absence data) in the study by Araujo, which also included the tortoise populations introduced in Doñana (Huelva), and they did not appear later in the potential distribution area; the use of a 100-fold less detailed spatial resolution; adjustment to three preselected variables.

The results obtained by the cited authors implied maintaining 63% and 80% of the current distribution area for the 2040-70 period, for which they considered that Iberian spur-thighed tortoise populations did not require any measure in this matter. However according to our work, at the bioclimatic level this effect would end in extreme reduction, with a maximum 2% presence of the present area. When higher quality biological and physical data were employed, climate change effects appeared to be varied and the current area was more resistant, and was around 10% for the distribution model with all the variables, and between 40% and 70% for the abundance model with all the variables. This gradual increase in resistance to climate change was consistent with the improvement in SDM when local factors were included (Heikkinen *et al.* 2006, Luoto & Heikkinen 2008, Austin & Niel 2011). Lack of these local or micro-environmental factors could overestimate extinction risks due to climate change (Suggitt *et al.* 2018). Other studies have also emphasised the influence of non-climatic factors on species sensitivity to climate change (Acevedo *et al.* 2017). Furthermore, the role of local conditions is expected to increase in species' geographical limits, which falls in line with these Iberian spur-thighed tortoise populations as suggested by the general theory of size and specificity of habitat by Neilson & Wullestein (1981). Araujo *et al.* (2011) suggested that the climate change effect would even be overestimated for Ibero-African species, which could withstand severer aridity conditions than the currently existing conditions in the Iberian Peninsula. This possible modelling bias can be largely overcome by precisely comparing the degree of coincidence of present (used to generate the SDM) and future (in which it is projected) climate spaces. However, it is very important to note that each specific *T. graeca* subspecies responds to a different bioclimatic model (Anadón *et al.* 2015), and we still do not have either high-quality biological data (at least presence-absence or abundance data on a detailed scale), or data on the role of local factors in North African *T. graeca* populations.

A reduction in the potential habitat does not translate directly and immediately into changes in populations, but can increase local extinction risks (Thomas *et al.* 2004; Thuiller *et al.* 2005), depending on other factors like land use and spatial connectivity of landscape (Heller & Zavaleta 2009; Felton *et al.* 2009), the existence or not of overlapping present and future habitats (Pearson & Dawson 2005; Heller & Zavaleta 2009) and species' dispersal capacity in relation to climate change pace (Opdam & Wascher 2004; Pearson & Dawson 2005). None of these factors seems to favour *T. graeca* populations remaining in the southeast Iberian Peninsula. However, it is important to verify the results suggested by these models with population monitoring programmes in the mid and long terms. The combination of models and experimental data is a key to help to cope with climate change effects (Esteve *et al.* 2017). Extreme events, such as forest fires and prolonged severe drought periods, can have significant impacts on local *T. graeca* populations, as previously verified (Sanz-Aguilar *et al.* 2011, Rodríguez-Caro *et al.* 2014). Both pressures will undoubtedly intensify with climate change. In fact the negative effects of droughts on the *T. graeca* reproductive pattern could even affect this species' ability to colonise in and/or withstand low-quality habitats due to habitat transformation, where the compensatory strategy involves greater reproductive activity (Rodríguez -Caro *et al.* 2014), which could be understood as an insidious or negative synergy between climate and land-use changes.

Acnowledgments

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Tortugas en cautividad: expolio, comercio, gestión y dimensiones sociales

El expolio y el tráfico de tortugas representan una de las principales amenazas para su conservación, siendo de los animales más traficados a nivel mundial. Las tortugas mediterráneas del género *Testudo* sufren especialmente el expolio debido a su aprecio como animales de compañía, con un fuerte arraigo cultural en las zonas donde existen poblaciones naturales. Además, estas tortugas también son objeto de tráfico internacional, siendo demandadas en lugares muy alejados de sus áreas de distribución natural. La extracción de tortugas del medio natural tiene un impacto directo sobre la abundancia poblacional y, por tanto, compromete su viabilidad a largo plazo. No obstante, la amenaza para las poblaciones silvestres no termina ahí.

Las tortugas mediterráneas se crían con relativa facilidad en cautividad y, con frecuencia, quienes las albergan las reproducen de manera ilegal. Como consecuencia, el número de tortugas mantenidas en cautividad es muy elevado. En general, estos ejemplares no son adecuados para contribuir a la conservación de las poblaciones naturales, ya que su estado sanitario suele ser deficiente y su origen genético es incierto.

La gestión de esta elevada cantidad de tortugas en cautividad plantea importantes desafíos, tanto desde el punto de vista de la conservación como del bienestar animal, dada la dificultad de ofrecer condiciones adecuadas a tantos individuos. En cualquier caso, la gestión de estos animales no debe, en ningún supuesto, comprometer la conservación de las poblaciones silvestres. Antes de plantear refuerzos poblacionales o reintroducciones, es imprescindible analizar cada caso de forma rigurosa y llevar a cabo únicamente aquellas acciones que hayan sido específicamente diseñadas y justificadas por tener un impacto positivo en la conservación.

La cautividad constituye una amenaza compleja en la que las dimensiones sociales juegan un papel relevante. Las tortugas no deberían ser consideradas mascotas por la población general y, del mismo modo, las tortugas mantenidas en cautividad no pueden ser asumidas por los gestores del medio natural como individuos a priori aptos para reforzar poblaciones silvestres. En este sentido, los programas de cría en cautividad han de ser ambiciosos y garantizar la conservación del patrimonio genético y de la singularidad de las poblaciones silvestres.

Es fundamental promover la colaboración entre el ámbito académico, los gestores y las asociaciones conservacionistas para investigar y comprender el fenómeno de la cautividad, y así poder revertir sus consecuencias negativas sobre las poblaciones naturales.

Tortoises in captivity: poaching, trade, management and social dimensions

Poaching and trafficking of tortoises represent one of the main threats to their conservation, as these animals are among the most heavily traded worldwide. Mediterranean tortoises of the *Testudo* genus are particularly affected by poaching due to their popularity as pets and the strong cultural attachment to them in areas where natural populations still exist. Moreover, these tortoises are subject to international trafficking, being in demand far from their native range. The removal of tortoises from the wild directly reduces population abundance and threatens their long-term viability. However, the threat to wild populations does not end there.

Mediterranean tortoises breed relatively easily in captivity, and people often reproduce them illegally. As a result, the number of tortoises kept in captivity is extremely high. These individuals are generally unsuitable for supporting wild populations, as their health is often poor and their genetic origin uncertain. Managing such large numbers presents significant challenges for conservation and animal welfare. Any management must not compromise wild populations; reinforcements or reintroductions should be rigorously assessed and implemented only if they have a clear positive conservation impact.

Captivity constitutes a complex threat in which the social dimensions of tortoises play an important role. Tortoises should not be regarded as pets by the public, and captive individuals should not be assumed to support wild populations. Captive breeding programs should be ambitious, ensuring the conservation of the genetic heritage and uniqueness of wild populations. Collaboration between academia, environmental managers, and conservation organizations is essential to investigate captivity and mitigate its negative consequences for wild populations.

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Spur-thighed tortoise non-commercial collection and trade for pets: the sociological value and the ecological consequences through its distribution range

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Abstract

Collection for pet is one of the current and historically major conservation threats to the spur-thighed tortoise throughout its entire distribution range. Social drivers behind tortoise collection for pets have received limited attention, yet there has not been acquired a baseline demographic information, which allow to face the ecological consequences of this activity. In spite of the legal protection (CITES, 1975), spur-thighed tortoise is currently illegally sold in markets of Morocco, Tunisia and Algeria, exported to Europe, and often collected with non-commercial porpoises by local inhabitants. The recent magnitude of the trade, which is mainly domestic, and noncommercial collection (22-64% inhabitants own tortoises), might be large enough to potentially jeopardize populations, as it is evidenced by demographic parameters as regards population density and structure observed in Moroccan populations. Therefore, collection for pet might be considered as an additive effect to other population decline drivers such as predators or diseases, and might receive further attention in research agenda. We recommend appropriate actions that can facilitate the conservation of this species according to a socio-ecological approach that will focus on pet owners' values, perceptions and attitudes, and demographic parameters of wild tortoise populations affected by non-commercial collection and trade for pets.

Resumen

La recolección de ejemplares de Tortuga mora para su tenencia como mascota es una de las principales amenazas a lo largo de su rango de distribución. Tanto las motivaciones sociales que llevan a adquirir estas tortugas como mascotas, como la adquisición de información demográfica de las poblaciones silvestres afectadas por esta recolección han recibido poca atención. A pesar de su protección legal (Convenio CITES 1975), la Tortuga mora es actualmente vendida ilegalmente en mercados de Marruecos, Túnez y Algeria, exportada ilegalmente a Europa y a menudo recogida sin fines comerciales por la población local. La magnitud del comercio de esta especie como mascota y su recogida sin fines comerciales (entre el 22-64% de los habitantes poseen una tortuga como mascota), es suficientemente elevada como para amenazar su conservación. Así lo evidencian, en las poblaciones silvestres de Marruecos, parámetros demográficos como la densidad y la estructura poblacional. Consequentemente la recolección de tortugas como mascotas tanto con o sin fines comerciales, podría considerarse un efecto aditivo a otros factores reguladores de las poblaciones como la depredación o las enfermendades, y por lo tanto debería ser investigado en mayor profundidad. Por todo ello recomendamos acciones apropiadas que favorezcan la conservación de esta especie mediante un enfoque conjunto socioecológico que se centrará por una parte en adquirir conocimiento de los valores, percepciones y actitudes de los propietarios de las tortugas como mascotas y por otra del estudio de los parámetros demográficos de las poblaciones silvestres, que se ven afectados por la tenencia de esta especie como mascota.

Keywords: demographic parameters, conservation, welfare, perceptions, human dimension
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1. Introduction

The collection and trade of wildlife are cause for concern as regards animal welfare, public and health safety, species conservation and ultimately environmental degradation (Warwick 2004; Robinson *et al.*, 2016). The use of live reptiles destined as pets has been reported as one of the major threats to their conservation (Böhm *et al.*, 2013). For example, 355 species of reptiles were intentionally targeted by collectors, including 160 CITES-listed species (Auliya *et al.*, 2016). Despite there are growing scientific and policy debates over which types of conservation strategies are most effective to stop this threat, including increased enforcement, demand reduction strategies, and provision of alternative livelihoods, as well as market-based and incentive-oriented approaches (Duffy, 2014), policy enforcement in several countries, such as Thailand, India and Morocco has demonstrated to be inefficient as regards preventing the overexploitation of species (D´Cruce *et al.*, 2015; Auliya *et al.*, 2016). The complexity of wildlife harvest requires new socio-ecological approaches that address both the roles and motivations of diverse involved actors (not only poachers/ perpetrators/ criminals) and the demographic and physical characteristics of the species. In particular, it is necessary to attain more in-depth knowledge of pet owners' values, perceptions and attitudes towards wildlife pets (Liu *et al.*, 2016) as well as to obtain empirical data of population dynamics as regards reproduction and survival rates in the different age stages in wild populations affected by collection and trade for pets.

Among reptiles, certain species and groups of tortoises and turtles, such as Testudines (341 species), are preferred by pet owners (Czech *et al.*, 1998) and their collection and trade are considered the highest (Nijman & Sheperd, 2007; Luiselli *et al.*, 2016). One of these species is the spur-thighed tortoise (*Testudo graeca*), whose collection as pets has been historically considered a major threat for its conservation. This species is protected by the Convention on International Trade in Endangered species of Wild Fauna and Flora since 1975 and is also protected by European and national conservation laws (e.g. Spanish, Moroccan) that ban its sale. In spite of this, it is currently illegally sold openly in markets of Morocco, Tunisia and Algeria, and exported to Europe (Nijman & Bergim, 2017). In addition, it is also often collected with noncommercial purposes from wild populations by local inhabitants (Perez *et al.*, 2004; Tiar *et al.*, 2019; Segura *et al.*, 2020). Here, we review spur-thighed tortoise pet trade and non-commercial collection (hereafter pet collection), drawing from the literature in order to 1) define the historical and recent characteristics and magnitude of pet trade, 2) highlight the role played by noncommercial collection as a possible relevant cause of tortoise decline, 3) discuss the implications of pet collection for tortoise conservation and welfare, and 4) discuss social awareness importance. In basis to aims 1-4, we 5) recommend actions that can facilitate the conservation of this species.

2. Historical and recent magnitude and characteristics of pet trade

Keeping spur-thighed tortoises in captivity is a long-established custom in Europe but also in many local cultures along the tortoise distribution range in Africa. North African countries has traditionally supplied the European tortoise pet trade, for example Morocco peaked with 100 000 tortoises per year in the sixties (Lambert, 1969). Nowadays, it is difficult to know the extent to which tortoises are currently traded to Europe, as it is an illegal activity and consequently most of the tortoise trade is not documented. Nevertheless, some evidences suggest that the number of tortoises involved, which come from Algeria and Morocco and to a lesser extent from Tunisia (Mundy-Taylor, 2013), must still be high. For example, the illegal importation of 1400 tortoises from North Africa to Sicily was recently reported (Brianti *et al.*, 2010) and hundreds tortoises captured for illegal export were documented in the Moroccan cities of Tangier and Rabat (Nijman & Bergin, 2017). Furthermore, the spur-thighed tortoise is currently by far the most frequently wild traded species on Moroccan markets; in 2013, 94% of the 2113 wild animals surveyed by Bergin and Nijman (2018) in Moroccan markets were of this species.
Tortoises used for pet trade in Morocco are captured in the wild near the cities (Bergim & Nijman, 2017; Segura *et al.*, 2019). Tortoises that were traded in Moroccan markets in 2013-2014 had different age structure than those traded in 1969 (preference for younger individuals increased). In addition, the domestic rather dominated in 2013-2014, while international trade was more frequent in the 1960s (Nijman & Bergim, 2017; Lambert, 1979). This pattern likely corresponds to Morocco trade, therefore further studies undertaking fieldwork in markets of north African countries are necessary to quantify the pet trade overall magnitude.

3. The non-commercial collection

Local inhabitants in the species distribution range frequently collect with non-commercial porpoises spur-thighed tortoises to use them as pets. Animals are found in the natural areas through chance encounters (Perez *et al.*, 2004; Tiar *et al.*, 2019; Segura *et al.*, 2020). For example, in the Maamora forest of Morocco, an area with the highest population density of tortoises (Segura & Acevedo, 2019), a weekly presence of 30 000 visitors has been reported on sunny days (Fennane & Rejdali, 2015), therefore the likelihood of tortoise encounter and collection could be high. In central Morocco, as occurs in northern Algeria and southern Spain, most people that hold tortoises as pets results from direct collection from wild populations (see Table 1). The number of tortoises owned is high and ranges from 1.4 to 3.1 tortoises (see Table 1). The whole estimate of captive tortoises, mostly from non-commercial collection in those countries -tens of thousand of animals- is an indication of the magnitude of this threat for this tortoise species (see Table 1; Perez *et al.*, 2004; Tiar *et al.*, 2019; Segura *et al.*, 2020). Nevertheless, it would be desirable to quantify the magnitude of tortoise non-commercial collection in other localities and countries, since it is difficult to understand the overall relevance of this activity without further estimations.

Table 1: Summary of the studies reflecting the numbers of interviewed people, the percentage of captive tortoises, the percentage of tortoises from wild populations, the number of captive tortoises per person and the whole estimate of captive tortoises.

	Interview people	Captive	Wild origin	Captive/person	Captive estimate	Reference
Spain	796 children	64%	66%	2.9	10 000	Pérez *et al*., 2004
Algeria	5659 children	22%	57%	1.4	15 300	Tiar *et al*., 2019
Morroco	480 adults	55-61%	42-68%	2.1-3.1	85 000	Segura *et al*., 2020

4. Implications of pet collection for conservation and welfare

Spur-thighed tortoise collection as pet has been invoked as one of the main factors contributing to the decline of the species, yet there has not been assessed its associated consequences for demographic parameters (e.g. density, fecundity). Indeed, Lambert (1995) observed a decline in their populations of 90% during the period 1900-1984 in some areas in northwestern and central Morocco, probably owing to the pet trade.

Pet collection might reduce adult survival and juvenile recruitment and hence population growth rate (Znari *et al.*, 2008). In this sense, Segura & Acevedo (2019) showed that tortoise population density is higher in the protected area of the Moroccan Maamora forest (17-23 tortoises ha^{-1}) than in a close non-protected area of the same forest (5 tortoise ha^{-1}), and this might be, at least in part, a consequence of tortoise collection for pets. In addition, the population in the non-protected area shows an unbalanced size carapace length (CL)/age structure as regards lower number of old males (<115 mm CL) and young females (<155 mm CL), the lack of very old females (>215 mm CL) and unbalanced sex ratio (female biased 1.2 *vs.* 2.5). Particularly, this low number of old females (highly detectable due to size) might reduce the population reproduction success (Díaz-Paniagua *et al.*, 2011), with a strong effect on population dynamics. In addition, the higher number of juveniles found in both, homes surrounding Maamora cities and villages and Moroccan markets (see Table 2), might reduce the number, age class and sex of juveniles in wild, likely acting as a selective pressure, and potentially increasing the effect of the juvenile mortality rate in wild, deeply documented as very high (95%; Díaz-Paniagua *et al.*, 2001). Therefore, pet collection might be considered as an additive effect to other population declines drivers like predators or diseases. The release of captive tortoises into the wild without any control, which is frequent in Morocco can contribute to disease spread and, therefore, affect negatively wild populations (Jacobson, 1993; Esperón *et al.*, 2019) and possibly might contribute to outbreeding depression if the released animals contact among genetically distant populations (Templeton, 1994). In the most extreme scenario, tortoise collection might cause local extinction in wild populations that is especially relevant close to villages and large cities or new exurban areas, along their distribution range area (Perez *et al.*, 2004, 2012a).

Table 2: Juvenile and adult captive tortoises from Morocco: Moroccan markets (Nijman & Bergim, 2017), Rabat markets, and homes of Rabat city and Sidi Allal Bahraoui village (30 km apart). All the captive tortoises did not have CITES certificates.

	Juveniles	Adults	Ownners
Maroccan markets	630	370	
Rabat market	61	51	
Rabat homes	185-279	154-228	167
Sidi Allal Bahraoui homes	146-204	99-137	103

The welfare conditions of tortoises kept in Moroccan markets and homes are far from adequate since markets are busy, hot, and overall stressful places for wild animals (Mellor & Beausoleil, 2015). Bergim & Nijman (2018) documented that the vendors possessed little knowledge and advised poor care and transportation practices. Also, people holding tortoises in the Rabat region (Morocco) showed a low basic understanding about tortoise reproduction or its adequate environment; e.g. flats were often considered adequate environments (Segura *et al.*, 2020). Therefore, both captive tortoises from markets and homes are likely affected by discomfort in thermoregulation and might suffer distress.

5. Social awerness matters

The long-established use of tortoises as pets is especially important in North Africa because the demand for tortoises has changed, e.g. dominating now the domestic trade and the non-commercial collection, and continues to do so across social contexts. The high demand for tortoises might be related to the easiness of obtaining them in certain areas close to villages and cities, and the very low cost of its maintenance (Perez *et al.*, 2004; Segura *et al.*, 2020). Social drivers behind the tortoise collection demand of this tortoise species for pets have received relatively limited attention in the literature despite it is by no means a new phenomenon (e.g. Phelps *et al.*, 2016; van Uhm, 2018).

There is a gap of knowledge of tortoise owners' perceptions and preferences (e.g. certain age/sex classes, which might be associated to aesthetical values as regards the cuteness of the first years´ tortoises or the strength or even breeding ability of the adults) and of the social factors as regards people attitudes towards wildlife, their education or the urban/rural origin. In addition, the species biological and ecological requirements, its conservation and legal status, and the zoonotic diseases associated with it remained unknown (but see Pérez *et al.*, 2011, 2012a; Segura *et al.*, 2020). This information is necessary to improve our understanding of the motivations for acquiring wildlife species as pets, and thus to design conservation strategies; these should include comprehensive educational campaigns that might be focused on the potential illegality of owing tortoises and the possibility of contracting zoonotic diseases that might risk human safety (Moorhouse *et al.*, 2017). In this line, in North African countries, the poor knowledge of the protection of this species by CITES (95% of people in central Morocco; A. Segura unpublished) suggests that a special effort should be done to inform local people that owning this species is illegal. Perez *et al.* (2012a, 2012b) and Segura *et al.* (2020) pinpointed that effectiveness of the education program depends on the quality and the speed with which the educational campaign is initialized and the characteristics of the target people (tortoise owners are usually mature people who live in a house with garden and also own other pets).

6. MANAGEMENT AND CONSERVATION RECOMMENDATIONS

Motivations for acquiring wild pets and the diverse roles of actors that move beyond the pet collection should be studied in much greater depth to inform more appropriate and effective conservation actions.

International conservation strategies might integrate socio-ecological policies, for example by the development of dynamic models that integrate both ecological data and social information (Pérez *et al.*, 2012a). Particularly such strategies might: 1) put more efforts on the monitoring of tortoise non-commercial collection, quantifying with greater precision how many animals are actually being removed from wild populations; 2) increase the number of protected areas in African countries; 3) include scientific unified and systematic protocols in the relocation of captive tortoises to allow public take right actions -e.g. in north African countries there are no wildlife protection institutions, which would allow the deposit of captive tortoises; 4) disconnect the management of captive populations with the conservation of wild populations – e.g. avoid the use of government and ONG translocations of captive tortoises into wild populations; and 5) encourage a stronger investment in educational campaigns that focused to change the social representation of tortoises as pets to tortoises as wild protected species that need to live in wild, mainly in North Africa due to the changes from international to domestic trade.

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Challenges in the management of *Testudo hermanni* in wildlife recovery centers in Catalonia

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Abstract

The widespread presence of Mediterranean tortoises in Spanish households as pets has been common since the 1960s. The laws that banned their trade and possession from 1973 onwards led to a significant influx of individuals into wildlife recovery centers. At the CRARC wildlife center, 11 577 *Testudo hermanni* individuals were admitted over a ten-year period, causing a major management challenge. Therefore, developing dedicated projects to address the fate of these tortoises is a priority for recovery centers.

Resumen

La presencia masiva de testudínidos mediterráneos en los hogares españoles como animal mascota es un hecho desde la década de los sesenta. Las leyes que prohibieron su comercio y posesión desde 1973, tuvieron como consecuencia un gran ingreso de ejemplares los centros de recuperación de fauna salvaje. En el centro de recuperación CRARC, ingresaron 11 577 ejemplares de *Testudo hermanni* en 10 años, creando un problema de gestión. Disponer de proyectos para dar destino a estos ejemplares es una prioridad.

Keywords: hermann's tortoise, *Testudo graeca*, trade, captivity, projects
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1. Introduction

The Hermann's tortoise (*Testudo hermanni*) became a highly popular reptile in Spanish, and particularly Catalan, households from the 1960s onward. For decades, this species was considered a pet. Combined with the destruction of its most suitable habitats, this led the species to the brink of extinction (López Jurado *et al.*, 1979). As a result, *T. hermanni*, together with *T. graeca* (the other species present in Spain), was under legal protection since the 1970s. In Catalonia, the entry into force of the Animal Protection Law, or "LLei de protecció dels animals" in 1988 marked the prohibition of trade, capture, and possession of the species. However, keeping this tortoise remained, and still remains, widespread among the general population. Therefore, a significant portion of the population found itself in breach of the law, which has led to ongoing conflicts between citizens and the administration.

The CRARC wildlife center (Centre de Recuperació d'Amfibis i Rèptils de Catalunya) has been operating in Catalonia since 1992 and serves as a leading facility for the reception of captive tortoises—whether acquired through purchase, as gifts, bred in captivity, or of known or unknown origin. CRARC releases hundreds of *T. hermanni* tortoises across Catalonia each year. The only area excluded from this management measure is the Albera Mountains, where the last wild population of Hermann's tortoises (unintroduced and located near the French border) survives. In this area, a breeding center, the Centre de Reproducció de Tortugues de la Albera (CRT), is responsible for managing and conserving this population.

2. Methods

For each animal entry at CRARC, a detailed form is completed. To track the evolution over time of the large number of *T. hermanni* entries, the forms were reviewed for the last decade (2010–2019). Each tortoise was categorized by subspecies or hybridization between the two subspecies, based on the morphological characteristics of the shell (Soler *et al*., 2012). Moreover, for the year 2019, records of entries of other Mediterranean tortoises from the *Testudo* genus were also counted to better illustrate the burden the center faces in receiving tortoises.

3. Results

Based on data from the last decade of *Testudo hermanni* entries at CRARC, a total of 11 577 specimens were recorded (Table 1). As shown in the annual totals, the number has increased steadily over the years. Most animals (92,6 %) belonged to the western subspecies (*T. h. hermanni*) although specimens of the eastern subspecies (*T. h. boettgeri*) and hybrids were also recorded. While the eastern subspecies remains variable, hybrids appear to be on the rise.
In 2019 alone (Table 2), a total of 2863 tortoises were received, including other Mediterranean species of the *Testudo* genus (*T. graeca* and *T. marginata*). Entries of the native lineage *T. h. hermanni* accounted for 83.4%.

Table 1: Specimens of Hermann's tortoise (*Testudo hermanni*) admitted to the CRARC Rehabilitation Center over the past decade, with details on subspecies and hybrids.

Specie	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	Total
T. h. hermanni	191	551	596	814	1193	871	1186	1081	1851	2390	10724
T. h. boettgeri	4	7	21	32	15	19	8	8	17	21	152
T. hermanni hybrid	11	0	30	180	40	98	15	76	3	248	701
Total	206	558	647	1026	1248	988	1209	1165	1871	2659	11577

Table 2: Tortoises of the *Testudo* genus admitted to the CRARC in 2019, with details on subspecies and hybrids.

Specie	Specimens/individuals
Testudo hermanni hermanni	2390
Testudo hermanni boettgeri	21
T. hermanni hybrid	248
Testudo graeca whitei	189
Testudo graeca ibera	4
Testudo marginata	11
Total	2863

4. Discussion

The implementation of the protection law more than 30 years ago (1988) aimed to halt the trafficking of this species in Catalonia. However, the number of entries at CRARC has not decreased over the past decade. This trend, observed in tortoises, aligns with the increasing number of exotic specimens from other species described in a similar period at the same center (Maceda-Veiga *et al*., 2019). Wild populations of *T. h. hermanni* are now extremely threatened, with very few animals living freely, currently concentrated in the Sierra de la Albera (the easternmost stretch of the Pyrenees), where the only natural population, numbering approximately 7000 individuals, survives (Félix, 1985; Budó *et al*., 2002; Bertolero, 2017). Other areas where the species has been introduced include the Parque Natural del Garraf, with 7868 individuals introduced since 1992 (Parellada, 1997; Soler *et al*., 2019); the Parque Natural del Macizo de Montsant, with 5754 individuals introduced since 2005 (Soler & Martínez Silvestre, 2011); the Parque Natural del Delta del Ebro, with 277 individuals introduced since 1989 (Bertolero, 2002; 2019); the Macizo de Llaveria, with 200 individuals introduced since 2008 (D. Martínez, Pers. Comm.); and Vall Major de Bovera, with 172 individuals introduced since 2015 (D. Sanchez, Pers. Comm.). The number of introduced Hermann's tortoise specimens in natural areas in Catalonia exceeds 14 500 since 1989. However, in the last decade alone, over 11 500 tortoises have entered CRARC. Given these numbers, it can be inferred that the captive population of *T. hermanni* in Catalonia greatly surpasses the wild population. These numbers highlight the need for protected areas where all these tortoises can be released. Furthermore, it is important to note that Catalonia has another official tortoise recovery center, CRT, whose annual entries would further contribute to the issue of the captive population (Budó *et al*., 2017).

The Hermann's tortoise has virtually disappeared from its entire distribution range in Catalonia, which was potentially located in the coastal and pre-coastal mountains (Bertolero, 2015). Although the introduction of 14 500 tortoises in Catalonia over the past few decades may appear significant, it is relatively modest when compared to the estimated 80 000 individuals inhabiting the Maures Massif in France (Devaux, 2005), a population classified as "in serious danger of extinction".

One reason for the high number of specimens in captive populations is that reproduction in captivity presents no significant challenges for keepers (Soler & Martínez Silvestre, 2005). As a result, *T. hermanni* is widespread in captivity, despite being rare and highly threatened in the wild. This paradox is also observed in other herpetofauna species facing habitat loss, such as the Chinese giant salamander (*Andrias davidianus*) (Wang *et al*., 2004) and the axolotl from the Xochimilco canals (*Ambystoma mexicanum*), which is considered nearly extinct in the wild, yet has large captive populations (Wilson & Johnson, 2010). This situation is exemplified by data provided by López Jurado *et al*. (1979), who highlighted: "more than 55 000 Hermann's tortoises were imported into Barcelona from Menorca by a single exporter between 1965 and 1976". Assuming that only 17.19% survived, based on mortality data from Blatt & Müller (1974) for tortoises imported from Germany in the 1970's, this would represent approximately 9454 tortoises. The actual number may be even higher when considering the additional specimens bred in captivity, and it is worth considering what the numbers would look like if we had data on all the imports made during the years when their sale was legal.

The wildlife recovery centers are the primary depositories for tortoises that citizens voluntarily donate upon learning of the punitive nature of the law. These centers also receive specimens seized by the police, who are responsible for enforcing the law. The proper management of the large number of tortoises entering these centers, given the aforementioned circumstances, is a challenge that must be addressed in the design of laws aimed at the conservation of native chelonians (Martínez-Silvestre & Franklin, 2019). Housing such a large volume of tortoises for extended periods is unfeasible (from both a sanitary, logistical, and economic standpoint). One solution to this problem is the development of introduction or reintroduction projects in natural habitats. For instance, in 2018, 2530 *T. h. hermanni* entered CRARC, and in the same year, 2021 specimens were released from the same center in collaboration with three conservation projects.

The prolonged presence of the Hermann's tortoise as a pet in many Spanish (and particularly Catalan) households has led to a significant population of captive tortoises that are challenging to assess and manage. The traditional view of tortoises as pets complicates the strict enforcement of protective measures. This issue does not arise with other species under the same protection status, such as the otter (*Lutra lutra*) or the bearded vulture (*Gypaetus barbatus*), since these animals are not found in private homes.

Although direct public involvement in the *ex-situ* conservation of the Hermann’s tortoise is considered a viable option, this approach presents numerous challenges, including the management of captive stocks, individual identification, health monitoring, subspecies and ecotype selection, and securing the financial resources necessary for its implementation. Finally, the lack of suitable release sites poses a significant inconsistency in the management of the species. On one hand, it would undermine the rationale for asking citizens to surrender their animals to the authorities. On the other hand, it would lead to the paradoxical necessity of sacrificing a large number of tortoises from a protected species (in cases where euthanasia is required for sick individuals to prevent the spread of disease to healthy ones). These factors underscore the need to identify new protected areas for introduction, rather than population reinforcement, to avoid compromising existing, stable populations in their designated habitats. Therefore, habitat protection should be prioritized. Maintaining a population of thousands of captive tortoises serves little purpose—and may even pose a risk— for the conservation and recovery of *T. hermanni* in Catalonia.

5. Management proposals

Considering the high volume of tortoises entering the CRARC center, the authors of this article propose the following points for the management of the Hermann's tortoise in Catalonia.

1. The conservation of the natural habitats that host Hermann's tortoises must be prioritised.
2. The recovery of old areas within the distribution range should be carried out using animals specifically bred for this purpose, considering native genetics.
3. New areas that meet the ecological requirements must be found where captive tortoises would be released in the future.
4. Scientific studies must be carried out to monitor the population with released individuals to evaluate whether these populations are well consolidated.
5. The sacrifice of sick, silent carriers of diseases, hybrids, or unrecoverable specimens is sometimes the only measure to protect healthy individuals.
6. Breeding Hermann's tortoises in captivity by private keepers is unnecessary, as there is a specialized breeding center focused on native genetics, and too many captive tortoises are already available.

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When should we reintroduce tortoises? Collection, captivity, and translocations of spur-thighed tortoises in southeast Spain

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Abstract

The spur-thighed tortoise in Southeastern Spain has been traditionally collected for its maintenance in captivity by local inhabitants and without any commercial aims. Here, I present some important results from previous studies about the tradition of keeping tortoises in captivity, the consequences for tortoise population conservation, and some management recommendations. I show the results of surveys and in-depth interviews to quantify this problem in the Southeast of Spain as well as qualitatively analyze the existing discourses and visions of tortoises that justify this type of behavior (domestic, pet, in need of protection). Through a dynamic model I simulate the risk that non-commercial collections may have for the local extinction of the species. One of the measures used to counteract the negative effect of collections and solve the problem of the captivity is the reintroduction of tortoises. I will present a decision-making system to assess whether the species should be reintroduced to ensure conservation benefits while reducing risks to the wild population and increasing the chances of successful actions.

Resumen

La tortuga mora en el sureste ibérico ha sido capturada y mantenida en cautividad de manera tradicional y sin fines comerciales por las poblaciones locales. En este estudio presento resultados previos relevantes sobre la tradición de mantener tortugas en cautividad, las consecuencias para su conservación y algunas recomendaciones para la gestión de poblaciones de tortugas. Se muestran resultados de encuestas y entrevistas realizadas con el fin de profundizar y cuantificar el problema ibérico y el análisis cualitativo de los distintos discursos y versiones sobre la tendencia en cautividad, mascotismo o necesidad de protección de las tortugas en el sureste. A través de un modelo dinámico se simula el riesgo de las recolecciones no comerciales en campo y como éstas pueden conllevar la extinción local de la especie. Una de las medidas llevadas a cabo para contrarrestar el efecto negativo de la captura de tortugas y una solución al problema de la cautividad es la reintroducción de individuos en poblaciones silvestres. Presentaré un sistema de toma de decisiones para evaluar si la especie debe reintroducirse con el fin de garantizar los beneficios para la conservación, así como la reducción de riesgos para las poblaciones silvestres de estas reintroducciones y las posibilidades de éxito de este método.

Keywords: pet trade, management, conservation, reintroductions, social perception, *Testudo graeca*
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1. Collection and captivity of tortoises in Southeast Spain

In southeast Spain, keeping tortoises as pets is a long-established custom. Based on surveys to school children, Pérez *et al.* (2004) revealed that this practice continues to be a common activity. Surveys showed that 61-68% of the families living in the municipals of the distribution area of the species keep tortoises in captivity, with an average of three tortoises per family. The estimation is in the order of tens of thousands of tortoises maintained in captivity in this area. Tortoises are collected as a result of chance encounters with wild animals by local inhabitants, and without any commercial objectives. While 60-68% of inhabitants with tortoises collected them from the wild populations, only 5-10% stated to have bought them. The captive breeding and the release of tortoises without any institutional control is also common.

In addition to the collection of tortoises by local inhabitants, new rural residents, mainly from the North of Europe, continue with the practice of collecting tortoises from the wild as pets. The number of new residents in the municipals of the distribution area of the species has rapidly increased in the last decades. Based on a survey to new residents, Pérez *et al*. (2012) showed that most of the 9-16% of new residents who keep tortoises in captivity have collected them from the wild (36-66%). Reintroductions and captive breeding were also frequent practices (24% and 26%, respectively).

The conservation consequences of these practices include local extinctions due to tortoise collection, and inbreeding and outbreeding depression, and disease transmission due to un-intentional and uncontrolled translocations of captive tortoises.

2. Social representations of tortoises

Understanding how people view, think and talk about tortoises (i.e., social representation of tortoises) is important for management to be effective. Based on a qualitative study, Pérez *et al.* (2012) studied social representations relating to the practice of keeping tortoises in captivity in southeast Spain. The 13 in-depth interviews with rural–agrarian, urban and neorural dwellers who kept tortoises in captivity, allowed them to distinguish three types of social representations coexisting in southeast Spain: tameable animal, pet and safeguarded animal. All these social representations encourage dwellers to keep tortoises in captivity, but motivations varied according to each social representation.

Pérez *et al.* (2012) showed that rural– agrarian respondents represented tortoises as a 'tameable animal', and kept tortoises on farmyards with other domestic animals for certain benefits, such as eating fleas. Urban dwellers mainly viewed tortoises as pets, did not connect these animals with wildlife, and did not consider them to be wild in nature. Neorurals viewed tortoises as a safeguarded animal representation of the tortoise. The practice of keeping tortoises in captivity was justified by ideas about safeguarding them in a domestic environment. The people who reported this representation interact with the spur-thighed tortoise in a conservationist manner.

For the species conservation, the vision that this tortoise is a wild animal that should not be kept in captivity should be promoted. To this end, one important message to be highlighted is the importance of conserving wild populations rather than protecting single animals in captivity. In addition, taking advantage of neorurals' positive environmental attitudes, an interesting action may be to promote participation in conservation actions and citizen science for tortoise population monitoring.

3. Local extinction due to non-commercial collections

To study the long-term effect of pet collection on tortoise populations by residents of exurban areas in southeast Spain, Pérez *et al.* (2012b) built a system dynamic model. Their model included two submodels (tortoise population and households dynamics) interconnected through a tortoise collection submodel. The population dynamics submodel included variables such as survival rate and reproductive capacity of tortoises. The households dynamics submodel included variables such as occupation dynamic capacity, % households with occupants who want to have tortoises. The tortoise collection submodel included variables such as density of tortoises, probability of encountering a tortoise, effort in searching tortoises.

The model simulations showed that pet collection may cause a significant population reduction, and even local extinction of isolated population, in medium to high-size urbanized areas. Their model showed that the size of a housing development is key in determining the intensity and speed of tortoise population decrease. Collection of tortoises in areas around medium to large housing developments (> 650 houses) may cause the extinction of tortoise populations. Housing development with less than 650 houses also causes an important reduction of tortoise population levels (> 14%).

The model simulation allowed to assess the effectiveness of environmental education program, with the aim of reducing the willingness of new residents to keep tortoises in captivity, and urban development policies. Pérez *et al.* (2012b) conclusion is that the integration of policies of educational programs and development constraints are the most effective way to reduce the impact of exurban development on tortoise populations in southeast Spain.

4. When should we reintroduce tortoises?

Reintroduction of captive tortoises to wild populations is a controversial but common practice. Reintroduction have often been used as simple actions to solve complex conservation problems without first considering the root causes of population declines. Also, some reintroduction projects respond to philosophical, aesthetic, or sociopolitical reasons rather than for restoring the long-term viability of target populations. Finally, reintroductions are often unsuccessful may even be harmful in the long term as a result of unexpected associated impacts, like disease transmission, reduced population fitness caused by decreases in genetic diversity (inbreeding depression), or the introduction of maladaptive alleles (outbreeding depression) in managed populations.

In order to improve the likelihood of success in translocation projects and to better contribute to conservation efforts, Pérez *et al.* (2012c) proposed a hierarchical decision-making system for translocations. This system nests 10 criteria for translocations (Table 1) into successive decision levels (Figure 1). The first level, “Necessity of the translocation” (criteria 1–3), assesses whether the project will be carried out with adequate conservation objectives and whether translocation is the most adequate tool for conserving the threatened species or population. The second level, “Risk evaluation” (criteria 4–6), aims to ensure that impacts will be mitigated. The third level, “Technical and logistical suitability” (criteria 7–10), attempts to maximize the likelihood of success in terms of establishing new viable populations.

Table 1: Ten criteria for tortoise translocation (adapted from Pérez *et al*., 2012c).

Criteria
(1) Is the tortoise species or population under threat?
(2) Have the threatening factors been removed or controlled, or were they absent in the release area?
(3) Are translocations the best tool to mitigate conservation conflicts?
(4) Are risks for the target tortoise species acceptable?
(5) Are risks for other species or the ecosystem acceptable?
(6) Are the possible effects of the translocation acceptable to local people?
(7) Does the project maximize the likelihood of establishing a viable population?
(8) Does the project include clear goals and monitoring?
(9) Do enough economic and human resources exist?
(10) Do scientific, governmental, and stakeholder groups support the translocation?

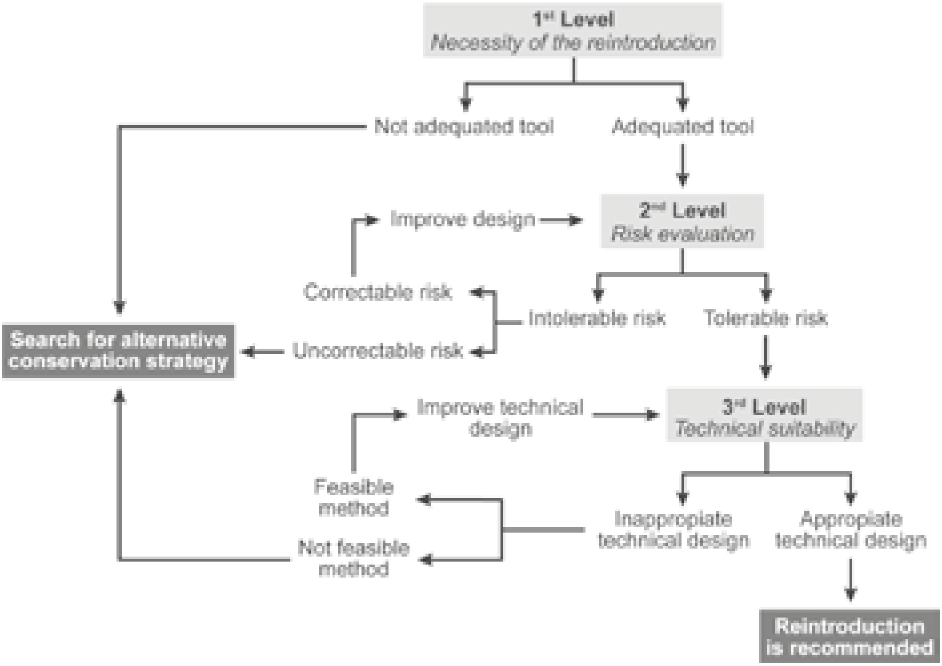

Figure 1: Hierarchical Decision-making System for tortoise translocations (adapted from Pérez *et al*. 2012c).

For the effectiveness of tortoise translocations, each criterion within the three levels should be evaluated. If a tortoise reintroduction proceeds without fulfilling the first level, the project would not be of much value to the intended species and could even have a negative effect if resources are spent on the translocation project instead of on more appropriate actions, such as habitat conservation. If a tortoise reintroduction proceeds without fulfilling the second level, the project could have unintended or negative effects on other elements of the ecosystem (e.g. disease transmission, genetic outbreeding). If a tortoise reintroduction proceeds without fulfilling the third level, the project could fail; in this case, however, there would be no direct negative consequences for conservation, apart from the waste of economic resources.

When the hierarchical decision-making system for translocations is used to assess the reintroductions of captive tortoises to wild populations in southeast Spain (Giménez *et al*., 2004), the conclusion is that reintroductions cannot be justified for the conservation of wild population (level 1), they might cause negative effect to wild population, mainly disease transmission and decreases in genetic diversity (level 2), and their effectiveness is doubtful (level 3). Other conservation strategies, like habitat conservation, educational programs and urban development constraints seems to be the most efficient to conserve spur-thighed populations in southeast Spain (Pérez *et al*., 2010).

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30 years of study, conservation, captive breeding and population reinforcement of Hermann's tortoise (*Testudo hermanni hermanni*) in the Sierra de l'Albera (Eastern Pyrenees, Catalonia)

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Abstract

Description of the main regression causes of Hermann's tortoise (*Testudo hermanni hermanni*) population in the Sierra de la Albera (easternmost stretch of the Pyrenees in Catalonia) in recent decades, along with the conservation actions taken in the last 30 years.

Resumen

Se describen las principales causas de regresión de la población de tortuga mediterránea (*Testudo hermanni hermanni*) de la sierra de la Albera (Pirineo oriental, Catalunya), en los últimos decenios y las diferentes acciones de conservación realizadas en los últimos 30 años.

Keywords: captive breeding, conservation, declining population, habitat protection, reintroduction, threats
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1. Introduction

A unique natural population of Hermann's tortoise (*Testudo hermanni hermanni*) remains in the Iberian Peninsula, in the Sierra de la Albera (easternmost stretch of the Pyrenees) in Catalonia. This small population (~80 km^2) has been studied from the end of the 1980s to the present-day. Its declining population size seems to have slowed down in the last few decades (Bertolero *et al*., 2020). The last censuses show certain population stability, albeit at a low population renewal rate. Currently, the average population is 0.8 tortoises/ha. However, densities are 4 tortoises/ha in some small valleys in the area (Bertolero, 2019).

This study aims to compile not only the main causes of *T. h. hermanni* population decline in the Sierra de la Albera, but also the conservation actions that have been taken in the last 30 years.

2. Causes of species regression in the Sierra de la Albera

The causes of this declining population are varied. They began being detected and studied more than 35 years ago (Félix, 1984; Budó *et al*., 2004).

- Loss of habitat due to agriculture.
  For more than 300 years, the substitution of forest and Mediterranean scrub to favour vineyard and olive cultivation has severely transformed thousands of hectares of Her-

mann's tortoise habitat, which has reduced this species' distribution, and has continued until the present-day (Vilardell *et al*., 2007a). Unfortunately, all the studies are based on approximations and no accurate data about this habitat change are available.

- Forest fires.
  Within the species' range, wildfires have been very common in the Sierra de la Albera in the last 70-80 years. The high recurrence of these disturbances has been an important cause of species regression (Félix *et al*., 1989; Félix, 1999; Badiane *et al*., 2017).
- Habitat disturbance due to human activities.
  Certain areas of the distribution range have been highly frequented by people in recent years because some architectural, cultural or leisure elements have attracted the public. These activities with marked human presence disturb the tortoise population in this area (Budó, 2001).
- Poaching and trade.
  Since the first quarter of the 20th century, having tortoises in house gardens became very fashionable in Catalonia (Fèlix, 1995). As tortoises did not benefit from any kind of protection measures, poaching and trade have been very common until 1986. In 1986, Catalan legislation prohibited specimen possession and commercialisation.
- Predation.
  Given the uncontrolled increases in wild boar (*Sus scrofa*) populations in recent years, high tortoise predation rates have been found, especially in tortoise nests (Vilardell *et al*., 2007b). Numbers of other predatory species have also increased, with a similar impact on tortoise survival, such as beech marten (*Martes foina*) and badger (*Meles meles*). Different studies indicate that the predation of these three species can be as high as 73% of the nests in the area (Budó *et al*., 2003; Budó & Capalleras, 2006).
- Low genetic diversity.
  Genetic studies carried out on different Hermann's tortoise populations in Europe indicate that the Albera population is the least diverse of all those studied. This narrow genetic variability may be one of the factors that hinders population growth (Zenboudji *et al*., 2016).
- Emerging diseases.
  Another factor to take into account is the detection of a certain load of pathogens, such as Herpervirus and Mycoplasma, in Albera population specimens. The origin of these emerging diseases is unknown because no studies support whether these diseases have developed in natural populations or been transmitted by uncontrolled released specimens of any land tortoise species, which are regularly detected in the distribution area (Budó *et al*., 2018).

3. Conservation actions

In order to preserve the Hermann’s tortoise population in the Albera, different actions and legislative measures have been taken by the Catalan administration over the years. These measures have led to conservation and preservation actions being taken with the last Iberian tortoise population.

- Legislation

  To international and European legislation, another has been added since 1986, which the Catalan government has more forcefully applied to eliminate poaching, trade and possession in private homes. It has had a very positive impact.
- Habitat protection (the Sierra de la Albera Natural Park)

  In 1986, the Catalan government declared a large part of Hermann’s tortoise’s habitat and distribution area in the Sierra de la Albera to be Natural Park and Nature Reserves. In 2006 with Natura 2000 Network approval, almost the entire species’ distribution area in Catalonia was protected. However, the management of agricultural, forestry and recreational practices in this area continues to be a key aspect for species conservation.
- Captive breeding (Centre de Reproducció de Tortugues de la Albera).

  In 1984, a naturalist association created a captive breeding centre for Hermann’s tortoises called the Centre de Reproducció de Tortugues de la Albera (CRT La Albera). This centre encouraged the administration to act by taking subsequent measures to recover the tortoise population. Nowadays, the CRT La Albera develops conservation programmes with captive Hermann’s tortoise breeding, and also with the two native freshwater turtles of Catalonia: the Mediterranean turtle (*Mauremys leprosa*) and the European pond turtle (*Emys orbicularis*). After several years of study to know the various biology, ecology and habitat aspects of Hermann’s tortoise, juveniles born in captivity have been released to reinforce the wild population (Figure 1).

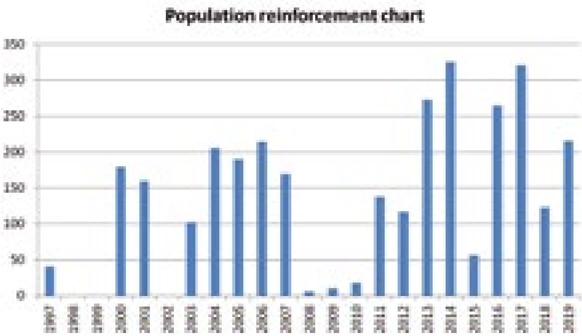

Figure 1: The number of specimens released in the population reinforcements conducted in the last 23 years. During this period, 3135 Hermann's tortoise juveniles have been released.

- Environmental education and awareness campaign.
  Along with the captive breeding work, the CRT La Albera opted to open its facilities to the general public. Thousands of visitors come to the centre every year, mostly families and groups of schoolchildren. Visitors can learn about the work done by the CRT La Albera to conserve tortoises and, above all, can be made aware about conservation problems and about not having turtle and tortoises as pets.

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Projects on research and protection of tortoises and freshwater turtles: cooperation of central european associations

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Abstract

Mediterranean tortoises suffer from population declines in their natural habitats. Therefore, several associations, institutions, and authorities in Central Europe are working on the research and protection of reptiles, including freshwater turtles and tortoises. These groups form a network for the exchange of information and expertise and collaborate to combat illegal wildlife trade. Such projects may include collecting information about captive breeding and trade, as well as supporting the management of ex-situ populations of species that are threatened in their natural habitats. This is carried out in line with the IUCN's One Plan Approach. Here we present some of the rules and regulations pertaining to *Testudo* in Germany, current and future collaborative projects for the conservation of tortoises, specifically of *Testudo hermanni* and *Testudo graeca*.

Resumen

Las tortugas mediterráneas están amenazadas en sus hábitats naturales. Por ello, varias asociaciones, instituciones y autoridades de Europa Central trabajan en la investigación y protección de los reptiles, incluyendo galápagos y tortugas terrestres. Estos grupos forman una red de intercambio de información y conocimientos, y colaboran para combatir el comercio ilegal de fauna silvestre. Estos proyectos pueden incluir la recopilación de información sobre la cría en cautividad y el comercio, así como el apoyo a la gestión de poblaciones ex situ de especies amenazadas en sus hábitats naturales. Todo ello se lleva a cabo en línea con el enfoque "One Plan" de la UICN. Aquí presentamos algunas de las normativas relacionadas con *Testudo* en Alemania, así como los proyectos colaborativos actuales y futuros para la conservación de las tortugas, en particular de *Testudo hermanni* y *T. graeca*.

Keywords: captive-bred *vs.* wild-caught, legal and illegal trade, laws and regulations, captive breeding, genetic diversity
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1. Introduction

In Central Europe there are no natural populations of tortoises (neither *Testudo hermanni* nor *T. graeca*), but keeping and breeding them in zoos and private collections in Central Europe has a long history (Kirsche, 1984). Thus, good amateur books on the health, behaviour and husbandry at private captive breeders exist, see for example Geier (2022). Some original founder stock of these tortoises was imported into Central Europe before they had been listed as protected species under CITES (Weerth, 2014). In Germany, self-sustaining captive populations of both species exist, and since it is often not easy to find good new homes for the offspring of the common local forms, it is recommended to incubate their eggs only on demand, as explained for example in the Ethics Code of the German Society for Herpetology and Herpetoculture (DGHTa).

Legal imports from large tortoise farms in southern Europe or elsewhere continue to regularly occur in Germany and the EU. There are indications that these imports sometimes involve wild-caught tortoises of particularly sought-after local forms, which are laundered as captive-bred (see, for example, species reviewed under the Review of trade in animal specimens reported as produced in captivity under Res. Conf. 17.8 -Rev. CoP18-). Often, end consumers are unaware of this fact or would question the validity of CITES permits issued by the exporting country. Therefore, it is crucial to unravel the network and identify the poachers and forgers behind these transactions. We here present current projects that assist to prove the real origin of tortoises in human care.

2. Rules and regulations concerning tortoise keeping in Germany

It is well known that Mediterranean tortoises are experiencing population declines in their natural habitats, which has led to the implementation of various rules and regulations. Both *Testudo* species, *T. graeca* and *T. hermanni*, are listed in CITES Appendix II and Annex A of EU Council Regulation 338/97. Within the EU, a certificate of exemption from the prohibition of commercial use (Art. 8 of Council Regulation (EC) 338/97) is required for each transfer of specimens listed in Annex A for every transaction. This is accompanied by the obligation to individually mark live animals. In Germany, specimens of species listed in Annex 6 of the BArtSchV must be individually marked from hatching or acquisition onward. One accepted method for marking tortoises in Germany is photo documentation (Bender, 2001), which must be updated at regular intervals, with the photos being submitted to the relevant authorities. The rules and regulations for Germany are published on the BfN website (BfNa; BfNb). German law currently prohibits the use of transponders in tortoises weighing less than 500 g. Additionally, in Germany, both tortoise species are classified as "strictly protected" under the German Federal Nature Conservation Act, meaning that each individual must be registered with local enforcement authorities. Violations of these laws and regulations are prosecuted (see, for example, Kecse-Nagy *et al*., 2006). Other countries in Central Europe may have different recommendations regarding photo documentation (Tichý & Kintrová, 2010) or may require the implantation of transponders, even in very small tortoises.

Reptile traders and the organizers of reptile trade fairs have to acquire a certificate of competence (Proof of expertise by the German society VDA/DGHT, see references), which can be acquired by private breeders, too. In order to acquire such a certificate, participants complete a seminar on special rules and regulations, biosecurity measures (quarantine, deworming, etc.), as well as husbandry requirements for their animal species and need to pass a final test.
The rehoming of abandoned or confiscated tortoises is also regulated under article 16.3 of Regulation (EC) No 338/97. In many cases, there is not possible or reasonable to reintroduce these captive-bred tortoises from Central Europe in their region of origin as this is mostly unknown. Also, not all specimens are of value for a conservation breeding program. While most pet animal shelters have few expertise/facilities on reptiles, there are some recognized reptile shelters, mostly managed by specialized veterinarians, such as the German "Auffangstation für Reptilien" and "Reptilienauffangstation Aachen", as well as the French "Centre *Emys*" in Switzerland. Some rescue centers offer tortoise keeper training before rehoming tortoises to new owners.

3. Current collaborative projects

Several associations and institutions in Central Europe manage, oversee, or support projects aimed at the conservation of Mediterranean tortoises. While an exhaustive overview of these associations and their projects is not provided here, given the dynamic nature of the field, we will highlight a few organizations and discuss some current projects and initiatives related to Mediterranean tortoises.
The German Society for Herpetology and Herpetoculture (DGHT) brings together private reptile and amphibian keepers, zoos, and herpetologists within a single organization, thus combining extensive expertise from diverse fields. Its members are involved in research, husbandry, and the conservation of species and their habitats. The DGHT publishes Salamandra (DGHTb), along with several other journals, to facilitate the exchange of knowledge. Together with Frogs & Friends and the VDZ – Verband der Zoologischen Gärten (Association of Zoological Gardens), the DGHT is also one of the three main coordinators of the Citizen Conservation network, which connects zoological gardens with professional and private animal breeders and caretakers (Citizen Conservation). This network enables scientifically based breeding programs to collaborate with individuals committed to protecting biodiversity. Currently, its focus is on breeding endangered species of fish, amphibians, and reptiles.
When it comes to the captive breeding of tortoises and freshwater turtles, the German Chelonia Group (Arbeitsgemeinschaft Schildkröten) within the DGHT compiles breeding statistics that include several hundred captive-bred *Testudo* spp. from many

private breeders each year. To manage *ex-situ* populations of selected local forms, the DGHT collaborates, for example, with the European Studbook Foundation (Bogaerts & Visser, 2019; Zwartepoorte & Bulsing, 2014). For tortoises and freshwater turtles, the most relevant publication is Radiata, the magazine of the German Chelonia Group, which features articles on field observations, captive husbandry, and breeding reports (Herz, 2013), as well as on research and species conservation, such as efforts to conserve tortoises in Catalonia (Pfau & Budó, 2019). In research projects on Mediterranean tortoises, the German Chelonia Group works closely with the Nederlands-Belgische Schildpadden Vereniging (NBSV) (Visser, 2022), the British Chelonia Group (Martínez-Silvestre *et al*., 2021), and several other similar organisations across Central Europe. The DGHT was commissioned now the third time by the German Federal Agency for Nature Conservation (Bundesamt für Naturschutz – BfN) in order to compile a guidance for authorities to assess the captive-breeding potential of reptiles and amphibians newly listed in CITES. One of those reports contains a fact sheet on captive breeding and farming of *Testudo horsfieldii* (BfNc). Both the German CITES Scientific and the national CITES Management authority are located at the BfN.

Within the project "Forensic Genetics of Species Protection – FOGS" (LIB), specific SNPSTR markers have been developed for certain local forms of *Testudo hermanni* (Mozer *et al*., 2025). Using these markers, it is possible to determine —through genetic analysis of an oral swab— whether an imported tortoise from a highly valued local form has been falsely declared as captive bred with forged CITES documentation, regardless of the animal's morphology or coloration.

Species360 is a non-profit NGO with a global network of more than 1200 progressive zoos, aquariums, universities, research institutions, and governmental members across six continents and 99 countries, working to improve animal welfare and species conservation. The first joint publication project of the DGHT and Species360 concerning *Testudo* spp. was the compilation of the *Manual for the Differentiation of Captive-Produced and Wild-Caught Turtles and Tortoises (Testudines)* (Staerk *et al*., 2019). This manual is intended to assist customs officers who may not have experience with chelonian trade in inspecting shipments of tortoises or freshwater turtles labeled as "captive bred" and evaluating the credibility of such claims.

With overexploitation being one of the most significant direct threats to many species, WWF prioritizes the fight against illegal and unsustainable wildlife trade. Initiated by WWF Germany, several projects have employed stable isotope analysis to determine the origin of plant and animal products. The collaborative project "Stable Isotope Analysis for Tortoises" analyzes keratin samples from *Testudo hermanni* specimens of declared origin to test whether wild-caught and long-term captive-bred individuals can be distinguished by their isotopic signatures. The feasibility of this method has already been demonstrated in the Crocodile Lizard (*Shinisaurus crocodilurus*) (van Schingen *et al*., 2016). This methodology may also prove useful for verifying the geographic provenance of imported tortoises.

We hope that the aforementioned projects will help inspire broader cooperation among organizations of private keepers, zoological gardens, scientific institutions, and national enforcement authorities in the field of international species protection.

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Desafíos de bioseguridad en la conservación y el manejo de tortugas

Algunas enfermedades infecciosas amenazan la conservación de poblaciones silvestres de tortugas, y la morbilidad asociada a patógenos como herpesvirus y *Mycoplasma* en tortugas del género *Testudo* mantenidas en cautividad es elevada. Desde una perspectiva One Health, no se pueden obviar los efectos de estos patógenos pueden tener en la conservación de las poblaciones naturales de tortugas, especialmente porque el tráfico de especies incrementa el riesgo de brotes de enfermedades emergentes. La consideración de los riesgos debe ser amplia, incluyendo el análisis y control de bacterias, virus, protozoos, nematodos intestinales y ectoparásitos como las garrapatas, que además pueden actuar como vectores de patógenos a humanos.

El personal que trabaja con tortugas debe aplicar protocolos de higiene consensuados para evitar zoonosis y la transmisión de patógenos entre animales, tanto en el medio natural como en los centros de recuperación.

En la gestión de animales mantenidos en cautividad es fundamental establecer cuarentenas adecuadas y controles veterinarios para prevenir la transmisión de enfermedades a poblaciones silvestres.
No obstante, a pesar de los controles veterinarios y las pruebas diagnósticas, debe asumirse que el estado sanitario de las tortugas no puede determinarse con certeza absoluta. Por ello, los ejemplares mantenidos en cautividad deben considerarse siempre como potenciales vectores de patógenos para las poblaciones naturales. Las translocaciones de tortugas deben realizarse con extrema precaución, bajo criterios estrictamente científicos o de conservación, y siempre acompañadas de un seguimiento a largo plazo.
En general, se necesita más investigación para comprender cómo afectan los patógenos a las tortugas, en particular cómo actúan en poblaciones silvestres, un aspecto especialmente complejo de estudiar y monitorizar.

Biosecurity challenges in tortoise conservation and management

Some infectious diseases pose a threat to the conservation of wild tortoise populations, and the morbidity associated with pathogens such as herpesvirus and *Mycoplasma* in *Testudo* tortoises kept in captivity is high. From a One Health perspective, the potential effects of these pathogens on the conservation of natural tortoise populations cannot be overlooked, especially since wildlife trafficking increases the risk of emerging disease outbreaks. Risk assessment should be broad, encompassing the detection and control of bacteria, viruses, protozoa, intestinal nematodes, and ectoparasites such as ticks, which may also act as vectors of pathogens transmissible to humans. Personnel working with tortoises must apply agreed hygiene protocols to prevent zoonoses and the transmission of pathogens among animals, both in the wild and in recovery centers. In the management of animals kept in captivity, it is essential to implement proper quarantine procedures and veterinary checks to avoid the spread of diseases to wild populations.

However, despite veterinary examinations and diagnostic tests, it must be acknowledged that the health status of tortoises cannot be fully assessed. Therefore, captive individuals should always be regarded as potential vectors of pathogens for wild populations. Tortoise translocations must be carried out with extreme caution and long-term monitoring.

Overall, further research is needed to understand how pathogens affect tortoises, particularly in wild populations —an especially challenging aspect to study and monitor.

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Emerging diseases and biosecurity in the conservation of Mediterranean Tortoises *Testudo graeca* and *T. hermanni*

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Abstract

Many diseases have been described to affect Iberian testudines, but those of concern are those that affect or threat their conservation programmes. Nematodes and hemoparasites: although their presence is normal (all tortoises have these parasites in ecological balance), the conditions that facilitate the breakdown of this balance are worrying (captures, periods in captivity, stress or mixtures with North African tortoises). *Mycoplasma*: it has vastly variable presence percentages in both species and it is difficult to interpret the real impact on their conservation. Herpesvirus: seems to be more present in *Testudo graeca* populations than in *T. hermanni*, and causes rhinitis and glossitis. Torchivirus: is difficult to detect due to oscillating virus shedding. It is associated with mortalities in *T. hermanni* neonates with renal disease and shell softening. It gives negative results in adults.

Health controls are especially recommended in selected tortoises, as are health tests in all the parks where these animals live in the wild. It is necessary to take minimum biosecurity measures (using disinfectants) in the field and sampling materials. Avoid releasing animals not belonging to official centres or without permits from the involved administrations is emphasised.

Resumen

Se han descrito múltiples enfermedades que afectan a los testudíneos ibéricos, y las de interés son las que afectan o ponen en peligro sus programas de conservación. Nematodos y hemoparasitos: Si bien su presencia es normal (todas las tortugas de tierra tienen estos parasitos en equilibrio ecológico), son preocupantes aquellas condiciones que facilitan la ruptura de este equilibrio (capturas, periodos en cautividad, estrés o mezclas con tortugas norteafricanas). *Mycoplasma*: tiene porcentajes de presencia muy variables en ambas especies y de difícil interpretación, desconociéndose el impacto real en la conservación de las mismas. Herpesvirus: parece estar más presente en las poblaciones de *Testudo graeca* que en *T. hermanni*, provocando rinitis y glositis. Picornavirus: es de difícil detección debido a la eliminación oscilante del virus. Se describe asociado a mortalidades en neonatos de *Testudo hermanni* con problemas renales y reblandecimiento del caparazón. En los adultos dan resultados negativos.

Se recomiendan especialmente controles sanitarios presuelta en las tortugas seleccionadas así como análisis sanitarios en todos los parques donde estos animales están presentes. Es necesario tomar medidas de bioseguridad mínimas (uso de desinfectantes) en los materiales de campo y muestreo. También se enfatiza en evitar la suelta de animales no procedentes de centros oficiales o sin permisos de las administraciones implicadas.

Keywords: Herpesvirus, *Mycoplasma*, Picornavirus, *Ascaris*, release, biosecurity
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1. Introduction

The first descriptions of emerging diseases in turtles and tortoises appeared in the 1990s. Particularly, the first fibropapillomatosis description was provided in the green turtle in 1990, *Mycoplasma agasizii* was found in desert tortoises (*Gopherus* sp.) in 1994 and ranavirus in box turtles (*Terraene* sp.) in 2004. However, the number of described diseases in the last 15 years is 10-fold higher.

The confirmation that a pathogen is the cause of a disease in a tortoise must follow Koch's postulates (Paries *et al.*, 2019). Therefore the response, treatment and prevention capacity of wild species management might slow down due to the need for previous research work.

Wildlife diseases are characterised by usually being asymptomatic (the species does not show symptoms), benign (it causes unapparent disease that does not compromise the individual), low mortality, self-limiting (disease causes a certain percentage of deaths in a population) and related to environmental variations (variation of pathogenicity depending on habitat disturbances. Whereas the first ones do not have effects on populations, the last ones (e.g. self-limiting) might affect populations, which can compromise species conservation due to non-common outbreaks. Indeed infectious diseases have serious implications due to the propagation of pathogens in introduction programsme of threatened/endangered tortoises (Jacobson, 1993).

This work aimed to: i) summarise the diseases caused by the main pathological processes that affect wild *Testudo* tortoise populations, including parasitic, viral and bacterial diseases; ii) discuss their effects on conservation and recommended actions for adequate disease detection and tortoise populations management.

2. Description of parasitic, bacterial and viral diseases

Parasitic diseases: haemoparasites and nematodes

There are different species of haemoparasites of the families Apicomplexa and Hemogregarinidae (mainly: *Hemolivia*, *Haemoproteus*, *Haemogregarina*).

Although the presence of haemoparasites in snakes and saurians has been known since the beginning of the last century, Lawrence and Hawkey (1985)

Figure 1: Macroscopic appearance of a large amount of *Mehdiella* adult parasites in *Testudo hermanni* cloaca. In the inserted square, details of the egg of this species from a coprological microscopic analysis.

detected them in Mediterranean tortoises later. The ecological relation between the presence of haemoparasites and ticks has been verified in Mediterranean tortoises in both the field (Siroky *et al.*, 2009; Harris *et al.*, 2013) and experimental infections (Siroky *et al.*, 2007). Most studies performed in wild populations of Mediterranean Testudinidae have focused on the description of haemoparasite species and their phylogenetic relation (Harris *et al.*, 2013, Ibañez *et al.*, 2013). Despite haemoparasites usually not being related to diseases or alterations in body condition (Marzal *et al.*, 2017), Segura *et al.*, (2019) describe a bad body condition in Moroccan tortoises with haemoparasites related to high tick infestation. Haemoparasites are detected during routine haematology tests, including blood smears on slides. They stain well with usual dyes (Diff Quick or May Grunwald Giemsa).
In several infected tortoises, macroscopic findings primarily include the presence of bleeding in the skin of inguinal and axillary regions, with carapace and plastron necrosis in some tortoises.
On many occasions, the disproportionate presence of haemoparasites might be considered an indirect indicator of tortoise health status, which may suggest another worse disease (Stacy *et al.*, 2011).
In the Mediterranean pond turtle (*Mauremys leprosa*), Marzal *et al.* (2017) report an immune reaction and a decrease in haematocrit associated with high parasitisation or mixed parasitisation, in addition to generic reactive eosinophilia. In deeply affected European pond turtle *Emys orbicularis*, Jozsef (2016) describes morphological changes in tissues, as well as a significant drop in the number of erythrocytes,

haemoglobin levels and iron values according to an examination of blood haematological and biochemical parameters.
The qualitative evaluation can be carried out by means of diagnostic cytology in blood smears from routine analyses in both captive and wild populations. Species detection can be performed by PCR techniques.
Hundreds of species of nematodes (intestinal parasites) have been described in tortoises. The main ones in Mediterranean tortoises are ascaris (*Angusticaecum* spp.) and pinworms (*Tachigonetria* spp., *Mehdiella* spp.; Figure 1). Generally, all reptile species tolerate the common digestive parasitism of these species, even with a 100% degree of parasitisation (Miñana & Ponce, 2018).
Serpinema and other parasites belonging to the Camallanidae family have been found in the Mediterranean pond turtle (*Mauremys leprosa*) and invasive water turtles (e.g. North American slider *Trachemys scripta*) with a different degree of pathogenicity according to their immunological condition (Martínez-Silvestre *et al.*, 2015). Nematode species and tortoise origin (captive or wild), or even environmental conditions, might have different consequences for tortoise health status (Benítez-Malvido *et al*., 2019).
Clinical signs are evident when the balance is broken when, for example, wild tortoises are kept in captivity, overstressed or overcrowded. The specific signs are the elimination of parasitic structures through faeces sightseeing (whole roundworms) or by microscopic (eggs or larvae in stool tests) (Figure 2). Nevertheless, there are non-specific symptoms, such as thinness, apathy, inactivity, or even discomfort or poor digestion, and intestinal retention if parasitic accumulation becomes disproportionate (Ras-Noriska & Sokol, 2015).
Carrying out three representative stool analyses is recommended (one every 2 weeks) in tortoise breeding groups because a single negative result does not imply that the reptile is "parasite-free". To also check for viviparous parasites (e.g. *Serpinema*, that does not leave eggs in stools), a coprological analysis 2 and 5 days after administering fenbendazole (100 mg/kg) should done to detect larvae in faeces.
There is a wide variety of coprological analyses depending on nematode species and research goals. Sedimentation (Teleman's method) or flotation is used in recovery programmes, but other currently recognised methods are: direct examination (detection of living forms of coccidians), DiffQuick (stain to detect cells in faeces), Gram (detection of predominant flora in faeces), Ziehl Neelsen (detection of *Cryptosporidium* or *Mycobacterium* in gastric lavages or faeces). If the approximate number of parasites is required, quantitative tests can be used (McMaster or counting chamber methods). Additionally, PCR approaches (eDNA) are employed to detect *Aspiculuris* in *Testudo graeca* (Huggins *et al.*, 2017).

Bacterial diseases: Mycoplasma

The *Mycoplasma* species detected in Mediterranean tortoises are *M. testudinis* and/or *M. agasizii*, which cause chronic rhinitis (nasal discharge) and other upper respiratory tract symptoms.

It has been documented as one of the causes of declining populations of other Testudinidae, such as Desert tortoises (*Gopherus* spp.) in the southwest USA in the past 30 years, in addition to other threats (Jacobson *et al.*, 2014). The disease is related to the release or escape of captive desert tortoises, which later come into contact with wild populations (Aiello *et al.*, 2014).

In Europe, *Mycoplasma* positives have recently been recorded in tortoise rescue centres that affect *Testudo hermanni* and *Testudo graeca* (Marschang, 2015; Maurin, 2019; Ballouard *et al.*, 2022; Marenzoni *et al.*, 2022).

In Spain, the diagnosis of rhinitis caused by *Mycoplasma* is not easy because of the multiple bacteria found in *Testudo graeca* (Martínez-Silvestre & Mateu-de-Antonio, 1997). Nevertheless, the first results in a wild (not reintroduced) *Testudo hermanni* population in Catalonia (northern Spain) show *Mycoplasma* positivity of more than 50% (n=59) (Martínez-Silvestre *et al.*, 2021), which contrasts with the lesser presence of *Mycoplasma* in wild populations of the same species in France (8.1%, n=347; Jourdan, 2013; Ballouard *et al.*, 2022) or Italy (2.17%, n=168; Marenzoni *et al.*, 2022).

Symptoms are similar to herpesvirus and cause "runny nose syndrome" by the production of mucus and nasal bubbles (Figure 3) in sick terrestrial tortoises. However, unlike Herpesvirus, *Mycoplasma* is not related to mortality outbreaks in Spanish wild tortoises, which makes it difficult to interpret its presence.

Figure 2: Runny nose syndrome in a *Testudo graeca* positive for *Mycoplasma*.

For a preliminary diagnosis, carrying out nasal or lingual cytology is recommended. Moreover, to confirm aetiology, performing histology, PCR, serology (ELISA; EIA), nasal cytology and microbiological cultures of accompanying flora might be advisable.

Viral diseases: alpha-herpesvirus and picornavirus

Four genetically distinct Alpha-herpesviruses have been described in tortoises and turtles (Marschang, 2019): TeAHV 1 in Russian tortoises (*Testudo horsfieldii*) and Pancake tortoises (*Malacochersus tornieri*), among others; TeAHV 2 in desert tortoises (*Gopherus agassizi*); TeAHV 3 in Greek tortoises (*T. marginata*), Herman's tortoises (*T. hermanni*), Moorish tortoises (*T. graeca*) and Russian tortoises (*T. horsfieldii*), with a special incidence in the last two); TeAHV 4 in South African Angulate tortoises (*Chersina angulate*). Alpha-Herpesviruses TeAHV 1 and TeAHV 3 have been detected in Aldabra tortoises (*Aldabrachelys gigantean*), African spurred tortoises (*Centrochelys sulcate*) and leopard tortoises (*Stigmochelys pardalis*).

In Mediterranean Testudinidae, detection mainly lies in TeAHV1 and TeAHV3 (Marschang, 2019). Heldstab & Bestetti (1989) described the first detection of herpesvirus to cause glossitis in *T. hermanni*. The first cases of herpesvirus in Spain were detected in wild *T. graeca* populations in Murcia (Martínez-Madrid, 1989; Eguia & Jimenez, 1994) and in captive individuals of the same species (Muro *et al.*, 1998; Martínez-Silvestre *et al.*, 1999; Martínez-Silvestre *et al.*, 2001). Herpesvirus has also been detected in *Testudo hermanni* and might be a threat for reintroduction conservation programes (Martínez-Silvestre *et al.*, 2012). It has also been docu-

Figure 3: Necropsy picture of a typical Herpesvirus stomatitis in a *Testudo hermanni.*

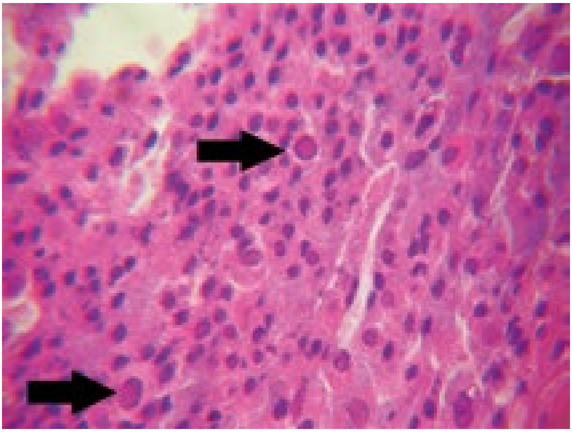

Figure 4: Histological picture of tongue epithelial cells in *Testudo hermanni*, where arrows highlight herpesviral intranuclear inclusion bodies.

mented in wild populations of both *T. graeca* in Morocco and *T. hermanni* in southern France (Mathes, 2003).

Symptoms are associated with the upper respiratory tract and are complicated by opportunistic bacteria, which also cause stomatitis/glossitis (Figure 4). In initial stages, it causes the production of mucus and nasal bubbles (similar to the symptoms caused by *Mycoplasma*, or even Ranavirus) and noisy breathing. It can later cause depression, stomatitis, glossitis, inability to feed, cachexia and death.

Nasal or lingual cytology is recommended for a preliminary diagnosis. Histology (mouth, tongue, pharynx and respiratory system), PCR (from mucus swabs or oro-pharyngeal smears), serology, microbiological culture (of accompanying flora to perform antibiograms), haematology (heteropenia and lymphocytosis) have been described), and can be performed to confirm disease. PCR, ELISA and serum neutralisation (SNT) tests have a confidence factor of at least 97%. Repeating tests at intervals of 10-12 weeks apart can increase overall reliability to an acceptably high degree of confidence.

At the start of this century, the presence of a virus from the Picornaviridae group was described in some studied Mediterranean tortoises (Marschang, 2000). Although it was initially described in tortoises as "virus X" (Marschang & Rümenapf, 2002), it was later reclassified as Picornavirus. It has been recently incorporated into a different subgroup of the Picornaviridae family (Farkas *et al.*, 2014), and Marschang (2019) classified it as a new genus: Torchivirus (Tortoise X virus).

It has been recently described in an *in situ* breeding programme of *T. hermanni* in Tarragona (northern Spain) and 77% of young tortoises died (Martínez-Silvestre

et al., 2020). By a serologic analysis, the virus has been detected in *Testudo hermanni* in an introduced population in Tarragona (13.3% positive asymptomatic adults; n=15) (Martínez-Silvestre *et al.*, 2022), and also in a non-introduced wild population innorthern Spain (Gerona; 24.4% positive asymptomatic adults; n=41; Martínez-Silvestre *et al.*, 2021).
The virus causes the mortality of hatchlings and young tortoises (< 5 years) (Paries *et al.*, 2019). Sick animals show softening shells, which seem fragile and deformable, and slow down growth and lead to apathy. Later young tortoises are characterised by no longer feeding, closed eyes, no limb movement and bulging shells (Figure 5a). If cystocentesis is involved, profuse urine retention can be detected. The commonest findings in necropsy are nephromegaly, urine retention and carapace decalcification.
Diagnosis requires running a PCR because histology is not conclusive despite showing alterations of the possible viral cytopathic effect (degeneration of renal tubules –Figure 5b– and osteodystrophy. However, the virus involves intermittent excretion that makes its detection difficult (Paries *et al.*, 2019). Working with PCR samples only in adults can result in false-negatives. In this case, a serologic analysis (ELISA/EIA/EA) is advisable to detect asymptomatic adult carriers. Serological positive

Figure 5: a) Soft shell in a *Testudo hermanni* hatchling caused by Torchivirus infection. b) Histological picture of a kidney in *Testudo hermanni*, where arrows highlight vacuolisation and degeneration in renal tubules, which is well related to Torchivirus infection.

picornavirus adult carriers should be isolated to see if virus clearance disappears over time or they are still chronic carriers.

3. General discussion and recommended actions

The ability of viruses to activate and spread under natural conditions is not well understood (Reinnauer & Marschang, 2005; Martínez-Silvestre & Soler, 2019b) and the host immune system might play an important role in these ecological relations (Riera-Romo *et al.*, 2016). Further studies in wild *T. graeca* and *T hermanni* populations are necessary, which will include the detection of Herpesvirus and *Mycoplasma* in adults, and of Torchivirus in young and adult tortoises. While haemoparasites and nematoda might not have population repercussions in tortoise health status, this is not the case of Torchivirus or Herpesvirus, which might cause population die offs. In reintroduction conservation programmes, preventive measures should be immediately taken to detect the latter.

In addition, there are other virus associated with rhinitis like ranaviruses (still not reported in *T. graeca* and *T. hermanni*), but present in American box turtles (*Terrapene* sp.) and tropical turtles (*Astrochelys radiata*). Other emerging diseases described in other wild tortoises and turtle populations that might be cause of concern are intranuclear coccidiosis, cryptosporidiosis and mycosis due to *Emydomyces testuvorans* (Adamovicz *et al.*, 2020). Although other diseases have not been described in the wild in Spain, they have been described in captivity and could be detected in wild tortoises through spillover if appropriate biosecurity measures are not taken, such as atypical tuberculosis *Mycobacterium* (Muro *et al.*, 2020). Thus including them in the future in sanitary protocols is recommended.

It is necessary to carry out a disease analysis of wild populations and those for reintroduction conservation programmes, and to maintain field biosecurity measures in the field when manipulating individuals.

Carrying out group PCRs (several individuals in only one PCR) allow the detection of positives in suspicious populations. They do not have a reliable quantitative value, but are useful (positive/negative) in population management. It also allows economic costs to be reduced. However, it is not advisable to mix more than four individuals in a single sample. Mixing too many individuals also lowers the concentration and presence of the pathogen, and may not be detected due to the sensitivity of the followed method. When sampling recently died specimens, PCRs include internal tissues (kidney, bone, spleen) and tongue. If tortoises are seized, following the rules established in the Kiten protocol to implement the accurate and reliable taxonomic, sanitary and geographical identification of all the animals intended to be released (Kornilev *et al.*, 2017) is advised. Moreover, the animals that belong to a known location close to its natural habitat have never been exposed to other conspecifics of

unknown origin. Any animals from a large group of mixed species, of unknown origin or from the pet trade have to be considered to be at extremely high risk.
The application of minimum biosecurity measures during fieldwork and in centres when sampling is necessary: in the former, using gloves when handling tortoises; in the latter, by adding disinfectant foot baths, disposable shoe coverings when moving through different enclosures or handling different tortoises, or wash hand and vehicles with disinfectants. Movements of humans or other animals between enclosures should be minimised.
In reintroduction programmes, which sometimes favour overcrowding, the pathogen spread risk is higher. For example, environment persistence studies show that Torchivirus endures lengthy periods in cold water and viral inactivation in soil at summer temperatures, which may take more than 3 weeks (Marschang, 2019). In line with this, tortoise behaviour, characterised by burying for extended periods in their lives, is an important potential mode of transmission.
Prevention is, thus, based on three principles: control of carriers (analysing asymptomatic hatchlings, young and adult individuals); avoiding overcrowding and adopting biosecurity measures in breeding facilities; during field work. In these programmes, a considerable quarantine period (at least 6 months) is advisable before releasing tortoises, and always from official centres or with permits from public administrations (Highfield & Highfield, 2015).
Particularly in rescue centres, and in conservation programmes for future release, to minimise the effect of these described pathogens or those that may appear in the future, several measures are recommended to prevent disease from spreading:
In the captive status, it is necessary to take the necessary samples to insure correct animal clinical status (microbiology, histology, haematology, cytology, PCR, ELISA/EIA/EA, and even imaging diagnostics). Deworming 2 to 3 times a year in captive tortoises. Euthanasia of confirmed positive Herpesvirus or Torchivirus is recommended.

Before releasing:

1) Deparasitisation (against worms) 2 to 3 weeks in advance.
2) Final check to determine good body condition (weight) and health.
3) Tagging, microchips or other individual identification for long-term survival monitoring.
4) PCR or ELISA/EIA tests for Herpesvirus, *Mycoplasma*, Picornavirus and Ranavirus at least 2 months in advance.
5) Minimum sample of one random analysis every 5 to 10 animals, or one group analysis (with a maximum of four animals in the same sample) in multitudinal releases.

After releasing:
Monitoring release sites in the first 15 to 25 years: population density and random

PCR testing (minimum for these three diseases: *Mycoplasma*, Herpesvirus and Torchivirus) with a minimum sample of 20% of the population density.

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Health screening protocol for the relocation of *Testudo hermanni hermanni* to the wild: the proposed model of the Italian guidelines

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Abstract

Testudo hermanni hermanni is irregularly distributed in western Mediterranean Europe. In Italy, the species is found mainly on the Peninsula, and on Sardinia and Sicily. The species is affected by several threats, including habitat loss, illegal collection and trade. The Italian relocation of confiscated individuals is supported by the Italian Ministry of the Environment. A health screening, to avoid introducing potentially dangerous infectious agents into the wild, was implemented and performed on confiscated animals, including: 1) clinical evaluation and collecting biological samples; 2) a parasitological analysis for endo- and ectoparasites; 3) biomolecular and serological tests for Testudinid-Alphaherpesvirus (TeHV); 4) a biomolecular test for *Mycoplasma* spp. This protocol defines the necessary sanitary criteria to release a tortoise to the wild, which include a clinically healthy status, lack of detection of TeHV and *Mycoplasma* spp. Individuals testing positive for infectious agents or without an appropriate genetic profile cannot be released.

Resumen

Testudo hermanni hermanni se distribuye de forma irregular en el Mediterráneo occidental europeo. En Italia se distribuye principalmente en la península, en Cerdeña y Sicilia. La especie se ve afectada por varias amenazas (incluida la pérdida de hábitat, la recolección y el comercio ilegales, todos ellos vinculados con la actividad humana) responsables de su declive. El Ministerio Italiano de Medio Ambiente está promoviendo la gestión adecuada de la reubicación de ejemplares intervenidos o confiscados. En consecuencia, se ha implementado un sistema de vigilancia sanitaria para evitar la introducción de agentes infecciosos mortales, potencialmente peligrosos o aún no bien caracterizados en las poblaciones silvestres. Para tal fin, se implementó un examen de salud en animales confiscados para realizar: 1) una evaluación clínica y recolección de muestras, 2) un análisis parasitológico para endo- y ectoparásitos, 3) pruebas biomoleculares y serológicas para alfa-herpesvirus de las tortugas (TeHV), 4) un ensayo biomolecular para *Mycoplasma* spp . Como resultado de la encuesta, definimos los criterios necesarios para liberar una tortuga en la naturaleza, que incluyen un estado clínicamente saludable, falta de detección de TeHV y *Mycoplasma* spp. y un perfil genético adecuado que permita una correcta reubicación geográfica. Los ejemplares que son positivos para los agentes infecciosos mencionados o que no tienen un perfil genético apropiado no deberían ser liberadas.

Keywords: *Testudo hermanni*, Testudinid-Alphaherpesvirus, *Mycoplasma agassizii*, parasites
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Introduction

Testudo hermanni is included as Near-Threatened on the Red List of the International Union for Conservation of Nature (IUCN). The nominal subspecies, *T. hermanni hermanni*, is distributed in western Mediterranean Europe. It is found on the peninsular and insular Italy, and is considered Endangered by the Italian IUCN Red List (Rondinini *et al*., 2013, 2022). Habitat loss, illegal collection and trade of wild individuals are major threats.
The Italian Ministry of the Environment (Ministero dell'Ambiente e della Tutela del Territorio e del Mare – MATTM) promotes rigorous management for the relocation of confiscated tortoises to the wild. As a result, potentially dangerous, fatal or even poorly characterised pathogens, yet of concern, were selected for monitoring. The following protocol originates from the need to relocate the numerous confiscated individuals hosted by Italian State Forestry recovery centres (Reparti Biodiversità dei Carabinieri Forestali), despite very limited resources in both staff and logistics terms.
The relocation of clinically and (for known selected pathogens) microbiologically healthy animals with an appropriate genetic profile would help recovery centres by reducing maintenance costs and avoiding overcrowding (and correlated health problems), while respecting captive animal welfare.

Protocol Methodology

Monitoring activity on selected micro-/macroorganisms has been carried out since 2014 on confiscated *T. hermanni* hosted by institutional recovery centres. The assessment of the mandatory health status for each rescued tortoise follows the steps listed below.

1- Clinical evaluation and biological sampling

A clinical examination is performed to assess the general health status, presence of visible alterations of mucous membranes, gross lesions and ectoparasites. A pit tag for individual recognition is implanted between the plastron and the left pectoral muscle.

Biological samples are collected and stored as follows: a) an oral swab preserved in PBS (phosphate-buffered saline, pH 7.4); DNA extracted within 24 h (Qiagen, Milano, Italy - 200 µL); b) 1 ml of whole blood collected by phlebotomy from the subcarapacial plexus in lithium heparin tubes (LP-Italiana, Milano, Italy) and centrifuged at 1600 rpm for 10 minutes to separate plasma from cellular fractions; c) fresh faecal samples and ectoparasites collected and kept at 4ºC until examined.

2- Parasitological examination

Faecal samples are processed by traditional and immunological copromicroscopic techniques to detect the eggs and larvae of helminths, (amoeba) cysts and oocysts of protozoa (Wolf *et al*., 2014).

Three faecal material fractions are used for a qualitative examination:

- The first fraction is concentrated by flotation with sucrose solution (Sheather solution s.g. 1.250) (copromicroscopic examination);
- The second fraction is analysed and concentrated by formol-ether sedimentation (Ridley & Hawgood, 1956);
- The third fraction is used for the simultaneous detection of *Giardia* spp. and *Cryptosporidium* spp. co-antigens by the ProSpecTTM *Giardia* / *Cryptosporidium* microplate assay, a qualitative enzyme immunoassay (EA) that identifies the presence of specific antigens of *Giardia* spp. and *Cryptosporidium* spp. (GSA 65 and CSA) in aqueous faecal material extracts.

All the positive samples undergo a quantitative coproparasitological examination following a modified McMaster technique to define the number of excreted eggs per gram (e.p.g.) of faeces (sensitivity equals 50 e.p.g.) (Sloss *et al*., 1994).

The ectoparasites collected during the physical examination are identified to the species level (Guglielmone *et al*., 2014; Sloss *et al*., 1994).

3- Herpesvirus detection/exposure

3.1- Biomolecular analysis

An array of biomolecular assays, including PanHerpesvirus nested cPCR (consensus) (VanDevanter *et al*., 1996) and additional s[[pecific protocols (Murakami *et al*., 2001; Origgi *et al*., 2004; Marenzoni *et al*., 2018), are carried out using the DNA extracted from the oral swab to detect Testudinid-Alphaherpesviruses. More specifically, the PanHerpesvirus positive tests are repeated for confirmation purposes. Double positivity implies excluding relocation. Positive samples are further characterised by specific PCR (protocols in progress):

a. nested PCR for TeHV-1 (Murakami *et al*., 2001);
b. nested PCR for TeHV-3 (Origgi *et al*., 2004; Marenzoni *et al*., 2018);

If the amplicons obtained using the PCR protocols at points a or b are not of the expected size or negative (no amplification), the product amplified by PanHerpesvirus PCR is sequenced to characterise it.

3.2- Serology

A serological test using plasma is performed to determine *T. hermanni* exposure to TeHVs:

1. ELISA (Enzyme-Linked Immunosorbent Assay) for the detection of specific anti-TeHVs antibodies (Origgi *et al*., 2001);
2. If the OD reading obtained with 1. comes close to the cut-off, but is still negative, a Western blot is carried out to determine if any specific anti-TeHV antibodies are present.

4- *Mycoplasma* sp. detection

A series of biomolecular assays is performed to detect mycoplasmosis on oral swabs:

1. PanMycoplasma: PCR using two independent PCRs (Lierz *et al*., 2007; Vojdani & Franco, 1999);
2. *Mycoplasma agassizii*: species-specific PCR (Brown *et al*., 1995 – concurrently performed with 1.);

If the amplicons obtained from the previous molecular protocols are not of the expected size, amplicon sequencing is performed for confirmation purposes.

Some critical points faced during the health screening, which are an opportunity for additional considerations, are listed below.

1- The clinical examination assesses the general health status. The individuals showing locomotor disorders, severe integument injuries or respiratory/gastrointestinal signs cannot be relocated. The veterinarian is in charge of assessing whether to continue with further investigations or not (MATTM, 2019).

2- The proposed parasitological screening allows the presence of various genera of protozoa (e.g., *Entamoeba* spp., *Balantidium* spp. and *Nyctotherus* spp.), and of eggs and adults of pinworms (Nematoda: Oxyurida: Pharyngodonidae), to be detected. EA targets are specific protozoa (*Giardia* and *Cryptosporidium*) of potentially concern for *Testudo* sp. In the event of positive results, the following criteria are used:

a) A drug treatment followed by screening after 15 days is required; if the copromicroscopic test is negative, then the tortoise can be relocated;
b) Positivity for pinworms requires antiparasitic treatment to reduce the parasite load; after 15 days, if the parasite load is low or moderate, relocation can take place;
c) Ectoparasites must be treated and reevaluated after 15 days. Only if they are negative can animals be relocated.

3- This screening allows Testudinid-Alphaherpesvirus (TeHV-1, 2, 3 and 4) to be detected in both acutely and chronically infected individuals in a complementary manner.

More specifically, the molecular test (PCR) is the test of choice during acute infection when the viral load is presumptively higher and shedding occurs along with the absence of, or the presence of a few, antibodies. Serology is the test of choice in the chronic (latent) infection stage when replicating and shedding virus might not be present. Accordingly, the PCR test would be negative (Origgi *et al*., 2001, 2004; Origgi, 2012). Infected tortoises can be asymptomatic virus carriers/hosts. Viruses can reactivate and be shed by the tortoise following immunosuppression often before or after hibernation. Molecular and serological tests are complementary and, when used in synergy, increase the robustness of assessments and provide a more reliable result about the tested tortoise's actual microbiologic condition. Therefore, the individuals that test positive to at least one of these assays should not be relocated to the wild. They should remain isolated from other susceptible tortoises for the rest of their lives because their condition is likely a permanently infected status, which is known for many other herpesviruses, and, consequently, as possible sources of infection for other susceptibly uninfected individuals.
4- An array of PCR tests is applied to detect the bacteria of the genus *Mycoplasma*, particularly *M. agassizii*, regardless of the clinical presentation at the time of testing. It is believed that the principal transmission route of mycoplasmosis is horizontal by direct contact, and infected tortoises may shed bacteria, especially when they show clinical signs (Jacobson *et al*., 2014). PCR assays play a pivotal role in preventing the release of infected tortoises to the wild. Accordingly, any positive test for *Mycoplasma* spp. excludes the individual's relocation.

3. Discussion

The relocation of clinically and microbiologically (selectively - for known selected pathogens-) healthy animals is crucial to avoid introducing pathogens into the wild. Captivity, frequently characterised by a mix of species and overcrowding, by definition favours pathogen spread. Different *Testudo* species also show distinct and apparent susceptibility and resistance to various pathogens.
The identification of micro-/macroorganisms that threaten *Testudo* spp. is still far from complete. However, current scientific knowledge allows to draw a baseline.
Along these lines, two well-known and characterised conditions in tortoises, tortoise herpesvirosis and mycoplasmosis, need to be investigated as part of a proper health assessment of those tortoises expected to be relocated.
Herpesvirosis may have significant negative impacts on wild tortoise populations. At least four herpesvirus genotypes can infect tortoises: the most widespread and putatively pathogenic is TeHV-3, reported in both Italy (e.g., Marenzoni *et al*., 2018) and elsewhere (reviewed in Origgi, 2012). Latency, a common dormant condition to

many herpesviruses, poses challenges for clinical diagnosis (Origgi, 2012). Moreover, infections may be characterised by high mortality depending on the affected species: *T. graeca* is considered more resistant to the disease and is a presumptively carrier, whereas *T. hermanni* is highly susceptible with high mortality rates following exposure to herpesvirus (Origgi & Rigoni, 2003; Origgi, 2012; Marenzoni *et al*., 2018). The introduction of herpesvirusinfected tortoises into the wild may seriously threaten the survival of virus-free wild populations (Martel *et al*., 2009).

Although *Mycoplasma* spp. is often considered a commensal (e.g., Hill, 1985 [*M. testudinis*]; Di Ianni *et al*., 2015), *M. agassizii* and *M. testudineum* cause upper respiratory tract diseases (URTD) associated with mortality in North American tortoises (Jacobson *et al*., 2014), likely due to the impairment of olfactory and respiratory functions (Jacobson *et al*., 2014).

Data on the impact of *Mycoplasma* spp. on free-ranging *Testudo* sp. are relatively scarce (e.g., Lecis *et al*., 2011; Ballouard *et al*., 2022; Marenzoni *et al*., 2022). However, the precaution principle is crucial when it comes to relocation. Antibiotic treatment of *Mycoplasma*-infected tortoises has been attempted, but conclusively and definitively clearing bacteria is a matter of debate. Therefore, the relocation of positive tortoises, even when treated for *Mycoplasma*, is not recommended in the light of current knowledge.

Some protozoans (e.g., *Giardia* spp., *Nyctotherus* spp., *Balantidium* spp.) and metazoans (oxyurids) are often detected in tortoises and considered commensal and symbiotic given their described role in the digestion of cellulose and carbohydrates (Barnad & Upton, 1994; Greiner & Mader, 2006). Nevertheless, a heavy load of these parasites has been associated with anorexia, loss of weight, diarrhoea and enteritis (Bardi *et al*., 2019; Eatwell & Richardson, 2019). Many Testudinidae are parasitised asymptomatically. For instance, tortoises are traditionally considered to be reservoirs of *Entamoeba* sp. (Bardi *et al*., 2019), which can be transmitted to other susceptible reptile species (e.g., lizard and snakes) with fatal consequences (García *et al*., 2014; Eatwell & Richardson, 2019). However, reports of pathological findings associated with *Entamoeba* sp. are also found in chelonians (e.g., Jacobson, 2007; Bardi *et al*., 2019). A search for *Cryptosporidium* sp. in our protocol aims to help to clarify the potential pathogenic role of this parasite, which is less frequently associated with diseases in *Testudo* (e.g., Traversa *et al*., 2008; Bardi *et al*., 2019) than in other reptiles (Thompson, 2004). Anti-parasitic treatments are considered relevant for the relocation of candidate clinically and (selectively) microbiologically healthy individuals without a high parasitic load.

Other known pathogens not presently considered in our protocol, but that are currently being evaluated for health screenings for chelonian relocation, are:

- Ranavirus (reviewed in Wirth *et al*., 2018);
- Tortoise Picornavirus (ToPV – Heuser *et al*. 2010; Paries *et al*., 2019);
- *Chlamydia* sp. (Mitura *et al*., 2017), including *Candidatus Chlamydia testudinis* (Laroucau *et al*., 2020);
- *Mycobacterium* spp. (e.g., Muro *et al*., 2020);
- Intranuclear coccidia of tortoises (Hofmannová *et al*., 2019);
- *Hexamita* spp. (Bardi *et. al*, 2019).

In addition to the above health-related conditions, other items must be considered when managing relocation (MATTM, 2019; see also Martínez-Silvestre & Franklin, 2019):

1. morphological and/or behavioural alterations that can compromise survival in nature;
2. tortoises must be relocated within their original geographical area;
3. presence of suitable relocation sites;
4. the individuals that can be relocated must be released away from areas where autochthonous free-ranging populations are present to avoid "immediate" contact;
5. the confiscated tortoises that cannot be relocated must remain in institutional recovery centres.

An additional suggestion is that pharmacologically treated individuals should not be relocated to avoid spreading drug-resistant organisms in the environment, unless lack of evidence for emerged antibiotic resistance has been thoroughly assessed.

Despite using thorough and well-thought protocols, relocation cannot completely exclude any risk for wild populations. Therefore, any relocation programme shall be followed by stringent monitoring activity to detect any long-term effects occurring on relocated populations and to implement conservation measures.

The protocol is conceived to be easily improved and updated so that it adapts to constantly growing knowledge on reptiles' infectious diseases and disease ecology (e.g., Marini *et al*., 2019).

The relocation to the wild of individuals that are as healthy as can be feasibly assessed is crucial for the long-term success of relocation actions and for preserving free-ranging populations, which is the cornerstone of conservation.

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Clinical Trial of *Mycoplasma* treatment in captive *Testudo graeca* populations

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Abstract

The spur-thighed tortoise (*Testudo graeca*) is considered a threatened species in Spain and is listed as vulnerable internationally on the IUCN Red List. Since 2015, FIEB has led the Firmus Graeca project. This project aims to study the health status of captive *Testudo graeca* populations and to compare them to wild populations to obtain the necessary information for following a health protocol to help to make appropriate decisions about the destination of captive specimens. Three years of sampling revealed that 71.4% of the specimens kept at the FIEB facilities were positive for *Mycoplasma agassizii*. Consequently, we proposed this clinical trial with three treatments in three *Testudo graeca* groups (Marbofloxacin IM, Enrofloxacin IM and Ceftazidime IM + Tobramycin IN -intranasal-) and a control group. The presence of *Mycoplasma agassizzi* was determined before treatment, 1 week and 6 months after treatment, and immediately before hibernation to check whether animals stopped being carriers of *M. agassizii* after any of these time points. Although the results are not statistically significant due to sample size, they suggest that there is no totally effective treatment and more factors exist that affect disease evolution because the control group is that with the most negatives in the last sampling.

Resumen

La tortuga mora (*Testudo graeca*) es una especie catalogada como amenazada en España y como vulnerable a nivel global por la lista roja de la IUCN. Desde 2015, FIEB lidera el proyecto Firmus Graeca, que tiene como objetivo realizar un estudio global del estado sanitario de la población cautiva de *Testudo graeca* y compararlo con el estado sanitario de las poblaciones libres con el fin de obtener la información necesaria para realizar un protocolo sanitario que ayude a tomar las decisiones oportunas en torno al destino de los ejemplares cautivos. Tras tres años de muestreos se observó que el 71.4% de los ejemplares mantenidos en las instalaciones de FIEB eran positivos a *Mycoplasma agassizii*. Como consecuencia, se planteó el siguiente ensayo clínico, basado en tres tratamientos en tres grupos de *Testudo graeca* (Marbofloxacina IM, Enrofloxacina IM y Ceftazidima IM + Tobramicina IN -intranasal-) y un grupo control. Se determinó la presencia de *Mycoplasma agassizzi* antes del tratamiento, una semana después y cinco meses después, justo antes de la hibernación, para comprobar si tras alguno de ellos los animales dejaban de ser portadores de *M. agassizii*. Aunque los resultados no son estadísticamente significativos debido al tamaño muestral, sí que revelan que no existe un tratamiento totalmente eficaz y que hay más factores que afectan a la evolución de la enfermedad ya que el grupo control es el que mostró un mayor número de negativos en el último muestreo.

Keywords: spur-thighed tortoise, rhinitis, desease, enrofloxacin, marbofloxacin, ceftazidime
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1. Introduction

The spur-thighed tortoise (*Testudo graeca*) is catalogued as a threatened species in Spain and as vulnerable internationally on the IUCN Red List due to the plundering and destruction of habitats. It is also included in Appendix II of CITES, Annexe A. To recover wild populations, the reintroduction of captive specimens as a conservation strategy has been considered. However, upper respiratory tract disease (URTD) prevalence is high for captive populations, which can lead to death.

In this context, and to design a Health Protocol that allows animals to be released without posing a risk to wild populations, FIEB has led a Firmus Graeca. Since 2015, FIEB leads a research project that aims to isolate the main pathogens causing rhinitis, and to determine the pathogenicity and treatment of the disease. To date *Mycoplasma agassizii* has been identified as the main pathogen to cause rhinitis (with a 71.4% prevalence in captive populations).

In Spain, plundering in the environment and the abandonment of many specimens have caused animals to accumulate in wildlife recovery centres. Many of them develop rhinitis, a disease of the upper respiratory tract, which ends up causing pneumonia, septicaemia in many cases and, consequently, death.

2. Objectives

A clinical trial is proposed to establish the best therapeutic protocol based on collected data and previous treatments in both this species and other tortoise species and, for the first time, a medium-term follow-up to be carried out (6 months) to rule out the condition of an individual being a carrier of the pathogen.

The objectives of this study are to:

1) Find the most effective treatment for URTD caused by *Mycoplasma agassizii* in spur-thighed tortoises in captivity
2) Show whether it is possible for an individual to become a non-carrier of *Mycoplasma agassizii*.

To fulfil these objectives, the taken actions are to:

1. Determine the presence of *Mycoplasma agassizii* by PCR techniques after hibernation (April).
2. Separate positive animals into four different treatment groups (including symptomatic and asymptomatic individuals) and apply the corresponding treatment to each one for 1 month (April-May).
3. Determine the presence of *Mycoplasma agassizii* and clinical status in animals immediately after the end of treatment and 6 months later (October).

3. Methodology

The employed animals were 32 adult *Testudo graeca* individuals housed in the FIEB facilities that were positive before entering hibernation in 2018.

Four groups of eight individuals each were formed: one control group and three groups with three different treatments. The first two are described in the bibliography as being successful to treat *Mycoplasma agasizii* in other tortoise species. The third one is Ceftazidime IM, a bactericidal cell wall synthesis inhibitor that should not act against *Mycoplasma* (bacteria with no cell wall), plus intranasal Tobramycin application. The intranasal application route was used to ensure the better elimination of pathogens locally. This treatment was chosen for the growth inhibitory effect of possible concomitant bacteria in rhinitis (Martinez-Silvestre *et al*. 1997, 1999), and allows the tortoise immune system to focus on controlling *Mycoplasma*.

a) Control Group (8 individuals): there is no treatment.
b) Group 1 (7 individuals): positive individuals with Marbofloxacin treatment IM (intramuscular) 5 mg/kg/24 h/30 days.
c) Group 2 (8 individuals): positive individuals with Enrofloxacin treatment IM 10 mg/kg/24 h/30 days.
d) Group 3 (8 individuals): positive individuals with Ceftazidime treatment IM 20 mg/kg/48 h/30 days + Tobramycin Intranasal/24 h/30 days.

Adult individuals were randomly selected for each treatment group after bearing in mind that the selection criteria were development of exudative rhinitis after posthibernation. By adopting strictly ethical criteria however, the control group was made up of individuals that, despite being positive, did not have a runny nose upon sample extraction. The different groups were placed in separate places to avoid them coming into direct contact. It should be noted that individuals were not tested for concomitant non-mycoplasma-related bacteria.

Samples were obtained by nasal lavage and introducing sterile physiological serum through nostrils and aspirating it again, or by direct swab to directly collect nasal discharge.

Real-time PCR was performed to detect and quantify the presence of *Mycoplasma agassizii* DNA. Total DNA was extracted from the wash samples by a pressure filtration method (S DNA Tissue Kit, Fujifilm®). A real-time PCR was performed based on Sybr Green, developed at the INIA-CISA. This method amplifies 162 bp of the 16S RNA gene from *Mycoplasma agassizii* and *Mycoplasma testudineum*. It allows identification by the analysis of the fusion curve (manuscript being prepared). Relative quantification was adapted from the delta ct (cycle threshold) method. In summary, quantification was performed using this formula:

$$RQ_{(sample,\ time)} = 10^{[-0.303\times(ct_{sample}\ -\ ct_{mena\ CG})]}, \text{ where:}$$

- $RQ_{(sample,\ time)}$: is the relative quantification for a given sample at a given time.
- ct_{sample}: is the threshold of the sample cycle at the given time.
- ct_{mean} CG: is the mean of the control group cycle thresholds at the given time. Negative samples were considered to be RQ=0.

Statistical analysis

The clinical trial aims to decrease *Mycoplasma agassizii* prevalence in treated turtles by up to 0% and is, therefore, a dichotomous endpoint. A chi-square test was performed for the qualitative results (positive/negative), while a Mann-Withney U was carried out for relative quantification. Statistically significant differences were considered if $p < 0.05$.

The Table below shows the number of individuals per treatment and time.

Treatment	**t0**	**t1**	**t2**
0 (CONTROL)	8	8	6
1	7	6	7
2	8	6	7
3	8	8	7

4. Results

Figure 1 shows *Mycoplasma agassizii* evolution over time with the different treatments. It reveals that treatments do not seem to lower *M. agassizii* prevalence in relation to the negative control.

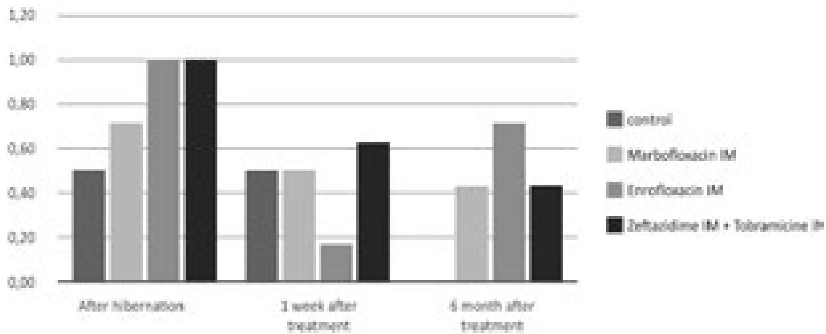

Figure 1: *M. agassizii* prevalence per treatment and time. All the animals in the control group were negative 6 months later.

5. DISCUSSION

Despite the fact that, according to the analysis performed immediately after treatment ended, the animals treated with Enrofloxacin have the lowest *M. agassizzi* prevalence, the treatment based on intramuscular Ceftazidime combined with the application of intranasal Tobramycin seems more effective in the long term. However, differences are not statistically significant. It should be noted that in no case is long-term 0% prevalence achieved. In addition, the prevalence of the pathogen lowers in the control group despite not receiving any treatment.

Some previous studies have highlighted that variations in the competence of tortoises' immune system are given by each animal's intrinsic factors (sex, age, nutritional status, present diseases, etc) and external factors, such as climate and season. In this study, samples are taken at very different times and are, therefore, another variable to consider.

Thus several possible explanations for these results are suggested.

First of all, it is important to note that a previous bacteriological culture of animals' nasal secretions is not included to determine the present bacterial flora. Consequently, we do not have any information on the real effect that treatment may have had by inhibiting other possible concomitant bacteria. Perhaps by eliminating them or reducing the number with the third treatment, tortoises' immune system can focus on fighting *Mycoplasma* much more effectively than in the other groups. In this third treatment, one remarkable feature is that a bactericidal antibiotic is used intranasally unlike the other two treatments. Therefore, efficacy may be conferred by both the synergy of both antibiotics and the exclusive effect of local tobramycin. This enables to add another study group, one treated exclusively with IN tobramycin, in the future.

Another consideration is that all the animals were treated as positive at the start of the study using the prehibernation results from the previous year. It is true that they started with different prevalences: control: 50%; treatment 1: 71.4%; treatment 2: 100%; treatment 3: 100%.

Another factor to remark on is lack of stress. Captivity provides them with low population density, remaining calm (in the control group for not being handled daily like other groups), and good nutrition and hydratation. All these factors are immunostimulants and can help tortoises to fight against *Mycoplama*.

In the control group, the fact that there are no positive animals in the last sample might suggest that the presence of *M. agassizzi* in nasal secretions can be cyclical regardless of the applied treatment. For this reason, recommendations are: expanding n for future studies; extending the tests of the antimicrobial treatments described to treat mycoplasmosis in other species, such as doxycyclin, florfenicol and tildipirosin; adding a group treated only with IN tobramycin.

Finally, another factor to take into account is that the presence of individuals with symptoms that are negative for detecting *M. agassizii* continues. So there might be other infectious agents involved. To do this, as these samples have already been checked for herpesvirus and are also negative, Ranavirus spp. and Picornavirus spp. can be screened on the file samples when these PCRs are ready to be used.

These results suggest that, to establish the basis for a new release programme based on a risk analysis of the disease, it is necessary to extend not only this study by adding the aforementioned viruses, but also the sample by adding individuals from other centres.

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Prophylaxis protocol for tortoise fieldwork

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Abstract

Parasites and disease are major threats for wildlife tortoise conservation and for people who work with these reptiles. Here we describe a hygiene and safety protocol developed by the Ecology Area of the Miguel Hernández University to minimise health risks associated with tortoise fieldwork. This protocol contains advice and details about the procedures and materials needed to perform fieldwork safely for humans and tortoises. We also describe some instructions for disinfecting and cleaning work material in the field and the laboratory. We quantify investments made in time and money to accomplish this hygiene and safety protocol. Following a protocol like this is especially necessary when handling tortoises from threatened populations.

Resumen

Las enfermedades y parásitos constituyen una importante amenaza para la conservación de las poblaciones silvestres de tortugas y para las propias personas que entran en contacto con estos animales. Aquí se presenta un protocolo higiénico desarrollado por el Área de Ecología de la Universidad Miguel Hernández para minimizar los riesgos asociados al trabajo en campo con tortugas terrestres. El protocolo contiene todas las recomendaciones y equipamiento necesario para llevar a cabo los trabajos de campo de una manera segura tanto para tortugas como humanos. Además, se describen las directrices de limpieza y desinfección del material de trabajo tanto en campo como en laboratorio y cuantificamos el coste de su implantación en términos temporales y económicos. Es especialmente necesario llevar a cabo protocolos como éste cuando se trabaja con poblaciones amenazadas de tortugas animales de poblaciones amenazadas.

Keywords: health risks, hygienic protocol, disease, handling animals, VIRKON™
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1. Introduction

Biodiversity research focuses on conservation, but the research activity itself can become a conservation problem. Handling animals during fieldwork entails important biosecurity risks. There are so many pathogens and parasites that can spread through animals (e.g. amphibian chytridiomycosis) (HACC 2004; Kilpatrick & Randolph, 2012) and some can be potentially transferred to humans and cause zoonotic infections (MAPA, 2019; SEZ, 2019; UW-Madison, 2020). This issue is important in recovery and breeding centres, but is crucial in wildlife populations. Unknown animal health conditions and lack of control of many variables during fieldwork increase the health risk. All this renders the development of hygiene and safety protocols to ensure animal and human security extremely necessary. Safety protocols are commonplace for cattle management (GREFA, 2019), and for recovery and breeding centres (Chomel, 2003; SEC, 2016; VCF, 2019), but they are scarcer for fieldwork issues, except for amphibians (globally imperilled by pathogens) (AHE 2016; BsalEurope 2019a,b; HACC, 2004). To the best of our knowledge, no protocol has been disseminated for tortoises. Tortoises face lots of spreadable diseases, such as rhinitis, fungal and protozoal infections, or intestinal parasites, among others. In relation to tortoises, it is known that zoonosis increases the human health risk (e.g. salmonellosis) (Lecis *et al.*, 2011). In this work we provide advice and details about the procedures and materials needed to perform hygienic fieldwork that is safe in health terms. We also quantify the time and money investments made to follow this protocol.

2. Safety and hygiene protocol description

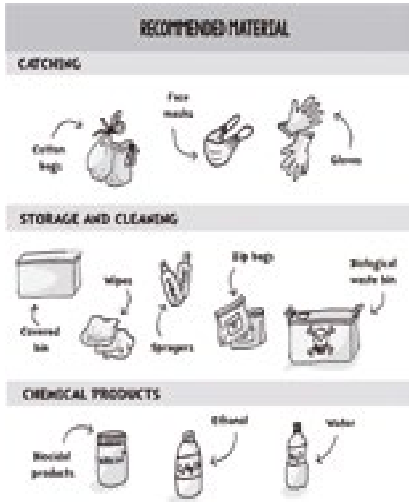
Safety and hygienic protocols should address two principal objectives: (i) animal protection from handling (avoiding pathogens and parasites spreading among individuals and populations); (ii) human safety against zoonotic transmission. A precautionary principle must govern scientific work by assuming that any animal is likely to be a carrier of pathogens and parasites, and these could intraspecifically spread through animal populations, and interspecifically via human-animal contact. It is also important to implement bearable and realistic measures to perform protocols *in situ*.

According to the field research methodology usually undertaken for tortoises, there are three main steps: catching; data and sample collection; releasing animals into the wild (Figure 1). Our hygiene and safety protocol involve these fieldwork phases by focusing on two essential issues: animal and worker protection during manipulation and material disinfection to reduce the disease spread risk. Finally, all equipment must be disinfected and dirt material disposed and/or appropriately stored.

For tortoise handling, we detail the recommended material (Figure 1b):

- Breathable cotton bags. Small and breathable cotton bags (preferably uncoloured) are desirable for catching and transporting animals until sample collection. Afterwards, the same bag is to release the tortoise. It is noteworthy that each bag must be exclusively used with each animal to avoid contact risks between individuals. The use of these bags allows a covering-catching system. Handlers

CATCHING: When we find a tortoise, we wrap it with an exclusive cotton bag, and avoid direct human-tortoise contact and among individuals.



HANDLING: One worker handles the tortoise (wearing disposible gloves and a face mask). The other worker records all the data (without gloves, but avoiding direct contact).

RELEASING: Each tortoise must be released at the same point where it was found. After fieldwork, all material must be properly cleaned, and biohazard waste and material must be properly disposed.

Figure 1: Safety and health protocol description for the different research fieldwork phases with tortoises. The recommended material is detailed (1b). 1.a Catching: Detecting the tortoise, catching it with a white cotton bag and marking the location (with any viewable element or GPS point). 1.c Handling: Measuring, weighing, photographing, blood sampling, among other tasks; 1.d Releasing: Releasing the tortoise at exactly the marked location.

place their hand inside the bag, wrap it around the tortoise and keep it inside the bag without touching the animal. During fieldwork, we attempt to minimise the time animals remain in bags to avoid animal stress, protect the bags from the sun and avoid their movement as much as possible. After fieldwork, bags must be washed at above 60ºC.

- Disposable gloves. Handlers must wear them to avoid coming into contact with tortoises while sampling and taking measurements. Sample and data collection require at least two workers. One worker works exclusively on handling the tortoise and taking samples and measures according to the sampling protocol. In this case, workers must always wear gloves and change them after use or, instead, disinfect with ethanol (70%) or biocidal solution (see Chemical Products on Figure 1.b and description below). The other worker records all the data and does not need to wear gloves. As the collected data material (tablets, notebooks, notepads, etc.) is very difficult to disinfect, this material should remain clean throughout the process (without coming into contact with tortoises).
- Face masks. Masks are optional but recommended to avoid the aerosol inhalation of pathogens.

After fieldwork, all material must be properly cleaned, and biohazard waste and rubbish must be removed and disposed in suitable storage containers until proper waste management. We suggest the following material (Figure 1b):

- Covered bin. Dirty materials should be placed in these containers to avoid human or animal contact. Containers should be big enough for them.
- Self-closing bags. The purpose of these bags is to store and isolate those materials that are reusable after being disinfected.
- Biohazard waste bin. We make biohazard waste, such as syringes, gloves, cotton, and needles inside a specific bin for this. This bin should be airtight, resistant and impermeable, and should always be closed. All hazardous waste is properly managed after fieldwork.
- Wipes. Preferably in two colours: one for simple cleaning with water (general soiling, e.g. dust) and the other for sensitive material with electronic components or others that need special management (e.g., dry cleaning).
- Sprayer. Applied to disinfect agents simply and effectively to all material that has come into contact with animals either directly or indirectly. Preferably, disinfection should be performed among individuals during sampling. If this is not possible, all material must be always cleaned and disinfected between populations. This step is even more important if we have noticed the presence of parasites, rhinitis, and any other disease symptoms or health decline in animals.

Some chemicals solutions are detailed below for efficient cleaning and disinfection purposes (Figure 1b).

- Ethanol 70% or hydroalcoholic solutions for hand washing.
- Water. Simple cleaning water is used to clean general dirty (non-biohazard) or organic waste before disinfecting materials.
- Biocidal or disinfecting solution. It is particularly important for this product to be safe, effective, inexpensive and enviro-friendly. Many disinfectant agents can be corrosive to equipment and harmful to people and animals. Therefore, we recommend using organic biocides and oxidising agents under acidifying conditions to enhance their effect. Our group has selected a triple salt of peroxigenic acid, with brand name VIRKON S (Copyright © BAYER AG, Bayer Hispania) in 1% solution, as recommended by the manufacturer (Bayer, 2019). The fieldwork application mechanism should be to spray all contaminated equipment or any material that had come into contact with animals or handlers (including operators' shoes or boots).

3. Economical and time investment

To fulfil these requirements, the suggested protocol implies a cost of approximately 260 €/season (by assuming a season involving 30 work days, 4 workers and 10 animals sampled per day) (Table 1). It is important to highlight that some equipment is reu-

Table 1: Estimated cost of selected products.

Product	Cost/Unit (€)	Unit/Season[1]	Cost/Season (€)
Breathable cotton bags	1	100	100
Disposable gloves (*)	4	240	9.6
Face masks	0.65	120	78
Cover bin	7	2	14
Self-closing bags (*)	13.5	1	13.5
Biological hazard waste bin (*)	3	4	12
Wipes (*)	3	1	3
Sprayers	0.65	1	0.65
Ethanol (70%) or hydroalcoholic solution	2	4	8
Water	-	-	-
Biocidal dissolution (*)	-	-	10
Laundry (*)	5	3	15
		Total	**263.75**

(*) Gloves: 1 box=100 gloves; Self-closing bags: 1 pack = 100 bags; Biological waste bin: 5L; Wipes: 1 pack= 10 units; Biocidal dissolution (Virkon S, in our case): approximate cost of 1% dissolution is 0.5-1 €/L, dissolution is feasible for 5-7 days (expected use of 0.5 L/3 days) sable and washable (cotton bags, wipes, sprayers, bins). The chemicals selected for this protocol are sold in large volumes to allow their continuous use throughout the season. Therefore, many of the listed materials can be used for the following seasons. Our protocol estimates that it takes 2-3 minutes to disinfect among individuals during sampling, and roughly 10 minutes to disinfect and clean after fieldwork. The cleaning of cotton bags and wipes must be done by specialised laundries.

4. Conclusions

In order to protect wildlife populations, limit the transmission risk and prevent zoonotic infection to humans, it is necessary to adapt a hygiene and safety protocol for fieldwork in economical, temporal, and operational terms. Disinfection and cleaning advice should be followed as safely and effectively as possible. Animals and workers' security must be made a priority during fieldwork. Safety protocols should be economically assimilable, and fast and simple to implement. Here we describe an assumable protocol which has been perfectly adapted to fieldwork conditions for tortoise research.

Acknoledgments

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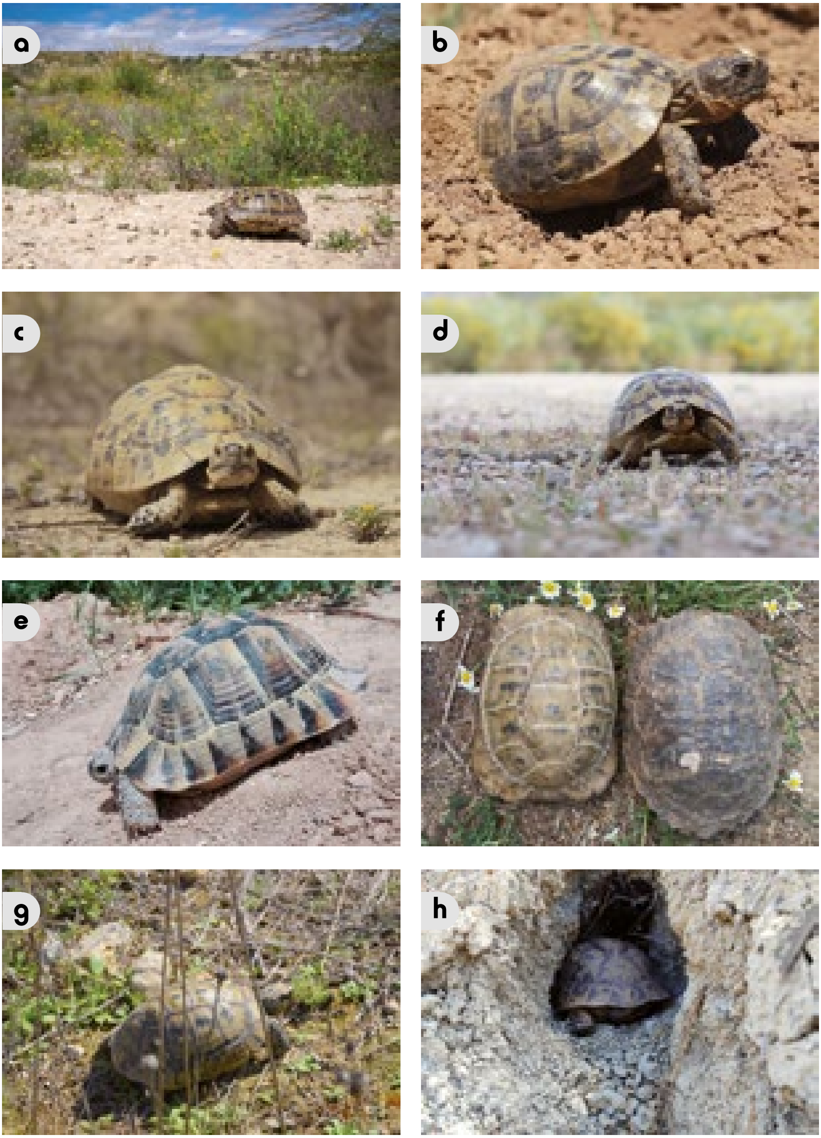
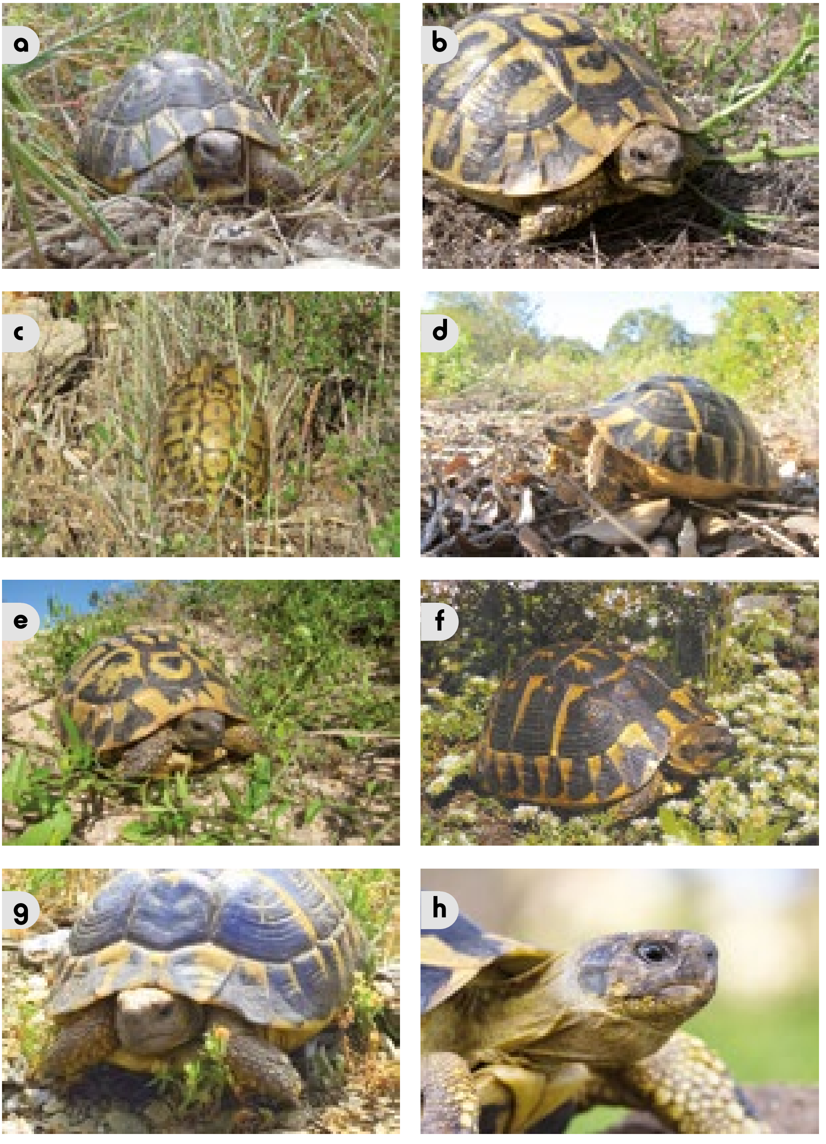
Colección fotográfica
Photographic collection

Tortugas silvestres / Wild tortoises

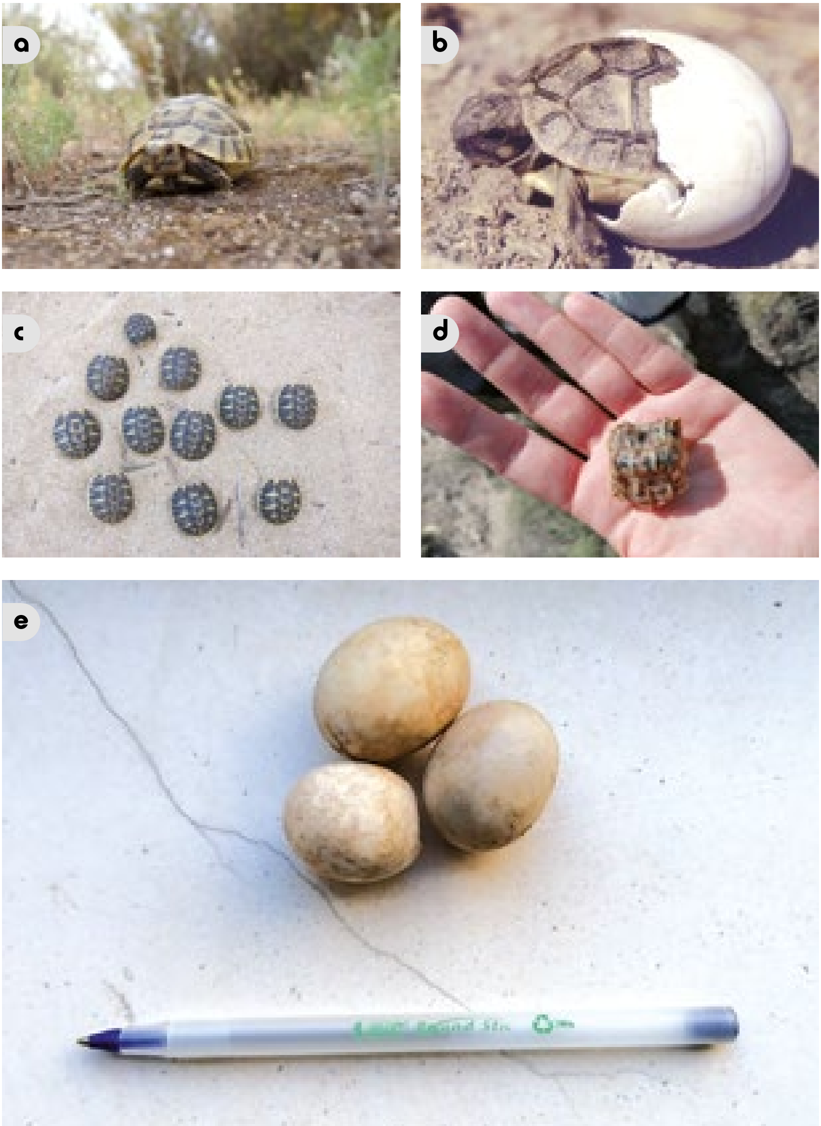


Tanto *Testudo graeca* (pag. 162) como *T. hermanni* (pag. 163) muestran patrones morfológicos distintos entre individuos, y no siempre es posible capturar con fotos su belleza, ni sus interesantes comportamientos.

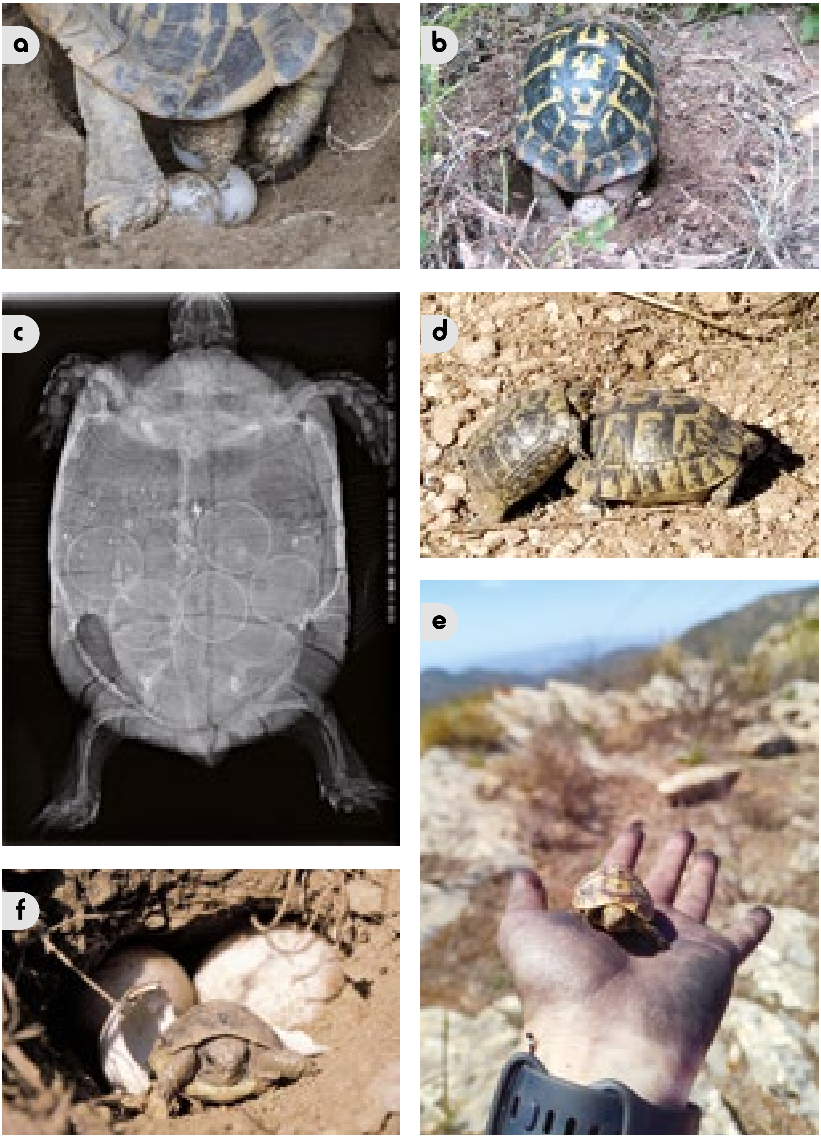


Both *Testudo graeca* (pag. 162) and *T. hermanni* (pag. 163) display different morphological patterns among individuals, and it is not always possible to capture their beauty or their fascinating behaviours in pictures.

Reproducción y juveniles / Reproduction and juvenile



La reproducción y la supervivencia de los juveniles constituyen un gran desafío para las tortugas, pues en cada etapa se enfrentan a amenazas como la depredación de nidos y crías, la escasez de alimento o las alteraciones de su hábitat.



The reproduction and survival of juvenile tortoises pose a major challenge, as at each stage they face threats such as nest and hatchling predation, food scarcity, o habitat disturbances.

Paisajes / Landscapes

Cerca de Relizane, Argelia / near Relizane, Algeria

Menorca, España / Menorca, Spain

Menorca, España / Menorca, Spain

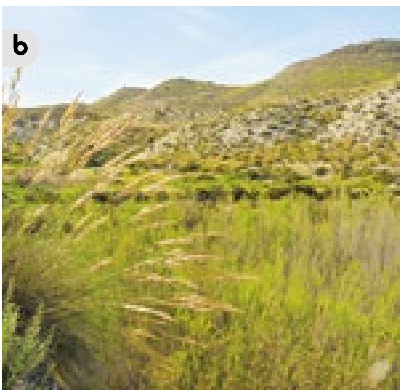
Menorca, España / Menorca, Spain

Derdara, Marruecos / Derdara, Morocco

Derdara, Marruecos / Derdara, Morocco

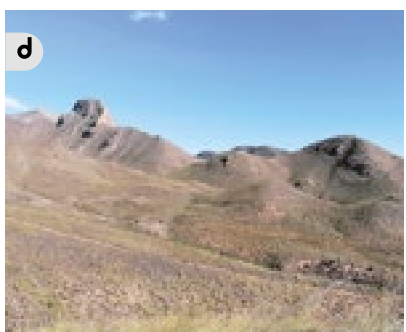
Las tortugas del género *Testudo* habitan una gran variedad de ambientes en el Mediterráneo, desde bosques de coníferas o caducifolios hasta zonas semiáridas, incluyendo matorrales, pastizales y áreas agrícolas de secano. Estos hábitats, además, experimentan cambios significativos entre estaciones y años, dependiendo de la pluviometría anual.

El Rif, Marruecos / The Rif, Morocco



Sureste de España / Southeastern Spain

Sureste de España / Southeastern Spain



Sureste de España / Southeastern Spain

Tortoises of the genus *Testudo* habitan a wide range of environments in the Mediterranean, from coniferous or deciduous forests to semi-arid areas, including shrublands, grasslands, and dry-farmed agricultural lands. These habitats also undergo significant changes between seasons and from year to year, depending on annual rainfall.

Trabajos de campo y laboratorio / Field and laboratory work

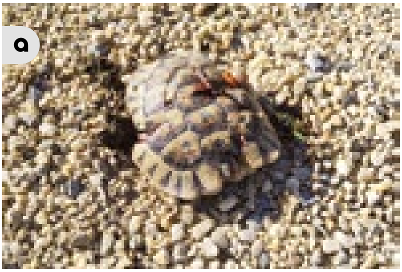
Localizar tortugas en el campo no es tarea sencilla, por lo que son necesarias muchas personas e incluso perros entrenados. Cada tortuga aporta valiosa información, siempre bajo estrictas medidas de bioseguridad. Otra parte del trabajo se realiza en instalaciones veterinarias, así como en centros de recuperación y de cría en cautividad.

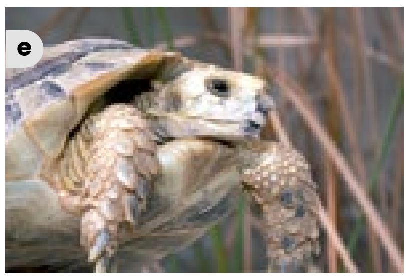
Finding tortoises in the field is not an easy task, so it often requires many people and even trained dogs. Each individual provides valuable information, always collected under strict biosecurity measures. Work with tortoises is also carried out in veterinary facilities, as well as in recovery and captive breeding centers.

Amenazas e impactos / Threats and impacts

La pérdida y fragmentación del hábitat (izquierda: a,b,c), junto con su consideración como mascota, que conlleva el expolio de poblaciones silvestres y el tráfico ilegal (izquierda: d,e), constituyen las principales amenazas para la especie.

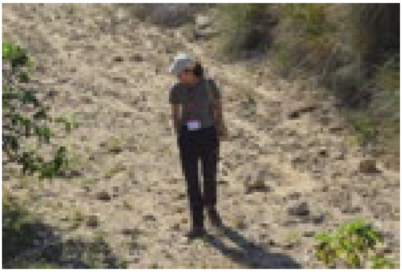
Otros factores relevantes, cuyas consecuencias requieren un estudio más profundo, son el cambio climático, que incrementa la frecuencia de sequías, los incendios forestales (derecha: f), las enfermedades emergentes como la rinitis (derecha: e) y los impactos ocasionados por infraestructuras humanas como carreteras o balsas de riego (derecha: a,b,c).





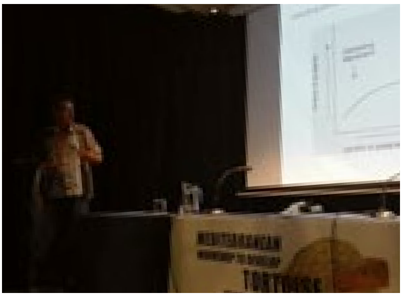
Habitat loss and fragmentation (left: a,b,c), together with their consideration as pets, which leads to the poaching of wild populations and illegal trafficking (left: d,e), are the main threats to the species.
Other relevant factors, whose consequences require further study, include climate change, which increases the frequency of droughts, wildfires (right: f), emerging diseases such as rhinitis (right: e), and the impacts caused by human infrastructures such as roads and irrigation ponds (right: a,b,c).

Participantes del workshop / Workshop participants



Durante el *Mediterranean Workshop to Develop Tortoise Conservation Strategies* tuvimos la oportunidad de visitar la población de *Testudo graeca* del sureste ibérico y disfrutar de ponencias y conversaciones muy enriquecedoras. Fue un gran encuentro y esperamos que pronto se repita una ocasión similar.

During the Mediterranean Workshop to Develop Tortoise Conservation Strategies, we had the opportunity to visit the *Testudo graeca* population in southeastern Iberia and enjoyed very engaging talks and conversations. It was a great meeting and we hope for a similar occasion to take place again soon.



MEDITERRANEAN
WORKSHOP TO DEVELOP
TORTOISE
CONSERVATION STRATEGIES

MEDITERRANEAN WORKSHOP TO DEVELOP TORTOISE CONSERVATION STRATEGIES

El Mediterranean workshop to develop tortoise conservation strategies fue organizado por la Asociación Herpetológica Española, el área de Ecología de la Universidad Miguel Hernández de Elche y la red de mujeres científicas de Casa Mediterráneo, con la financiación de la Generalitat Valenciana a través de las subvenciones para la organización y difusión de congresos, jornadas y reuniones científicas, tecnológicas, humanísticas o artísticas de carácter internacional (AORG/2019).

La publicación de este libro ha sido posible gracias a los proyectos del área de Ecología de la Universidad Miguel Hernández de Elche, PID2019-105682RA-I00 y TED2021-130381B-I00, finaciados por el Ministerio de Ciencia e Innovación de España (MICIU/AEI/10.13039/501100011033), siendo el segundo de ellos cofinanciado por la Unión Europea a través de "NextGenerationEU"/PRTR.

The Mediterranean Workshop to Develop Tortoise Conservation Strategies was organized by the Asociación Herpetológica Española, the Ecology group of the Universidad Miguel Hernández de Elche, and the network of female scientists of Casa Mediterráneo, with funding from the Generalitat Valenciana through grants for the organization and dissemination of international scientific, technological, humanistic, or artistic conferences, workshops, and meetings (AORG/2019).

The publication of this book was made possible thanks to the projects of the Ecology group of the Universidad Miguel Hernández de Elche, PID2019-105682RA-I00 and TED2021-130381B-I00, funded by the Ministry of Science and Innovation of Spain (MICIU/AEI/10.13039/501100011033), with the second project also co-financed by the European Union through "NextGenerationEU"/PRTR.

Fotografía de contraportada: Marcos Ferrández
(*Testudo graeca*, Guertoufa, Argelia)
Back cover photograph: Marcos Ferrández
(*Testudo graeca*, Guertoufa, Algeria)

Las tortugas, especialmente las terrestres, se encuentran entre los vertebrados más amenazados del planeta, enfrentándose factores como la pérdida de hábitat, el tráfico ilegal, el cambio climático y las enfermedades emergentes. A pesar de su delicada situación, en el Mediterráneo aún no existen estrategias comunes y coordinadas para su conservación. En 2019, un encuentro internacional en Alicante reunió a expertos de varios países para debatir sobre el estado de las tortugas mediterránea (*Testudo hermanni*) y mora (*T. graeca*), y proponer acciones conjuntas. Este libro recoge algunas de las ponencias presentadas, centradas en la conservación de poblaciones silvestres, la gestión de ejemplares en cautividad, el tráfico de especies, y los riesgos sanitarios. Su publicación busca mantener vivo el espíritu de aquel encuentro y contribuir a la creación de una red de colaboración para proteger a las tortugas de tierra en el entorno Mediterráneo.

Turtles—especially tortoises—are among the most threatened vertebrates on the planet, facing factors such as habitat loss, illegal trade, climate change, and emerging diseases. Despite the fragile status of tortoises, no common or coordinated conservation strategies currently exist in the Mediterranean region. In 2019, an international meeting held in Alicante brought together experts from several countries to discuss the conservation status of the Mediterranean tortoise (*Testudo hermanni*) and the spur-thighed tortoise (*T. graeca*), and to propose joint actions. This book compiles several of the presentations given at that meeting, focusing on the conservation of wild populations, the management of captive individuals, species trafficking, and health-related threats. Its publication aims to preserve the spirit of that meeting and contribute to the creation of a collaborative network to protect terrestrial tortoises across the Mediterranean.

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