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Research paper

Successful reintroduction of the European pond turtle *Emys orbicularis* with a small number of founders: results from a 20-year, small-scale experiment

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Abstract

Successful reintroduction of the European pond turtle Emys orbicularis *with a small number of founders: results from a 20-year, small-scale experiment.* Reintroduction programs can be an effective management tool for conservation of threatened chelonians, yet the success of such programs relies highly on numerous factors, such as the number of founders and the size of introduced individuals. We conducted a reintroduction experiment of the European pond turtle *Emys orbicularis* in a pond located within its historical range, using a small number of founders (N = 20), most of which were hatchlings maintained over winter and then released in June 2004 (mean weight about 33 g, head-started cohort, N = 7), and others were released within one month after hatching in October 2006 (mean weight about 8 g, control cohort, N = 8). One female contributed two hatchlings (out of seven) to the 2004 cohort and the eight hatchlings released in 2006, so most hatchlings were half siblings. Two adult males and three females from nearby recovery centres were also released in order to increase the genetic variability of the population. At the end of 2023, at least five founder individuals were alive and their weight was about 500 g. The growth rate was similar for both cohorts and is among the highest recorded for the species, suggesting that the habitat in the main pond is optimal. Based on Jolly-Seber and Manly-Parr estimates, reproduction within this population was confirmed by the increase to 42-44 individuals by 2023, respectively. Genetic analysis confirmed the expected low level of genetic diversity, the lowest for the subspecies *E. o. occidentalis* distributed across the Iberian Peninsula and northern Morocco.. For this population to thrive, a management plan with long-term monitoring is needed to maintain a favourable habitat and genetic diversity, and thus minimize the risk of extinction. Our findings suggest that a small number of *E. orbicularis* hatchlings can be used as founders to successfully reintroduce the species in suitable habitats, even without headstarting.

Key words: Captive breeding, Freshwater turtles, Headstarting, Reintroduction, Survival rate

Resumen

Reintroducción exitosa del galápago europeo Emys orbicularis *con un pequeño número de fundadores: resultados de un experimento en pequeña escala de 20 años de duración.* Se ha sugerido que los programas de reintroducción son una herramienta de gestión eficaz que contribuye a la conservación de los quelonios amenazados; sin embargo, el éxito de dichos programas depende en gran medida de numerosos factores, como el número de fundadores y el tamaño de los individuos introducidos. Llevamos a cabo un experimento de reintroducción del galápago europeo *Emys orbicularis* en un estanque situado dentro de su área de distribución histórica, utilizando un pequeño número de fundadores (N = 20), la mayoría de los cuales eran neonatos que se habían mantenido durante el invierno y luego se liberaron en junio de 2004 (peso medio aproximado de 33 g; cohorte de fundadores criados en cautividad [headstart], N = 7) y otros que se liberaron al cabo de un mes desde la eclosión en octubre de 2006 (peso medio aproximado de 8 g; cohorte de control, N = 8). Una hembra contribuyó con dos crías a la cohorte de 2004 (de un total de siete) y las ocho crías liberadas en

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2006, por lo que la mayoría de las crías liberadas eran medio hermanas. También se liberaron dos machos y tres hembras adultos procedentes de centros de recuperación cercanos, para aumentar la variabilidad genética de la población. A finales de 2023, al menos cinco individuos fundadores seguían vivos y su peso era de unos 500 g. La tasa de crecimiento, que fue similar en ambas cohortes, es una de las más altas registradas para la especie, lo que sugiere que el hábitat en el estanque principal es óptimo. La reproducción de esta población se confirmó a partir de 2010, ya que en 2023 el número estimado de individuos fue de 42 y 44, según las estimaciones de Jolly-Seber y Manly-Parr, respectivamente. El análisis genético confirma la baja diversidad genética prevista, que es la más baja en la subespecie *E. o. occidentalis* distribuida por la península Ibérica y norte de Marruecos. La persistencia futura de esta población requiere un seguimiento a largo plazo y un plan de gestión para aumentar tanto la disponibilidad de hábitats favorables como la diversidad genética, a fin de minimizar el riesgo de extinción. Este experimento sugiere que se puede utilizar un pequeño número de neonatos de *E. orbicularis* como fundadores para reintroducir la especie de forma exitosa en los hábitats adecuados, incluso sin haber sido criados en cautividad.

Palabras clave: Cría en cautiverio, Galápagos, Cría en cautividad de neonatos (headstarting), Reintroducción, Tasa de supervivencia

Introduction

Estimates of extinction rates suggest that we are confronting a mass extinction due to anthropogenic causes, namely habitat loss, overexploitation, the introduction of exotic invasive species, and global change. This scenario could yield extinction rates a thousand times higher than natural background rates (De Vos et al 2014). To confront these biodiversity threats, it is vital to identify the causes of population decline and, once these causes are controlled, direct management of populations might be used to restore lost biodiversity, unless global extinction occurs.

The reintroduction of species to their historical ranges is a common management strategy in vertebrate conservation (Seddon et al 2007) and is used to restore ecological interactions when key species have been extirpated (Polak and Saltz 2011). For example, the reintroduction of wolves into Yellowstone National Park produced a series of cascading effects on major vertebrate species, but also on vegetation, effectively rewilding the Yellowstone area (Boyce 2018). Long-term monitoring and a higher effort in publishing the results of relocations are needed to improve our knowledge of the factors that affect the success of these projects (Fischer and Lindenmayer 2000).

Chelonians (turtles and tortoises) are among the vertebrate groups with the highest extinction risk, with 35 % of the recognized species considered Critically Endangered or Endangered (CR + EN) according to IUCN criteria (Rhodin et al 2018) due to a variety of anthropogenic causes, and thus in need of human intervention to prevent the expected extinctions in coming decades (Stanford et al 2020). The release of captive-bred turtles to reinforce natural populations is a widely used conservation strategy with Chelonians (e.g., Mitrus 2005, Bona et al 2012, Mullin et al 2020, Golba et al 2022). For example, it has been proposed as a management strategy for the Vietnamese pond turtle *Mauremys annamensis*, threatened by overcollection and habitat loss (McCormack et al 2014). This technique has several potential vulnerabilities because animals maintained in captivity may become habituated to human presence, have less ability to survive without food supplementation, may not recognize predators once released, or may serve as vectors of diseases and parasites.

The European pond turtle *Emys orbicularis* (Linnaeus, 1758), is considered near threatened by the IUCN across its wide distribution in the Western Palaearctic (Tortoise and Freshwater Turtle Specialist Group 1996). Captive breeding and the release of reared turtles have been used in conservation programs aimed at reinforcing local *E. orbicularis* populations across its range (Fritz and Chiari 2013). As these programs often used abandoned or confiscated individuals of unknown origin, maintained in zoos and recovery centres. Good practices in conservation programs were not always followed (see Velo-Antón 2013), accounting for possible national and international translocations via the pet trade market identified in *E. orbicularis* (Velo-Antón et al 2007, 2011a, 2021).

Hatchlings maintained in captivity during the first months (or years) and thereafter released into natural habitats are expected to show higher survivorship if this limits predation during the first ages, and these animals are said to be 'headstarted' (Heppell et al 1996). Mitrus (2005) evaluated the results of an experiment where 123 one-year-old *E. orbicularis* were head-started and released into their original population. He concluded that this technique only increases population size if a large percentage of the hatchlings are artificially reared, and suggested that, until the long-term effects of headstarting are known, these programs cannot be considered as useful management strategies for this species. Subsequently, Mitrus (2008) simulated the effect of headstarting in *E. orbicularis* populations in Poland and concluded that the most relevant parameter for population persistence is adult survival, and did not recommend captive breeding as it was ineffective. Population viability analyses of the same species in NW Spain also concluded that juvenile and adult survival was the critical parameter for population persistence (Cordero-Rivera and Ayres Fernández 2004). Another experiment released 14 hatchlings of *E. orbicularis*. These hatchlings were head-started for one year, and it was found that their survival was higher than that of hatchlings that remained in the nest for overwintering. Two of the females were found nesting after 11 years, suggesting that headstarting could help to increase population size in this species (Bona et al 2012). However, given the limited experimental evidence on the effectiveness of headstarting (Golba et al 2022), our knowledge of the benefits of this technique for *E. orbicularis* reintroduction is limited. The success of reintroductions is also highly variable (for an unsuccessful example see Bertolero and Oro 2009), but a large of number of founders is normally associated with successful projects (Fischer and Lindenmayer 2000).

Here we present the results of a small-scale, long-term experiment, the main objective of which was to test the appropriateness of an area for the reintroduction of *E. orbicularis*, because there were historical records of the species in that area from the second half of the XIX century (López Seoane 1877). Given the scarcity of *E. orbicularis* in the region, translocation of adult individuals was considered too risky for the remnant populations, and therefore only hatchlings from three females from the nearest population, and five adults from nearby recovery centres, were introduced. Secondarily, following the availability of European pond turtles raised in captivity, we observed whether there were changes in survival potentially attributable to the maintenance time of hatchlings in captivity

Methods

Study area and data collection

We released hatchlings of *E. orbicularis* in a man-made pond (about 0.3 ha estimated from GoogleEarth images, fig. 1), excavated in the 1980's to obtain sand for construction works. The area is behind the largest mobile sand dune in NW Spain, situated in the locality of Olveira (Ribeira, A Coruña province), which since 1992 has been protected as a Natural Park ('Complexo Dunar de Corrubedo e Lagoas de Carregal e Vixán'; abbreviated here as N. P. of Corrubedo), and it has been protected as a Ramsar site since 1993. The pond became colonised by vegetation and is now home to a diverse community of aquatic flora (Pulgar Sañudo 2010) and fauna, including one of the very few populations of Iberian spadefoot toads *Pelobates cultripes* in the region (Galán Regalado 2006), and a large population of a regionally rare dragonfly *Brachytron pratense* (Cabana et al 2018).

Emys orbicularis occidentalis is the westernmost subspecies of the European pond turtle. It is characterized by mitochondrial DNA lineage VI (Stuckas et al 2014), and distributed throughout most of the Iberian Peninsula (Pereira et al 2018) and northern Morocco (Velo-Antón et al 2015). This subspecies recolonised the Iberian Peninsula from Morocco and rapidly expanded northwards, decreasing genetic diversity and causing genetic drift through allele-surfing processes (Velo-Antón et al 2008, Pereira et al 2018), and an increase in the carapace malformations (Velo-Antón et al 2011b). The European Pond turtle is a rare species in NW Spain (Galicia), where small populations (Louro, Arnoia, Barbaña and Avia rivers) persist despite showing the lowest genetic diversity patterns (Velo-Antón et al 2008) and facing numerous threats (i.e., habitat loss, pollution). It is thus considered endangered in the regional list of protected species (Decree 88/2007 that regulates the Galician catalogue of endangered species). Consequently, this experiment was constrained by the limited availability of hatchlings, which were obtained for an experiment aimed at evaluating the effect of incubation conditions on the presence of carapace anomalies (Cordero-Rivera et al 2008, Velo-Antón et al 2011a). The first cohort of seven newborns was released in the N. P. of Corrubedo on 5th June 2004. These were the descendants of three females (numbers 63, 66 and 92) from Gándaras de Budiño population (Pontevedra province) about 60 km to the South. This first group of newborns was obtained from eggs laid in the laboratory and hatched in September 2003, constituting the head-start group. They were maintained in captivity with food ad libitum in a climate-controlled chamber, with a temperature of 20 °C and a photoperiod of 15:9 h (day:night). Their weight was 32.6 ± 2.5 g on the release date (mean ± SE), when they were nine months old.

The second cohort consisted of eight hatchlings, all descendants of female 63, and therefore half-siblings of two of the newborns released in 2004. They hatched in September 2006 and were released in the N. P. of Corrubedo on 27th October 2006, with an average weight of 7.8 ± 0.2 g (control cohort). Therefore, hatchlings were not randomly assigned to headstarted and control groups, and any factor that might have differentially affected both cohorts is confounded with the treatment. This fact must be considered when interpreting the results.

With the aim of increasing the genetic variability of this small population, we released, in the same pond, two adult males in 2004, and one adult female in each of the years 2006, 2011 and 2012. These three turtles were from local recovery centres whose exact geographical origin was unknown, but they showed a phenotype similar to autochthonous *E. o. occidentalis*. Genetic assignment analysis recently conducted to captive individuals from Galician recovery centres indicated a Galician origin for most of the samples (Velo-Antón et al 2021), suggesting that the adults used in this experiment probably came from one of the remnant Galician populations. The males had disappeared by 2008, but the females were found in subsequent years.

All specimens were measured and weighed before their release, and a photo of the carapace and the plastron was taken for future identification. Identification was possible because the number of specimens was low, and because most of them had unique anomalies

Fig. 1. Sites sampled during 2022-2023 to check for the presence of *Emys orbicularis*, in the Natural Park of Corrubedo and surrounding areas. The distribution of *E. orbicularis* in the Iberian Peninsula (white squares; map obtained from the Spanish and Portuguese Herpetological Atlas) is shown in the top left embedded figure (the red circle indicates the location of the N. P. of Corrubedo). *Emys orbicularis* was established in the two Olveira ponds (yellow circles) but not in any surrounding water bodies (red circles). Pictures of the traps and the habitat characterising the Olveira ponds are shown in the middle and bottom left embedded figures. An adult of *E. orbicularis* is represented in the top right embedded figure.

Fig. 1. *Sitios muestreados durante 2022-23 para comprobar la presencia de* Emys orbicularis *en el Parque Natural de Corrubedo y alrededores. La distribución de* E. orbicularis *en la península ibérica (cuadrados blancos; mapa obtenido de los Atlas Herpetológicos de España y Portugal) se muestra en la imagen incrustada superior izquierda (el círculo rojo indica la ubicación del P. N. de Corrubedo).* Emys orbicularis *se estableció en los dos estanques de Olveira (círculos amarillos), pero no en ninguno de los cuerpos de agua circundantes (círculos rojos). Las trampas y el hábitat que caracteriza a los estanques de Olveira se muestran en las imágenes incrustadas central e inferior izquierda. En la imagen incrustada superior derecha se muestra un adulto de* E. orbicularis.

in their carapace (Cordero-Rivera et al 2008) which were maintained over time. We did not mark newborns with shell notches until they were about one year old. Each year since 2004 to 2013, in 2017-2018 and 2022-2023, we sampled this pond with traps, using marine fish as bait (normally sardines), because this would impede possible parasites from being introduced with the bait. The pond showed large fluctuations in water level, but never became completely dry. We used four to seven traps distributed over the deeper parts of the pond. In 2022 and 2023, we increased the number of water bodies sampled by situating 1-2 traps at eight additional sites (fig. 1). However, only one pond, situated 170 m from the first pond, showed presence of *E. orbicularis* individuals.

Traps were checked every 1-2 days and were maintained in the pond for 5-7 consecutive days in spring (March-June) and autumn (September-November). To avoid drowning of the animals, we introduced one empty plastic bottle in each trap (see fig. 1) to act as a floating device.

Captured animals were measured to the nearest 0.1 mm using callipers. They were then sexed, weighed, and photographed, and released in the same pond after biometric measurements were obtained. Sex was determined by the presence of concavity in the plastron of males, combined with brownish-red colouration in the eyes, whereas females show a flat plastron, and their eyes are not red (Fritz 1998). Individuals under 100 mm in carapace length were considered juveniles because, at that size, the secondary sexual characters become visible (Escoriza et al 2020). We recorded the presence of carapace anomalies, and marked all new individuals with marginal notches using the method of Pérez et al (1979). In 2022-2023 all animals were also marked with a Passive Integrated Transponder (PIT) under the skin of the hindlimb, to allow for long-term identification.

Population size

To estimate the number of individuals present in the population over the study period, recapture histories were analysed using the POPAN 5 program (Arnason et al 1998), with two different models, the Jolly-Seber method (Jolly 1965), which includes births and deaths during the study period, and the Manly-Parr method (Manly and Parr 1968), which in essence is similar to the above but assumes that there is heterogeneity in the probability of survival (i.e., it is not the same for all individuals).

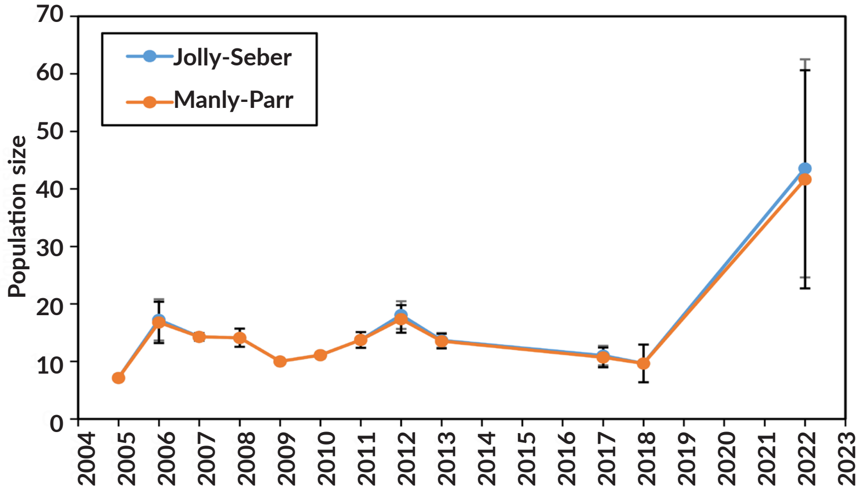

Fig. 2. Estimates of population size (± SE) for *Emys orbicularis* at the Natural Park of Corrubedo since the release of the first seven newborns in 2004, using the methods of Jolly-Seber and Manly-Parr. Reproduction was first confirmed in 2010.

Fig. 2. Estimación del tamaño poblacional (± DE) de Emys orbicularis *en el Parque Natural de Corrubedo, desde la liberación de los siete primeros recién nacidos en 2004, utilizando los métodos de Jolly-Seber y Manly-Parr. La reproducción se confirmó por primera vez en 2010.*

Survival, recapture and growth rates

To estimate survival (Phi) and recapture (p) rates, we analysed the histories of recaptures of each individual using Cormack-Jolly-Seber models with the software MARK 10 (White and Burnham 1999), using pre-defined models that include variation in both parameters in relation to year and group (male, female, juvenile). First, we estimated the degree of fit of the saturated model (which includes time, groups and their interaction) by means of the program Release from within Mark, because if the saturated model did not fit the data, any reduced model would be worse. The saturated model was appropriate to explain the variability of the data (Test 2 + Test 3 by groups, χ^2 = 10.40, df = 26, p = 0.997). Standard errors and confidence intervals were corrected by the overdispersion parameter, c-hat, which was estimated as 1.378, by dividing the deviance of the saturated model by the mean deviance of 100 bootstrap runs, using the option implemented in Mark. Models were ranked by QAIC corrected for overdispersion (Burnham and Anderson 1998).

In a second step, we compared the survivorship of the two cohorts, using only the recapture histories of the newborns that were released in 2004 and 2006. In this case, the saturated model (time dependent) also explained the variability of the dataset in a satisfactory way (Test 2+ Test 3 by groups, χ^2 = 3.06, df = 13, p = 0.998). Models were ranked by QAIC corrected by the overdispersion parameter, which was estimated as 1.472, using the same method as above.

We estimated growth in carapace length and weight by averaging the values for all the individuals of each cohort found in each month. Means are presented with their standard errors.

Genetic analysis

During the 2022-23 fieldwork, we obtained blood samples from 25 unmarked (new) individuals. Genomic DNA was extracted from blood samples following the manufacturer's protocols of the EasySpin commercial kit (iNtrON Biotechnology, WA, USA), which were later amplified with seven microsatellite markers, following PCR protocols in Pereira et al (2018) and Velo-Antón et al (2021), and genotyped in an ABI 3130xl genetic analyzer (Applied Biosystems, Foster City, CA, USA). GeneScan™-500 Liz was used as size standard and allele scoring was performed in Geneious Prime 2023 (www.geneious.com).

The presence of null alleles was assessed in MICRO-CHECKER 2.2.3 (Van Oosterhout et al 2004) and the genetic data were analysed in GenAlex 6.5 (Peakall and Smouse 2012) to calculate the following genetic diversity indices: the average number of alleles per locus (Na), the number of effective alleles (Ne), the observed heterozygosity (Ho), the unbiased expected heterozygosity (He), and the inbreeding coefficient (Fis).

Results

Population size

The estimates of population size ranged from seven to 18 individuals until 2018, with a value of around 13-15 individuals per year, but the population increased to three times that number in 2022, reaching 42-44 animals (Jolly-Seber and Manly-Parr, respectively). The two models used to estimate population size provided similar results (fig. 2). Reproduction was first confirmed in 2010 when the first newborn was found. A second newborn was found in 2011, and two more hatchlings were found in 2012. By the end of 2023, a total of 50 individuals had been recorded, and 30 of these were born in place.

Survival and recapture rates

Animals were grouped as: males, females, and juveniles. The most supported QAICc model is {Phi(g) p(.)}, with

Table 1. Estimates of survival (Phi) and recapture (p) probabilities according to the model {Phi(g) p(.)}. (Values corrected by c-hat = 1.378117).

Tabla 1. Estimaciones de la probabilidad de supervivencia (Phi) y recaptura (p) según el modelo {Phi(g) p(.)}. (Valores corregidos por c-hat = 1.378117).

			95% Confidence limits	
Parameter	Estimate	SE	Lower	Higher
Phi (males)	0.9547	0.0284	0.8535	0.9870
Phi (females)	0.9664	0.0237	0.8734	0.9917
Phi (juvenile)	0.7192	0.0818	0.5366	0.8500
p	0.6845	0.0495	0.5805	0.7728

three values for survival (Phi, one for each group) and a single recapture value (p) for all groups and years (table 1s). This model had a weight of 0.799, and was 4.23 times more statistically supported than the following model, so we used it to estimate survival and recapture rates (table 1). This analysis suggested that survival was similar in females and males and that juveniles had lower annual survival. The annual recapture rate was 0.68 ± 0.05 irrespective of the group.

The analysis by cohort (table 2s) indicates that the best model was the reduced model, with only one survival and recapture rate for all animals (model {Phi(.) p(.)}). The second most supported model included a different survival rate for each cohort and a common recapture rate (model {Phi(g) p(.)}). The relative weight of the first model compared to the second was only 1.13 (0.342/0.303), so both are well supported. The second model estimated a survival rate of 0.89 ± 0.05 for the 2004 cohort and 0.96 ± 0.02 for the 2006 cohort. Two further models, including different recapture rates but a common survival ({Phi(.) p(g)}), and different survival and recapture rates for both cohorts ({Phi(g) p(g)}), also have some support (table 2s).

Individual growth rate

Using carapace length (CL) and weight values of recaptured individuals, we reconstructed the growth of the two cohorts, with the limitation that in the case of the 2004 cohort there was only one recapture in 2022-23 (fig. 3). In 2023, the two cohorts reached very similar length, around 145 mm, but the 2006 cohort reached a higher mean weight value (between 560 and 580 g) than the only living individual in the 2004 cohort, weighing 460-495 g in the period when it was recaptured (a male and therefore smaller in size). The maximum growth rate occurred in the first 6 years after release (fig. 3), with a mean of 11.9 mm/year and 53.7 g/year for the 2004 cohort for the period 2004-2010, and then the rate slowed down to an average growth rate of 0.9 mm/year (8.8 g/year) over the period 2010-2023. Corresponding values for the 2006 cohort were 17.3 mm/year and 65.0 g/year for the period 2006-2012 and 1.1 mm/year and 13.2 g/year for the period 2012-2023.

Genetic diversity

Seven out of 25 samples were removed from the analysis due to missing data in more than two loci. We found no evidence of null alleles and the total number of alleles was 32. Genetic diversity indexes were as follows: Na = 4.57 ± 0.52, Ne = 2.46 ± 0.28, Ho = 0.57 ± 0.08, He = 0.55 ± 0.06, and Fis = -0.06 ± 0.09.

Discussion

Our findings suggest that it is possible to reintroduce *E. orbicularis* in optimal habitats using a small number of founders. Our observations also suggest that headstarting practices do not necessarily increase the survival of newborns. However, because our sample size is too small for meaningful statistical comparisons, further experiments are needed to test whether headstarting, and under which conditions, might be a useful strategy in this species.

Population size and growth

Population size estimates (fig. 2) indicate that the population remained small for a long period, at around 13-15 individuals, and therefore natality and mortality were similar during that period. It should be noted that the population began to grow in 2010, when the first hatchling, born in the N. P. of Corrubedo, was detected. In the South of Spain, males reach sexual maturity at around 110-120 mm in length, or approximately 4 years, while females need to reach 130 mm, or 6 years of age (Keller and Andreu 2002). In various regions of France, several authors report much older ages for sexual maturity. For males, estimates range from 8-9 years to 12-13 years, while for females the corresponding values are 11-12 years or 18-20 years (Girondot and Pieau 1993, Baron and Duguy 2000). Mitrus (2004) indicates that males mature at 11 years of age and females after 15 years in Poland. Bozhansky and Orlova (1998), for Russian populations, and Kotenko (2000) for Ukrainian populations, suggest that males and females mature at the same age, 5-8 years. Given these data, it seems unlikely that the first new specimens were descendants from the 2004 cohort, and is more plausible that the adult female released in 2006 was reproducing, given that two adult males were also released in 2004, although none of them was found after 2008. Unfortunately, we were unable obtain genetic data for the previously released hatchlings and adults; this would have helped to determine the parents of the sampled individuals while providing insights into the sexual maturity of this population.

The high number of individuals in 2022 in our study may be due to the start of reproduction of the first individuals born in the park, that would be about 10-12 years old in 2020. Although the error of the 2022 estimate is large (because the last sampling was in 2023) our results point to a population growth in the most recent period. The density of *Emys orbicularis* populations ranges between minimum values of 3-7 individuals per hectare (Naulleau 1991; Mazzotti 1995; Servan 1998; Duguy 2000) to very large values of 80-

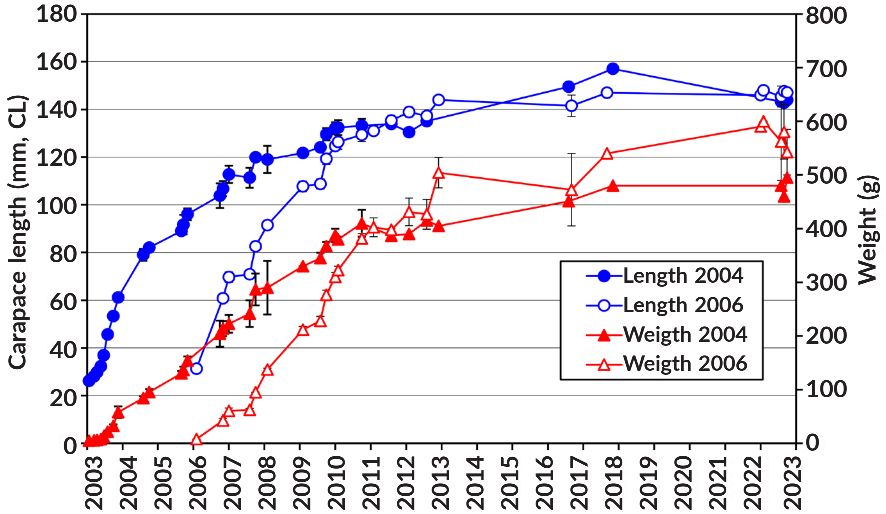

Fig. 3. Growth rates (mean ± SE) for the headstarted cohort (2004) and the control cohort (2006), for carapace length and weight, with their standard errors. After 2006 values represent the mean of the recaptured animals per month, and are plotted as if they were obtained the first day of the month.

Fig. 3. *Tasa de crecimiento (media ± DE) de la cohorte de fundadores criados en cautividad (2004) y la cohorte de control (2006) respecto de la longitud y el peso del caparazón, con sus errores estándar. A partir de 2006, los valores representan la media de los animales recapturados por mes y se representan como si se hubieran obtenido el primer día del mes.*

100 individuals per hectare (Szczerbak 1998; Kotenko 2000; Ayaz et al 2007), or even more than 250 ind/ha (Liuzzo et al 2021). The main Olveira ponds have a limited area of 0.3 ha, implying that the density is currently more than 140 individuals per hectare (43/0.3). These estimates suggest that the maximum capacity may have already been reached, and the population would be limited in growing further in this ponds.

Survival and individual growth

Our results indicate that survival and recapture rates were high (table 1), with annual survival rates of 0.95-0.97 for adults, similar to estimates in Central Poland (Mitrus 2004). In Doñana National Park (southern Spain), Keller et al (1998) estimated an annual survival of 0.663 for juveniles, 0.787 for males, and 0.795 for females. In NE Spain, survival was estimated as 0.91-0.92 for females and 0.84-0.86 for males (Escoriza et al 2020). We found that juveniles had lower survival rates than adults, as reported in other studies. However, this is partly because some of the individuals died before reaching the minimum size to be sexed, and this inevitably reduces the survival estimate of the juvenile group. According to the formula of Cook et al (1967), an annual survival of 0.9664 for females indicates a life expectancy of 67.3 years. This means that a small increase in annual survival (for instance by only 0.05 per year) drastically changes life expectancy, given that the process is exponential. In fact, *E. orbicularis* is an extremely long-lived species, as some individuals have lived up to 120 years (Jablonksi and Jablonska 1998). The results of our experiment thus agree with the known biology of the species.

Both cohorts showed good acclimatation to the conditions of the pond, judging by their fast growth (fig. 3), which reached 12-17 mm and 54-65 g per year in the first 6 years. Hatchlings maintained in captivity with *ad libitum* food during one year grew 19.5 mm, a value similar to our findings (Zuffi et al 2017). However, captive growth data for this species in France indicate much slower growth: 5-6 g at year 1 of age, 13-20 g at 2 years, 22-26 g at 3 years, 30-33 g at 4-5 years, and 60-90 g at 6 years (Rollinat 1934). Estimation of survival of both cohorts suggests that both groups survived equally well, and, if any difference existed, it was in favour of the non headstarted group. Again, the evidence suggests that the pond of Olveira provides an optimal habitat for *E. orbicularis*.

Genetic diversity

Our results indicate low levels of genetic diversity in this small, reintroduced population. The Ho obtained for the N. P. of Corrubedo is the lowest reported for *E. o. occidentalis* (see Pereira et al 2018) while the He, Na and Ne are similar to the values observed in two NW Iberian populations, Ribadavia and Zamora (Pereira et al 2018). As expected, since the number of founders was very low and mostly related, this reintroduced population showed lower genetic diversity than that of the source population of the hatchlings (Gándaras de Budiño; Ho = 0.72, He = 0.67; Ne = 3.4; Na = 5.8). However, our results do not indicate signs of inbreeding in this population (Fis near 0). This might be explained by the possible contribution of adults from recovery centres to the reproduction of the species in these ponds, which would help enhance the genetic diversity of this small population.

The success of introduced populations from a small number of founders is known for invasive species (e.g., Ficetola et al 2008, Kinziger et al 2011) and avian reintroductions (Jamieson 2011). However, reduced genetic diversity can decrease fitness and reduce adaptive potential (Willi et al 2006), which may lead to an increased extinction risk. Reintroduction guidelines recommend an adequate number of founders to minimize loss of genetic diversity, although this number also depends on the population growth (Tracy et al 2011), among other factors (e.g., age to maturity, clutch size, adult and juvenile survival rates). Indeed, the number of founders in reintroduced populations is often small, and although the resulting populations seem to be stable, the strong population bottlenecks of reintroduced individuals are associated to increased rates of inbreeding and loss of genetic diversity (Grossen et al 2018) and thus likely affecting the long-term viability of the introduced population. Predicting the fate of this small population in the N. P. of Corrubedo is premature due to the characteristics of chelonians (i.e., longevity, extended fertility and delayed sexual maturity). Although our demographic and growth parameters showed positive results, the reduced genetic diversity does not bring optimistic expectations for the long-term viability of this population. Consequently, future efforts should be allocated to increase the genetic variation for the success of this reintroduced population.

Conclusions

Our small-scale and long-term experiment adds to the cases of captive breeding success in reintroduction programs. Despite the low levels of genetic diversity and founders, this experiment was successful in reintroducing the European pond turtle to its historic range and suggests that a small number of hatchlings can serve as founders for this purpose. Reintroductions from a limited number of founders in captive breeding programs have been successful in a chelonian species rescued from the brink of extinction, the Española giant Galapagos tortoise *Chelonoidis hoodensis* (15 founders, see Gibbs et al 2014). As the limitations of our experimental design prevented confirmation of whether head-starting is necessary, further experiments are needed.

An increase in the availability of suitable habitat might be the best measure to enhance the long-term maintenance of this population. The fact that some turtles have started to use a second pond in Olveira may contribute to this goal, although the species was not detected in the remaining water bodies sampled, suggesting it failed to naturally occupy other ponds and expand its limited range in the N. P. of Corrubedo. Current plans to improve the habitat quality of the newly colonised pond in order to favour the establishment of the species should be followed by a study of the species dispersal in the Park so as to obtain critical information for its management. This would involve the use of biologging (e.g., Gutowsky et al 2017, Robichaud et al 2022), which would provide detailed information on the habitat use and connectivity patterns within the Park.

References

Arnason NA, Schwarz CJ, Boyer G, 1998. *POPAN-5. A data maintenance and analysis system for mark-recapture data.* Department of Computer Science, The University of Manitoba, Manitoba, Canada.

Ayaz D, Fritz U, Tok C, Mermer A, Tosunoğlu M, Afsar M, Çiçek K, 2007. Population estimate and body size of European pond turtles (*Emys orbicularis*) from Pazarağaç (Afyonkarahisar/Turkey). *Biologia* 62(2), 225-227. DOI: 10.2478/s11756-007-0035-2

Baron J-P, Duguy R, 2000. La Cistude d'Europe, *Emys orbicularis*, dans le marais de Brouage (Charente Maritime, France): croissance, reproduction et déplacements. *Chelonii* 2, 53-54.

Bertolero A, Oro D, 2009. Conservation diagnosis of reintroducing Mediterranean pond turtles: What is wrong? *Animal Conservation* 12(6), 581-591. DOI: 10.1111/j.1469-1795.2009.00284.x

Bona M, Novotný M, Danko S, Burešová A, 2012. Headstarting in a small population of European pond turtles (*Emys orbicularis*) in Central European conditions: first results. *Herpetology Notes* 5, 547-550.

Boyce MS, 2018. Wolves for Yellowstone: dynamics in time and space. *Journal of Mammalogy* 99(5), 1021-1031. DOI: 10.1093/jmammal/gyy115

Bozhansky AT, Orlova VF, 1998. Conservation status of the European pond turtle, *Emys orbicularis* (Linnaeus, 1758), in European Russia. *Mertensiella* 10, 41-46.

Burnham KP, Anderson DR, 1998. *Model selection and inference. A practical information-theoretic approach*. Springer, New York.

Cabana M, Cordero-Rivera A, Romeo A, 2018. *Brachytron pratense* (Odonata: Aeshnidae) en la Península Ibérica: distribución, fenología y estado de conservación. *Boletín de la Sociedad Entomológica Aragonesa* 63. 343-347.

Cook LM, Brower PP, Crozer HJ, 1967. The accuracy of a population estimation from multiple recapture data. *Journal of Animal Ecology* 36, 57-60.

Cordero-Rivera A, Ayres C, Velo-Antón G, 2008. High prevalence of accessory scutes and anomalies in Iberian populations of *Emys orbicularis*. *Revista Española de Herpetología* 22, 5-14.

Cordero-Rivera A, Ayres Fernández C, 2004. A management plan for the European pond turtle (*Emys orbicularis*) populations of the Louro river basin (Northwest Spain). *Biologia* 59(Suppl. 14), 161-174.

De Vos JM, Joppa LN, Gittleman JL, Stephens PR, Pimm SL, 2014. Estimating the normal background rate of species extinction. *Conservation Biology* 29(2): 452-462. DOI: 10.1111/cobi.12380

Duguy R, 2000. Cycle d'activité de la Cistude, *Emys orbicularis*, dans le marais de Brouage (Charente-Maritime, France). *Chelonii* 2, 55-57.

Escoriza D, Franch M, Ramos S, Sunyer-Sala P, Boix D, 2020. Demographics and survivorship in the European Pond Turtle (*Emys orbicularis*): a 31-Year Study. *Herpetological Conservation and Biology* 15(1), 41-48.

Ficetola GF, Bonin A, Miaud C, 2008. Population genetics reveals origin and number of founders in a biological invasion. *Molecular Ecology* 17(3), 773-782. DOI: 10.1111/j.1365-294X.2007.03622.x

Fischer J, Lindenmayer DB, 2000. An assessment of the published results of animal relocations. *Biological Conservation* 96(1), 1-11. DOI: 10.1016/S0006-3207(00)00048-3

Fritz U, 1998. Introduction to zoogeography and subspecific differentiation in *Emys orbicularis* (LINNAEUS, 1758). *Mertensiella* 10, 1-27.

Fritz U, Chiari Y, 2013. Conservation actions for European pond turtles - a summary of current efforts in distinct European countries. *Herpetology Notes* 6, 105.

Galán Regalado P, 2006. *Guía dos anfibios e réptiles. Parque Natural do complexo dunar de Corrubedo e lagoas de Carregal e Vixán*. Xunta de Galicia. Consellería de Medio Ambiente e Desenvolvemento Sostible, Santiago de Compostela.

Gibbs JP, Hunter EA, Shoemaker KT, Tapia WH, Cayot LJ, 2014. Demographic outcomes and ecosystem implications of giant tortoise reintroduction to Española Island, Galapagos. *PLoS ONE* 9(10), e110742. DOI: 10.1371/journal.pone.0110742

Girondot M, Pieau C, 1993. Effects of sexual differences of age at maturity and survival on population sex ratio. *Evolutionary Ecology* 7(6), 645-650. DOI: 10.1007/BF01237827

Golba CK, Glowacki GA, King RB, 2022. Growth and survival of wild and head-started Blanding's turtles (*Emydoidea blandingii*). *Ichthyology and Herpetology* 110(2), 378-387. DOI: 10.1643/h2021005

Grossen C, Biebach I, Angelone-Alasaad S, Keller LF, Croll D, 2018. Population genomics analyses of European ibex species show lower diversity and higher inbreeding in reintroduced populations. *Evolutionary Applications* 11(2), 123-139. DOI: 10.1111/eva.12490

Gutowsky LFG, Stoot LJ, Cairns NA, Thiem JD, Brownscombe JW,

Danylchuk AJ, Blouin-Demers G, Cooke SJ, 2017. Biologgers reveal post-release behavioural impairments of freshwater turtles following interactions with fishing nets. *Animal Conservation* 20(4), 350-359. DOI: 10.1111/acv.12323
Heppell SS, Crowder LB, Crouse DT, 1996. Models to evaluate head-starting as a management tool for long-lived turtles. *Ecological Applications* 6(2), 556-565. DOI: 10.2307/2269391
Jablonksi A, Jablonska S, 1998. Egg-laying in the European pond turtle, *Emys orbicularis* (L.), in Leczynsko-Wlodawskie Lake District (East Poland). Mertensiella 10, 141-146.
Jamieson IG, 2011. Founder effects, inbreeding, and loss of genetic diversity in four avian reintroduction programs. *Conservation Biology* 25(1), 115-123. DOI: 10.1111/j.1523-1739.2010.01574.x
Jolly GM, 1965. Explicit estimates from capture-recapture data with both death and immigration: stochastic model. *Biometrika* 52, 225-247.
Keller C, Andreu AC, 2002. *Emys orbicularis* (Linnaeus, 1758). Galápago europeo,. In: *Atlas y Libro Rojo de los Anfibios y Reptiles de España:* 181-186. Ministerio de Medio Ambiente. Organismo Autónomo Parques Nacionales, Madrid.
Keller C, Andreu AC, Ramo C, 1998. Aspects of the population structure of *Emys orbicularis hispanica* from southwestern Spain. In: *Proceedings of the EMYS Symposium Dresden, October 1996:* 147-158 (U Fritz, U Joger, R Podloucky, J Servan, JR Buskirk, Eds). Deutsche Gesellschaft für Herpetologie und Terrarienkunde, Rheinbach, 1998. *Mertensiella* 10.
Kinziger AP, Nakamoto RJ, Anderson EC, Harvey BC, 2011. Small founding number and low genetic diversity in an introduced species exhibiting limited invasion success (speckled dace, *Rhinichthys osculus*). *Ecology and Evolution* 1(1), 73-84. DOI: 10.1002/ece3.8
Kotenko TI, 2000. The European pond turtle (*Emys orbicularis*) in the Steppe Zone of the Ukraine, In: *Die Euroäische Sumpfschildkröte*: 87-106 (Hödl W, M Rössler Eds). Landesmuseums, Linz.
Liuzzo M, Borella S, Ottonello D, Arizza V, Malavasi S, 2021. Population abundance, structure and movements of the European pond turtle, *Emys orbicularis* (Linnaeus 1758) based on capture-recapture data in a Venice Lagoon wetland area, Italy. *Ethology Ecology and Evolution* 33(6), 561-575. DOI: 10.1080/03949370.2020.1870567
López Seoane V, 1877. Reptiles y anfibios de Galicia. *Anales de la Sociedad Española de Historia Natural* 6, 349-358.
Manly BFJ, Parr MJ, 1968. A new method for estimating population size, survivorship, and birth rate from capture-recapture data. *Transactions of the Society for British Entomology* 18, 81-89.
Mazzotti S, 1995. Population structure of *Emys orbicularis* in the Bardello (Po Delta, Northern Italy). *Amphibia-Reptilia* 16, 77-85.
McCormack TEM, Dawson JE, Hendrie DB, Ewert MA, Iverson JB, Hatcher RE, Goode MJ, 2014. *Mauremys annamensis* (Siebenrock 1903) Vietnamese Pond Turtle, Annam Pond Turtle, Rùa Trung Bộ. *Conservation Biology of Freshwater Turtles and Tortoises* 5, 081.1-081-14. DOI: 10.3854/crm.5.081.annamensis.v1.2014
Mitrus S, 2004. Body size and survivorship of the European pond turtle *Emys orbicularis* in Central Poland. *Biologia* 59(Suppl. 14), 103-107.
Mitrus S, 2005. Headstarting in European pond turtles (*Emys orbicularis*): Does it work? *Amphibia-Reptilia* 26(3), 333-341. DOI: 10.1163/156853805774408504
Mitrus S, 2008. The headstarting technique is an ineffective method for conservation of the European pond turtle, Elasticity analysis. *Ecological Questions* 10, 51-55. DOI: 10.2478/v10090-009-0006-z
Mullin DI, White RC, Lentini AM, Brooks RJ, Bériault KR, Litzgus JD, 2020. Predation and disease limit population recovery following 15 years of headstarting an endangered freshwater turtle. *Biological Conservation* 245, 108496. DOI: 10.1016/j.biocon.2020.108496
Naulleau G, 1991. Adaptations écologiques d'une population de cistudes (*Emys orbicularis* L.) (Reptilia, Chelonii) aux grandes variations de niveau d'eau et á l'asséchement naturel du mileu aquatique fréquenté. *Bulletin de la Societé Herpetologique de France* 58, 11-19.
Peakall R, Smouse P, 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research - an update. *Bioinformatics* 28(19), 2537-2539. DOI: 10.1093/bioinformatics/bts460
Pereira P, Teixeira J, Velo-Antón G, 2018. Allele surfing shaped the genetic structure of the European pond turtle via colonization and population expansion across the Iberian Peninsula from Africa. *Journal of Biogeography* 45(9), 2202-2215. DOI: 10.1111/jbi.13412
Pérez M, Collado E, Ramo C, 1979. Crecimiento de *Mauremys caspica leprosa* (Schweigger, 1812) (Reptilia, Testudines) en la Reserva Biológica de Doñana. *Doñana, Acta Vertebrata* 6(2), 161-178.
Polak T, Saltz D, 2011. Reintroduction as an ecosystem restoration technique. *Conservation Biology* 25(3), 424-424. DOI: 10.1111/j.1523-1739.2011.01669.x
Pulgar Sañudo I, 2010. *Guía da Flora do Parque Natural do complexo dunar de Corrubedo e lagoas de Carregal e Vixán*. Xunta de Galicia. Consellería do Medio Rural e do Mar, Santiago de Compostela.
Rhodin AGJ, Stanford CB, Dijk PPV, Eisemberg C, Luiselli L, Mittermeier RA, Hudson R, Horne BD, Goode EV, Kuchling G, Walde A, Baard EHW, Berry KH, Bertolero A, Blanck TEG, Bour R, Buhlmann KA, Cayot LJ, Collett S, Currylow A, Das I, Diagne T, Ennen JR, Forero-Medina G, Frankel MG, Fritz U, García G, Gibbons JW, Gibbons PM, Shiping G, Guntoro J, Hofmeyr MD, Iverson JB, Kiester AR, Lau M, Lawson DP, Lovich JE, Moll EO, Páez VP, Palomo-Ramos R, Platt K, Platt SG, Pritchard PCH, Quinn HR, Rahman SC, Randrianjafizanaka ST, Schaffer J, Selman W, Shaffer HB, Sharma DSK, Haitao S, Singh S, Spencer R, Stannard K, Sutcliffe S, Thomson S, Vogt RC, 2018. Global conservation status of turtles and tortoises (Order Testudines). *Chelonian Conservation and Biology* 17(2), 135. DOI: 10.2744/CCB-1348.1
Robichaud JA, Bulté G, MacMillan HA, Cooke SJ, 2022. Five months under ice: biologging reveals behaviour patterns of overwintering freshwater turtles. *Canadian Journal of Zoology* 101(3), 152-162. DOI: 10.1139/cjz-2022-0100
Rollinat R, 1934. *La vie des reptiles de la France Centrale. 50 années d'observations biologiques.* Delagrave, Paris.
Seddon PJ, Armstrong DP, Maloney RF, 2007. Developing the science of reintroduction biology. *Conservation Biology* 21(2), 303-312. DOI: 10.1111/j.1523-1739.2006.00627.x
Servan J, 1998. Ecological study of *Emys orbicularis* in Brenne (Central France). *Mertensiella* 10, 245-252.
Stanford CB, Iverson JB, Rhodin AGJ, Paul Van Dijk P, Mittermeier RA, Kuchling G, Berry KH, Bertolero A, Bjorndal KA, Blanck TEG, Buhlmann KA, Burke RL, Congdon JD, Diagne T, Edwards T, Eisemberg CC, Ennen JR, Forero-Medina G, Frankel M, Fritz U, Gallego-García N, Georges A, Gibbons JW, Gong S, Goode EV, Shi HT, Hoang H, Hofmeyr MD, Horne BD, Hudson R, Juvik JO, Kiester RA, Koval P, Le M, Lindeman PV, Lovich JE, Luiselli L, McCormack TEM, Meyer GA, Páez VP, Platt K, Platt SG, Pritchard PCH, Quinn HR, Roosenburg WM, Seminoff JA, Shaffer HB, Spencer R, Van Dyke JU, Vogt RC, Walde AD, 2020. Turtles and tortoises are in trouble. *Current Biology* 30(12), R721-R735. DOI: 10.1016/j.cub.2020.04.088
Stuckas H, Velo-Antón G, Fahd S, Kalboussi M, Rouag R, Arculeo M, Marrone F, Sacco F, Vamberger M, Fritz U, 2014. Where are you from, stranger? the enigmatic biogeography of North African pond turtles (*Emys orbicularis*). Organisms Diversity and Evolution 14(3), 295-306. DOI: 10.1007/s13127-014-0168-4
Szczerbak NN, 1998. The European pond turtle (*Emys orbicularis*) in Ukraine. *Mertensiella* 10, 259-266.
Tortoise and Freshwater Turtle Specialist Group, 1996: *Emys orbicularis*. *The IUCN Red List of Threatened Species 1996*, e.T7717A97292665. DOI: 10.2305/IUCN.UK.1996.RLTS.T7717A12844431.en
Tracy LN, Wallis GP, Efford MG, Jamieson IG, 2011. Preserving genetic diversity in threatened species reintroductions: how many individuals should be released? *Animal Conservation* 14(4), 439-446. DOI: 10.1111/j.1469-1795.2011.00448.x
Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P, 2004. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes* 4(3), 535-538. DOI: 10.1111/j.1471-8286.2004.00684.x
Velo-Antón G, 2013: Towards better management of European pond turtles (*Emys orbicularis*) in conservation programs. *Herpetology Notes* 6, 381-382.
Velo-Antón G, Becker CG, Cordero-Rivera A, 2011a. Turtle carapace anomalies: the roles of genetic diversity and environment. *PLoS ONE* 6(4), e18714. DOI: 10.1371/journal.pone.0018714
Velo-Antón G, García-París M, Cordero-Rivera A, 2008. Patterns of nuclear and mitochondrial DNA variation in Iberian populations of *Emys orbicularis* (Emydidae): Conservation implications. *Conservation Genetics* 9(5), 1263-1274. DOI: 10.1007/s10592-007-9457-1
Velo-Antón G, Godinho R, Ayres C, Ferrand N, Cordero-Rivera A, 2007 Assignment tests applied to relocate individuals of unknown origin in a threatened species, the European pond turtle (*Emys orbicularis*). *Amphibia-Reptilia* 28, 475-484. DOI: 10.1163/156853807782152589
Velo-Antón G, Marnisi B, Fritz U, Fahd S, 2015. Distribution and conservation status of *Emys orbicularis* in Morocco. *Vertebrate Zoology* 65(1), 131-134. DOI: 10.3897/vz.65.e31514
Velo-Antón G, Pereira P, Gonçãlves DV, 2021. Genetic assignment of captive European pond turtles (*Emys orbicularis*) increases conservation value of recovery centres. *Journal for Nature Conservation* 59, 125953. DOI: 10.1016/j.jnc.2020.125953
Velo-Antón G, Wink M, Schneeweiß N, Fritz U, 2011b. Native or not? Tracing the origin of wild-caught and captive freshwater turtles in a threatened and widely distributed species (*Emys orbicularis*). *Conservation Genetics* 12, 583-588. DOI: 10.1007/

s10592-010-0141-5
White GC, Burnham KP, 1999. Program Mark: Survival estimation from populations of marked animals. *Bird Study* 46, S120-S139. DOI: 10.1080/00063659909477239
Willi Y, Van Buskirk J, Hoffmann AA, 2006. Limits to the adaptive potential of small populations. *Annual Review of Ecology, Evolution and Systematics* 37, 433-458. DOI: 10.1146/annurev.ecolsys.37.091305.110145
Zuffi MAL, Mangiacotti M, Masucci GD, Sacchi R, Scali S, Sannolo M, 2017. Stable or plastic body shape? *Emys orbicularis* hatchlings-juveniles growth patterns under different ecological conditions. *North-Western Journal of Zoology* 13(2), 262-270. Article No: e161510.

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Adolfo Cordero-Rivera: data curation, formal analysis, funding acquisition, methodology, project administration, resources, roles/writing–original draft, writing–review and editing; **César Ayres:** data curation, investigation, methodology, roles/writing–original draft, writing–review and editing; **Guillermo Velo-Antón:** data curation, formal analysis, investigation, methodology, roles/writing–original draft, writing–review and editing.

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Supplementary materials

Table 1s. Results of model selection using QAIC corrected for overdispersion. Animals were divided into males, females, and juveniles (g, group; t, time). The most supported model is indicated in bold. (Values corrected by c-hat = 1.378117).

Tabla 1s. *Resultados de la selección de modelos mediante QAIC, corregidos por el exceso de dispersión. Los animales se dividieron en machos, hembras y juveniles (g, grupo; t, tiempo). El modelo más apoyado se indica en negrita. (Valores corregidos por c-hat = 1,378117).*

Model	QAICc	Delta QAICc	AICc Weights	Model Likelihood	Num. Par.	QDeviance
{Phi(g) p(.) PIM}	188.678	0.000	0.799	1	4	139.396
{Phi(g) p(g) PIM}	191.561	2.883	0.189	0.237	6	137.907
{Phi(.) p(g) PIM}	198.335	9.657	0.006	0.008	4	149.053
{Phi(.) p(.) PIM}	198.450	9.772	0.006	0.008	2	153.399
{Phi(g) p(t) PIM}	205.820	17.142	0.000	0.000	16	127.920
{Phi(.) p(t) PIM}	214.565	25.887	0	0	14	141.860
{Phi(t) p(.) PIM}	217.046	28.368	0	0	14	144.341
{Phi(t) p(g) PIM}	218.668	29.990	0	0	16	140.768
{Phi(t) p(t) PIM}	238.486	49.808	0	0	25	134.660
{Phi(.) p(g*t) PIM}	271.199	82.521	0	0	40	112.105
{Phi(g) p(g*t) PIM}	271.903	83.225	0	0	42	103.948
{Phi(g*t) p(.) PIM}	276.519	87.841	0	0	39	121.702
{Phi(g*t) p(g) PIM}	284.156	95.478	0	0	41	120.684
{Phi(t) p(g*t) PIM}	319.439	130.761	0	0	51	105.764
{Phi(g*t) p(t) PIM}	319.623	130.945	0	0	50	111.563
{Phi(g*t) p(g*t) PIM}	504.662	315.984	0	0	75	90.178

Table 2s. Results of model selection using QAIC corrected for overdispersion. Animals were divided into 2004 (headstarted) and 2006 (control) cohorts (g, group; t, time). The two most supported models are indicated in bold. (Values corrected by c-hat = 1.471618).

Tabla 2s. *Resultados de la selección de modelos mediante QAIC, corregidos por el exceso de dispersión. Los animales se dividieron en cohortes de 2004 (fundadores criados en cautividad) y 2006 (de control) (g = grupo; t = tiempo). Los dos modelos más apoyados se indican en negrita. (Valores corregifdos por c-chat = 1,471618).*

Model	QAICc	Delta QAICc	AICc Weights	Model Likelihood	Num. Par	QDeviance
{Phi(.) p(.) PIM}	127.518	0.000	0.342	1.000	2	84.120
{Phi(g) p(.) PIM}	127.759	0.241	0.303	0.887	3	82.194
{Phi(.) p(g) PIM}	128.783	1.265	0.182	0.531	3	83.218
{Phi(g) p(g) PIM}	128.885	1.366	0.173	0.505	4	81.093
{Phi(.) p(t) PIM}	147.911	20.393	0.000	0.000	14	73.901
{Phi(g) p(t) PIM}	149.335	21.816	0.000	0.000	15	72.229
{Phi(t) p(.) PIM}	151.880	24.361	0.000	0.000	14	77.869
{Phi(t) p(g) PIM}	153.231	25.713	0.000	0.000	15	76.126
{Phi(.) p(g*t) PIM}	180.868	53.350	0.000	0.000	25	66.141
{Phi(g) p(g*t) PIM}	183.734	56.215	0.000	0.000	26	64.417
{Phi(t) p(t) PIM}	187.950	60.432	0.000	0.000	26	68.634
{Phi(g*t) p(.) PIM}	188.019	60.501	0.000	0.000	25	73.293
{Phi(g*t) p(g) PIM}	190.911	63.393	0.000	0.000	26	71.595
{Phi(t) p(g*t) PIM}	245.926	118.408	0.000	0.000	37	60.587
{Phi(g*t) p(t) PIM}	248.702	121.184	0.000	0.000	37	63.363