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Assessment of the health status of a reintroduced headstarted population of *Emys orbicularis galloitalica* through the analysis of weight and size growth curves and allometries

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ABSTRACT
Biometric measures can serve as proxies for the health condition of wildlife populations, and deviations from their expected trends may signal stress, disease, or resource scarcity. This study evaluates the success of the reintroduction of a headstarted European pond turtle population by analysing individual growth rates and allometries. From 2016 to 2018, three groups of turtles that had experienced two different captive-rearing protocols were released, totalling 32 individuals. To collect biometric data, eight capture sessions were conducted from 2017 to 2022. Twenty-nine out of 32 individuals were recaptured, with 17 identified as males and 9 as females. The first group, which hibernated during the winter before release, was smaller and lighter at release but grew faster than the other two, which avoided hibernation; in addition, we found a significant difference in the allometric relationship between weight and carapace length when comparing the first group with the other two, due to a sex-dependent differential weight gain among groups. Despite limitations related to sample size and imbalance issues, we found evidence suggesting that the successful reintroduction of multiple stocks of individuals released at different times may be attributable to the simultaneous action of the rearing strategy and the intraspecific within-sex competition for food or space.

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Introduction

Static allometries describe the within-species evolutionarily-constrained relationship between morphological traits (Voje et al. 2014) and are a crucial tool for evaluating the health status of wild animal populations (Smith 1984; Kophamel et al. 2022; Balaguera-Reina et al. 2023). By analysing static (within-species) allometric patterns, researchers can assess growth rates, metabolic efficiency, and the overall fitness of individuals within a wild population. Deviations from expected allometric relationships may indicate environmental stress, possibly signalling habitat degradation (Delgado-Acevedo & Restrepo 2008; Lazić et al. 2015), overcrowding (Pafilis et al. 2009), or resource scarcity (Castro et al. 2021) and health issues, such as nutritional deficiencies and disease occurrence (Kophamel et al. 2022; Balaguera-Reina et al. 2023). Thus, incorporating allometric analysis into wildlife health assessments enhances the ability to detect early warning signals of population health status, guiding effective conservation and management strategies (Kophamel et al. 2022).

In this study, we aimed to evaluate the effectiveness of a reintroduction project of a European pond turtle population (Masin et al. 2020) by analysing weight gain, carapace growth and their allometries in 32 individuals released from 2016 to 2018. The project aimed primarily to raise public awareness about the impact of habitat loss and invasive species on wildlife, highlighting the importance of biodiversity conservation and, secondarily, to establish a self-sustaining population that can thrive in the medium term, serving as both an educational tool and a concrete step towards ecosystem restoration for this endangered and protected species (Zuffi 2004; Rondinini et al. 2022).

Since young turtles in the wild are typically subject to high predation rates, which would hinder the success of reintroduction efforts (Tetzlaff et al. 2019; Wijewardena et al. 2023; Lanszki et al. 2024), most of the

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released individuals underwent an headstarting phase, which is beneficial in reintroduction projects as it significantly enhances the survival rate of young turtles during their most vulnerable early stages (Janzen et al. 2000; Tetzlaff et al. 2019). This technique involves raising hatchlings in controlled ex situ environments until they reach a size that reduces their susceptibility to predators and other environmental stressors, such as difficulties in finding adequate food. By releasing headstarted individuals, the project may boost population growth and increase the likelihood that more individuals survive to adulthood, ultimately supporting the success of the reintroduction and the establishment of a self-sustaining population (Golba et al. 2022; Mullin et al. 2023).

Materials and methods

The study was conducted in Lombardy, northern Italy, within the boundaries of the "Montevecchia e della Valle del Curone" Regional Park, specifically in a flat valley floor at about 300 m a.s.l. Here, a population of the European pond turtle belonging to the subspecies *Emys orbicularis* ssp. *galloitalica* Fritz, 1995 was reintroduced, and monitored in the following years by the authors and the park authority. Individuals were purchased from an authorized nursery that rears individuals for reintroduction purposes. Purchasing, rearing and releasing has been authorized by the Lombardy Regional Administration, Directorate General for Environment and Climate (T1.2016.0007767), according to national regulations.

A dedicated pond with permanent water, covering an area of 300 m^2 (Figure 1), was constructed by the protected area administration close to two smaller interconnected ponds, measuring 150 and 50 m^2, respectively. The banks of the main pond, as well as those of the smaller ponds, were covered with riparian vegetation, including sedges (*Carex* spp. L., 1753), bulrush (*Typha latifolia* L., 1753), and common reed (*Phragmites australis* (Cav.) Trin. ex Steud., 1841). In the main pond, there was a small central islet with riparian vegetation, and in the deepest central area, logs had been placed to provide basking spots (Vignoli et al. 2015). The main pond was built in a large clearing that ensures good sun exposure, with partial shading provided by surrounding trees. The land adjacent to the ponds gently sloped towards the banks, preventing water stagnation. The soil had a sandy or sandy-loamy texture, facilitating easy excavation and proper ventilation of turtle nests (Meeske 1997; Zuffi and Rovina 2006; Kiss et al. 2024). Vegetation of slope land was periodically controlled by mowing to maintain suitable conditions for nesting (Liuzzo et al. 2024). For reasons of public safety all ponds were enclosed by a wooden fence that allowed the turtles to move under the lower crossbar, positioned about 30 cm above the ground. To protect the released turtles from bird predation (e.g. herons, cormorants, and hooded crows), the main pond was covered with a plastic-coated metal wire net, with a 50 cm mesh size, attached to the top of the wooden fence. Released animals were free to disperse into the surrounding area, where large deciduous forests were interspersed with meadows and small, extensive agricultural areas. A dense network of small to medium-sized rivers and small ponds provided suitable freshwater habitats for the turtles.

Individuals were released into the main pond over a period of 3 years. The first group (I), consisting of seven individuals, was born from captive breeders and grew in natural ponds within a fenced area, fed by animal curators, but without the control of temperature and hygrometry, allowing young turtles to hibernate. This group was purchased and immediately released in July 2016 at 10 months of age (born in September 2015). The second group (II), composed of nine individuals born in September 2016, underwent an 8-month headstarting period before being released in July 2017. The final group (III), consisting of 16 individuals born in September 2017, was subjected to a 10-month headstarting period before being released in July 2018. During the rearing phase (headstarting), turtles from groups II and III were randomly divided into three small groups (5–6 individuals) and reared in plastic tanks to avoid any potential overcrowding issue. For further details on rearing conditions, such as temperature control to prevent hibernation, see Masin et al. (2020).

Each released individual was uniquely marked using the edge shell notching technique (Plummer & Ferner 2012) to enable identification during subsequent capture sessions. Up to four notches for each side were cut into the left (tens) and right (units) marginal scutes of the shell, viewed dorsally, allowing the composition of a unique identity code ranging from 1 to 99. Periodic captures were conducted to estimate health status and growth rates. Overall, eight capture sessions were conducted from September 2017 to September 2022 (one session per year in spring/summer, except for 2019 and 2022, when a second session

Figure 1. (a) Pond constructed in the "Montevecchia e della Valle del Curone" Regional Park for the reintroduction of the European pond turtle (*Emys orbicularis galloitalica*); (b) an individual European pond turtle on top of a hoop net, during a capture session.

was conducted in late summer). Capture sessions lasted 1 or 2 days, for a total of 11 capture days, during which 15 hoop nets, baited with shrimp and small fish, were placed in both the main pond and in the surrounding smaller ponds. Captured individuals were kept in a tank with shallow water until the end of the session to avoid multiple recaptures and were measured by experienced observers, who collected the weight (W) and the linearized or straight carapace length (SCL). The weight was measured with an electronic scale (resolution: 0.1 g), while length measures were recorded using a calliper (resolution: 0.1 mm). Since SCL was measured once or twice, according to the availability of one or two experienced observers, we averaged repeated measures over individuals and capture events as input for the following analyses. Sex was assessed by observing the plastron concavity and the preanal tail length (Readel et al. 2008).

We tested whether the growth rates of W and SCL differed among groups after release by fitting linear models on biometric measures of the sex-determinable individuals, with time (number of days elapsed from release of the pertaining group) as the independent variable. We tested for differences in growth rates between groups and sexes by including the interaction of time with group and sex. We selected the best model by a stepwise Akaike information criterion (AIC) selection procedure, and we evaluated the marginal effect of contrasts using the *emtrends* function of the package "emmeans" 1.10.7 (Lenth 2025) in R v. 4.4.2 (R Core Team 2024). We assessed the linear covariation between the relative growth rates of SCL and W by estimating the intercept ("a") and slope ("b") parameters of the power-law function $Y = aX^b$ (Klingenberg 2016), where Y and X were the log-transformed values of SCL and W, measured from all 32 recaptured individuals. We tested whether the two measurements covaried isometrically (null hypothesis, $b = 1$) or allometrically (alternative hypothesis, $b \neq 1$) using the standardized major axis (SMA) method implemented in the "SMART3" R package (Warton et al. 2012) and we compared results to the parameters found by Meek for *E. orbicularis* (Meek 1982; $r_{Pearson} = 0.99$; $N = 7$) (Equation (1)):

$$SCL[mm] = 10.84 \times W[g]^{0.41 \pm 0.06} \quad (1)$$

Finally, we tested for differences in allometric relationships among groups by comparing the slope of the best-fit lines of log-transformed W and SCL of each group (common slope hypothesis) by a pairwise likelihood ratio test (Warton et al. 2012). Significantly higher slopes would indicate a larger body weight at equal length.

Results and discussion

Among the eight capture sessions, the recapture success varied between 6.3% and 56.3% of the released turtles in the pond. Twenty-nine out of the 32 released individuals were recaptured at least once, 5 out of 7 in group I (range of single session recapture rate: 14.3–57.1%), all individuals of group II (0–55.5%) and 14 out of 16 in group III (6.2–56.2%). The introduction of hatchlings or individuals subjected to headstarting, hatched in facilities where breeders are kept in natural conditions (as in our case), did not allow for determining the sex of the turtles at the time of release. This may hinder the establishment of a population with a sex ratio close to 1:1. However, during subsequent recaptures, the growth of the individuals made it possible to determine the sex of the turtles. Within the pool of released turtles, 17 were identified as males and nine as females, while six were not sex-determinable due to ambiguous sexual characteristics. The sex ratio of sexed individuals was 1:4 in group I, 6:2 in group II and 10:3 in group III. The best model explaining the SCL growth rate was a linear model with time [days], group, and their two-way interaction as predictors. The best model performed on W was a linear model with time [days], group, sex, and their two-way and three-way interactions as predictors (Table 1). The growth rate of SCL was significantly higher in group I than in group II and group III, while no differences were found between group II and III (Table 2, Figure 2a). The female W growth rate was significantly higher in group I compared to group II and III, while the growth rate of group II and III did not differ. Moreover, the female W growth rate in group I was significantly higher than that of males of the three groups (Table 2, Figure 2b). A slight signal ($0.05 < p < 0.1$) for a faster W growth of male in group II respect to females of the same group and males of group III was also detected.

As documented in other studies (Meek 1982; Ramos et al. 2009) the relative growth of SCL and W of all individuals across the whole period was significantly allometric (H0: slope = 1; $p < 0.001$; $R^2 = 0.994$; $N = 323$) and the following power function can be derived from estimates (Equation (2)):

$$SCL[mm] = 11.57 \times W[g]^{0.383 \pm 0.003} \quad (2)$$

The overall allometric relationship according to the power function did not substantially differ from that found by Meek (1982), while allometric relationships differed by group, with group III showing significantly lighter individuals, at the same SCL values, than groups I and II (I vs II, $p = 0.248$; I vs III, $p < 0.001$; II vs III, $p = 0.007$).

Different factors could have driven differential growth rates between groups and sexes. The growth curve for freshwater turtles is expected to behave exponentially, and individual growth should slow as body size increases and sexual maturity approaches (Miranda Garcez et al. 2025). Group I was released at an average SCL of 40.80 mm (standard deviation [SD] = 8.271), while groups II ($avg_{SCL} = 67.96$ mm, SD = 7.038) and III ($avg_{SCL} = 71.15$ mm, SD = 6.329) were about 30 mm longer at release (see Figure 2a). Hence, individuals of

Table 1. Linear regression models estimating the growth rate of straight carapace length (SCL) and weight (W) of *Emys orbicularis galloitalica* released in the wild after headstarting. Time is expressed as the number of days elapsed from the release of the relevant group for each individual. Individuals in group I ($N = 7$) were reared in a natural environment with food supply and hibernated before release. Groups II ($N = 9$) and III ($N = 16$) were captive-reared in the lab with food supply and temperature control and avoided hibernation. Estimates of group and sex effects are expressed as difference from the reference levels, group I and female, respectively. SE is the standard error of the estimated regression coefficient. SCL model: adjusted $R^2 = 0.797$, df: 67; W model: adjusted $R^2 = 0.842$, df: 61.

Biometry	Parameter	Group	Estimate	SE	t value	*p* value
SCL	Intercept	I	51.00	3.425	14.889	<0.001
		II	22.31	4.333	5.149	<0.001
		III	18.74	3.996	4.691	<0.001
	Time [days]	I	0.043	0.003	15.701	<0.001
		II	−0.019	0.004	−4.879	<0.001
		III	−0.021	0.004	−5.205	<0.001
W	Intercept	I	10.83	13.28	0.816	0.417
		II	57.45	23.30	2.466	0.016
		III	45.83	19.92	2.301	0.024
		Male	20.89	34.01	0.614	0.541
		II × Male	−16.83	40.45	−0.416	0.678
		III × Male	−21.72	38.02	−0.571	0.569
	Time [days]	I	0.173	0.010	17.30	<0.001
		II	−0.121	0.021	−5.721	<0.001
		III	−0.107	0.028	−3.854	<0.001
		Male	−0.088	0.042	−2.088	0.040
		II × Male	0.126	0.048	2.638	0.010
		III × Male	0.082	0.051	1.614	0.110

Table 2. Pairwise contrasts of time effect (growth rate) between factor levels (sex [F: female, M: male] and group) included in the linear regression models for straight carapace length (SCL) and weight (W) of *Emys orbicularis galloitalica* from the time of their release into the wild. SE is the standard error of the estimated regression coefficient.

Biometry	Contrast	Difference	SE	t ratio	*p* value
SCL	Group I – Group II	0.020	0.004	4.879	<0.001
	Group I – Group III	0.021	0.004	5.205	<0.001
	Group II – Group III	0.002	0.004	0.403	0.688
W	F Group I – F Group II	0.121	0.021	5.721	<0.001
	F Group I – F Group III	0.107	0.028	3.854	<0.001
	F Group II – F Group III	−0.015	0.032	−0.459	0.648
	F Group I – M Group I	0.088	0.042	2.088	0.040
	F Group I – M Group II	0.083	0.016	5.263	<0.001
	F Group I – M Group III	0.113	0.015	7.344	<0.001
	F Group II – M Group I	0.033	0.045	0.736	0.464
	F Group II – M Group II	−0.038	0.022	−1.694	0.094
	F Group II – M Group III	0.008	0.022	−0.387	0.699
	F Group III – M Group I	0.018	0.048	0.383	0.703
	F Group III – M Group II	0.023	0.029	0.814	0.418
	F Group III – M Group III	0.006	0.028	0.215	0.830
	M Group I – M Group II	−0.005	0.043	−0.110	0.913
	M Group I – M Group III	0.025	0.043	0.578	0.565
	M Group II – M Group III	0.029	0.017	1.734	0.087

group I were still in the first phase of rapid growth when released in the wild, while individuals of the other groups anticipated that growth during the headstarting period and their growth rate had already begun to decline. According to estimated growth rates, group I should have matched the SCL of the other groups in 1 year after release. The rapid linear growth of group I may also be attributable to the initial lack of competition for habitat, basking sites, and trophic resources (Lebboroni & Chelazzi 1991; Hamer et al. 2018), which progressively increased with the addition of new individuals in subsequent years (Matte et al. 2020). Moreover, density-dependent intraspecific competition may have been exacerbated by dominance hierarchies established by the older individuals (Zedrosser et al. 2006; Woodman et al. 2024). This hypothesis is supported by the group- and sex-dependent gain in weight, which determined differential allometric relationships among the three groups. Deviations in allometric relationships may be a symptom of

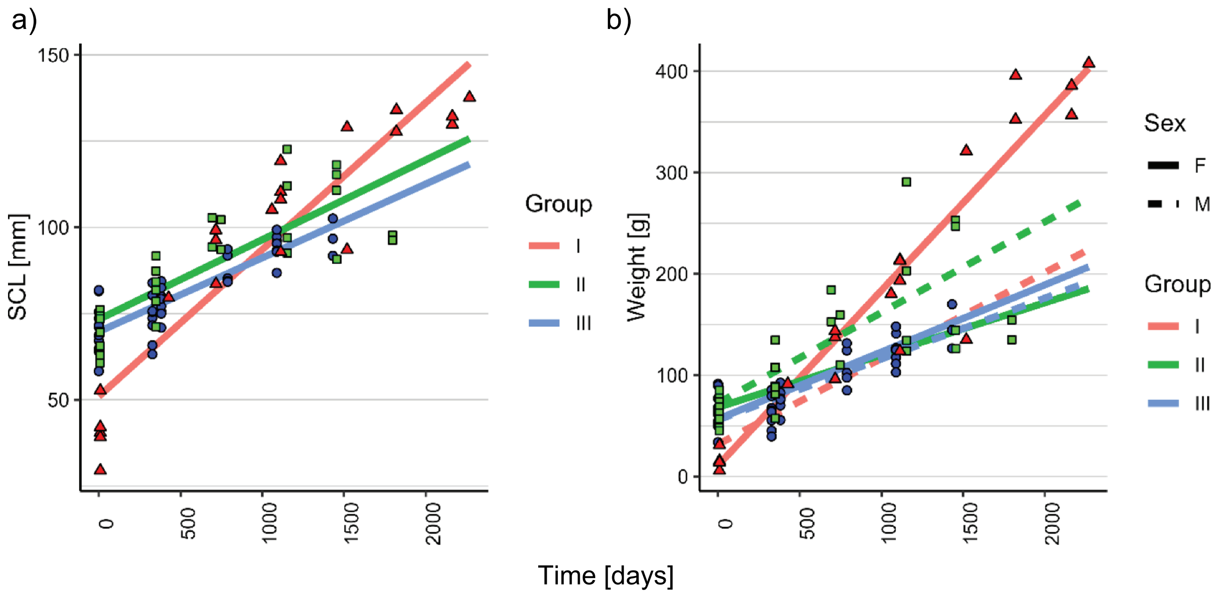

Figure 2. Estimated growth curves for (a) straight carapace length (SCL) and (b) weight (W) of individuals of *Emys orbicularis galloitalica*, from the time of their release into the wild. Zero on the x-axis corresponds to the date of release of individuals into the wild. Individuals in group I ($N = 7$) were reared in a natural environment with food supply and hibernated before release. Groups II ($N = 9$) and III ($N = 16$) were captive-reared in the lab with food supply and temperature control and avoided hibernation. Sex: F, females - M, males.

limitations in resource acquisition (Lazić et al. 2015), and mechanisms regulating turtle growth allometry may be disrupted by intraspecific competition. The rapid growth in weight of group I females, two of which were found with eggs, signalled a sudden reach of sexual maturity (SCL ≥13 cm, Zuffi et al. 2006) and the possible establishment of a hierarchical structure that could have penalized females of the other two groups. Similarly, we can hypothesize that males of the second group did not find many competitors at release, as only one male was present in group I, and could have established dominance hierarchies over males of Group III, which showed the slowest body weight gain among males. Moreover, the high density of males could have established strong competition for pairing during the breeding season (Rovero et al. 1999; Poschadel et al. 2006) and could have determined the temporal departure of some of the non-breeding males towards suboptimal habitats with limited availability of basking and trophic resources.

Conclusion

The reintroduction of a population of headstarted European pond turtle as a part of a conservation and awareness-raising project is considered successful, as evidenced by the high recapture rate and the linear growth in size and weight of released individuals, indicating local establishment within a functional ecosystem that provides adequate trophic resources (Ottonello et al. 2018). Further confirmation of the project's success comes from the recapture of sexually mature females carrying eggs, as well as the capture and observation of hatchlings and young individuals estimated to be between 1 and 2 years old. Moreover, the overall good health of the reintroduced animals can be inferred from their alignment with the typical allometric relationships of the species known from established populations (Meek 1982).

Based on Spencer et al. (2017), our results confirm headstarting as a crucial tool for the success of reintroduction projects of freshwater turtles. However, the finding of group- and sex-dependent growth after release suggests possible constraints derived from multiple restocking events which could vary in numerosity, sex ratio and protocol experienced by headstarted individuals (Cordero-Rivera et al. 2024). Avoidance of hibernation during ex situ captive rearing was found to strongly affect size at release and subsequent growth rates. Moreover, sex-dependent weight gain suggests a potential increase in intra-sex competitive pressure over time. If the sex of reintroduced individuals can be controlled in advance, a potential strategy in reintroduction projects could involve prioritizing the release of females, the limiting reproductive sex in viable populations, to promote their growth under conditions of low population density. However, a higher proportion of males in subsequently released stocks should be planned to balance the sex ratio (Girondot & Pieau 1993). Furthermore, to ensure the long-term conservation of metapopulations that are resilient to climate and environmental changes (Roberts et al. 2023), it would be beneficial to establish networks of suitable biotopes that can support the movement of non-breeding males and the recruitment of young individuals dispersing from the main source population.

This study also demonstrates the importance of long-term morphological monitoring of reintroduced freshwater turtles as a tool for assessing the success of reintroduction and habitat restoration efforts. However, further research is needed to monitor body-shape allometries (Balaguera-Reina et al. 2024) and health status by more specific methods (McCaffrey et al. 2023), as well as to complement the understanding of population dynamics by investigating nest-site selection (Spencer 2002), hatching success and predation rates (Liuzzo et al. 2024), individuals' use of surrounding habitats, diet (Meyer et al. 2025), and competition for natural resources.

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Data availability statement

The data that support the findings of this study are available from the Montevecchia e della Valle del Curone Regional Park administration. Restrictions apply to the availability of these data, which were used under licence for this study. Data are available from the authors with the permission of the Park administration.

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