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DOMENIUL BIOLOGIE

DOCTORAL THESIS SUMMARY

ECTOTHERMS POPULATIOS DYNAMICS

(joint PhD thesis)

Supervisors,

Dr. Dan Cogălniceanu (Ovidius University of Constanta, Romania)

Dr. Miguel Angel Carretero (Universidade do Porto, Portugal)

Doctoral student,

Ochiană (Vlad) Elena-Sabina

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There is no vertebrate group facing greater survival problems today. Turtles saw the great dinosaurs come and go and are now facing their own extinction crisis.

— John L. Behler

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KEYWORDS: demographic parameters, climate change, age and growth-related parameters, anthropogenic impact, sexual selection, invasive species, biotic interactions.

INTRODUCTION

There are 12000 species of reptiles worldwide of which 3% are represented by chelonians and 34% are represented by lizards (Uetz et al., 2023). Factors such as habitat loss, pollution, poaching, invasive species and diseases lead to a worldwide decline, at an alarming state (Gibbons et al., 2000, Böhm et al., 2013, Stanford et al., 2020).

Preventing chelonians extinctions in the 21st century requires protection of the remaining natural habitats, particularly hotspots of species diversity (Stanford et al., 2020). In this context long-term monitoring studies are essential for revealing trends in population size or health (Gibbons et al., 2000). According to Turtle Conservation Coalition (2018) 10 taxa of chelonians have gone extinct in recent human history.

Lizards constitute a heterogenic group of reptiles, many of them facing a serious risk of extinction, having fragmented or very reduced distributions. However, the available information for adequately assessing this risk for all species is insufficient (Meiri et al., 2018). Therefore, lizards require more attention in taxonomic, ecological and research efforts.

We are currently facing a severe biodiversity crisis at global level (Ceballos et al., 2015; Rull, 2022). The most vulnerable are populations living at the edge of their range, which have limited capacity to spread and colonize new territories (Foden and Young, 2016). Global change threatens in special the ectothermic organisms, these being more vulnerable to climate warming. The effects are amplified by the anthropogenic alterations which can affect multiple aspects of the ecosystem function and structure, causing habitat damage (Mona et al., 2019) and species decline (Gibbons et al., 2000; Botejue and Wattavidanage, 2012; Janiawati et al., 2016).

The main goal of the thesis is to bring insights regarding life history adaptations in ectothermic vertebrates inhabiting areas close to the range limits. For this I have chosen two model species, well distributed among the central zone of the western Palearctic and I have focused on populations inhabiting areas close to either the latitudinal range limit or the altitudinal limit. The thesis is structured in five chapters, each designed around an independent aim:

(i) Characterize tortoise population dynamics in the proximity of the northern distribution limit;

(ii) Evaluate the correlation between growth rate and body size parameters in tortoises.

(iii) Estimate the anthropogenic impact on tortoises inhabiting protected areas.

(iv) Infer if the polymorphism in populations of wall lizards living in the proximity of the altitudinal limit can be maintained by alternative strategies of interaction.
(v) Propose a series of measures for efficient management and conservation of reptiles.

GENERAL PRESENTATION OF THE MODEL SPECIES

Testudo graeca (Linnaeus, 1758) - The Spur-thighed Tortoise (Figure 1A) is a priority species for conservation within the European Union (Habitats Directive, 1992), being included in Annex II as “Animal and plant species of community interest whose conservation requires the designation of special areas of conservation”. It is considered vulnerable according to the International Union for Conservation of Nature (IUCN; Cox and Temple, 2009), highlighting the necessity of specific conservation and management activities.

Podarcis muralis (Laurenti, 1768) - The common wall lizard (Figure 1B) is a species of community interest within the European Union, being included in Annex IV along with other “Animal and plant species of community interest in need of strict protection” (Habitats Directive, 1992). It currently has a stable trend, being most recently assessed in 2009 as least concern by the IUCN (Böhme et al., 2009; Cox and Temple, 2009).

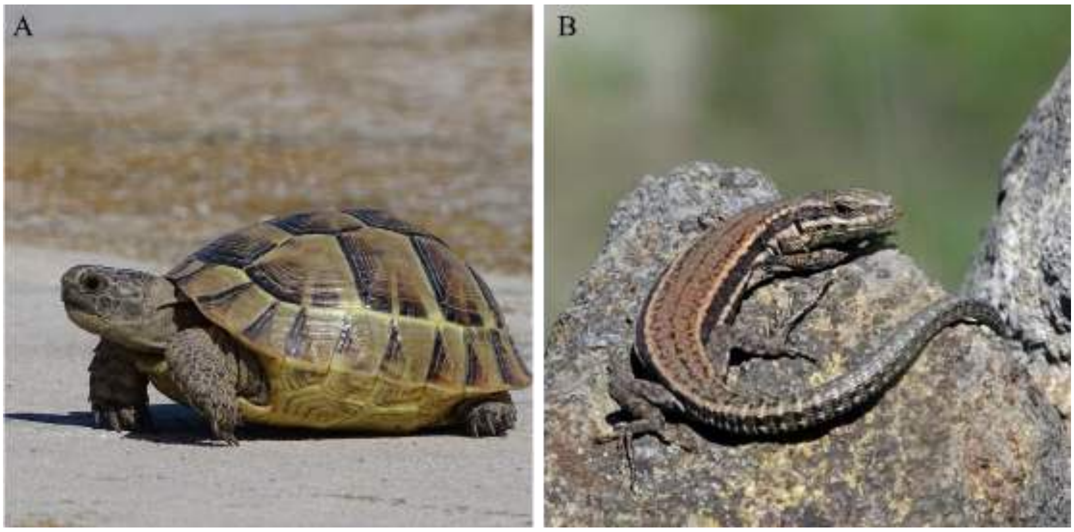

Figure 1. Target species. A - Spur-thighed tortoise (*Testudo graeca*) photographed in Northern Saele Levee, Dobrogea region, Romania. B - Common wall lizard (*Podarcis muralis*), photographed in refugi del Fornet, Central Pyrenees, Spain.

GENERAL PRESENTATION OF THE STUDY SITE

Multiple populations of the two target species, i.e., the Spur-thighed tortoises (*Testudo graeca*) and the common wall lizards (*Podarcis muralis*), were studied in two distinctive geographic areas of Europe, close to the latitudinal or the altitudinal range limit, respectively.

We inventoried Spur-thighed tortoise populations, in different habitats, most of them located within the Natura 2000 network and situated at altitudes ranging between sea level, in the Danube Delta Biosphere Reserve, to 455m above sea level, in the Hercinic mountains, Măcin Mountains National Park (Figure 2A).

Common wall lizards were inventoried in 12 populations from Central Pyrenees (North-eastern Spain). Individuals were sampled within the Natura 2000 site Baish Aran (ES5130004) protected under both Birds and Habitats Directives, in Aran Valley, at elevations ranging between 995-2072 m above sea level (Figure 2B).

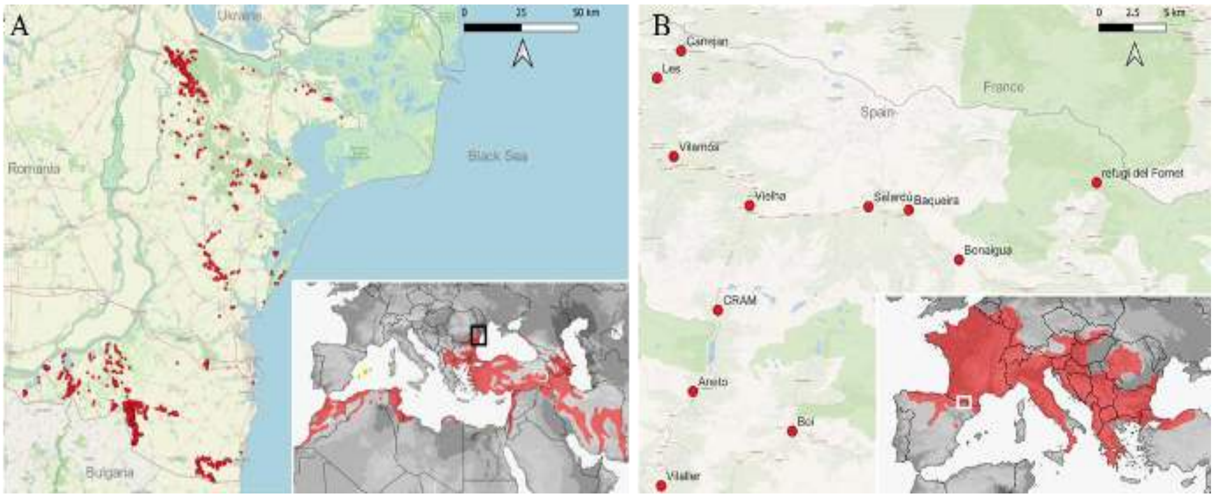

Figure 2. Sampling sites of the target species (original) and distribution of the species within the western palearctic (red - native range, yellow - introduction; source: Midtgaard, 2019). A – Distribution and sampling sites of the Spur-thighed tortoise (*Testudo graeca*) in Dobrogea region, South-eastern Romania. B – Distribution and sampling sites of the Common wall lizard (*Podarcis muralis*) in Aran Valley, Central Pyrenees, Spain.

Chapter I. Population dynamics in tortoises inhabiting areas close to the distribution limit

INTRODUCTION

Long-term studies focusing on Chelonians benefit from the advantage given by the longevity of these reptiles. One of the most important traits of reptiles is the body size. It determines individual life history and fitness (Bailey et al., 2020) and it is commonly used as an indicator of the environmental conditions' effect on populations (Chamaillé-Jammes et al., 2006; Daly et al., 2018; Frank and Dudás, 2019; Moldowan et al., 2022). Thus, it can bring insights on population stability, distribution patterns and extinction risk (Peters 1983; Calder 1984). Since traits are influenced by temperature, reptiles are particularly sensitive to temperature variation (Gatten Jr, 1984; Angilletta et al., 2004; Vitt and Caldwell, 2013). According to Bergmann's rule (Angilletta et al., 2004), a decrease in temperature, which naturally occurs with increasing altitude or latitude, determines an increase in body size.

We used a large dataset collected within Dobrogea Region, Romania, for determining whether (i) differences in body size and sexual size dimorphism exist between populations, (ii) assess the population size, structure and density of a long-term monitored population and (iii) determine which climatic variables can explain the variation in size of the tortoises.

MAIN RESULTS AND DISCUSSION

Differences in body size and sexual size dimorphism

In Dobrogea region the body size of the tortoises (CCL, see Figure 3) varied regardless of the latitudinal gradient, not supporting the generally accepted rule in chelonians proved for the Spur-thighed tortoise, but at a larger scale (Werner at al., 2016 and *Annex 1*).

However, in this species, body size variations can be determined by factors such as annual rainfall or thermal amplitude (Carretero et al., 2005). The tortoises from Southern Dobrogea had an increased body size, but a lower body condition comparing to the populations from central and northern Dobrogea. Sexual size dimorphism was present in all three regions, but in South an opposite trend was highlighted, males being larger than usually. This could be interpreted in the light of sexual selection, larger male size evolving as an adaptation for increasing the success in combats or for establish forcible mating with females (Berry and Shine, 1980).

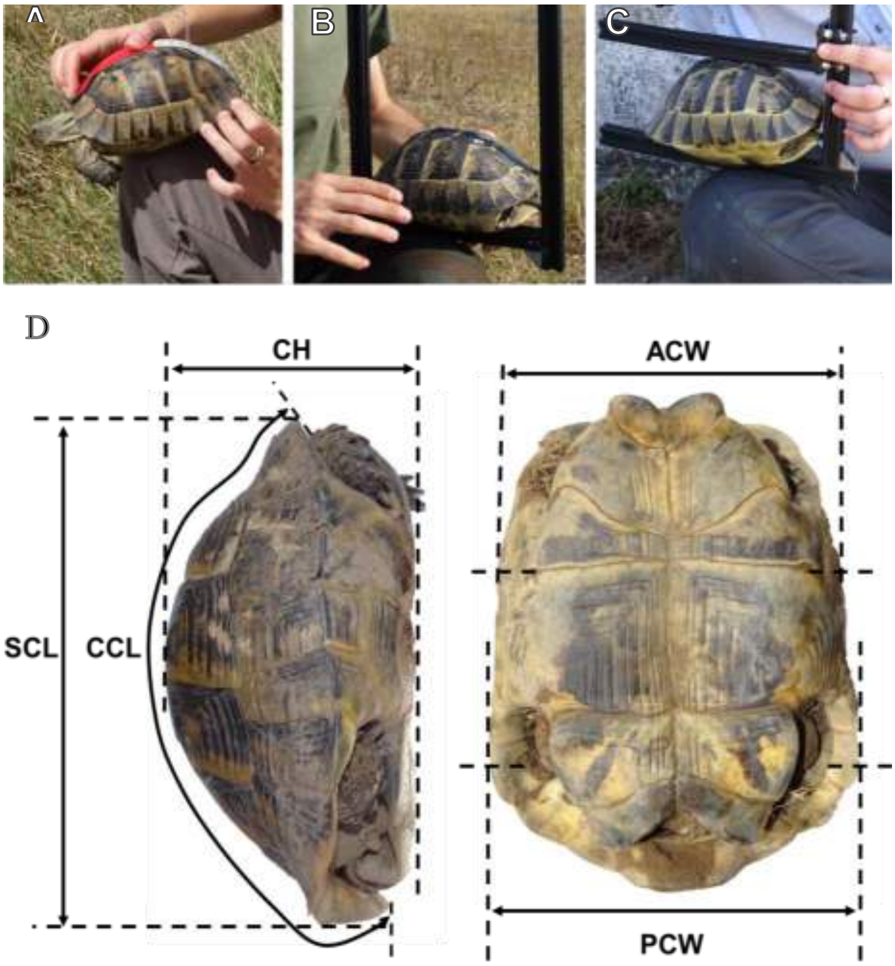

Figure 3. The methodology of measuring a tortoise on the field – A, B and C, and morphometric measurements recorded – D: straight carapace length (SCL), curvilinear carapace length (CCL), carapace height (CH), anterior carapace width (ACW) and posterior carapace width (PCW).

Population parameters in southern Danube Delta

At Histria, an intensively monitored population from central Dobrogea, the available morphometric measurements allowed exploring the sexual dimorphism on a larger data set than previously (no dimorphism previously reported by Buică et al., 2013) and allowed confirming a normal sexual size dimorphism. The current observed density of about 14 adults/ha and 20 tortoises/ha exceeds the previously observed density for this population, of 5 tortoises/ha (Buică et al., 2013). The model estimated a population of 748 adults, with a density of 23 adults/ha, higher than previously reported.

Increasing body size in response to climate change

The data collected overtime at Histria showed an increase in tortoises' body size along with the aridization of the habitat. In this population, the increase in body size can be explained by phenotypic plasticity in size, driven by the "resource rule", which means that body size is fundamentally influenced by food availability (Aragón and Fitze, 2014). Thus, an increase in the value of the climatic variables (i.e., warmer and wetter winters) influenced the increase in size, probably as a consequence of an increase in the active growing season of terrestrial plants (Ottersen et al., 2001; Vespremeanu-Stroe and Tătui, 2005).

Chapter II. Age assessment in tortoises: an evaluation of methods

INTRODUCTION

Age-related parameters are key to a better understanding of life-history adaptations and trade-offs (Caswell, 1982; Medica et al., 2012). These can be studied through capture-mark-recapture (CMR) and sclerochronology (i.e., skeletochronology and counting growth annuli on the shell). While the applicability of skeletochronology in chelonians is limited to carcasses, age can be determined by counting shell growth annuli (SGA) on the keratin plates of either live or dead tortoises.

A long-term monitoring study of a large population of *T. graeca*, started in 2010, provided us with the opportunity to test and compare all three known different methods for age estimation in tortoises: CMR, skeletochronology and SGA counts. We used CMR to (i) assess if the growth annuli on the shell are deposited annually. We have collected a variety of bones from tortoise carcasses, which allowed us to (ii) test the usefulness of skeletochronology in various skeletal elements and, when keratin scutes from the same individuals were available, (iii) to compare age assessments obtained through skeletochronology with SGA counts. Finally, we (iv) characterised the growth trajectories in the tortoise population based on the different age assessment methods tested.

MAIN RESULTS AND DISCUSSION

Counting growth annuli on the tortoise shell is a reliable, non-invasive method for age assessment in tortoises up to 26 years. Photographs collected during capture-mark-recapture surveys showed that a new keratin annulus formed annually in 71% of the sampled tortoises, similarily with the results reported in populations of *T. hermanni* from northeastern Spain

(Bertolero et al., 2005). Deviations from the expected age (i.e., inaccuracy) increased significantly with the time elapsed between captures. The highest inaccuracy (i.e., 10 years) was observed in only one individual with the expected age of 40 years old.

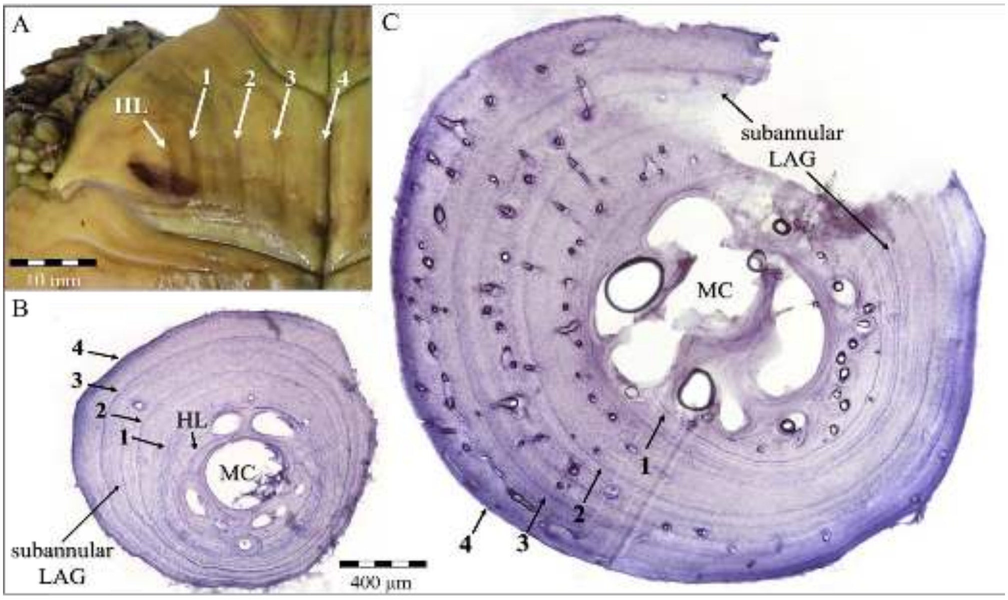

Figure 4. Age estimation in a four-year old *Testudo graeca*. Shell growth annuli on the plastron and lines of arrested growth from within the osseous tissue are indicated with arrows. A. photograph including details of the keratin humeral scute, B. cross-section through the radius and C. cross-section through the femur of the same individual. HL – hatching line, MC - marrow cavity, subannular LAG – subannular line of arrested growth.

Age assessment based on skeletochronology was similar between skeletal elements in 58% of the animals (Figure 4) and could not be performed on vertebrae, mandibles and phalanges. Our results showed that the endosteal resorption and reshaping occurred with a higher rate in the pubis, coracoid, ischium and tibia, preventing the observation of the LAGs formed during the first years of life. The highest number of LAGs were visible in ulna, radius and femur, followed by the humerus, scapula, fibula and ilium. Our results are similar to the study conducted by Ehret (2007) in *Gopherus polyphemus*, who stated that humeri and femurs are best suited for skeletochronology. Skeletochronology provided few comparable results, overall underestimating the age assessed by SGA, and the differences appeared to increase with age. The oldest age assessed by skeletochronology alone was 28 years, by SGA 30 years, and by SGA and CMR 40 years.

Von Bertalanffy's growth model fitted equally the age estimated from either method with the straight carapace length (Table 1). The age of sexual maturity in this Spur-thighed tortoise population is attained the earliest at 6-7 years old. In comparison, Díaz-Paniagua et al.

(2001) found that the earliest maturation in *T. graeca* from southern Spain was 5 and 6 years old in males and females, respectively. However, our growth models indicated a growth trajectory that started to decline only after the first 10 years of life. This growth strategy may provide an advantage under optimal conditions, allowing tortoises to attain larger sizes and thus maximize survival and reproductive output (Heino and Kaitala, 1999; Ejsmond et al., 2010; Armstrong et al., 2018).

Table 1. Von Bertalanffy growth parameters in *Testudo graeca* (n = 30) using age assessment based on lines of arrested growth (LAGs) and shell growth annuli (SGA). SE = standard error, CI 95% = 95 % confidence intervals, SCL_{max} = asymptotic body size (cm), k = growth coefficient.

Method	Parameter	Estimate	SE	CI 95%
LAGs	SCL_{max}	20.46	1.07	18.25-22.67
	k	0.20	0.03	0.12-0.28
SGA	SCL_{max}	19.58	0.69	18.19-21.40
	k	0.17	0.03	0.10-0.21

Chapter III. Shell scars and malformations in tortoises as an archive of human impact

INTRODUCTION

Tortoises are slow moving animals that are often impacted by human activities (Meek, 2007). Compared to mammals of similar size, tortoises have a more limited dispersal ability (Hailey, 1989), acquiring either permanent or temporary scars on the shells, as a result of the direct and indirect interactions with humans. Although wildlife in protected areas benefits from conservation measures, the pressure of anthropogenic impact is still present there, animals often suffering both within and outside these sites (e.g., Anderson and Mammides, 2020). Thus, while protected areas tend to mitigate human impacts, a considerable part of the global wilderness is affected by anthropogenic activities and is in a process of decay (Watson et al., 2016).

Tortoises are also affected by natural factors, the most dangerous being the steep slopes or habitat abrasions, However, direct interactions or factors derived from anthropogenic activities, e.g., vandalism (i.e., intentional hitting), road traffic, and/or mechanized interventions in grasslands (Hailey, 2000; Biaggini and Corti, 2018; Vlad et at., 2020) are more likely to lead to killing or wounding the tortoises. Fire has the potential to lead to a decline of up to 50% of tortoise density (Chergui et al., 2019). Environmental factors (temperature and humidity) can influence the proliferation of pathogens such as viruses, bacteria or fungi and lead to infections (Hatt, 2010).

The aim of this study was to evaluate the extent of human impact on three Spur-thighed Tortoise populations, inhabiting nature reserves from Dobrogea region, Romania. For this, I have hypothesized that (i) the scars on carapace and plastron, quantified based on digital photographs, are indicators of direct impact (natural or man-caused) and that (ii) the malformations are indicators of both developmental instability and environmental stressors.

MAIN RESULTS AND DISCUSSION

The studied populations are located within natural habitats, protected within the Natura 2000 Network, but are situated in different regions of Dobrogea and tortoises are exposed to different types of human interactions.

The percentage of tortoises unaffected by scars or malformations was different between populations. The highest percentage of tortoises with healthy, non-affected shells was in Histria, southern Danube Delta (H-DD), suggesting that individuals inhabiting this site benefit of a higher degree of protection comparing with the other two populations. Overall, only 15% of the animals had healthy shells, with no evidence of scars (neither natural or anthropogenic) or malformations. Most of these animals were young adults, this being in accordance with the findings of Buică et al. (2014). In the rest of the tortoises scars had a different pattern among populations, different shell regions (Figure 5) being affected.

Scars as indicators of direct impact

Natural scars were present in all populations in a high proportion. As stated by Buică et al. (2014) scars' abundance was higher on the carapace. The most affected tortoises were the ones inhabiting *Dunele marine de la Agigea* (DMA). Overall, less scars were present on the the tortoises from H-DD and *Podișul Nord Dobrogean* (PND), but when looking only at scratches determined by habitat abrasions, tortoises from PND were the most affected. Tortoises from DMA had the highest percentage of dermatomycoses and canid marks.

Anthropogenic scars were present in all populations, but in a different proportion. While in DMA only three tortoises had wounds on their shells, in PND almost one quarter were injured. In H-DD we found five tortoises with shells impacted by vegetation fires, while in PND only one and in DMA none.

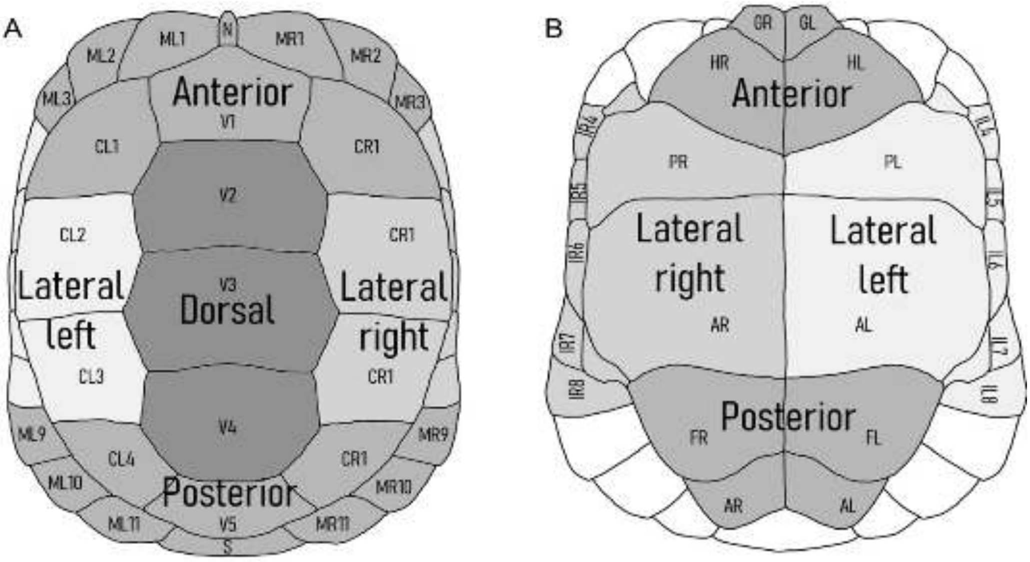

Figure 5. Tortoise shell plates: A. Carapace: N – nuchal, M – marginal, V – vertebral, C – costal, S – supracaudal and B. Plastron: G – gular, H – humeral, P – pectoral, A – abdominal, F – femoral, A – anal, I – inframarginal. Shell regions are marked with grey shades for anterior, posterior and lateral.

Malformations as indicators of stressors

Tortoises inhabiting PND had a higher percentage of malformation compared to the other two populations, even though the difference was not statistically significant. Considering the positive correlation found between malformations and the scars of natural origin, and also that this population was the most affected by anthropogenic impact, this could be considered an indicator for the presence of environmental stressors which might determine developmental instability in young individuals.

Chapter IV. Using scars to infer sexual selection in a polymorphic lizard

INTRODUCTION

Environmental pressures acting on organisms may determine plastic responses, selection or both, resulting in phenotypic variation within the individuals of the same population (White and Kemp, 2016). Among reptiles, colour polymorphism has been documented for numerous species, being described for at least seven families of lizards (see review by Stuart-Fox et al., 2021). In lacertid lizards, intraspecific interactions during the breeding time and agonistic encounters within the activity season result in temporary body marks (Galeotti et al. 2010, Carretero et al., 2018), which usually disappear within the following shedding. These marks can provide reliable insights regarding the selection pressure to which the animals are subjected.

The polymorphic European wall lizard (*Podarcis muralis*) displays alternative discrete ventral colours, and within the same population, morphs have different frequencies and vary in microhabitat use, home range size and success in staged agonistic encounters, fact which was often interpreted in the light of sexual selection. Yet, no correlation was assessed between the colour and the level of aggression suffered by certain morphs, inferred through quantifying the body marks.

Based on digital images taken during the breeding season, we aimed to infer if the polymorphism in Central Pyrenean populations is maintained by alternative strategies of interaction, specific to each color morph, by quantifying the biting marks from the body. For this, we hypothesised that (i) in males the frequency of interactions could signal different behavioral strategies and that (ii) the number of copulation attempts does not depend on female color morph, but on the size.

MAIN RESULTS AND DISCUSSION

The pattern of bite marks revealed as an indirect but informative inference on the intensity of sexual selection in the common wall lizard. As such, sexes strongly differed in their scar patterns, while size and colour morph accounted for substantial variation within each sex.

Table 2. Results of the fitted linear mixed model showing the effects of phenotype (MORPH), snout-vent length (SVL), relative head size to the body (rPL), sampling week (WEEK) and two ways interactions with the level of scars present in the cephalic and chest for males and inguinal for females. Site was used as random factor in all models. MS – mean square, $F_{n, d}$ – F statistic with degrees of freedom for numerator and denominator.

Fitted model	MS	$F_{n, d}$	p
Male agonistic marks			
MORPH	**53.25**	**2.73 $_{4, 481}$**	**0.028**
SVL	5.70	1.17 $_{1, 481}$	0.27
rPL	**76.85**	**15.79 $_{1, 481}$**	**< 0.001**
WEEK	**413.37**	**16.99 $_{5, 481}$**	**< 0.001**
MORPH*SVL	**52.97**	**2.72 $_{4, 481}$**	**0.029**
Female copulation marks			
MORPH	**12.82**	**4.74 $_{4, 346}$**	**< 0.001**
SVL	5.98	2.21 $_{1, 344}$	0.13
WEEK	5.58	2.06 $_{5, 11}$	0.14
MORPH*SVL	**12.80**	**4.73 $_{4, 346}$**	**< 0.001**

Male scars as potential proxy of male-male competition

According to the bite marks, the level of aggression was explained by the relative head size to the body, phenotype and by the interaction between phenotype and length (Table 2). The sampling week was also a significant factor, with time animals gaining more marks, as expected. Most males had more agonistic marks with increasing size. Evidence suggests that males identify potential competitors based on size, or familiarity and the aggressive behavior could be allocated as a response to a potential threat, raised by nonresident conspecifics, based on the scent marks (Carazo et al., 2008), but chromatic signals also mediate the intensity of the combats (Briffa, 2014) as well as females' presence (López and Martín, 2002).

Female scars as potential proxy of the intensity of males choice

The presence of copulation marks was explained by the sampling time and by the interaction between phenotype with SVL, contradicting the preferentially courting in the presence of scent marks, irrespective of body coloration identified in other *Podarcis* species (López et al., 2002). Most of the females had more copulation marks with increasing size, suggesting multiple mating and providing the chance for the multiple paternity (Oppliger et al., 2007).

Overall, results confirm that bite marks can be informative on the patterns of sexual selection mediated by either male-male competition or by male-female choice in lacertid lizards, while also provide an indirect but solid indication that such selective forces differ among colour morphs in a polymorphic *Podarcis* species. Hence, future studies should be conducted i. to validate these findings in other populations and species, and ii. to infer the subjacent behavioral differences involved by means of manipulative experiments in realistic conditions.

Chapter V. Management and Conservation of reptiles

INTRODUCTION

Existing protected areas networks are currently struggling to ensure species viability due to unsustainable land use, rampant urban expansion and climate change (Popescu et al., 2013; Devitt et al., 2015). However, biodiversity goes beyond protected areas and since many species require broader ranges than any protected area can provide, the degree of protection is insufficient to prevent disturbance and declining. Archaeological sites exhibit a high level of reptile diversity (Báez et al., 2016, Attum et al., 2022) and therefore could be used for inferring the nature of the relationship between humans and these ectothermic vertebrates.

A real threat to local wildlife is represented by the alien species, a part of which may become harmful for native biota (Corti, 2006). In behavioral terms, invasive animals are frequently more proactive and aggressive than their native equivalents, which become outcompeted and eventually extinct. One way of anticipating the potential impact of alien species on the wildlife within the invaded areas is by looking at how they interact in both native and invaded regions.

In this chapter we focused on several case studies for highlighting (i) the public perception and shortcomings of human interactions with reptiles within Histria archaeological site, (ii) issues raised by deficient management within the same site, included in Southern Danube Delta Biosphere Reserve, and (iii) approaches related to the management of alien species, which have the potential to threaten the biodiversity at global scale.

MAIN RESULTS AND DISCUSSION

1. Human and wildlife coexistence

How people perceive the reptiles within an archaeological site

Significant differences were identified in visitors' perception on the reptiles inhabiting Histria archaeological site, a positive perception being directed towards tortoises and freshwater turtles and negative or neutral on the nonvenomous species of snakes. (Table 3). This confirms the findings of Barthwal and Mathur (2012) who documented a negative perception towards species that have the potential to raise conflicts with humans. Women were more likely to be disturbed by snakes, as presented in other studies (e.g., Alves et al., 2014).

Table 3. Summary of the parameter estimates that evaluate the determinants of the disturbance extent towards chelonians and snakes, and the emotional response, based on the questionnaire survey held at Histria archaeological site.

	Disturbance extent			Emotional response		
Parameter	SE	β	p	SE	β	p
Age (scale)	0.01	0.01	0.296	0.01	−0.01	0.223
Gender = 'man'	0.23	−1.04	**<0.001**	0.19	−0.13	0.496
Nationality = 'Romanian'	0.42	1.1	**0.009**	0.35	0.7	**0.048**
Education level = 'graduated College'	0.27	−0.52	0.054	0.25	0.24	0.344
Visit = 'second time or more'	0.24	−0.58	**0.016**	0.2	0.13	0.511
Guided visit = 'conducted by guide'	0.39	−0.65	0.100	0.31	0.52	0.101
DDBR = 'aware of protection area'	0.23	−0.59	**0.013**	0.21	−0.56	**0.009**
Reptile categories = 'Chelonians'	0.53	0.11	0.839	0.43	−0.49	0.256
Reptile categories = 'Snakes'	0.42	3.66	**<0.001**	0.38	2.87	**<0.001**

Most visitors revealed interest in wildlife conservation, this being in accordance with other studies suggesting that humans wish to protect the natural and cultural resources (e.g., Holtorf and Ortman, 2008). It was shown that coexistence or prior interactions with snakes might decrease the negative perceptions of people (Pinheiro et al., 2016). Therefore, it can be considered appropriate to create a favorable educational context to contribute to diminishing the repulsion towards snakes at Histria.

Anthropogenic impact in protected areas

Several anthropogenic factors affect the reptiles inhabiting the same site and the adjacent areas from northern Saele levee (Figure 6). Many tortoises were found dead after mechanized mowing. Although reducing the vegetation height maintains a proper habitat for tortoises and diminish the potential vegetation fires (Chergui et al., 2019), caution must be taken, and traditional techniques are recommended. Due to the demographic pattern of tortoises, which assumes slow growth, delayed sexual maturity, mortality of adults constitutes a high risk of population extinction. Thus, adults, and particularly large females, must be prioritized in conservation (Bertolero et al., 2007).

Another risk is represented by the recent archaeological diggings. These must be constantly checked to ensure that no animals are trapped. However, this could be avoided by installing sloping ramps on which animals can exit. As the archaeological site attracts many visitors, the risk of animals being killed on the road increases. Another factor which might alter the population structure is collecting the tortoises as pets. Attempts have been witnessed within the studied population and were documented in other studies as well.

The two studies included within this subchapter provide evidence for the necessity of integrative management implementation for the joint protection of biocultural heritages. A stronger cooperation between the biosphere reserve administration and archaeological site could bring valuable benefit in this way (Vanderplank et al., 2014).

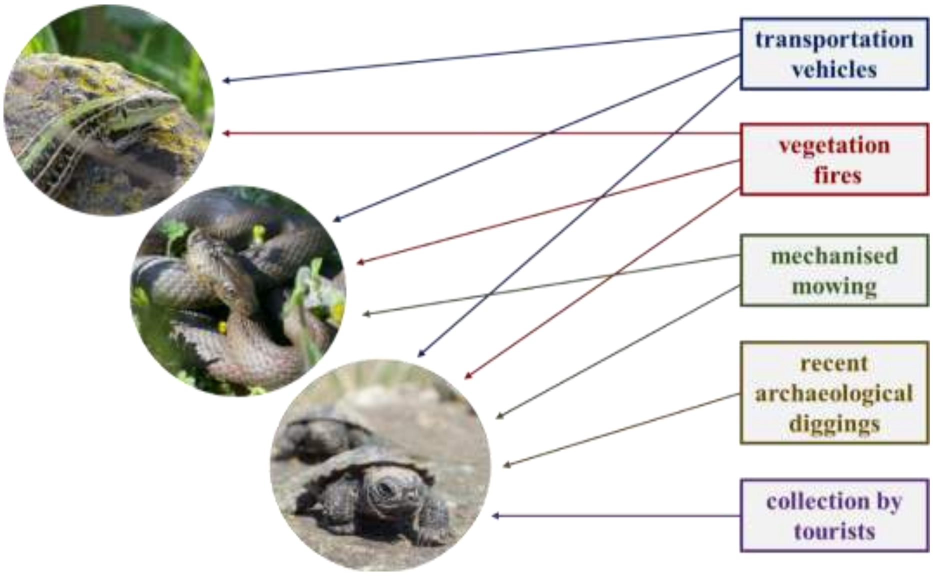

Figure 6. Main anthropogenic factors affecting the squamates and chelonians inhabiting northern Saele levee.

2. Alien species and their impact on local fauna

Mediated spread of alien species

We have showed that individuals of Italian wall lizards sampled in Alba Iulia, Romania, belong to a single clade, originating from Tuscany, so it is unclear whether repeated introductions existed. However, within the same study (Oskyrko et al., 2022b) we report the existence of admixed populations in Bucharest. The presence of young individuals at Alba Iulia supports the high ability of this species to establish populations outside the native distribution range, using not only rocky substrate for foraging, basking and finding shelter, but also vegetation (Corti, 2006). This might allow the species to be transported along multiple types of materials, from construction materials to plants and materials associated with agriculture and gardening (Silva-Rocha et al., 2014). While the presence of the species was rapidly reported, no measures of control, containment or eradication were carried out and no current monitoring of the potential spread and impact of the species is being done.

How can we use biotic interactions data for conservation

For a better understanding of how alien species interact with native wildlife, a general understanding of the focal species was needed. By conducting a literature review and constructing biotic interactions networks we were able to reveal the interactions occurring between the introduced Common Green Iguana (*Iguana iguana*) and the endemic and endangered Grand Cayman Blue Iguana (*Cyclura lewisi*) in the Cayman Islands. Competition might occur between the two species when looking at the trophic preferences (Vlad et al., 2022, 2023). Therefore, if the plants on which its diet relies are consumed by a competitor, the Grand Cayman Blue iguana will struggle. Habitat use is another factor which can affect the native species, as in the case of other squamates (Damas-Moreira et al., 2019; Limnios et al., 2021).

Halting the spread of alien species is possible through appropriate management and regulation (Falcón et al., 2013) as well as long-term monitoring (Burton and Ribera-Milán, 2014). Education campaigns are essential where alien species with invasiveness potential are promoted as pets in order to diminish the desire to own these animals, to reduce the risk of escaping from captivity or the negligence of releasing them into the wild when they no longer meet the expectations.

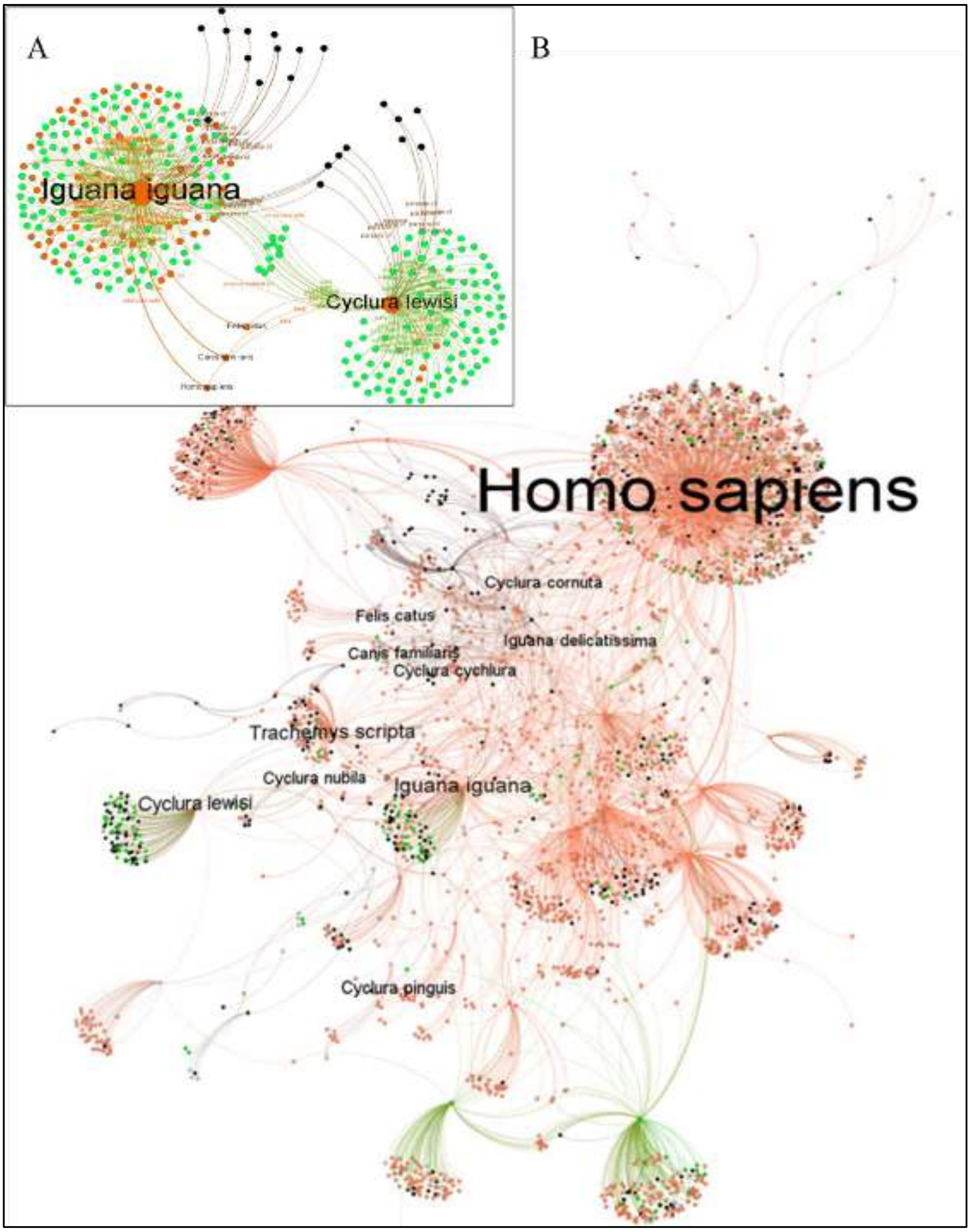

Figure 7. Interaction networks A. primary interactions between the two iguana species occurring in The Grand Cayman and other taxa and B. primary and secondary interactions of the Green iguana from its native and invasive range (purple – animals, green – plants, black – pathogens).

Conclusions

Chapter I. The body size of Spur-thighed tortoises varied, but not along a latitudinal gradient and there is a reversed sexual size dimorphism in some populations, at the northern distribution limit, in Dobrogea (Romania). In the southern Danube Delta, there is a higher density than previously reported and, along with the large number of young individuals, it indicates a thriving population. An increase in the value of the climatic variables which determined warmer and wetter winters influenced the increase in body length and body mass of the tortoises.

Chapter II. Growth continues in Spur-thighed tortoises from central Dobrogea (Romania) after attaining the sexual maturity at a faster pace at least for another three to four years, allowing tortoises to attain larger sizes and thus maximize survival and reproductive output. Within the same population, the longevity is of at least 40 years old. While age assessment in tortoises is difficult, the combination of methods significantly increases the accuracy.

Chapter III. The spur-thighed tortoises unaffected by scars or malformations varied significantly between populations at the northern distribution limit, in Dobrogea (Romania). This study shows that scar analysis is a valuable tool for inferring the protection status received by this species and suggests that the studied populations, located within Natura 2000 protected areas, are exposed to different types of human interactions and experienced different levels of aggression.

Chapter IV. In the Common wall lizard from the central Pyrenees (Spain), bite marks can be informative on the patterns of sexual selection mediated by either male-male competition or by male-female choice, while also provide an indirect but solid indication that such selective forces differ among color morphs in lacertid lizards.

Chapter V. Visitor perception at a touristic cultural and natural heritage site (Histria, Romania) was negatively biased towards snakes, but positive towards tortoises. The most dangerous anthropogenic factors affecting the reptiles inhabiting this site are the mechanized mowing, road traffic and recent archaeological diggings, with steep slopes, which trap animals. Our studies provide evidence for the necessity of integrative management for the joint protection of natural and cultural heritages, which can help educate the public and reduce the impact on wildlife in general.

Repeated introductions of alien species can lead to the establishment of new populations of single of admixed origins in the invaded areas. For a better understanding of how alien species interact with native wildlife, a general understanding of the focal species is needed. This can be achieved by building biotic interaction networks, which are useful tools for inferring the competition which might occur for foraging, habitat use or nesting sites, but also for the risk in pathogen transfer.

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