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Abundance and activity of *Chelonoidis chilensis* (Gray, 1870) in the Chaco-Monte ecotone of central Argentina region

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Abstract. Turtles represent one of the most threatened groups of vertebrates. Habitat loss by expansion of the agricultural frontier, exploitation and unregulated trade are the primary causes for sharp declines in many turtle species. *Chelonoidis chilensis* is a widespread turtle having the broadest distribution in South American continent, it has recently been reclassified from threatened to vulnerable. Considering this, it is necessary to search for non-invasive study methods that allow us to safely assess its ecological traits and monitor its populations. The objective of this work was to evaluate abundance and activity patterns of *C. chilensis*, using conventional and non-invasive methodologies in different years and vegetation types in Sierra de las Quijadas National Park. Conventional methodology consisted in sampling a study area through 5m width visual detection transects, placed one adjacent to another, involving a total of 168 transects (216 has). This sampling was repeated twice, with a one-year interval. Non-invasive novelty methodology consisted of using ten camera traps that were installed for two years. Turtle frequency in different vegetation types, activity patterns according to temperature and sex ratio were determined. The abundance of *C. chilensis* registered was 0.027 (in 2016) and 0.036 turtles/ha (in 2017). Temporal activity, observed through camera-traps, was detected between 25 to 40 °C and from 8 am to 8 pm. The results allow us to conclude that the abundance in the park is lower than what has been reported by several authors in other Chaco regions. In this sense, further studies should be made on this ecotonal region to assure the protection of this vulnerable species. This study is the first to report abundancy and activity patterns of *C. chilensis* in the ecotonal region with the novelty of using a non-invasive method for monitoring the species.

Keywords. Density, Activity patterns, Chaco tortoise, Non-invasive monitoring, ecotonal region, camera-trap

Introduction

Turtles represent one of the most threatened groups of vertebrates, including 10% of the ca. 317 currently recognised species considered critically endangered on the IUCN Red List of Threatened Species (Buhlmann et al., 2009; IUCN, 2018). Approximately 63% of the total of assessed species and near 42% of all known species are considered as threatened (Buhlmann et al., 2009; IUCN, 2018). Habitat loss by expansion of the agricultural frontier, exploitation and unregulated trade are the primary causes for sharp declines in many turtle species (Buhlmann et al., 2009; Sanchez et al., 2014). In Neotropical regions, one of the greatest representatives of this group is *Chelonoidis chilensis,* better known as the Argentinian or Chaco tortoise (Ruete and Leynaud, 2015). This species of terrestrial turtle has the broadest distribution in the continent and has been reported in 18 different provinces of the Argentinian territory, furthermore it can be found in Bolivia and Paraguay (Prado et al., 2012; IUCN, 2018). *C. chilensis* distribution extends to Dry Chaco and Monte ecoregions, it also inhabits ecotonal areas, reaching Espinal and Pampa ecoregions of Argentina (Cabrera, 1994; Richard, 1999; Prado et al., 2012; Romito et al., 2015; SIB, 2019).

Recent taxonomic unification of *C. chilensis* complex notably expands the range of distribution of the taxon. For this reason, the species conservation status has been changed from "Threatened" to "Vulnerable" in the recent categorisation of Argentinian turtles (Prado et al., 2012), by the Tortoise & Freshwater Turtle Specialist Group from the IUCN, and has been included in the Appendix II of CITES (Fritz et al., 2012; Romito et al., 2015; Ruete and Leynaud, 2015; IUCN, 2018). Factors for this species to be considered vulnerable include its

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use and exploitation for mascotism (being the most commercialised native reptile in Argentina), and habitat loss caused by the advance of agriculture frontier and extensive cattle breeding (Waller and Micucci, 1997; Buhlmann et al., 2009; Prado et al., 2012). There is evidence that *C. chilensis* populations in Argentina could suffer a dramatic reduction in the number of individuals if management programs for its conservation are not developed (Waller and Micucci, 1997; Romito et al., 2015). Some authors suggest that populations of the species are kept in a dozen of protected areas, depending on national and provincial governments (Prado et al., 2012; SIB, 2019). In addition, the species has been declared as a key interest species by the National Park Administration in Argentina, APN (2006). Nonetheless, only 3.7% of the total area of Monte and Chaco ecoregions, the main habitat of *C. chilensis*, is subject to some kind of government protection (Ruete and Leynaud, 2015).

The Sierra de las Quijadas National Park (SQNP) preserves the ecotonal area where Monte and Chaco ecoregions of central Argentina meet (Cabrera, 1994). There have been some studies regarding the species that predicted high probability of *C. chilensis* occurrence in this area (Ruete and Leynaud, 2015). Other authors suggest that research in this site should be intensified and standardised monitoring programs should be promoted along its distribution range (Prado et al., 2012). Studies focussing on the species, developed in similar areas, have registered a low number of individuals (Sanchez et al., 2014), which enhances the need of studying basic ecological traits of the species (specially its activity patterns), in order to develop efficient methods and improve the detection of *C chilensis*. Understanding the species´ use of the territory and its temporal variations would contribute to the interpretation of the population´s interactions with other species and with its habitat.

In this context, and with the knowledge that distribution of the native fauna in semiarid regions is a fundamental tool for assessing management and conservation decisions (Guerreiro et al., 2005); the objective of this work was to evaluate abundance and activity patterns of the Chaco tortoise (*Chelonoidis chilensis*) in the SQNP using conventional and non-invasive novelty methodologies in two different years.

Materials and Methods

Study area. The study was carried out in Sierra de las Quijadas National Park (SQNP). This park is located in the northwest of San Luis province in Argentina (from 32°20'46" to 32°47'00" S, and from 67°10'41" to 66°58'59" W), and comprises 735.3 km² (Fig. 1). This region has a broad annual temperature variation going from -10 °C to more than 40 °C, with a medium temperature of 13.7 °C. The area presents a low precipitation regime, with a mean rainfall of 500 mm per year, concentrated in the wet season (from November to March). The study site included 216.4 ha, encompassing shrubby vegetation typical of Monte of steppes and plains, Chaco intrusions, represented by impoverished forests of *Aspidosperma quebracho-blanco* Schltdl., and sierras dominated by *Mymoziganthus carinatus* (Griseb.). The area was characterised by four habitats according to vegetation types: (a) sierra; (b) open forest; (c) open shrubs; (d) dense shrubs.

"Sierra" vegetation type is characterised by *M. carinatus* and *Senegalia gilliesii* (Steud.) Seigler & Ebinger shrubs, occasionally accompanied by *A. quebracho-blanco*, *Larrea cuneifolia* Cav. and *Larrea divaricata* Cav. "Open forest" is composed by *A. quebracho-blanco*, accompanied by other woody plants like *Prosopis flexuosa* DC. "Open shrubs" is identified by the presence of *L. divaricata* and *L. cuneifolia*, with isolated individuals of *Prosopis chilensis* (Molina) Stuntz and *Bulnesia retama* (Gillies ex Hooker & Arnott) Grisebach, among other species, distributed in low and disperse patches, and finally, "Dense shrubs" presents higher density of *L. divaricata* and *L. cuneifolia,* constituting compact patches of low visibility (Gatica et al., 2019). This study was carried out under the permits provided by the Argentinian Administration of National Parks, no. DRC-262.

Abundance and activity pattern estimation. The study area was sampled through visual detection

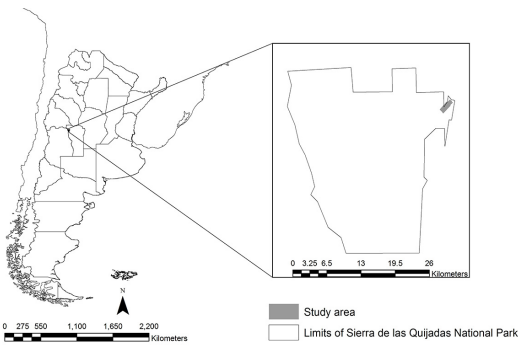

Figure 1. Studied area shaded with grey within the boundaries of the Sierra de las Quijadas National Park, San Luis, Argentina.

transects, this method is widely used for terrestrial chelonids and it allows estimation of abundance (Hofmeyr and Henen, 2016). Transects were placed one adjacent to another, and had a 5 m width; total number of transects was 168. These transects were used to document the presence and activity of *C. chilensis*. During 21 days, an average of eight observers walked simultaneously looking under shrubs, checking the ground surface and all types of refuges carefully. Two censuses were carried out, one in October 2015 (mean temperature 16.2 ± 6.1 °C) and another in 2016 (mean temperature: 18.5 ± 7.4 ºC). Each census required a total of 695.17 hours-person.

Turtles density per year was estimated in each vegetation type and for the total area. Sex of each individual was determined, and the presence of wounds was recorded (this last information was incorporated only for 2016). Geoposition and photographs of each individual were registered before liberation. Type of activity was determined following Sanchez et al. (2014), recording: 1) active (subject was found moving); 2) semi-active (individual was found still, but with signs of recent movement); and 3) inactive (found resting with head and paws inside it´s shell).

As a complement, in order to record presence and activity pattern, camera traps (Bushnell 119736c model and Moultrie MFH-DGW-5.0 model) were installed in front of *Dolichotis patagonum* (Zimmermann, 1780) dens, which could presumably constitute refuges for the species. These cameras were set for two years from October 2015 to October 2017, monitoring a total of 22 dens, using 10 camera traps. The first year, cameras were rotated between dens, and during the second year they were left fix, monitoring ten dens throughout the entire year. This non-invasive method has proved to be very efficient to register a broad number of species, including reptiles (Gompper et al., 2006; Ariefiandy et al., 2013; Welbourne et al., 2015; Ballouard et al., 2016) and could be useful as a novelty methodological tool for terrestrial turtles as *C. chilensis*.

Statistical analysis. A classification map was generated to visualise the different vegetation types found in the study site, from a satellite image. Maps were designed using ArcGIS 10.3.1 (License number ESU351868492). The density of turtles was then calculated for each vegetation type. The frequency of turtles between vegetation types and sex ratio between years was compared using Chi-square test.

Finally, to evaluate if activity patterns were associated to temperatures, correlation between maximum daily temperature and number of registers was assessed (using transects and camera trap data). Pearson correlation and Chi-square tests were made using R software version 3.5.2.

Results

Activity pattern and abundance estimations using conventional methods. During 2015 a total of six adults (2 males and 4 females) were registered, while in 2016, seven adults and one juvenile *C. chilensis* (5 males and 3 females) were recorded in SQNP (Fig. 2A-C). The total density for the study site was 0.027 and 0.036 turtles/ha in 2015 and 2016, respectively. Greater density was observed in dense shrubs (2015: 0.041 turtles/ha; 2016: 0.051 turtles/ha), followed by Sierra (2015: 0.029 turtles/ha; 2016: 0.029 turtles/ha) and low shrub (2015: 0.015 turtles/ha; 2016: 0.031 turtles/ha) (Table 1). No individuals were found in the open forest unit (Table 1). No significant differences were found between vegetation types when pooling both years (X^2 = 0.162, p = 0.922, GL = 2). Furthermore, results showed that sex ratio between years (M/H_{15} = 0.5 y M/H_{16} = 1.6)

Table 1. Registers of *Chelonoidis chilensis* in the monitored area for each year in Sierra de las Quijadas National Park, San Luis, Argentina.

		Type of habitat				
	Year	**Sierra**	**Open forest**	**Open shrubs**	**Dense shrubs**	**Total**
Area		34.41 (ha)	21.79 (ha)	63.02 (ha)	97.2 (ha)	216.42(ha)
Individuals	2015	1	0	1	4	6
	2016	1	0	2	5	8
Density	2015	0.029	0	0.015	0.041	0.027
	2016	0.029	0	0.031	0.051	0.036

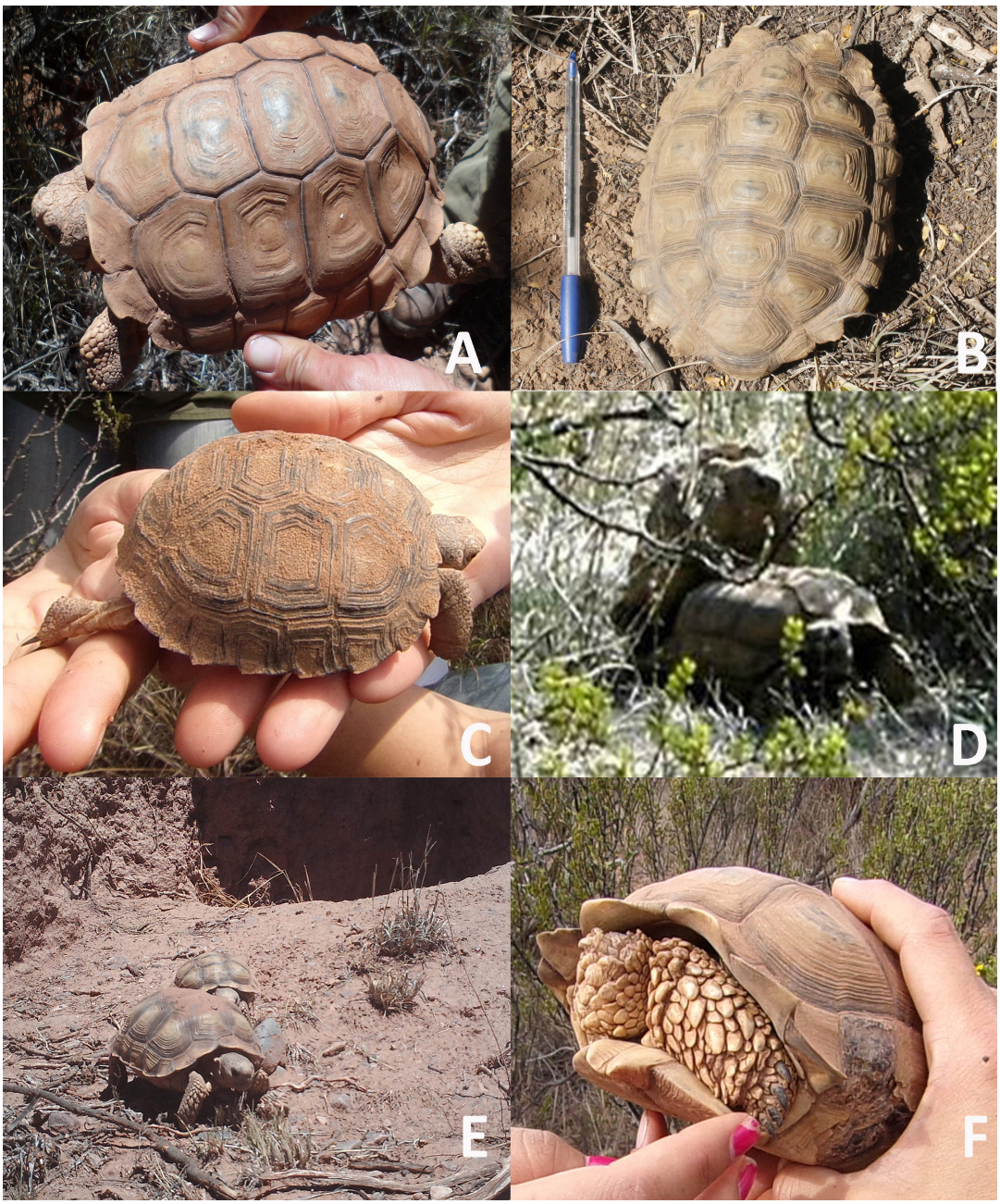

Figure 2. Photographs of specimens of *C. chilensis* in the Sierra de las Quijadas National Park. (A) male (TUJ3), (B) female (THJ4), (C) juvenile (THJ2), (D) copulate, (E) camera traps capturing individuals using dens, and (F) lesions in an individual. (Photo E was taken by Nicolás Seoane, the rest of the photographs by A. Gatica and A.C. Ochoa).

was not statistically significant ($X^2 = 1.166$, $p = 0.280$, $GL = 1$).

Considering activity patterns, 50% of the turtles were found active while 37.5% were inactive and 12.5% were semi-active. Regarding temperature of detection days, most observations were made on days in which maximum temperatures were between 26 and 30 ºC. Particularly, the monthly average maximum

temperatures during the sampling months were 30.2 and 28.4 ºC in 2015 and 2016, respectively. During 2016, 75% of individuals had wounds in their shells or bodies (Fig. 2F). Other information registered included a mating event outside the study area (Fig. 2D) and a total of six carcasses found (three in each year) making a total of six dead individuals.

Activity pattern using camera traps. The monitoring of the species with camera traps allowed registration of the turtles in 33 opportunities. Added to this, we observed the presence of *C. chilensis* using *D. patagonum* dens as a refuge (Fig. 2E). The species was detected in 8 of the 22 dens monitored (36%). A majority of these dens were located in dense shrub (75%), one in open shrub and one in Sierra. Turtles used dens principally from November to January in both years. This corresponds to the time of the year normally with the highest temperatures recorded in the park (records obtained from the park's meteorological station). Individuals presented diurnal activity with an activity peak at midday (Fig. 3).

When comparing turtles´ detection according to maximum temperature of the day (using transects and camera trap data) correlation was not significant (R^2 = -0.445, p_{slope} = 0.453, GL = 4).

Discussion

This work gives valuable information on the abundance and activity patterns of *C. chilensis* from the ecotonal region of Central west Argentina. Density of individuals detected in Sierra de las Quijadas National Park (0.027 to 0.036 turtles/ha) is lower than those reported by Sanchez et al. (2014) in Catamarca (0.117 turtles/ha) and Santiago del Estero (0.292 turtles/ha), but higher than what was found by the same authors in La Pampa. On the other hand, Ruete and Leynaud (2015) predicted a high probability of occurrence of *C. chilensis* in SQNP, due to its climatic conditions. However, these authors also remarked that despite the high and homogenous probabilities of occurrence predicted for the SQNP, the populations are confined to restricted areas and local heterogeneity in the species distribution is expected, due to the wide variability in habitat types and geographic accidents in the territory. This could also be related to the pressure of uncontained grasing activity by neighbour cattle (APN, 2006; Ruete and Leynaud, 2015). In this sense, our results are consistent with this prediction.

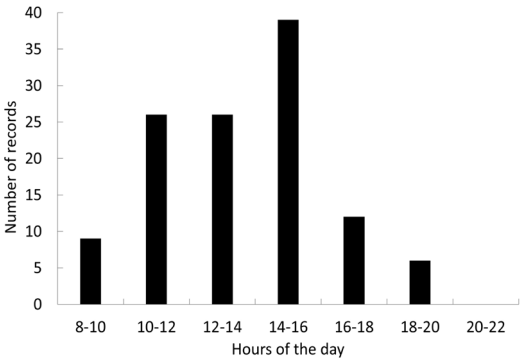

Figure 3. Activity patterns of *C. chilensis* obtained from camera traps in Sierra de las Quijadas National Park, San Luis, Argentina.

The majority of the specimens found in 2016 had shell and body injuries, being the first report of injured individuals in the park. These records contrast with the findings of Sánchez et al. (2014) who did not find injuries in *C. chilensis* individuals monitored for several areas in the Chaco and Monte regions. However, Waller and Miccuci (1997) reported similar injuries in *C. chilensis* that inhabit the Patagonian region. Furthermore, turtle populations with a high proportion of injured individuals could be reflecting an unhealthy population (Waller and Micucci, 1997). Comparing both years, we found predominance of females in 2015 and of males in 2016, however sex ratio did not vary significantly among years. Sanchez et al. (2014) reported more males than females for the Dry Chaco and Monte regions. Our results are not conclusive in this matter and suggest that one single sampling period could be insufficient to describe turtle populations´ sex ratio. It is important to note the high number of female individuals detected in this study (n = 7), which doubles the number previously registered by Sanchez et al. (2014). Even if comparison between studies is imprecise (mainly due to different sampling efforts), we suggest that the methodological approach of our study could be a good tool to develop comparable monitoring programs of this species populations along its distribution range, especially in low density areas.

SQNP management plan assessment (APN, 2006) highlights that there are many ongoing conservation problems in the park that could cause a particular impact in this species' conservation. Some of the conservation problems detected in the park include: habitat loss produced by tree clearing, free grasing of cattle inside the park (both determining the acceleration of erosive processes), poaching, commercial use and the presence of exotic species of flora and fauna. (APN, 2006). In general, our results support the hypothesis posed by other

authors of a low abundance of *C. chilensis* populations that inhabit SQNP (Ruete and Leynaud, 2015). This, and the poor sanitary condition detected in this study, could be due to the already mentioned conservation challenges that the species faces in the area. Also, the relatively large number of carcasses found, which could be related to a high mortality, indicate the need for the species urgent monitoring and assessment. Future studies should be carried out to deepen the knowledge of the sanitary state and sustainability of *C. Chilensis* populations in SQNP, determining whether or not this situation is related to the alteration of their habitats. However, the documentation of a reproductive event and the detection of a juvenile, constitute a rise in the expectations for the future of the species.

When evaluating activity of individuals detected through conventional methodologies our results are similar to the reports of Sanchez et al. (2014), using the same activity categories proposed by these authors. In general, they report that more than 50% of turtles found were active (Sanchez et al., 2014), we suspect that this could be related to higher detectability of active specimens. This is the first study to use camera traps to assess *C. chilensis* activity patterns. Considering the time of day in which turtles were detected with the cameras, it matches the hours of highest temperatures and solar radiation. We found a peak in detection during midday. This could be explained by the fact that cameras detected individuals occupying maras´ dens, and so, the animals could be avoiding extreme temperatures by moving to new habitats or restricting activity to certain micro-habitats, as shady refuges (Stevenson, 1985). This behaviour has been found for other herbivorous reptiles (Troyer, 1987; van Marken Lichtenbelt, 1992; Tracy et al., 2005).

The present work reports the use of *D. patagonum* dens by *C. chilensis* for the first time, highlighting that this was detected using camera traps. These data add to the well- known information that *D. patagonum* dens provide an important ecological function for several other vertebrate species (Alonso Roldán and Udrizar Sauthier, 2016). The use and refinement of non-invasive tools allows researchers to simultaneously study activity patterns of multiple species using a single study design and−assess temporal activity partitioning (O'Connell et al., 2011; Ariefiandy et al., 2013; Welbourne et al., 2015; Ballouard et al., 2016). In this sense, we consider that locations occupied by both *D. patagonum* and *C. chilensis,* could represent places of higher occurrence of turtles, especially in *D. patagonum* breeding areas. This shows that the use of *D. patagonum* refuges, in combination with camera trap-based methods, could be a useful resource to monitor the population of this turtle species and evaluate its temporal and spatial use.

Considering the categorisation of the species, it is essential to search for non-invasive methodologies that allow the study of their ecology and population monitoring. Our results are consistent with other authors, who state that this methodology provides a useful tool to observe animals of low abundance and elusive behaviour, as well as allowing for the study of activity patterns (Foster and Harmsen, 2012). The success of this non-invasive methodology for the study of *C. chilensis*, is in accordance to what was found by Hofmeyr and Henen (2016) in other species of terrestrial turtles that have a fixed home range or territory, for example those that build dens.

Turtle density was relatively low in this National Park; however, we detected a relatively large number of individuals, compared to other studies of the species (Sanchez et al., 2014) and especially a high number of females. This adds to the recording of a juvenile and a reproductive event. Therefore, our study provides evidence that the *C. chilensis* population may be able to recuperate in the park, if conservation actions are taken. However, there is little information on the resources and habitat quality that this species requires and of the predators that feed on it. We strongly recommend exhaustive studies and long-term monitoring programs to identify the habitat requirements of this species and its interactions.

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