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SHORT COMMUNICATION

Is the presence of eggs a relevant cue for predators of freshwater chelonian nests?

A presença de ovos é uma questão relevante para predadores de ninhos de quelônios de água doce?

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Abstract

Predation is considered a game between two players – predator and prey – where such pressure might affect the interactions, acting on prey and predator distribution and abundance. We evaluate whether the predation level in chelonian nests varies according to the presence of eggs in nests. Our hypothesis is that owing to the clues (visual and olfactory) of upturned soil left in newly constructed nests, predators attack nests regardless of the presence of eggs on nests. We constructed artificial nests organized in two treatments (15 with eggs, and 15 without eggs) in ESEC Taim, southern Brazil, and checked the nests during two consecutive days. We identified the possible predators through photographic traps installed near the nests, associated with the identification of footprints on disturbed nests. We verified high predation rates in both nests, which corroborate our hypothesis. We identified two canids (*Cerdocyon thous* and *Lycalopex gymnocercus*) as predators around the nests. Our results suggest that turtle nests are highly detectable by predators, and the location cues used by pampa fox to find newly constructed nests are related to soil disturbance and not to egg presence on nests.

Keywords: foraging, interspecific interactions, predation rate.

Resumo

A predação é considerada um jogo entre dois jogadores – predador e presa –, no qual tal pressão pode afetar as interações, atuando na distribuição e abundância de presas e predadores. Neste trabalho, avaliamos se a taxa de predação em ninhos artificiais varia em função da presença de ovos. Nossa hipótese é que, devido às pistas (visuais e olfativas) de solo revolvido deixadas em ninhos recém construídos, os predadores atacam ninhos independentemente da presença de ovos. Para testar essa hipótese, utilizamos ovos de codorna e construímos 30 ninhos artificiais divididos em dois tratamentos (15 com ovos e 15 sem ovos) na ESEC Taim, Brasil, e verificamos os ninhos durante dois dias consecutivos. Identificamos possíveis predadores por meio de registro em armadilhas fotográficas instaladas próximas aos ninhos, associadas à identificação das pegadas nos ninhos perturbados. Identificamos altas taxas de predação em ambos os tratamentos, corroborando nossa hipótese. Identificamos *Cerdocyon thous* e *Lycalopex gymnocercus* como predadores mais frequentes. Nossos resultados sugerem que os ninhos de quelônios são altamente detectáveis por predadores e que as pistas de localização usadas pelos graxains para encontrar ninhos recém-construídos estão relacionadas com a perturbação do solo, e não com a presença de ovos nos ninhos.

Palavras-chave: forrageamento, interação interespecífica, taxa de predação.

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Predation is considered a game between two players – predator and prey (Caro, 2005), where such pressure might affect interactions, acting on prey and predator distribution and abundance (Chase *et al.,* 2002; Chesson and Kuang, 2008; Lamarre-DeJesus and Griffin, 2015). Egg predation using artificial nests is a common method to study the predation relationships (Zanette, 2002; Martin and Joron, 2003; Bernstein *et al.,* 2015). These experiments are important to test ecological and behavioral hypothesis related to predation (Oja *et al*., 2015; Galvao *et al*., 2018), which can also be used to identify the potential predators and the factors that influences their activity (Roper, 1992; Burkey, 1993). Some predation patterns are not easy to observe in natural nests; therefore, investigations through artificial nests are essential (Major and Kendal, 1996).

Concerning Chelonia, nest predation rates may vary widely according to species and population. Nest predation levels are usually high in turtles (Congdon *et al.,* 1987; Burke *et al.,* 1998; Aresco, 2004; Dawson *et al*., 2016), which can cause low survival rates in juveniles and embryos (Hamilton *et al.,* 2002). In nests of *Emydoidea blandingii* Holbrook 1838 (Emydidae) the predation rate was 64% (Congdon *et al.,* 1983), whereas it was 81.9% and 86.5% in nests of *Malaclemys terrapin* Schoepff 1792 (Emydidae) in different years (Butler *et al.,* 2004). In nests of *Chelydra serpentina* Linnaeus 1758 (Chelydridae) the predation rate was wide, varying of 30% to 100% (Congdon *et al.*, 1987), as well as in *Chrysemys picta* Schneider 1783 (Emydidae), the variation of which was 19.7% to 76.2% (Kolbe and Janzen, 2002). Divergent nest predation rate was also found in *Trachemys dorbigni* Duméril and Bibron 1835 (Emydidae), reaching 98% in a conservation area (Gonçalves *et al.,* 2007) and only 18.1% in anthropic environment (Fagundes *et al.,* 2010). *Phrynops hilarii* Duméril and Bibron 1835 (Chelidae) nest predation also was high (82.35%) in a conservation area (Bujes, 1998). Such intraspecific divergence is possibly related with predator presence, highlighting the importance of predator behavior to comprehend the nest predation rates. Regarding freshwater turtles, the main predators found are foxes, lizards, possums, and hawks (Gonçalves *et al.,* 2007; Dawson *et al*., 2016), beyond human predation through hunting and habitat destruction (Norris *et al.,* 2018).

The location of chelonian nests by predators might be facilitated by sighting females during the spawning or by olfactory traces associated with females' secretions during the spawning, smell of eggs and/or soil revolved in nest construction (Wilhoft *et al.,* 1979). According to Moll and Legler (1971), the location of nests of *Trachemys scripta* Wied-Neuwied 1839 by lizards and armadillos occurred through the detection of odor of females' urine, but the visual vestiges could be act in the successful nest predation. In human-modified areas, *C. picta* and *C. serpentina* nests were more vulnerable to opportunistic predators, since nest discovery is facilitated in these environments (Wirsing *et al*., 2012). Therefore, the causes of high predation rates in chelonian nests remain poorly understood, besides its importance regarding to degraded environments and vulnerable species.

To understand the ecological mechanisms behind the predation in freshwater chelonian nests, it is necessary to understand the predator behavior. Predators can find nest incidentally, during the first day of nest establishment (Wirsing *et al*., 2012), or a week after the oviposition (Riley and Litzgus, 2014), showing a wide range of behavior according to predator species, prey species, and different environments. They can use the olfactory or visual signals to find the turtle's nests, but these mechanisms remain unclear. Since nests predation levels are usually high in turtles, understanding predator behavior during the nest predation is important especially for management of endangered species.

Possibly, predators attack turtle's nests when they find some signal (olfactory or visual) of upturned soil left in newly constructed nests. For example, goannas and crabs increase nest visitation after a nest had been opened by a goanna or after hatchlings had emerged from the nest (Lei and Booth, 2018). However, it is not clear whether is advantageous for the predators to attack a nest even when there are no eggs, dispensing energy to dig independently of egg presence on nests. To collaborate with the understanding about the predation relationship, we present in this paper a field experiment where we tested the chelonian nest predation, independently of eggs on nests. Our hypothesis is that owing to the clues (visual and olfactory) of upturned soil left in newly constructed nests, predators attack nests regardless of egg presence.

Our experiment was conducted at the Taim Ecological Station (ESEC Taim), in the coastal plain landscape of Rio Grande do Sul, extreme southern Brazil (S32°32'25.4" and W52°32'31.2"). Wide fields associated with wetlands are characteristics of this region, in which the predominant ecosystems are lagoons and wetlands colonized by aquatic macrophytes, sandy beaches, dunes, coastal fields and restinga vegetation (herbaceous vegetation in sandy soil) (Calliari, 1998). The weather is humid subtropical (Cfa, according to Köppen, 1948), with well-defined seasons (warm summers and cold winters).

At least four species of chelonians occur in the study region: *Phrynops hilarii, Trachemys dorbignyi, Acanthochelys spixii* Spix 1824 and *Hydromedusa tectifera* Cope 1869 (Gomes and Krause, 1982; Bager and Rosado, 2010). The sampling period (April 2016) coincided with the reproductive period of at least two of these species, *P. hilarii* and *A. spixii* (Bujes, 2010). There are some potential predators of the nests of chelonians in this area: Argentine giant tegu (*Tupinambis merianae* Duméril and Bibron 1839), Molina's hog-nosed skunk (*Conepatus chinga* Mo-

lina, 1782), six-banded armadillo (*Euphractus sexcinctus* Linnaeus 1758), southern caracara (*Caracara plancus* Miller 1777) and pampas fox (*Lycalopex gymnocercus* Fisher 1814, *Cerdocyon thous* Linnaeus 1766) (Gonçalves *et al.,* 2007).

We constructed 30 artificial nests at ESEC Taim in April 2016, simulating nests of chelonians, with two treatments: with eggs (n=15) and without eggs (n=15) (Figure 1). We excavate the nests with dimensions of 15 cm (depth) x 20 cm (width) (Bujes, 1998) in regions of low vegetation near water bodies, around 20 m distant from water (Bernstein *et al.,* 2015). We alternate the arrangement of treatments (nests without eggs and nests with eggs); the mean distance between treatment pairs was approximately 20 meters. For the treatment of egg nests, we added three commercially obtained quail eggs *(Coturnix coturnix japonica*). To mimic the water of the bladder thrown by chelonians during the excavation of the nests (Ernst *et al.,* 1994), we used water from a small artificial lake (Tamandaré Square, Rio Grande, RS, Brazil) that is inhabited by freshwater female chelonians *P. hilarii, Trachemys elegans* Wied-Neuwied 1839*, T. dorbignyi, A. spixii* (Loebmann, personal observation). This water was sprayed into all nests, namely at the bottom of the hole, on the surface of the nest, as well as above eggs when present.

We checked the nests every 24 hours for two consecutive days, always early in the morning, according to the methodology described by Marchand *et al.* (2002). After the first survey, the predated/excavated nests were reconstructed for reassessment after 24 hours. In this way, we work with the average of nests predated in the two surveys. The identification of the predators was done through observation, identification and registration of footprints in the disturbed nests and in one of the nests, we used a photographic trap (TRACE/MCS 12639) to capture images.

Considering that our response variable is qualitative (presence/absence of predation), a Chi-Square test was performed to compare differences between the frequency of predated and non-predated nests in the two treatments (with eggs and without eggs) ($p < 0.05$). Yates' continuity correction was applied, when necessary. We performed all analyses in R environment (R Core Team, 2017).

The rate of predation in both nests was 78% (23.5 ± 4.95 nests) (Figure 2), where 90% of the nests were predated in the first survey (27 nests) and 66.7% in the second survey (20 nests). There were no differences in the number of predated nests between treatments ($\chi^2 = 0.049$; df = 1; $p = 0.825$). The predation frequency in nests with eggs was 73% ± 9.43% (11 ± 1.41 nests), and in nests without eggs was 83% ± 9.43% (12.5 ± 3.54 nests). The photographic

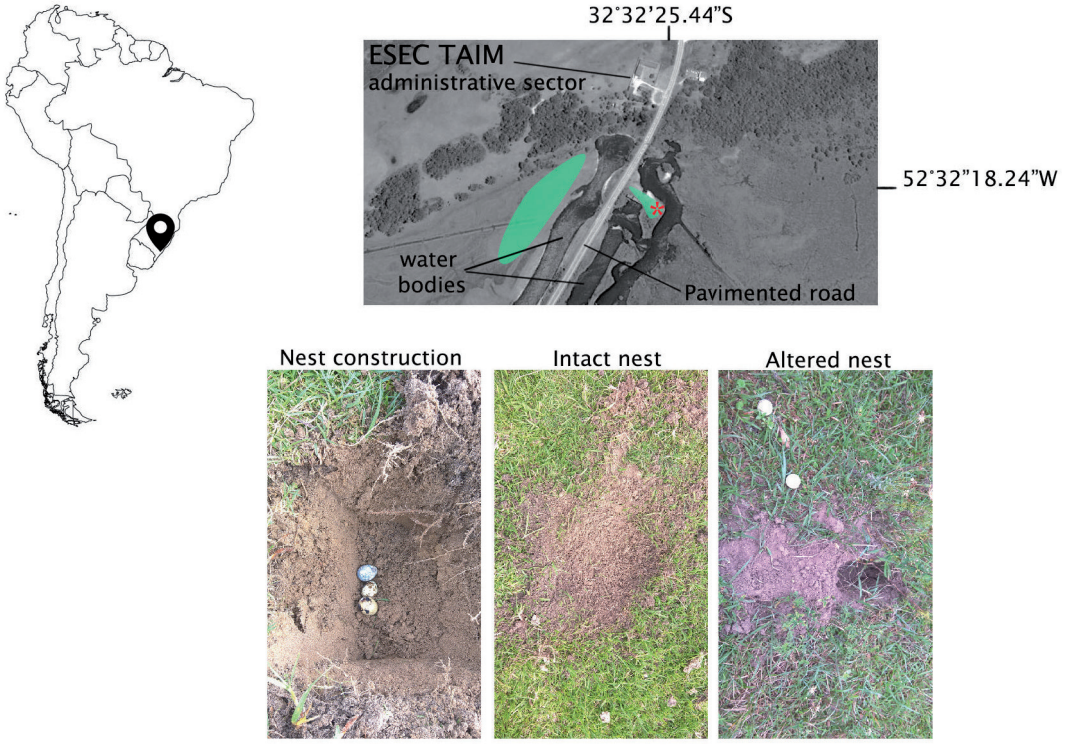

Figure 1. Study area: Taim Ecological Station (ESEC Taim), southern Brazil. Sites of nest construction are highlighted by green polygons. Asterisk indicates the place where the photographic trap was placed.

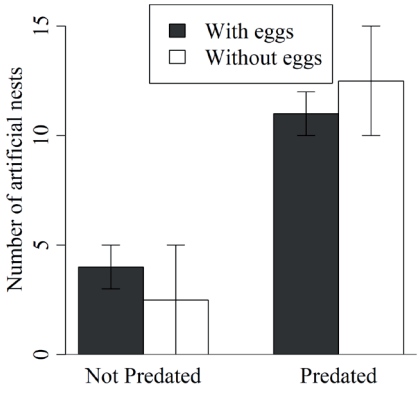

Figure 2. Number of artificial nests predated and not predated between two treatments, at Taim Ecological Station (ESEC Taim), southern Brazil.

Figure 3. Photographic trap image of two individuals of *Lycalopex gymnocercus* (pampas fox) near an artificial nest at the Taim Ecological Station (ESEC TAIM), southern Brazil. The arrow indicates the approximate position of the nest.

trap recorded the presence of *L. gymnocercus* in the artificial nests (Figure 3). Only two species footprints were recorded in some predated nests: *L. gymnocercus* and *C. thous* in nine and 14 predated nests, respectively, and both footprints were recorded simultaneously in five artificial nests.

Turtle nests are highly detectable by predators and mothers have little ability to influence predation risk of their nests (Voves *et al*., 2016). Probably, predators attack nests when they find any olfactory or visual cues from a possible nest. Olfactory cues thus could be important in locating nests, especially as a result from the female voiding her bladder prior to digging (Burke *et al*., 2005; Oddie *et al*., 2015). However, turtle-related olfactory cues are not necessary in nest predation (Wilhoft *et al*., 1979; Burke *et al*., 2005; Strickland *et al*., 2010; Bernstein *et al*., 2015; Geller, 2015). Other cues could be soil disturbance-related which are inherent in the construction of artificial nests and produces both visual and olfactory signals that have led to difficulties in resolving which are used as nest location cues (Geller, 2015). Our results showed a high predation rate in both artificial nests, with and without eggs, indicating that the presence of eggs was not important for predators. Combined with the high predation rates observed on artificial nests, the present findings indicate that the cues used by nest-foraging pampas fox are olfactory and related to disturbed soil, as this was the only component common to all depredated artificial nests and was responsible for high predation rates regardless of presence of eggs or not.

Revolved soil odor can be a strong draw for predators, since rates of predation in artificial nests in disturbed excavation soils are high, especially in the first 24 hours (Bernstein *et al.,* 2015). We also observed higher predation in the first 24 hours, highlighting the soil disturbance as an important cue used by nest predators. On the other hand, the visual attraction related with the soil revolved should also be considered (Fagundes *et al*., 2010). Artificial nests with visual soil disturbance are more likely to be depredated than artificial nests lacking soil disturbance (Strickland *et al*., 2010; Voves *et al.*, 2016). The absence of olfactory traces, such as the odor of eggs and the metabolites produced by the eggs inside the nests did not interfere in the probability to attract predators. Therefore, soil odor and soil irregularities due to excavation may have been one of the factors responsible for the found of these nests by predators (Bernstein *et al.,* 2015).

Furthermore, identification of predatory species is fundamental to understanding the predator-prey relationship (Gonçalves *et al.,* 2007). However, these authors argue that predators of chelonian nests are most often identified based on evidence and suggest as an alternative the use of photographic traps. According to the same authors, this methodology was appropriate, recording five of the six species that were identified as predators of *T. dorbigni* nests in the study area (Gonçalves *et al.,* 2007). We verified through the analysis of the footprints and with the aid of the images captured by the photographic trap that individuals of *L. gymnocercus* and *C. thous* predated the nests with and without eggs in our study. According to Bernstein *et al.* (2015), turtles are subject to high destruction rates of their nests by mesopredators, such as possums (*Mephitis mephitis* Schreber, 1776), red fox (*Vulpes Vulpes* Linnaeus, 1758) and, mainly, pampas fox, which are the most important.

Our results show that high predation rates in artificial nests were not related to the presence of eggs, and, thus, other factors should be responsible for the attraction and/or predation behavior of predators, corroborating our hypothesis. Then, it is plausible that the attraction of predators to turtle nests occurs due to the odor of upturned soil or even through the visualization of the altered soil. Therefore, we

emphasize the need for further studies on the multiple factors that affect the rates of predation of chelonian nests, to understand the prey-predator relationships and the role of each species in the trophic chains. In addition, we highlight the role of conservation units for the maintenance of biodiversity, which facilitates the verification of ecological relations with the minimum of anthropic intervention.

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