This final SRAS report has been translated directly using DeepL which may have caused translational grammatical errors. A minor review has attempted to correct any errors without changing the context.

SRAS Final Report: Influencia de la antropización en la nidificación del yacaré overo (*Caiman latirostris*)

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Abstract
Nesting is a critical period in the reproductive cycle of crocodilians, as it involves creating optimal conditions for offspring development. Populations of Broad-snouted caiman (*Caiman latirostris*) face increasing threats in their natural habitat, particularly in Santa Fe Province, Argentina. These include land-use changes such as extensive cattle grazing, watercourse channelization, and fires. This study aimed to: (1) Assess species richness, nesting area use, and visit frequency across habitats with varying anthropogenic impact; and (2) Describe nesting behavior and factors associated with maternal care. Over four seasons (2018–2022), camera traps monitored 64 nests with varying levels of anthropogenic disturbance. A total of 100 vertebrate species were identified with 62% interacting with nests. Maternal care was more frequent in floating vegetation habitats. Nesting areas serve as microhabitats for numerous species, highlighting their conservation value. Long-term monitoring of *C. latirostris* and *Sus scrofa* interactions is recommended to mitigate negative impacts on biodiversity.

1. Introduction

Nesting in crocodilians involves investment in a set of mechanisms aimed at protecting offspring over a certain period of time, defined as parental care (Shine, 2005; Gonzalez-Voyer and Kolm, 2010). These comprise pre-natal care, including nest site selection, nest building and nest care, and post-natal care, including assistance to hatchlings during birth and care of offspring during early life stages (Hénaut and Charruau, 2012; Royle et al., 2012; Martínez et al., 2013; Combrink et al., 2016; Torralvo et al., 2017; Merchant et al., 2018). Crocodilians stand out from other reptiles because of their maternal investment in terms of offspring care during incubation and into the juvenile stage (Refsnider, 2016). This pre- and post-natal care package consists of remaining in the vicinity of the nest during the egg incubation period, attending the hatching of their young and transferring them to the water, while maintaining caring behaviours towards the juveniles (Grigg and Kirshner, 2015).

Crocodilian nests play an important ecological role in the creation, modification and maintenance of habitat attributes, influencing various life history traits of other species (Somaweera et al., 2020). In this regard, numerous studies evidence a wealth of vertebrates associated with nests (Campos and Mourão, 2014; Merchant et al., 2014; Platt et al., 2014; Mazzotti et al., 2015; Combrink et al., 2016; Torralvo et al., 2017; González-Desales et al., 2020; Platt et al., 2021). Mound nests, according to the authors cited above, provide feeding, resting, breeding, nesting and sheltering areas for other species, so they could be considered as microhabitats.

Several factors are associated with the crocodilian nesting process in its complexity, and among the environmental or external factors are modifications in landscape configuration (Mainwaring et al., 2017). Individual nest builders must adjust their behaviour to adapt to environmental modifications (Mainwaring et al., 2017). Anthropisation is defined as the degree to which an ecosystem is modified by human activities (Martínez Dueñas, 2010). These modifications create new spatial configurations for biota (Haddad et al., 2015) and can influence the behavioural or phenotypic traits of an organism

(Fleming and Bateman, 2018), as well as generating a decreasing gradient of biological diversity (Suazo Ortuño et al., 2018).

Evaluating the effect of anthropisation on nesting behaviour and maternal care could provide useful information when planning strategies for the conservation of the caiman (*Caiman latirostris*), one of the crocodilian species within Argentina. Conservation efforts for *C. latirostris* are framed by more than 30 years of work in the province of Santa Fe on the management programme for the species, based on the technique of ranching or egg collection in the wild, incubation of the hatchlings in controlled conditions and their release into the wild (Larriera, 2022; Siroski et al., 2024).

In this context, the objectives were (1) to record species richness, nesting area use and nest visit frequency in environments with different degrees of anthropisation; and (2) to describe the nesting behaviour of *C. latirostris* females and determine the factors associated with maternal care in environments with different degrees of anthropisation.

2. Methods

2.1. Study area

The study was carried out at localities in the province of Santa Fe, Argentina, a region that corresponds to one of the extremes of the distribution of *C. latirostris*. The sites are located in the centre-north of the province, and include the localities of Cacique Ariacaiquín (Dto. San Javier, 30°38'09 ‘S 60°16'21 ’W), Alejandra (Dto. San Javier, 30°02'46 ‘S 59°58'29 “W), Calchaquí (Dto. Vera, 29°55'31 ”S 60°04'27 “W), Santurce, (Dto. San Cristóbal, 30°11'46 ”S 61°01'34 “W) and Huanqueros (Dto. San Cristóbal, 29°40'03 ”S 61°00'53 ’W).

The study sites have areas of savannah (Calchaquí, Santurce), bush (Alejandra, Santurce, Huanqueros) and reservoir (Cacique Ariacaiquín, Alejandra, Huanqueros), preferred by *C. latirostris* for nesting (Montini et al., 2006; Larriera and Imhof, 2006). Considering the pressures faced by both *C. latirostris* and the transformation of natural environments due to extensive cattle ranching and associated activities (canalisation of watercourses) (Larriera and Imhof, 2006; Prado et al., 2012, Pietragalla et al., 2019), sites were categorised using the Forest Landscape Integrity Index (FLII) according to Grantham et al. (2020). According to the quantitative valuation assigned, the ranking from more anthropised areas (less integrated landscapes) to less anthropised areas (more integrated landscapes) is: Calchaquí, Alejandra, Cacique Ariacaiquín, Huanqueros, and Santurce.

2.2. Nest monitoring

In December 2018-2022, at the start of each nesting season, *C. latirostris* nests were found and a camera-trap was set up, focusing on each nest. There were a maximum of four nests per site where possible, repeating the procedure each season. The cameras remained active for 24 h, recording date and time. The teams monitored each nest until the end of the season (end of March), so batteries and memory cards were changed every 25-30 days during January, February and March. Camera traps were programmed to take 1 photo every 5 minutes to obtain records of *C. latirostris*. In addition, 1 photo was programmed to be taken every 1 minute upon sensor activation, with a 10-second delay in activation due to movement, to record other vertebrates that could activate the PIR sensor (mammals and birds). A total of 64 camera-traps were installed, of which 22 were located in bush nests, 20 in dam nests and 22 in savannah nests.

2.3. Data analysis

To determine the richness of species visiting *C. latirostris* nests, sampling data reports (list of species, date and time of detection in the nesting area) were obtained using the CamTrapR package (Niedballa et al., 2020). To assess factors associated with species richness, the type of environment, the degree of anthropisation and the monitoring season were considered. Since the different nesting environments were not represented in all sites with different degrees of anthropization, the species richness detected

(response variable) was compared between sites with different degrees of anthropization (covariate) discriminating by environment type. Sites without a representative number of monitored nests were discarded for comparison using a t-student test. To assess the importance of environment type and nesting season on the species richness detected in the nesting area, generalised linear models with negative binomial distribution of residuals were used. The activity of each species in the nest was assessed to detect the type of interspecific interaction and the percentage of occurrence of each interaction. Interactions were categorised considering their beneficial (+), negative (-) or neutral (0) effect towards the nest: commensalism (+/0), competition (+/-) and predation (+/-). Neutral (0/0) was also included when an animal was detected in the nest, but no interaction was observed. The strength of interaction of each species with alligator nests was assessed to quantify patterns of alligator nest use. For this purpose, independent records and visit durations were determined for each species. The interaction strength index (IFI) was determined for each species at each nest, and species that were present at five or more nests were selected to establish an average IFI value. The packages data.table, tidyverse, reshape2 and dplyr in R 3.6.3 software were used to determine these indices. Species whose activities posed a risk to the nests (through competition or predation) were selected.

Finally, to meet objective (2), the importance of anthropization on maternal care of *C. latirostris*, quantified as nest visitation rate (ratio between total time -amount of hours- spent by *C. latirostris* in the nest and the number of active trap-nights -monitoring effort-), was assessed using a Wilcoxon test, among nests from sites coming from the same type of nesting environment. Generalised linear models with normal distribution of residuals were used to assess the importance of environment type, nesting season and nest-risk species visits (visitation rate) on maternal care of *C. latirostris*.

Nest risk species (nest-opening predators/competitors) with 50 or more independent records were selected to determine the coefficient of activity overlap with *C. latirostris* using the Overlap package in R 3.6.3.

We also compared the proportion of successful nests between sites with different degrees of anthropisation, discriminating by environment type, using a test of difference of proportions based on Fisher's exact test. To assess the importance of maternal care, environment type, nesting season and visitation by nest risk species on nest success, generalised linear models with binomial distribution of residuals were performed.

The Generalised Linear Models were simplified to the minimum adequate model by hypothesis testing, comparing the models through the ANOVA function, and all statistical and graphical analyses were performed using R 3.6.3 software (R Core Team, 2020), with a significance level of 0.05.

3. Results

Total sampling effort was 9,062 nights/chamber. Sixty-four nests were monitored in bush (22), reservoir (20) and savannah (22). A total of 100 vertebrate species were identified in *C. latirostris* nesting areas: 74 birds (74%), 23 mammals (23%), and 3 reptiles (3%, excluding *C. latirostris*). Because taxonomic identification of all reptiles was not possible (mainly due to their small size in the photos), the richness of this group is underestimated. Also, although anuran amphibians were detected in and around the nests, the images obtained were mainly nocturnal and the size of the anurans was too small for taxonomic identification. For this reason, amphibians were not included in the analyses.

Considering only those species that could be taxonomically classified, richness was similar in areas with different degrees of anthropisation in both bush ($t = 0.19$; $p = 0.8545$), reservoir ($t = 2.15$; $p = 0.050$) and savannah ($t = -0.24$; $p = 0.8144$) nests (Figure 1).

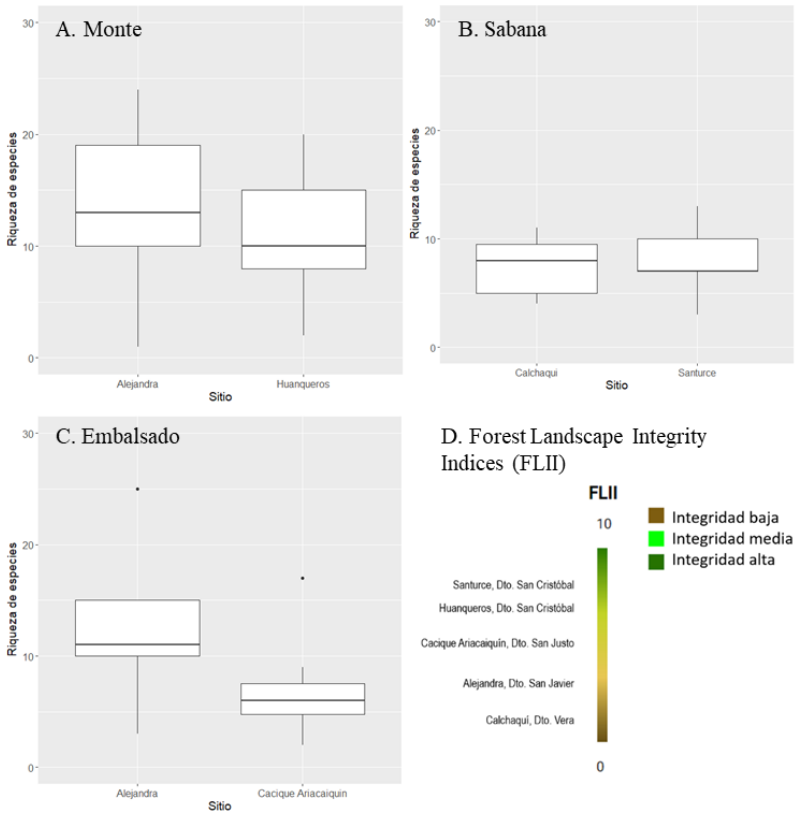


Figure 1. Species richness in (A) bush, (B) savanna and (C) reservoir nests, in sites with different degrees of anthropisation.(D) Forest Landscape Integrity Index (FLII) of the sites where the study was carried out (areas with low integrity represent higher anthropisation).

Species richness was associated with nesting season ($\chi2 = 10.200$; $p = 0.017$) and environment type ($\chi2 = 12.876$; $p = 0.002$). The number of species detected in 2021-2022 (the season with the lowest rainfall amounts) was significantly higher than that detected in the remaining seasons (Tukey multiple comparisons test: 2021-22 vs. 2018-19: $z = 4.727$; $p < 0.001$; 2021-22 vs. 2019-20: $z = 3.329$; $p = 0.005$; 2021-22 vs. 2020-21: $z = 2.634$; $p = 0.041$; Figure 2a). With respect to environment type, bush nests showed higher species richness than savanna and reservoir nests (Tukey's multiple comparisons test: bush vs. reservoir: $z = 2.775$; $p = 0.015$; bush vs. savanna: $z = -3.379$; $p = 0.002$; savanna vs. reservoir: $z = -0.471$; $p = 0.884$; Figure 2b).

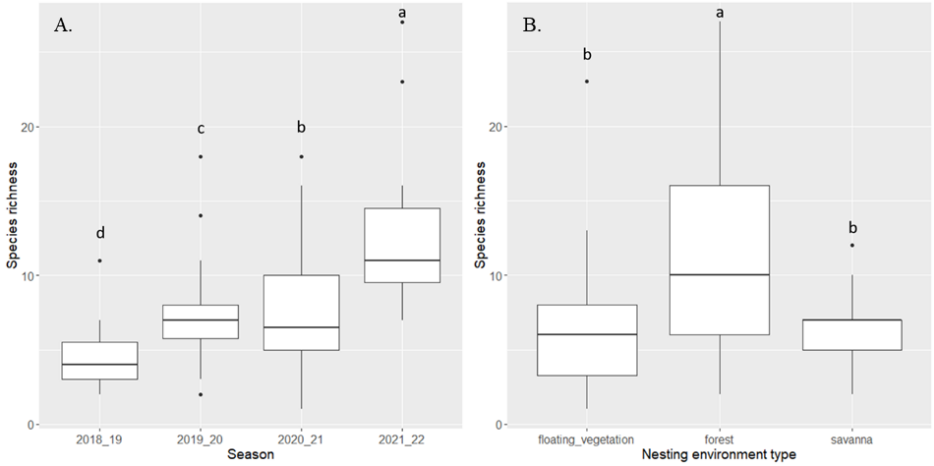


Figure 2. (a) Species richness in caiman nests monitored during four nesting seasons. (b) Species richness in different types of caiman nesting environments.

Sixty-two (59%) species were identified interacting with caiman nests through commensalism, predation and competition, in 1,928 independent events (Figure 3). A further 38 species were also identified whose nest visitation corresponded to species co-occurrence in the monitored area, with no interactions, and independent detection events were categorised as neutral (n = 412).



Figure 3. Number of events of each interspecific interaction detected in Caiman latirostris nests.

Predation occurred in 79% (50/64) of the monitored nests. In addition to the strong interaction detected between predators and nests, the bird group interacted strongly through commensalism. Among commensalist species, the red-crested coot (*Tigrisoma lineatum*) and the cuis (*Cavia aperea* and possibly *Galea* sp.) interacted strongly with the nesting area of *C. latirostris*, followed by omnivorous birds, whose interaction index with the nests was 0.1 to 0.3 stronger than insectivorous and granivorous birds (Figure 4a). On the other hand, among the species identified as competitors with *C. latirostris*, cattle interacted with caiman nesting areas at a rate between 0.1 and 0.15 higher than the other competing species (Figure 4b). Finally, the predators that interacted strongly with the caiman nests were the caiman lizard, the bush fox and the bighorn sheep, and the interaction strength index of the first species was double that obtained for the second and third species mentioned (Figure 4c).

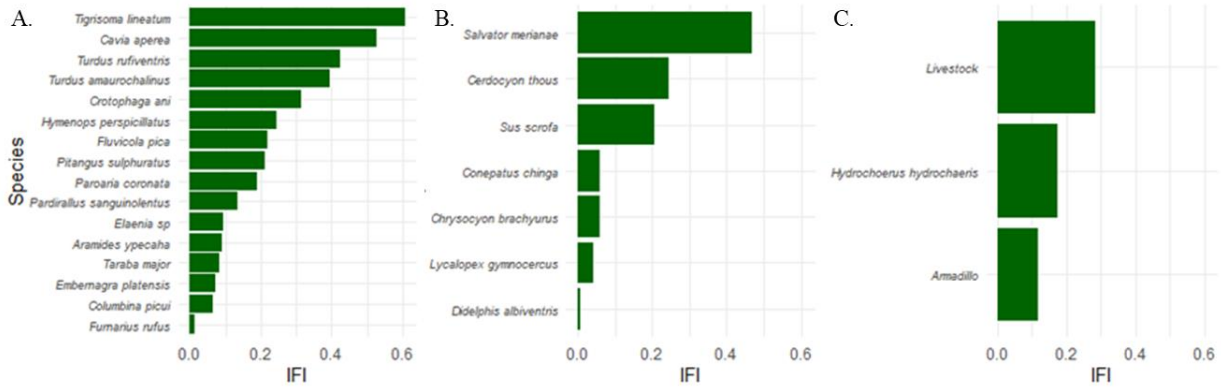


Figure 4. Interaction Strength Index (IFI) between: (a) commensalist species and alligator nests; (b) predator species and alligator nests; (c) competitor species and alligator nests. Values close to 1 indicate a strong interaction (the species frequently uses the area).

On the other hand, maternal care was related to the type of nesting environment (F=4.017; p-value=0.026), and reservoir nests received more care from females than bush nests (z= 2.527; p=0.0310; Figure 5).

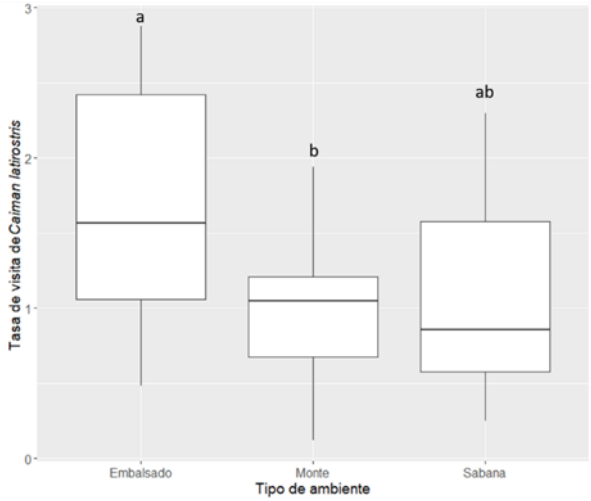


Figure 5. Maternal nesting care of *Caiman latirostris*, expressed as visitation rate, in dam, bush and savannah nests. Letters indicate groups obtained by Tukey's multiple comparisons test.

Less overlap of activity was found between *C. latirostris* and *S. merianae* (OVL=0.32), which showed a frequent period of activity (effective predation events) in the nest concentrated in daylight hours (Figure 6). Other predatory or nest-destroying species showed a greater overlap of activity with *C. latirostris* (armadillos, foxes, bighorn sheep, capybaras, cattle: OVL=0.50-078; Figure 6).

4. Discussion

Evidence of interspecific interactions in caiman nests reaffirms the role of caiman nests as microhabitats for a wide variety of species. To weight interspecific interaction events, we used the interaction strength index (IFI), which was a good indicator of vertebrate use of the nesting area. It is noteworthy that, through this study, it was possible to distinguish nest use by 62 species out of a total of 100 vertebrates detected.

Vertebrate richness is positively related to habitat heterogeneity, with bush areas fulfilling this characteristic in terms of vegetation strata. In addition, the lower specific richness in reservoir nests could be linked to the accessibility of these environments, while savannah nests were vulnerable to flooding in 2018-19 and predation by *Sus scrofa* in the monitored seasons. Their behaviour of destroying nests in one or two visits by rooting would be detrimental to the approach of other species to caiman nests.

On the other hand, Barão-Nóbrega et al. (2018) reported that the frequency of visitation by females of two crocodilian species was influenced by the distance from the nest to the nearby water body. The distance between nests and water bodies is reduced or non-existent in impoundment areas compared to bush and savannah nests, as these are aquatic areas with floating vegetation fragments. This would encourage frequent monitoring of impoundment nests.

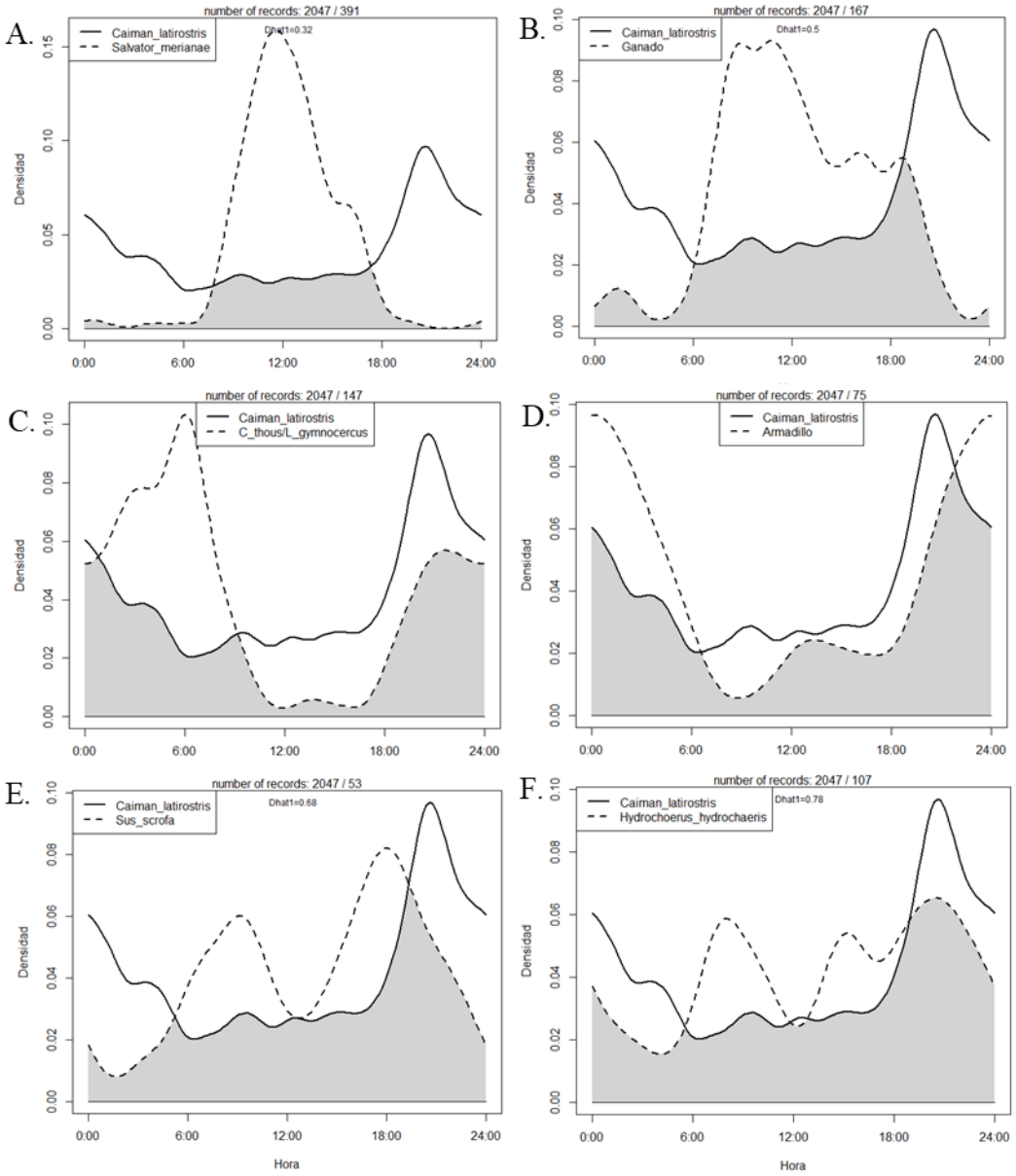


Figure 6. Activity patterns of *Caiman latirostris* and predators/competitors: (A) *Salvator merianae*, (B) Livestock (mainly *Bos taurus*), (C) Foxes (*Cerdocyon thous* and *Lycalopex gymnocercus*), (D) Armadillo (*Dasypus* sp. / *Euphractus sexcinctus*), (E) *Sus scrofa* and (F) *Hydrochoerus hydrochaeris*.

Finally, the vulnerability of the nests in savannah areas distinguishes these environments from the bush and reservoir areas because they are characterised by low flooded areas, due to the physiognomy of the landscape, but also due to the modifications of the natural water regimes of the Salado River basin, related to canalisations. In addition to the above, in savannah areas, predation of caiman eggs by *Sus scrofa* was detected.

In conclusion, the nests provided opportunities for numerous species to obtain resources mainly during hostile times, such as periods of drought. Given the important richness of species that actually interacted with the caiman nests, the information obtained validates the importance of caiman nests as microhabitats, so that in the planning of comprehensive conservation strategies, nesting areas should be a priority. Finally, savannah nests received little maternal care compared to nests in other types of environment, and were especially vulnerable to flooding and destruction by *S. scrofa*, so it is

recommended to monitor the interaction of the species with *C. latirostris* nests in the long term to understand the impact that its activity generates for other species that visit and interact with caiman nests.

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Bibliografía:

Barão-Nóbrega et al. (2018) Journal of Comparative Physiology B., 188:127−140. https://doi.org/10.1007/s00360-017-1103-8
Campos y Mourão (2014). Journal of Natural History. 49 (15–16):977–982. https://doi.org/10.1080/00222933.2014.930757
Combrink et al. (2016). Behavioural Processes, 133, 31-36. https://doi.org/10.1016/j.beproc.2016.10.014
Fleming y Bateman (2018). Animal Behaviour, 138, 145-155. https://doi.org/10.1016/j.anbehav.2018.02.011
González-Desales et al. (2020). Journal of Natural History, 54(29-30), 1813-1826. https://doi.org/10.1080/00222933.2020.1829723
Gonzalez-Voyer y Kolm (2010). Wiley & Sons Eds. https://doi.org/10.1002/9780470015902.a0021907
Grantham et al. (2020). Nature Communications, 11(1), 5978. https://doi.org/10.1038/s41467-020-19493-3
Grigg y Kirshner (2015). CSIRO Publishing, 671 pp. https://doi.org/10.1071/9781486300679
Haddad et al. (2015). Science Advances, 1(2), e1500052. https://doi.org/10.1126/sciadv.1500052
Hénaut y Charruau (2012). Animal Biology, 62(1), 29-51. https://doi.org/10.1163/157075511X597629
Larriera e Imhof (2006). https://www.produccion-animal.com.ar/produccion_yacares/33-proyecto_yacare.pdf
Larriera (2022). Ethnobiology and Conservation, 11. https://doi.org/10.15451/ec2022-03-11.07-1-5
Martínez-Dueñas (2010). Revista del Instituto de Investigaciones Tropicales, 5(1), 37-46
Martínez et al. (2013). Tlatemoani, (13)
Mazzotti et al. (2015). Biological Invasions, 17, 47-50. https://doi.org/10.1007/s10530-014-0730-1
Merchant et al. (2014). Herpetological Review. 45, 201–203
Merchant et al. (2018). Copeia, 106(3), 421-426. https://doi.org/10.1643/CH-17-709
Montini et al. (2006). Journal of Herpetology, 5(2), 91-96. https://doi.org/10.11606/issn.2316-9079.v5i2p91-96
Niedballa et al. (2020). https://github.com/jniedballa/camtrapR
Pietragalla et al. (2019). https://www.argentina.gob.ar/sites/default/files/nea_guias_buenas_practicas_1.pdf
Platt et al. (2014). Bull Texas Ornithological Society. 47(1–2):37–40
Platt et al. (2021). Journal of Natural History, 55(3-4), 133-149. https://doi.org/10.1080/00222933.2021.1895350
Prado et al. (2012). Cuadernos de Herpetología, 26, 403-410
R Core Team (2020). https://www.R-project.org/
Rainwater et al. (2024). Animals, 14(4), 620. https://doi.org/10.3390/ani14040620
Refsnider et al. (2015). Biological Journal of the Linnean Society, 116(4), 847-855. https://doi.org/10.1111/bij.12671
Royle et al. (2012). The evolution of parental care. Oxford University Press. ISBN: 9780199692583
Shine (2005). Annual Review of Ecology, Evolution and Sistematics, 36(1), 23-46. https://doi.org/10.1146/annurev.ecolsys.36.102003.152631
Siroski et al. (2024). Animals, 14(9), 1288. https://doi.org/10.3390/ani14091288
Somaweera et al. (2020). Biological Reviews, 95(4), 936-959. https://doi.org/10.1111/brv.12594
Torralvo et al. (2017). PloS One, 12(8), e0183476. https://doi.org/10.1371/journal.pone.0183476