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Nesting trends and predation risks among yellow-spotted river turtles in Essequibo River Basin

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ABSTRACT

The yellow-spotted river turtle (*Podocnemis unifilis*), inhabiting Amazon, Orinoco, and Essequibo River basins, is classified as vulnerable due to historical exploitation and current threats. Efforts have been made throughout the Amazon basin to understand the ecology of yellow-spotted river turtles and implement conservation measures, but the Essequibo River basin located in Guyana, remains the least studied part of the geographical range of the species. In this study we present data collected over a three-year period from 2020 to 2023, as part of a community driven conservation program by Yupukari village, in the North Rupununi, Guyana. We describe the nesting behavior of *P. unifilis*,and assess predation of eggs in the wild. A total number of nests in 9 beaches ranging from 59 (in 2021) to 76 (in 2023) (in averge 6.68 nest/beach, and 14.2 nests per ha) with an average number of 20.45 eggs per nest. Nesting locations were found to be predominantly situated within 1–20 m from the river, in proximity to vegetation, and on fine sand, with temperatures consistently falling within the 27–29 degrees Celsius range. Importantly, this study unravels the critical issue of nest predation, with lizards, human, and the birds emerging as the primary culprits, impacting nests located closer to vegetation to a greater extent. All nests in sites which potentially would be flooded were moved for a hatchery in the community of Yupukari in which the hatching success reached 83%.

1. Introduction

The yellow-spotted river turtle (*Podocnemis unifilis*), a species inhabiting the Amazon, Orinoco, and Essequibo River basins (Pearse, 2006), is currently categorized as Vulnerable by the IUCN Red List (2016), with potential reclassification to Endangered needed due to projections of further population declines (Rhodin et al., 2021; Norris et al., 2019). Historical exploitation in the 18th and 19th centuries led to severe declines (Freitas et al., 2020; Casal et al., 2013; El Bizri et al., 2020). Despite 20th-century regulations, illegal harvesting persisted due to inadequate enforcement (El Bizri et al., 2020; Kemenes and Pezzuti, 2007; Peñaloza et al., 2013). Additional challenges include deforestation (Fagundes et al., 2018), water pollution, increased boat traffic (Fachín Terán, von Mülhen, 2003), climate change, water level fluctuations (Páez et al., 2015; Eisemberg et al., 2016), and infrastructure development (Norris

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et al., 2018). Ineffective enforcement and insufficient environmental impact assessments hinder conservation efforts in certain regions (Norris et al., 2018).

The life cycle of *P. unifilis* is intricately linked to the region's hydrological patterns, with females embarking on migrations to nesting areas following the recession of waters during the dry season (Ponce de Leão et al., 2019). These nesting sites are often found on high coarse-sand riverbanks and the fringes of lakes, channels, and vegetated areas (Erickson and Baccaro, 2016), being nesting site selection of great ecological and evolutionary importance for turtles, because it is a key determinant of individual reproductive success (Erickson et al., 2020). The clutch size varies according to the female's body size and geographical distribution, typically ranging from 20 to 30 eggs (Hernández et al., 2010; Vanzolini, 2003).

Efforts to address the decline in yellow-spotted river turtle populations have focused on understanding the species' ecology, with numerous studies conducted in Brazil (Ferreira Júnior and Castro, 2003; Ferreira Júnior et al., 2010; Ponce de Leão et al., 2019), Venezuela (Thorbjarnarson et al., 1993; Escalona and Fa, 1998) and Bolivia (Carvajal-Bacarreza et al., 2021; Conway-Gómez, 2007). Conservation initiatives have emerged across the Amazon Basin, encompassing nest translocation, head-starting, protection of beaches and the monitoring of nests, turtle ranching, and in-situ and ex-situ conservation efforts (Páez et al., 2015; Balestra, 2016; Lima et al.,

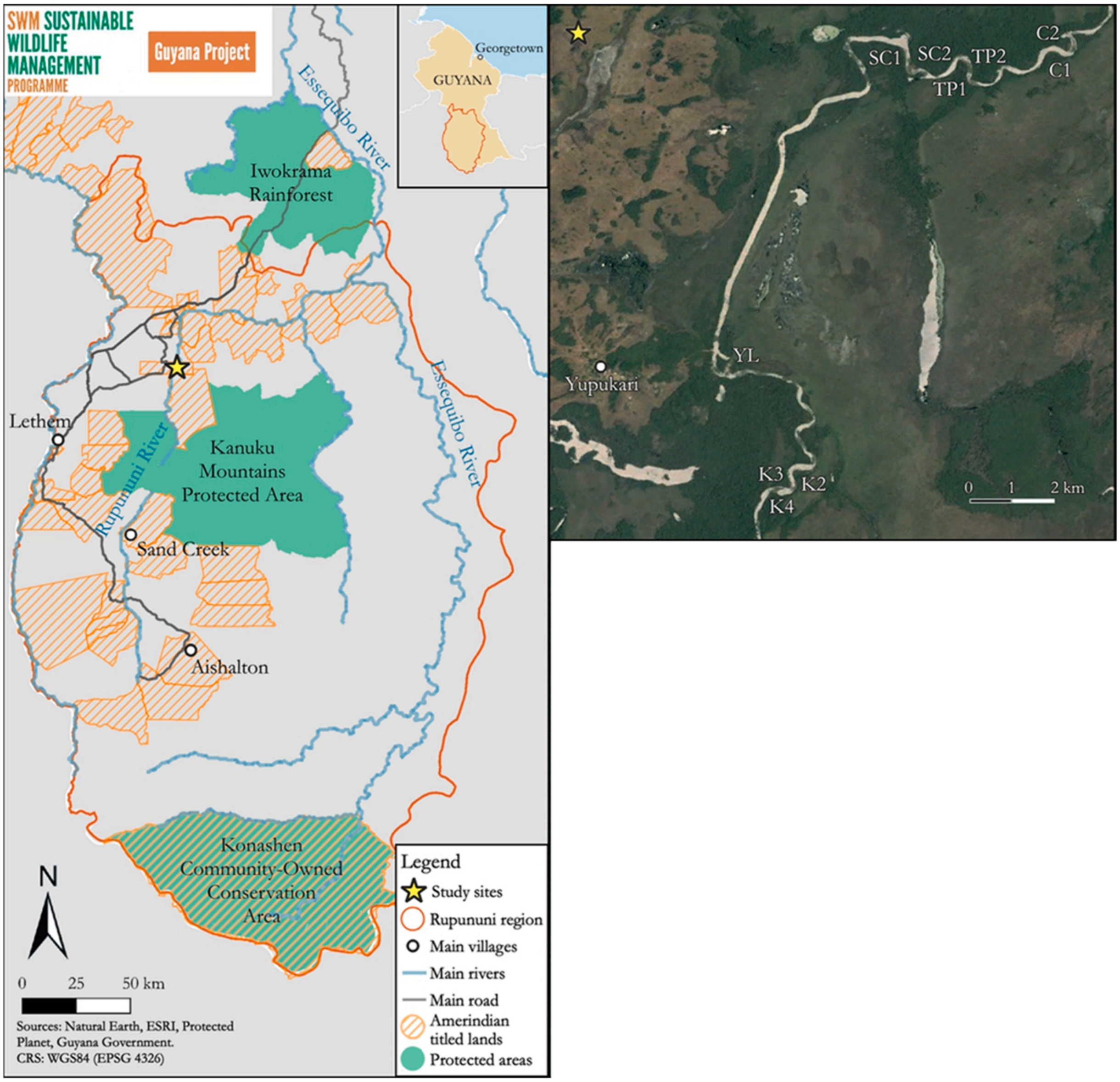

Fig. 1. Map of the study area. On the left, the Rupununi Region, with its three National Protected Areas, main rivers (Rupununi River a tributary of the Essequibo), and the location of the two study sites highlighted. Upper right, satellite image (source: Google Earth) of the North part (Yupukari) showing the ten monitored beaches along the Rupununi River, as follows: C2: Cadabai 2, C1: Cadabai 1, TP2: Thunder Pool 2, TP1: Thunder Pool 1, SC2: Steamer Creekmouth 2, SC1: Steamer Creekmouth 1, YL: Yupukari Landing, K3: Kumaka 2, K2: Kumaka 3, and K3: Kumaka. 4.

2008; Campos-Silva et al., 2018). These actions have not only led to reduced harvest rates but have also benefited other species and fostered a transition toward participatory conservation regimes, promoting social justice (Lopes et al., 2021).

Despite extensive research and conservation efforts, the Essequibo River basin, a critical region for the species, remains under-studied. This knowledge gap presents a significant challenge to effective conservation and management. Therefore, in response to the need for a comprehensive understanding of the yellow-spotted river turtle's ecology in the Essequibo River basin, the community of Yupukari and Caiman house, a community driven NGO, initiated a conservation program in 2011. This program includes *in-situ* conservation through the protection and monitoring of beaches, and *ex-situ* conservation with head-starting activities, monitoring of turtle consumption, and environmental education.

Since 2020, a research programme was implemented to systematically document nesting characteristics and address the critical knowledge gaps about the ecology of this species. In this study, we present data on nesting behaviour, predation in the wild and hatching success as part of the head starting programme. This information contributes to develop a comprehensive and participatory conservation program for this species while safeguarding the rich cultural and ecological heritage of the Rupununi region.

2. Material and methods

2.1. Study area

This study was conducted on 9 beaches located in Yupukari, a Makushi community, located in the North Rupununi (Fig. 1). The Rupununi Region, Guyana's largest administrative region, bordering Brazil, is inhabited by approximately 24,000 people from Indigenous ethnicities, primarily engaged in subsistence resource use (Henfrey, 2002). Situated within the biodiverse Guiana Shield, known for its ancient geological formations (de Souza et al., 2019), this region hosts three national protected areas, including the Kanuku Mountains Protected Area (KMPA), `the Kanashen Amerindian Protected Area (KAPA), and the Iwokrama Rainforest. Named after the Rupununi River, which connects to the Essequibo River to the north, the area predominantly comprises vast expanses of primary forest and roughly 20% savannah, featuring seasonally flooded wetlands. With its diverse habitats, sparse human population, and a history of conservation and traditional management, the Rupununi stands as the most conserved region in Guyana (Watkins et al., 2010).

2.2. Data collection

The data considered for this study was collected from December 2020 to May 2023 as part of the Yupukari turtle conservation programme.

i) *Nest surveys*

From 2021 to 2023 nesting seasons, a team composed of 4 or 5 community members surveyed 9 beaches. These selected beaches were known over the years to be important nesting sites nearby the village. These selections were made due to certain characteristics such as their close proximity to deeper pools and the larger size of their sandy beaches. In the study area, females usually begin laying eggs between end of December to end of March, when the water levels recede, and the banks are visible. As such, the beaches were monitored every day from January until April each year. Nest surveys were done from ~5:30 am to ~9:00 am. Based on the daily track records nesting dates were estimated, and the nests were identified using the indirect method of turtle tracks in the sand. Site characterization was recorded for all nests, including coordinate, sand grain size, distance to the river, distance to vegetation.

ii) *Nest depredation and destruction*

Naturally depredated nests were identified by the presence of broken eggshells and/or remains of partially eaten eggs outside the nest, disturbed/uncovered nests surrounded by animal tracks, and the presence of wildlife excavation marks (Norris et al., 2019). Human removal was identified when a hole with a mean depth of 10–15 cm was found, without eggs or with partially eaten eggshells. Human removal of eggs was also usually associated with signs of human activities, such as footprints, fire, charcoal, and campsite in the nesting areas. When a nest was marked as flooded, this meant that the nest was submerged due to a significant rise in water levels during the incubation or hatching period.

The determination of annual flooding onset relied on Local Ecological Knowledge, predictions, and monitoring changes in weather patterns upstream from the Kanuku Mountains. Because of the early floodings that occurred in the three consecutive years of monitoring, more than half of nests were translocated to the hatchery (see Fig. 3).

iii) *Hatchling Characteristics*

To ensure the protection and successful development of hatchlings, we implemented a protocol for collecting eggs from nests at risk of flooding (and not yet predated). Previously we were considering transport the nests within the first two days (48 h) of their existence, however, we have now modified the protocol to transfer the eggs within the first 12 h of nesting. These eggs were gently retrieved and stored for ex-situ management.

To further ensure the successful development of hatchlings, we diligently recorded the temperature, depth, and size of the nests, replicating these measurements in the hatchery. To ensure the same temperature, the hatchery has been constructed near to the beach, with beach sand and within native trees to ensure a sunshade and so the same environmental conditions such as air humidity, which will influence the nest temperature. Our monitoring shows that the hatchery closely replicates the conditions found in the natural nests. Our hatcheries were designed to mimic a natural habitat, with dimensions of 20 by 20 feet and a height of 3 feet. In order to replicate a sex ratio similar to that observed in nature, we collected eggs and carefully repositioned them at their original depth and orientation within the sand layer, utilizing sand from the same beach in the hatchery. This is crucial as the temperature varies at different depths within the nest, and this variation determines the sex.

The temperature of the sand in the hatchery were that natural in the area, and at the middle of the day the peak of the temperature were similar to that on the beach within a range of 32 to 33 degrees Celsius. In the controlled hatchery environment, the eggs completed their incubation period, and hatchlings were reared for one month before being released into the wild. Hatching survival rate was determined by measuring the number of turtles that successfully hatched from the eggs and produced viable hatchlings.

2.3. Data analysis

To compare the number of nests in each of the 9 monitored beaches between the monitored years we performed a Generalised Linear Model (GLM) with a negative binomial distribution, having the values of the year 2021 as a response variable and the values of 2022 and 2023 as predictor variables. We adjusted the nest number for the analysis based on the number of days of monitoring each year.

To verify if the nest site selection is influenced by environmental characteristics (sand temperature, sand grain size, distance to the river, and distance to vegetation) we performed a Regression Model considering the number of nests as the response variable and each above cited environmental characteristics as predictor variable of each single model. We used separated models to avoid collinearity.

To verify if the nest predation frequency is influenced by the environmental characteristics of the nest sites (sand grain size, distance to the river, distance to vegetation, slope, and height) we performed a Generalised Linear Mixed Model (GLMM) with the negative binomial distribution. We considered each beach as a random variable.

There was no collinearity ($p > 0.05$) among predictor variables. For GLMM and GLM, we used residual checks to verify whether our models were, in principle, suitable or not. We used the Akaike information criterion to select models of interest if ΔAIC values > 6 (ΔAIC obtained from the difference between a null and complete model AIC values; Harrison et al., 2018; Richards, 2008). All analyses were performed in R ver. 3.5.3 (R Development Core Team, 2019) using the LME4 (Oksanen et al., 2013) packages.

3. Results

Nest numbers varied across three years: 59 nests in 2021 (average 1.5 new nest identified per day, SD=0.4), 72 nests in 2022 (average 2.3, SD=4.1), and 76 nests in 2023 (average 4.2, SD=2.3). No significant changes were observed in nest numbers per beach between 2022 and 2023 compared to 2021 (Table 1). However, the overall number of nests in the study area exhibited an upward trajectory along the monitored years, there was a 2.1-fold increase in 2022 and a 2.6-fold increase in 2023 compared to 2021. The average number of nests per beach was 6.68 (SD=3.66), and the average density was 14.2 nests per hectare (SD=8.92) over the monitored years. For the years 2021, 2022 and 2023, a total of 1160, 514, and 1199 hatchings were recorded, respectively. We found an average of 20.45 eggs per nest (SD = 8.03; ranging from 1 to 31).

The majority of nests were found to be situated within a proximity of 1 to 20 m from the adjacent river ($p < 0.05$), within 0 to 8 m from the nearest vegetation, and were predominantly located in areas characterized by fine sand substrates. Furthermore, our data indicated that the temperature conditions of the nests predominantly fell within the range of 27 to 29 degrees Celsius ($p < 0.05$), as detailed in Table 2 and illustrated in Fig. 2.

All nests in sites which potentially would be flooded were moved for hatcheries in the community of Yupukari in which a hatching success of 83% occurred (Fig. 3A and B). After undergoing a one-month period in water tanks, the hatchlings were released into their natural habitat (Fig. 3C and D). A total of 127 nests were in flooding sites (Fig. 3E). However, those left in their natural habitat were subjected to predation.

In 2021 and 2023 most nests were transferred to the hatchery and the nest in the wild has a predation rate of 55.0% (n = 11) and 48.8% (n = 15), respectively. Conversely, during the year 2022, most nests were retained within the natural habitat, with a comparatively lower incidence of predation (17.7%; n = 8). Predation events were primarily attributed to the lizard (***Tupinambis teguixin***) (Fig. 3F), which was accountable for 66.6% of the nest predations, followed by human interference and the great black hawk (*Buteogallus urubitinga*) (Fig. 4). Our GLMM analysis unveiled a statistically significant correlation between the proximity of nests to vegetation and the predation rates ($p < 0.05$) (Table 3).

4. Discussion

Approximately 61% of all turtle species are threatened with extinction or already extinct (Lovich et al., 2018), and freshwater turtles are one of the most threatened vertebrate taxa (Gibbons et al., 2000). Therefore, understanding the condition of success of nest protection and hatching is essential to promote a turtle population increase. In the Rupununi region, the local community of Yupukari and Caiman house, have been pioneering the first river turtle conservation program in Guyana. This program includes *in-situ* and

Table 1
Details of the complete model and the null model using a generalized linear mixed model to compare the number of nests in 2022 and 2023, in relation to the number of nests in 2021.

Response variable	Predictor variables	Estimate	Std. Error	z value	Pr (>|z|)	AIC	AIC Null model	ΔAIC	Model
Nest number in 2021	Nest number in 2022	0.003662	0.008388	0.437	0.681	52.221	59.106	6.885	GLMM
	Nest number in 2023	0.082221	0.042079	1.954	0.108				

Table 2
Details of the complete model and the null model using the Regression model to verify the influence of environmental characteristics on the number of nests. The predictor variable showing a significant result is highlighted in bold.

Response variable	Predictors	Estimate	Std. Error	z value	$Pr(>\lvert z \rvert)$		$F_{1,16}$	R^2_{adj}	Model
Number of nests	**River distance (m)**	**-0.0429**	**0.0109**	**-3.94**	**1.62E-04**	***	F1,89: 15.52	0.1389	LM
Number of nests	Vegetation distance (m)	0.1725	0.1142	1.511	1.37E-01		F1,51: 2.282	0.02406	LM
Number of nests	**Sand temperature (°C)**	**-0.5562**	**0.1586**	**-3.508**	**0.001493**	***	F1,29: 12.31	0.2737	LM
Number of nests	Depth	0.1818	2.7411	0.066	0.949		F1,8: 0.0044	-0.1244	LM

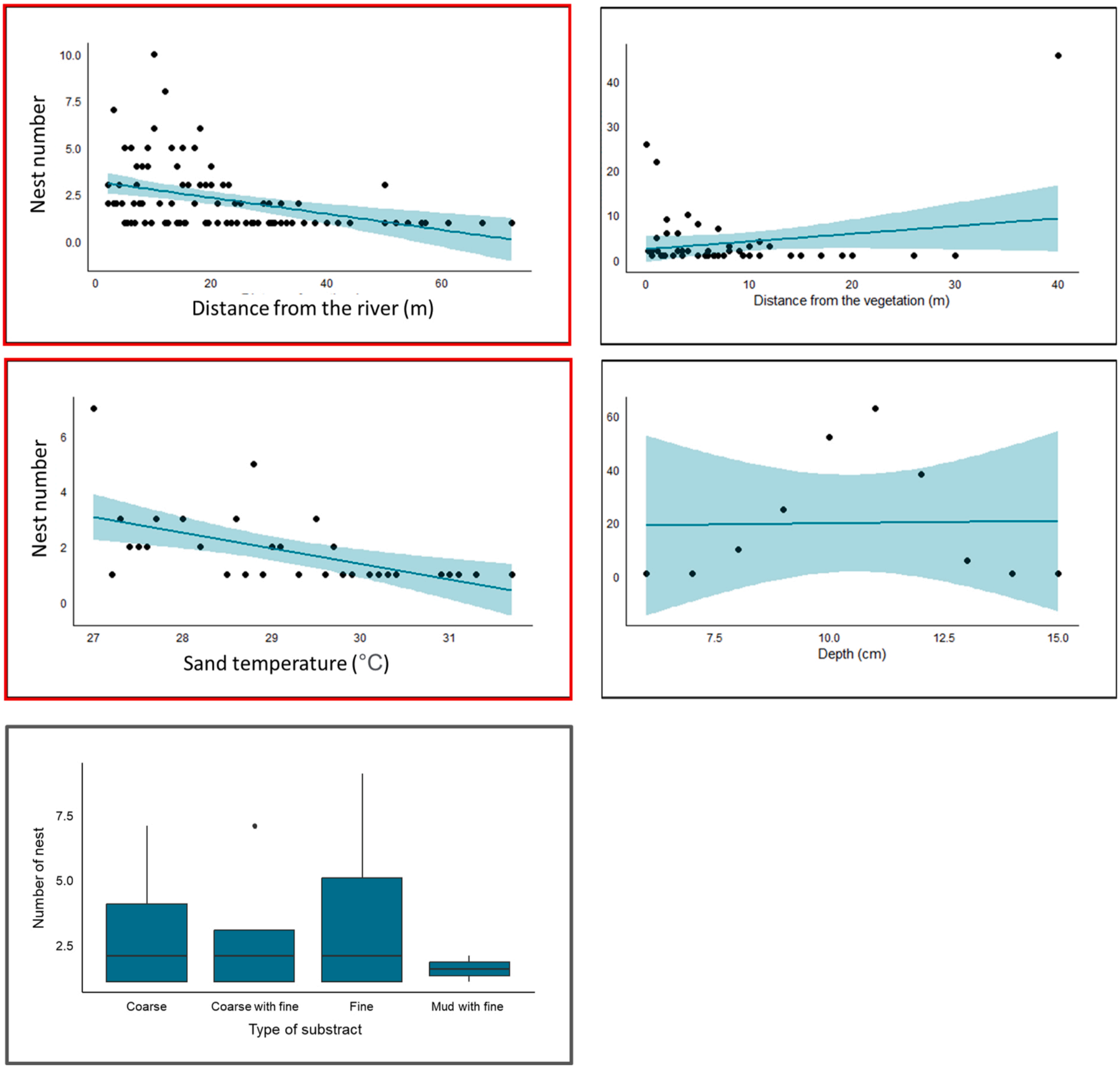

Fig. 2. Illustrates the relationship between each environmental characteristic and the number of nests. The highlighted red box indicates a significant relationship between the analyzed variables.

Fig. 3. A) Nest relocation to community hatcheries, B) hatching, C) hatchings in the water tanks, D) hatchlings being released into their natural habitat, E) nests in flooded sites having the eggs collected, F) egg being predated by *Tupinambis teguixin*. ©Luke McKenna/FAO.

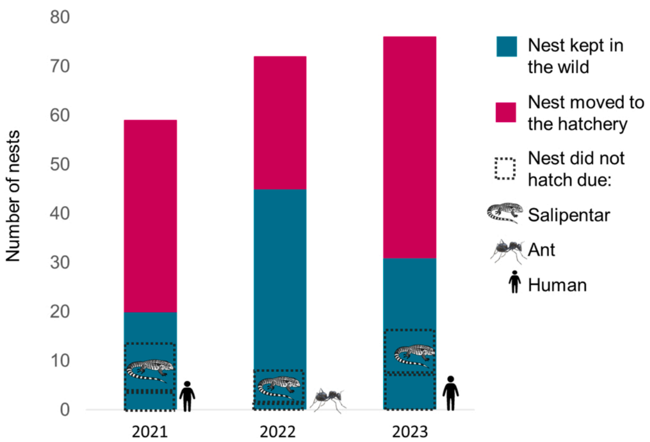

Fig. 4. Number of nests kept and monitored in the wild, moved to a hatchery in the community, and destroyed over the 3-year of nest monitoring.

Table 3
Details of the complete model and the null model using a generalized linear mixed model to verify the influence of environmental characteristics on the nesting predation rate. The predictor variable showing a significant result is highlighted in bold.

Response variable	Predictor variables	Estimate	Std. Error	z value	Pr (>|z|)		Model	AIC	AIC Null model	ΔAIC
Nesting predation	River distance (m)	0.1199	0.6562	0.183	0.8553		GLMM	167.56	194	33.29
	Vegetation distance (m)	**-1.1868**	**0.5545**	**-2.14**	**0.0336**	*				
	Substrate type (coarse: fine)	0.5532	0.5091	1.087	0.2785					
	Substrate type (coarse: fine/coarse mix)	0.6699	0.609	1.1	0.2727					
	Substrate type (coarse: fine/mud mix)	0.8033	1.1247	0.714	0.476					
	Depth (cm)	0.178	0.1522	1.169	0.2438					

ex-situ conservation actions, focusing on the eggs and hatchlings' stages of development in 9 sand beaches in the North part of Rupununi River.

5. Number of nests per beach and nesting characteristics

In our study, we observed a small variation in nest numbers across three consecutive years, but it is noteworthy that there was an average 2.1-fold increase in 2022 and a 2.6-fold increase in 2023 compared to 2021. The average of 6.68 nests per beach (SD=3.66) found in this study in the Essequibo basin is lower that recorded in the Orinoco River basin 60.28 (SD= 44.40) (Escalona and Fa, 1998), and in Jurua River basin 12 (SD=13.82) (Campos-Silva et al., 2018). Although we found a higher density in Essequibo than is those mentioned basins, we opt to compare raw numbers of nest per nesting sites (beaches) which provide us more useful information. However, distinctive nesting behaviors and habitat characteristics between the Essequibo and Jurua River basins underscore the need for caution in direct comparisons and more information on ecological nuances of each region, such as the total number and extension of nesting sites in each basin. This will ensure a more nuanced interpretation of the conservation status and total number of nests in the Essequibo River. In this study, we found an average clutch size of 20.45 eggs per nest (SD = 8.03). Similarly, the study conducted in the Orinoco River basin found an average clutch size of 20.1 ± 1.7 eggs (Escalona and Fa, 1998).

6. Nesting destruction and predation

The predation rate exhibited a decline in 2022 compared to both 2021 and 2023, attributable to the implementation of in-situ monitoring on the beaches. During 2022, the rangers were actively fortifying nests with wooden barriers to deter predators. Moreover, in 2022, a reduced number of nests were relocated to the hatchery due to a delayed onset of flooding in the season. The flooding occurred later, covering the sandy beaches with water within an 8-hour timeframe, enabling enough time to relocate all identified nests.

Our findings align with previous research, underscoring the significance of environmental factors in determining nesting success, particularly the distance from the river and vegetation (Pignati et al., 2013; Escalona and Fa, 1998). As also observed by MarinaTeófilo Pignati et al. (2013); Pignati et al. (2013), our study confirms that the main causes of nest loss include flooding, predation, and human collection. In the case of flooding, several river dynamics play a critical role in affecting hatching success for *P. unifilis*, including river level fluctuations, the timing of initial rises, nest height above the river, and the nesting period (Ferreira Júnior et al., 2010). Notably, a study conducted in the Trombetas River region in the eastern Brazilian Amazon found that the height of the nest above the river significantly influenced the likelihood of flooding during river level surges (Ponce de Leão et al., 2019).

It's worth mentioning that a study in the Orinoco River and other in the Amazonas river basin regions reported higher nest density in sites with higher elevations, further from the riverbank, and in proximity to vegetation (Escalona and Fa, 1998; Pignati et al., 2013). This may suggest that females tend to select areas with a lower risk of flooding. However, in our study, most of the nests were located in areas prone to flooding, especially in the years 2021 and 2023. Without the translocation action taken during those years, more than 50% of the nests would have been lost due to flooding. This contrasts with the Orinoco study, where only a small proportion of nests were affected by flooding (n = 8) or other environmental factors (n = 30) (Escalona and Fa, 1998).

Previous research suggested that fine sand is the primary substrate type explaining the proportion of *P. unifilis* nests removed by humans (Quintana et al., 2019; Michalski et al., 2020), because people can more easily find nests by visually following the nesting females or their characteristic tracks in the fine sand (Smith, 1979; Alho, 1985). However, our study did not find a significant influence of substrate type on nest depredation. Instead, the primary predator in our study area was the lizard *T. teguixin*, which appeared to rely on other environmental characteristics to detect nest presence, such as the proximity to vegetation, as indicated by our results. This finding is supported by a study conducted in Orinoco, which also observed a higher proportion of nests predated by animals located near vegetation, while those harvested by people were closer to the river (Escalona and Fa, 1998). Predation rates on turtle nests by the *T. teguixin* have been documented in other studies, being responsible for a predation rate of 89.1% of the *Phrynops geoffroanus* nests along the Guaporé River of the Brazilian and Bolivian Amazon (Schneider et al., 2011). Similarly, this predator was responsible for a significant proportion of nest predation incidents in our study, accounting for 66.6% of such events. Additionally, habitat loss for the lizard may contribute to shifting habitat utilization patterns and increased turtle nest depredation rates (Schneider et al., 2011).

While our study primarily observed nest losses due to animal predation rather than human interference, the research conducted in the Orinoco River basin reported a contrasting pattern. In the Orinoco study, human involvement accounted for 84.9% (n = 298) of all nest predation events, with the remaining 15.1% attributed to animals (n = 53), with animal predators destroying just 4.9% of the egg clutches, while 70.6% of the nests affected by human interference had all their eggs removed (Escalona and Fa, 1998). This disparity may be influenced by the time frame in which each study took place, considering that our study is more recent, and there may be enhanced conservation practices and systems in place compared to the study conducted in 1998.

7. Hatchling success *ex-situ*

In the pursuit of protecting turtle nests and promoting their successful hatching, various strategies have been employed in the Rupununi region over the past three years. Nest relocation was one such strategy required by the necessity to safeguard nests from flooding. An essential aspect of the discussion centers around nest predation. In 2021 and 2023, most nests were relocated to hatcheries, and in the wild, many of the remaining nests suffered predation (55.0% and 48.8%, respectively). On the other hand, in 2022, a significant number of nests were left in the wild, and the incidence of predation was notably lower.

The relocation of turtle nests, as observed in our study, has raised concerns regarding potential negative effects on hatchlings. These concerns include the alteration of sex ratios (Godfrey and Mrosovsky, 1999), reduced hatching success (Mortimer, 1999), potential distortions in gene pools (Mrosovsky, 2006), and the possibility of anatomical differences in hatchlings from relocated nests compared to in situ nests, such as variations in carapacial scute patterns, sizes, and weights (Türkozan and Yılmaz, 2007). However, once flooding emerges as the primary cause of nesting destruction, relocating turtle nests remains the most viable option for enhancing hatching success in areas susceptible to inundation. Moreover, with the looming threat of climate change, controlling temperature within the hatchery becomes an essential tool for regulating the sex ratio within the turtle population, ensuring its long-term health and stability. To mitigate the potential issues previously mentioned, our program focuses on collecting eggs exclusively from regions at risk of flooding, thereby safeguarding both hatchling survival and the ongoing conservation efforts. To further enhance the mitigation efforts, collected eggs were meticulously repositioned with their original orientation in plastic basins filled with beach sand to match the original nest depth. Additionally, our hatcheries are thoughtfully designed to closely mimic a natural habitat, with dimensions of 20 by 20 feet and a height of 3 feet, with strict temperature control within a range of 32 to 33 degrees Celsius at 12PM to ensure a balanced sex ratio. Furthermore, the eggs are transported to a hatchery located near the beach, constructed with beach sand and within native trees which provide sunshade. This is to maintain consistent humidity levels and other environmental characteristics that can impact temperature. Finally, considering hatching rate in the hatcheries is similar to the hatching rate in the wild this may suggest that the controls Caiman House are putting in place are effective.

The conservation efforts in the Rupununi region have made significant strides in protecting turtle nests and promoting successful hatching. The fluctuation in nest numbers across the years underscores the need for ongoing monitoring and adaptation of conservation strategies. The prevalence of nest predation by lizards and humans, highlights the importance of safeguarding nests from such threats. The practice of nest relocation, while raising concerns about potential consequences for hatchlings, shows effective hatchling success contributing to the release of several hundreds of turtles back to the river each year. Our next steps are twofold. First, we aim to support communities in their effort to raise awareness and influence governmental management for example, by promoting involvement of the Protected areas Commission and the Guyana Wildlife Conservation and management Commission in turtle monitoring activities. Second, we will continue to conduct research and monitor the effects of our conservation efforts, including the practice of nest relocation, to ensure that we are effectively protecting the fragile turtle populations.

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CRediT authorship contribution statement

Braga-Pereira Franciany: Writing – original draft, Formal analysis. **Roberts Rudolph Anthony:** Project administration, Methodology, Investigation, Data curation. **Millar Neal:** Writing – review & editing, Methodology, Investigation, Data curation. **van Vliet Nathalie:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Formal analysis.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

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