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Article

Factors Affecting Nest Site Selection by Freshwater Turtles: A Systematic Review

Andressa Estela Balbino Guimarães [1], Thays Nogueira Lobo Ribeiro[2], Ana Beatriz Nunes Ribeiro [3], Rodrigo Diana Navarro[4]

[1] MSc in Environmental Sciences, University of Brasília (UnB). ORCID: 0000-0002-0814-5592. E-mail: andressa.esb@gmail.com

[2] MSc in Environmental Sciences, University of Brasília (UnB). ORCID: 0000-0002-8199-3846. E-mail: thays.n.lobo@gmail.com

[3] MSc in Tropical Biodiversity**,** Federal University of Amapá (UNIFAP). ORCID: 0000-0001-8081-1986. E-mail: nunescpc@gmail.com

4 PhD in Zootechnics, Federal University of Minas Gerais (UFMG). ORCID: 0000-0001-8897-7163. E-mail: navarrounb@gmail.com

ABSTRACT

Nest site selection is an important behavior, particularly in species that lack parental care, as nest microclimate can greatly impact embryos' survival and offspring phenotype. Understanding which factors influence oviposition decisions is critical to identifying priority areas for conservation. This systematic review aimed to synthesize the factors that affect nest site selection by freshwater turtles. The search was conducted in the Web of Science database. In total, 35 publications met the eligibility criteria. The first study on the topic was published more than three decades ago, but only within the last ten years have studies started to be published annually. More than half of the studies were conducted in the United States and the most studied species was *Chrysemys picta*. Environmental factors, mainly vegetation characteristics, but also soil properties and beach morphology, are the most studied. Anthropogenic factors have been increasingly studied, especially residential areas and agricultural fields. Some studies suggest that human-altered habitats may act as ecological traps, emphasizing the need for further research on this topic. More studies are also needed on the influence of predation risk, co-species cues, female size, and offspring phenotype on nest site selection by freshwater turtles. We highlight that understanding the factors affecting nest site selection is important for identifying the habitat needs of nesting females, predicting possible ecological implications of nest site choice, assessing the adaptive potential of species to environmental change, and determining the vulnerability of populations to different threats.
Keywords: chelonian; testudine; habitat selection; spawning; oviposition.

RESUMO

A seleção do local de nidificação é um comportamento importante, particularmente em espécies que carecem de cuidados parentais, pois o microclima do ninho pode ter grande impacto na sobrevivência dos embriões e no fenótipo dos filhotes. Compreender quais fatores influenciam as decisões sobre a oviposição é fundamental para identificar áreas prioritárias para a conservação. Esta revisão sistemática visa sintetizar os fatores que afetam a seleção do local de nidificação pelas tartarugas de água doce. A pesquisa foi conduzida no banco de dados da *Web of Science*. No total, 35 publicações preencheram os critérios de elegibilidade. O primeiro estudo sobre o tema foi publicado há mais de três décadas, mas somente nos últimos dez anos os estudos começaram a ser publicados anualmente. Mais da metade dos estudos foi realizada nos Estados Unidos e a espécie mais estudada foi *Chrysemys picta*. Os fatores ambientais, principalmente as características da vegetação, mas também as propriedades do solo e a morfologia da praia, são os mais estudados. Fatores antrópicos têm sido cada vez mais estudados, especialmente áreas residenciais e campos agrícolas. Alguns estudos sugerem que habitats alterados pelo homem podem atuar como armadilhas ecológicas, enfatizando a necessidade de mais pesquisas sobre este tópico. Mais estudos também são necessários sobre a influência do risco de predação, pistas de indivíduos da mesma espécie, tamanho da fêmea e fenótipo dos filhotes na seleção do local de nidificação pelas tartarugas de água doce. Destacamos que a compreensão dos fatores que afetam a seleção do local de nidificação é importante para identificar as necessidades de habitat das fêmeas de nidificação, prever possíveis implicações ecológicas da escolha do local de nidificação, avaliar o potencial adaptativo das espécies às mudanças ambientais e determinar a vulnerabilidade das populações a diferentes ameaças.
Palavras-chave: quelônio; testudine; seleção de habitat; desova; oviposição.

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1. Introduction

Nest site selection can be defined as the female's non-random choice of a particular site for nesting within a certain area (Wilson 1998). In oviparous species, the decision about where to oviposit can have serious consequences for species reproductive success, as the nest site affect egg development and survival, offspring phenotype, post-hatching growth rate, locomotor performance, and female fitness (Refsnider & Janzen 2010; Delaney and Janzen 2018; Erickson et al. 2020). Nest site selection is especially important for species with minimal parental care since parents will not provide nest guarding, egg attendance, and thermoregulation after oviposition (Czaja et al. 2020).

The turtles do not provide parental care during the incubation period and nearly all species present temperature-dependent sex determination (Refsnider & Janzen 2015; Czaja et al. 2020). Hence, the nesting site will define the humidity and temperature of the incubating eggs, affecting not only the embryos' survival, but also the offspring sex ratio (Refsnider & Janzen 2010; Navarro & Alves 2021). Nest site selection can also affect the risk of depredation (Kolbe & Janzen 2002c) and inundation of the eggs (Castro & Ferreira Júnior 2008), the probability of hatchlings reaching the water (Kolbe & Janzen 2002a), and the survival of nesting females (Spencer 2002). Therefore, nest site selection plays a significant role in demographic parameters and in selective pressures acting on turtles' populations, and is of great ecological and evolutionary importance (Kolbe & Janzen 2002b; Refsnider & Janzen 2010).

Chelonians are one of the most endangered groups of vertebrates. There are about 356 species of turtles in the world, of which seven have become extinct and 179 are listed as endangered species by the International Union for the Conservation of Nature and Natural Resources (IUCN) (Turtle Conservation Coalition 2018). For that reason, suitable habitats for nesting turtles are important to increase the recruitment of new individuals to their populations (Micheli-Campbell et al. 2013). Chelonians search for suitable nesting sites according to various environmental and anthropogenic factors (Quintana et al. 2019; Mahbub et al. 2020). However, it is uncertain which factors can restrict the female's nest-site choice and which cues are more decisive (Silva et al. 2020).

Identifying the factors that influence nest site choice by chelonians is important for their conservation, since the nest location brings relevant consequences for the reproductive success of this group whose life history is characterized by low recruitment rates of individuals. With this information, it is possible to predict the adaptive potential of species to environmental changes and determine the vulnerability of chelonians to different threats (Refsnider & Janzen 2010). This knowledge can also assist in understanding the consequences of human disturbance for the reproduction of turtles (Kolbe & Janzen 2002b) and support the development of conservation actions and impact mitigation strategies for freshwater turtle populations. Although several studies have already identified environmental and anthropogenic factors that influence nest site selection by freshwater turtles (Quintana et al. 2019), there is still no synthesis of these data. For this reason, we performed a systematic review to synthesize the factors that affect nest site selection by freshwater turtles.

2. Methods

The systematic review was performed according to the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) statement (Page et al. 2021). The search was performed using the *Web of Science Core Collection database* and focused on scientific papers published until 10 January 2021.

To capture the greatest number of relevant publications, we searched for articles with the following combinations of terms in their title, abstract, and/or keywords: (turtle* OR tortoise* OR cheloni* OR testudin*)

AND ("nest* site*" OR "nest* place*" OR "nest* location*" OR "nest* area*" OR "nest* beach*" OR "nest* habitat") AND (choice OR select*) (Table 1). It was not specified in the search that the focus of this review is freshwater turtles because terms related to "freshwater" do not always appear in the title, abstract or keywords, since some articles mentioned only the species name and not their habitat. For the same reason, terms related to "influencing factors" were not included in the search.

Table 1. Search strategy for nest-site selection studies in turtles.

Descriptors	
Related to turtle	Turtle, tortoise, chelonian, testudine
Related to nest site	Nesting site, nesting location, nesting place, nesting area, nesting beach, nesting habitat
Related to selection	Selection, choice

As eligibility criteria, papers had to (1) investigate factors that influence the nest-site selection of turtles; (2) address freshwater turtles; (3) be a scientific research paper. Therefore, we excluded (1) articles that did not address factors that influence the nest-site selection of turtles; (2) publications that did not address freshwater turtles; (3) non-research articles. Two independent reviewers evaluated whether each article met the eligibility criteria based on their title and abstract contents. Designations were compared and, when discrepancies occurred, a third reviewer was consulted.

The selected publications were thoroughly read to ensure they met the above-mentioned eligibility criteria and to obtain the following information: (1) authors and year of publication, (2) turtle species, (3) evaluated factors influencing nest-site selection, (4) method used, and (5) study-area location. The data extracted from each article were tabulated and then processed by Excel to generate charts and map.

The factors evaluated by the articles were classified into: vegetation characteristics (vegetation, canopy and litter cover, vegetation height and type, and distance to vegetation); substrate characteristics (particle size, organic content, soil temperature and moisture); beach morphology (beach elevation, area, width, length and slope); river-related factors (water depth and proximity); climatic conditions (ice-off date, latitude, and environmental conditions); predation risk; conspecific cues (visual and olfactory cues from individuals of the same species); female size; offspring phenotype (egg size); residential areas; anthropogenic land use (agriculture and industrial use); and intensity of anthropogenic activities. Due to the wide variety of spatial scales, sample sizes, methods, and contexts among the reviewed articles, it was not feasible to perform a meta-analysis.

2. Results

The search retrieved 413 studies, of which 35 publications met the eligibility criteria (Figure 1). The search strategy was conducted comprehensively to include as many relevant articles present in the Web of Science database as possible. As such, it was possible to verify that most studies on nest site selection focus on the plasticity of this maternal behavior and the consequences for reproductive success, not evaluating the factors that may influence the turtles' choice. It also became evident that most articles investigating factors affecting nest site selection by turtles involve sea turtles (59 articles) and not freshwater turtles, which is the focus of this systematic review.

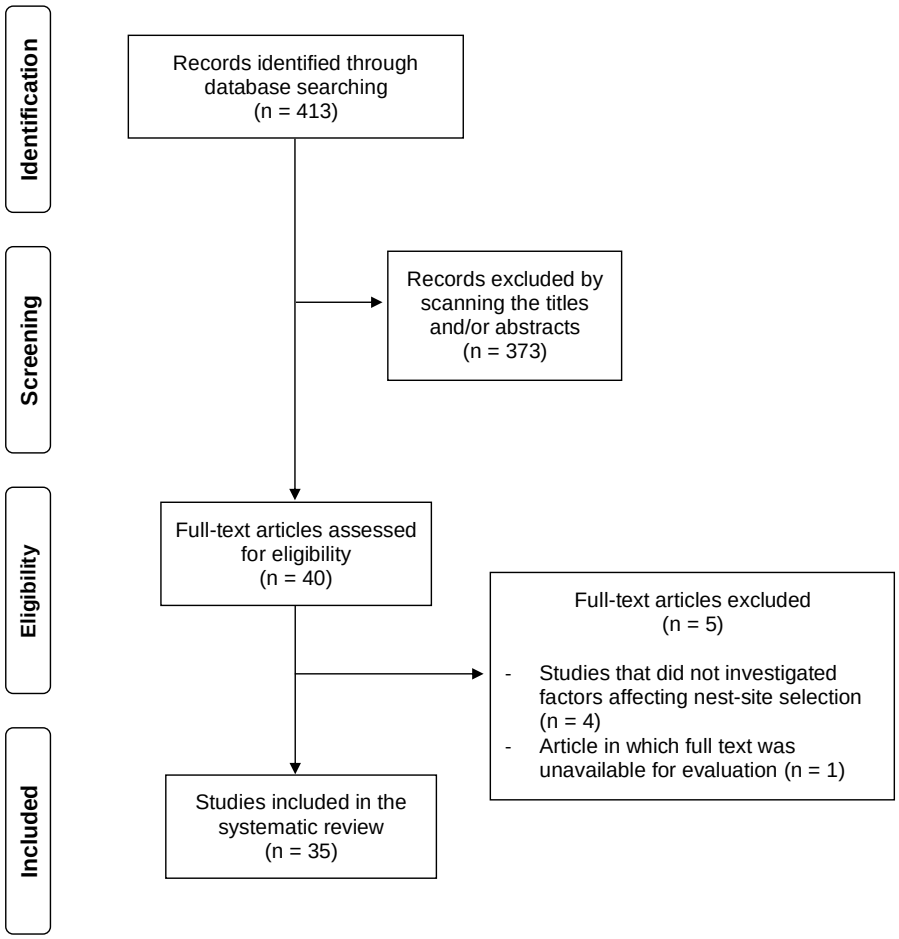

Figure 1. Flow diagram of the selection process of the studies that were included in the systematic review. Source: research data.

The earliest article on factors that influence nest-site selection by freshwater turtles was published in 1987 (Schwarzkopf & Brooks 1987). Publications continued only nine years later (Roosenburg 1996), and since 2012, articles on the topic have been published annually, reaching five publications on 2013 (Figure 2A). Most studies were conducted in the United States (20 articles), followed by Australia (5 articles), Canada (4 articles), and Brazil (3 articles) (Figure 2B). In total, fourteen species were studied. *Chrysemys picta* was the most studied species (13 articles), followed by *Podocnemis unifilis* (4 articles), *Chelydra serpentina* and *Malaclemys terrapin* (3 articles each) (Figure 2C). The studies evaluated a wide range of variables that can be affecting nest site selection (Table 2). The most studied ones were related to environmental factors, such as vegetation characteristics (19 studies), soil characteristics (12 studies), beach morphology (11 studies) and river characteristics (8 studies). The influence of anthropic factors, such as the influence of urban areas (4 studies), anthropic land use (3 studies) and intensity of human activities (2 studies), was also assessed (Figure 2D).

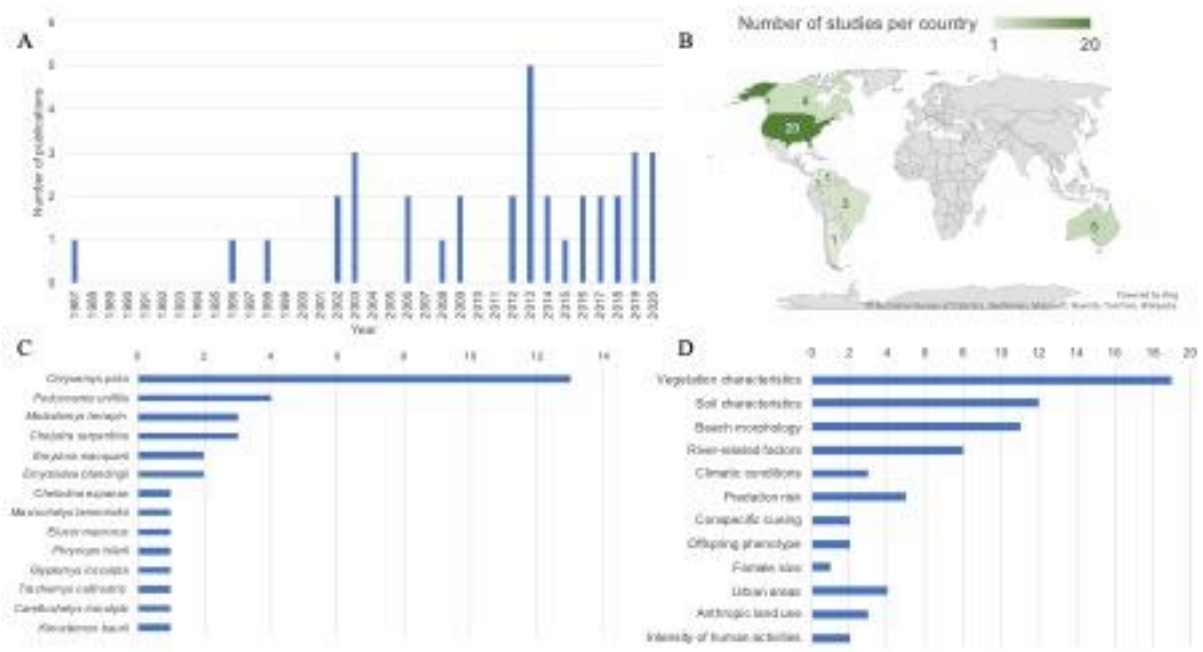

Figure 2. Descriptive characteristics of the 35 reviewed publications. Number of studies per (A) year of publication; (B) country; (C) species studied; and (D) categories of factors investigated. Source: research data.

Table 2. Description of publications investigating factors that affect nest-site selection by freshwater turtles.

Reference (year)	Turtle species	Investigated factors underlying nest site selection	Method	Country
Erickson et al. (2020)	*Podocnemis unifilis*	Nesting substrate, Female size	Comparison (nest sites on sand banks versus clay banks)	Brazil
Delaney and Janzen (2020)	*Chrysemys picta*	Predation risk	Experimental (simulated predation) and comparison (nest sites chosen before versus after)	USA
Czaja et al. (2020)	*Malaclemys terrapin*	Soil cover, Canopy cover	Comparison (nest sites versus random locations)	USA
Quintana et al. (2019)	*Podocnemis unifilis*	Riverine settlements, Different intensities in human activity, Size of nesting area, Nesting site type (island or bank), Presence of trees, Soil characteristics	Comparison (environmental and anthropogenic variables versus nesting patterns at different nesting sites)	Brazil
Pruett et al. (2019)	*Chrysemys picta*	Canopy cover, Soil temperature	Comparison (nest sites versus random locations)	USA
Byer et al. (2019)	*Emydoidea blandingii*	Predation risk, Temperature exposure	Comparison (nest sites versus random locations)	USA

Reference (year)	Turtle species	Investigated factors underlying nest site selection	Method	Country
Delaney et al. (2018)	*Chelydra serpentina*	Offspring dispersal ability	Experimental (artificial nest) and comparison (nest site location versus offspring dispersal)	USA
Petrov et al. (2018)	*Chelodina expansa*	Elevation, Water distance, Ground cover, Canopy cover, Habitat type	Comparison (nest sites versus non-nest sites)	Australia
Mitchell et al. (2017)	*Chrysemys picta*	Ice-off date	Experimental (simulated early and late ice-off) and comparison (nest sites versus available)	USA
Thompson et al. (2017)	*Chelydra serpentina*	Anthropogenic land use (soil contamination by mercury)	Comparison (nest site selection in mercury polluted versus reference sites)	USA
Iverson et al. (2016)	*Chrysemys picta*	Odors from previous nests	Experimental (urine-treated sites) and comparison (nest sites versus random locations)	USA
Mui et al. (2016)	*Emydoidea blandingii*	Agriculture	Comparison (nest site selection in a park versus an agricultural landscape)	Canada
Refsnider et al. (2015)	*Chrysemys picta bellii*	Predation pressure	Experimental (models to estimate predation) and comparison (nest distance to water versus nest and female survival)	USA
Refsnider et al. (2014)	*Chrysemys picta bellii*	Climatic conditions	Comparison (nest site choice in populations along a latitudinal gradient)	USA
Miller et al. (2014)	*Macrochelys temminckii*	Distance to the shoreline, Elevation, Slope at the site and at the water, Water depth, Canopy cover, Ground cover	Comparison (nest sites versus random locations)	USA
Pignati et al. (2013)	*Podocnemis unifilis*	Distance to river, Distance to vegetation,	Comparison (nest sites versus random locations)	Brazil

Reference (year)	Turtle species	Investigated factors underlying nest site selection	Method	Country
		Height, Slope, Grain-size		
Micheli-Campbell et al. (2013)	*Elusor macrurus*	Width and elevation of the bank, Slope angle, Lengths at the top and at the bottom of the bank, Slope aspect, Soil characteristics	Comparison (nesting versus nonnesting banks)	Australia
López et al. (2013)	*Phrynops hilarii*	Habitat categories: wetlands, natural terrestrial environment (grassland, forest, and savanna), agricultural fields and anthropogenic environment (cities, towns, streets, roads)	Comparison (nest sites versus random locations)	Argentina
Mitchell and Walls (2013)	*Malaclemys terrapin*	Canopy cover, Ground cover	Comparison (observed versus expected nesting frequency)	USA
Refsnider et al. (2013)	*Chrysemys picta bellii*	Shade cover	Comparison (nest sites versus availability)	USA
Refsnider and Janzen (2012)	*Chrysemys picta*	Environmental conditions	Experimental (transplanted populations)	USA
Foley et al. (2012)	*Chrysemys picta*	Ground cover in anthropic environment	Comparison (nest sites versus random locations)	USA
Escalona et al. (2009)	*Podocnemis unifilis*	Vegetation cover, Leaf cover, Soil characteristics (moisture content, particle size and temperature), Elevation, Slope, Distance to water, Distance to vegetation, Social facilitation	Comparison (nest sites versus random locations) a	Venezuel
Hughes et al. (2009)	*Glyptemys insculpta*	Soil characteristics (moisture content, organic content, grain- size distribution and temperature), Distance to water, Distance to vegetation,	Comparison (real nests versus false nests at nesting versus nonnesting beaches)	Canada

Reference (year)	Turtle species	Investigated factors underlying nest site selection	Method	Country
		Beach characteristics (elevation, vegetation)		
Bowen and Janzen (2008)	*Chrysemys picta*	Human recreation activity (number of recreational vehicles)	Comparison (daily number of recreational vehicles versus nesting patterns)	USA
Restrepo et al. (2006)	*Trachemys callirostris callirostris*	Ground cover, Soil type, Distance to the shoreline	Comparison (nest sites versus availability)	Colombia
Hughes and Brooks (2006)	*Chrysemys picta marginata*	Slope angle, Slope aspect, Understory vegetation cover, Canopy cover	Comparison (nest sites versus random locations)	Canada
Spencer and Thompson (2003)	*Emydura macquarii*	Predation risk, Vegetation height	Experimental (fox removal and grass mowing) and comparison (nest sites versus random locations)	Australia
Morjan (2003)	*Chrysemys picta bellii*	Climatic conditions	Comparison (nest sites versus random locations in New Mexico and Illinois)	USA
Doody et al. (2003)	*Carettochelys insculpta*	Substrate moisture, Sand temperature, Beach height, Beach slope, Beach aspect, Water depth at approach, Height of cohesive sand line, Solar exposure	Comparison (nesting versus nonnesting beach)	Australia
Spencer (2002)	*Emydura macquarii*	Predation risk	Experimental (fox removal) and comparison (control areas versus removal areas)	Australia
Kolbe and Janzen (2002)	*Chelydra serpentina*	Habitat types, Presence of houses, Slope, Slope aspect, Distance from the water, Canopy cover, Ground cover,	Comparison (nest sites versus random locations and observed versus expected nesting frequency)	USA

Reference (year)	Turtle species	Investigated factors underlying nest site selection	Method	Country
		Vegetation characteristics		
Wilson (1998)	*Kinosternon baurii*	Ground cover, Soil characteristics (water content, organic content, texture)	Comparison (nest sites versus random locations)	USA
Roosenburg (1996)	*Malaclemys terrapin*	Egg size	Comparison (nest sites versus random locations)	USA
Schwarzkopf and Brooks (1987)	*Chrysemys picta*	Distance, height, and type of the nearest vegetation, Density of vegetation cover, Slope and orientation of the slope	Comparison (nest sites versus random locations)	Canada

3. Discussion

Most studies investigating possible factors influencing nest site selection by freshwater turtles took a comparative approach and focused on determining whether differences exist between nest and random site variables. Other studies have used an experimental approach, which involves manipulating a variable to determine if alterations in that variable cause changes in nest site selection. According to Wilson (1998), the comparative approach allows the habitat needs of nesting females to be assessed, but the experimental approach provides more definitive information about the possible consequences of habitat selection, which helps researchers make more informed decisions about habitat management.

The first study evaluating possible variables affecting nest site selection in freshwater turtles was published more than three decades ago (Schwarzkopf & Brooks 1987). Only within the last ten years have studies started to be published annually, but there has not been a gradual increase in the number of studies over time. Most studies are concentrated in North America, mainly in the United States, where more than half of the studies have been conducted. No studies have been recorded in Europe, Asia, and Africa. The most studied species is *Chrysemys picta*, which has been the focus of most studies in the United States and two studies in Canada. The second most studied species is *Podocnemis unifilis*, which was the only species investigated by Brazil and Venezuela. Next, the most studied species were *Chelydra serpentina* and *Malaclemys terrapin*, both the focus of studies conducted in the United States.

These results show a geographical bias in the available scientific literature. However, this may be because the systematic review was restricted to scientific research articles published in the Web of Science database. This platform was selected due to its relevance (Cavacini 2015) and comprehensiveness (Bramer et al. 2017), but the use of a single database may have excluded some relevant research on factors influencing turtle nest site selection. Moreover, studies from grey literature were not selected.

Among the factors that may influence nest site selection, the most investigated is vegetation, which plays an important role in the thermal conditions experienced by the embryos. Studies in North America and Australia have shown that some freshwater turtles select nesting sites in open, sun-exposed areas with little or no vegetation (Schwarzkopf & Brooks 1987; Spencer & Thompson 2003; Hughes & Brooks 2006; Hughes et al. 2009; Mitchell & Walls 2013; Miller et al. 2014; Petrov et al. 2018; Pruett et al. 2019; Czaja et al. 2020).

This preference for sites with little or no vegetation is probably because it increases the likelihood that eggs will incubate at optimal temperatures for development (Wilson 1998; Miller et al. 2014; Pruett et al. 2019; Czaja et al. 2020) and because higher temperatures minimize the incubation period, which increases the chance of offspring survival (Spencer & Thompson 2003; Hughes et al. 2009; Petrov et al. 2018). In addition, areas away from vegetation reduce predation risk (Thompson et al. 2017), prevent nest invasion by roots (Hughes et al. 2009), and facilitate nest cavity construction (Petrov et al. 2018).

On the other hand, some studies conducted in South America have shown that some freshwater turtles select nesting sites close to vegetation and avoid open areas (Restrepo et al. 2006; Pignati et al. 2013; Quintana et al. 2019). The shade provided by trees allows nests to maintain adequate temperature and humidity levels during egg incubation (Pignati et al. 2013; Refsnider et al. 2013; Quintana et al. 2019). Besides that, vegetation cover helps reduce thermal stress to nesting female and detection by predators (Restrepo et al. 2006).

Wilson (1998) believes that nest site selection based on vegetation cover can be explained by turtles' body size. According to the author, smaller species prefer to nest in areas with vegetation cover to protect their embryos from thermal extremes, since they dig relatively shallower nests than larger species, which reach higher daily temperatures. Conversely, since larger species dig relatively deeper nests, they prefer to nest in open areas so that the eggs can reach the proper incubation temperature for embryonic development (Wilson 1998). Another study showed that in individuals of the same species the opposite happens: larger females select nesting sites closer to the vegetation. The researchers suggest that more mature females occupy the best sites and that smaller females avoid competition with the more dominant individuals in the population by nesting at sites away from the vegetation (Erickson et al. 2020).

Nest site selection based on vegetation cover may also be a means for turtles to control the sex ratio of their offspring (Roosenburg 1996; Czaja et al. 2020). Roosenburg (1996) demonstrated that females of *Malaclemys terrapin* that produce large eggs prefer nesting in open areas and those that produce smaller eggs select sites with vegetation. The author suggests that this is a mechanism to skew the offspring sex ratio because since it is advantageous for larger offspring to be female, turtles choose to lay large eggs at sites where the incubation temperature will be higher, increasing the chance of hatching females.

Another study corroborates this evidence that females alter nest site selection according to offspring phenotype. Delaney and Janzen (2018) demonstrated that females that produce heavier offspring, and therefore have greater dispersal ability, build nests farther from the water than those that produce lighter offspring. The authors suggest that females that produce good dispersers choose sites farther from the water to reduce the risk of nest predation, while females that produce lighter offspring nest closer to the water because their offspring are less able to survive during dispersal at greater distances (Delaney & Janzen 2018).

Substrate is also an important factor for turtles' nest site selection (Doody et al. 2003; Restrepo et al. 2006; Hughes et al. 2009; Quintana et al. 2019; Erickson et al. 2020). The selection of specific soil characteristics may be associated with the ease of nest construction (Doody et al. 2003; Restrepo et al. 2006) and the heat and moisture retention capacity of the soil (Restrepo et al. 2006; Hughes et al. 2009; Erickson et al. 2020). Clay-rich soils retain less heat and hold more water than sandy soils (Hughes et al. 2009; Erickson et al. 2020). These physical characteristics of different substrate types affect egg incubation duration, sex ratio, growth, and offspring survival (Erickson et al. 2020).

Therefore, some species choose sandy sites formed by relatively large grains, because the dry and low humic soil promotes the necessary warmth for egg incubation (Hughes et al. 2009). Other species, on the other hand, select sites with a more humid substrate to nest (Wilson 1998; Doody et al. 2003; Restrepo et al. 2006), avoiding that the loose consistency of the sand hinders nest construction (Doody et al. 2003).

As for beach characteristics, the most studied factors are elevation and slope. Beach elevation is an important characteristic for nest site selection by chelonians because nests located in lower areas may be more prone to flooding (Castro & Ferreira Júnior 2008). In this systematic review, all studies where beach elevation was considered a significant factor in nest site selection revealed that turtles select elevated regions for nesting (Hughes et al. 2009; Pignati et al. 2013; Miller et al. 2014; Petrov et al. 2018).

Slope aspect influence the thermal characteristics of the site, as it determines the amount of direct solar radiation received (Micheli-Campbell et al. 2013; Miller et al. 2014). A study conducted in the Southern Hemisphere showed that steep north-facing slopes are selected for nesting because they have higher mean daily temperatures than other sites (Micheli-Campbell et al. 2013). Accordingly, studies conducted in Northern Hemisphere countries demonstrated that turtles select nesting sites on south-facing slopes where nests experience warmer temperatures (Schwarzkopf & Brooks 1987; Miller et al. 2014). In addition, some turtles prefer to nest on steeper slopes to avoid nest flooding during periods of heavy rainfall (Miller et al. 2014).

Distance to the river is another important factor in nest site choice because it influences the risk of nest flooding, predation of females, and disorientation of hatchlings (Hughes et al. 2009; Pignati et al. 2013; Petrov et al. 2018). Moreover, Miller et al. (2014) demonstrated that water depth near the nesting beach is also a factor taken into consideration by *Macrochelys temminckii* females, as it provides additional protection, not only for females waiting near the beach before nesting, but also for offspring after hatching.

According to Restrepo et al. (2006), nest site preferences of a species can vary both geographically and temporally depending on climatic variation, indicating a certain flexibility on the part of females. Some researchers have corroborated this finding by demonstrating that populations of *Chrysemys picta* adjust nest site selection in response to environmental conditions by changing the amount of canopy cover, distance to water, and soil moisture content (Refsnider & Janzen 2012; Refsnider et al. 2014). However, other studies conducted with the same species have shown that females do not place nests under greater canopy cover to compensate for warmer weather conditions (Morjan 2003; Mitchell et al. 2017), but rather dig deeper nests closer to the water (Morjan 2003).

Another factor that may affect nest site selection by turtles is the predation risk. This factor was considered relevant in studies conducted with *Emydura macquarii*, leading females to nest closer to the water in areas of high risk of predation (Spencer 2002; Spencer & Thompson 2003). In contrast, Delaney and Janzen (2020) found no evidence that females nest closer to water in response to predation but reported that there was an increased probability of nest predation after simulating nesting female predation, suggesting that females altered nest site choice in a way that was not quantified. Still, other studies have found no evidence that predation risk significantly alters nest site choice, indicating that females more strongly select for other variables, such as suitable incubation conditions (Refsnider et al. 2015; Byer et al. 2019).

Certain species appear not to assess the characteristics of potential nest sites, relying instead on the nesting decisions of conspecific females through the use of visual and olfactory cues (Escalona et al. 2009; Iverson et al. 2016). Females of *Podocnemis unifilis*, for example, choose nesting sites by following other females of the same species rather than considering environmental factors on their own. Consequently, females nest close together, reducing predation risk and increasing hatching rates (Escalona et al. 2009). *Chrysemys picta* females, on the other hand, use olfactory cues from co-species individuals to choose a nesting site, building their nests where they detect the odor of other existing nests (Iverson et al. 2016).

The influence of anthropic factors, such as residential areas, agriculture, and tourism, on nest site selection by freshwater turtles has been increasingly studied (Kolbe & Janzen 2002b; López et al. 2013; Mui et al. 2016; Quintana et al. 2019). Human activity can lead to a reduction in the amount of available vegetation and

anthropogenic structures can act as barriers, modifying the nesting pattern of turtles, as well as increasing nest predation rates (Kolbe & Janzen 2002b).

One study already showed that *Phrynops hilarii* avoids agricultural fields and anthropic areas, preferring natural habitats (López et al. 2013). Another study found that *Podocnemis unifilis* females use sites near human settlements for nesting, but with increasing proximity to houses females nest closer to water, even though this may increase the risk of flooding (Quintana et al. 2019). Kolbe and Janzen (2002) also support the idea that anthropogenic disturbances can affect nest site selection in turtles. They reported that although *Chelydra serpentina* selects sandy substrate when nesting in protected areas, in residential areas females shift to grassy areas with greater canopy cover, near houses and fences, and with cooler temperatures. These differences in nest site selection alter offspring sex ratio and decrease nesting success, therefore, residential areas can act as ecological traps (Kolbe & Janzen 2002b).

According to Mui et al. (2016), agricultural fields can also act as ecological traps, since turtles cannot anticipate the rapid growth of plantations, which results in a significant increase in vegetation cover, changing critical environmental characteristics of nests: temperature and humidity. In addition, embryos are exposed to pesticides, nests can be damaged during planting or harvesting, and farm machinery poses threats to hatchlings and adults (Mui et al. 2016). Thompson et al. (2017) also state that agricultural fields are unsuitable nesting habitat for freshwater turtles, as it impairs the orientation of hatchlings to water (Congdon et al. 2015) and biases sex ratio towards males (Freedberg et al. 2011). In addition, the researchers showed that mercury soil contamination can affect nest site selection in turtles, which begin to select sites with higher soil water content (Thompson et al. 2017).

Although most studies state that areas modified by human activity can act as ecological traps for nesting turtles, one study states that golf courses can provide suitable nesting habitat in anthropogenic areas (Foley et al. 2012). This study showed that on golf courses *Chrysemys picta* females select areas covered with litter for nesting due to less human disturbance in these areas and higher soil temperature and moisture, which increases hatching success (Foley et al. 2012). Bowen and Janzen (2008) also did not observe negative impacts of anthropogenic activities on turtle nesting while investigating the influence of tourism on nest site selection. It was expected that as the level of human recreational activity increased, females would perceive a greater risk to themselves and the distance from the nests to the water would decrease (Spencer 2002) and that, consequently, the vegetation cover of the nests would also decrease (Kolbe & Janzen 2002c). However, human activity, as measured by the number of recreational vehicles on a nesting beach, does not appear to affect nest site selection by *C. picta* (Bowen & Janzen 2008).

4.Conclusion

Studies on factors influencing nest site selection by freshwater turtles are mainly concentrated in the United States and the most studied species is *Chrysemys picta*. Most studies investigated environmental factors, mainly vegetation characteristics, but also substrate characteristics and beach morphology. Females rely on these environmental factors to predict future nest conditions to ensure adequate temperature and humidity levels for embryo development and survival. The influence of anthropogenic factors on nest site selection has been increasingly studied, particularly urban areas and agriculture. Some studies suggest that human-altered habitats may act as ecological traps, emphasizing the need for further research on this topic. More studies are also needed on the influence of predation risk, co-species cues, female size, and offspring phenotype on nest site selection by freshwater turtles.

Understanding the factors affecting nest site selection is important for identifying the habitat needs of nesting females, predicting possible ecological implications of nest site choice, assessing the adaptive potential of species to environmental change, and determining the vulnerability of populations to different threats. Since a female's decision on where to build her nest may vary geographically and temporally in response to different environmental conditions and adaptive pressures, long-term research with species of wide geographic distribution has much to offer to inform the development of management strategies and conservation actions. Furthermore, future studies should include experimental manipulation of specific variables to identify the most relevant factors in nest site selection, as this is difficult to determine through observational studies.

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