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The Influence of Researcher Disturbance on Raccoon Predation of American Alligator Nests

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THE INFLUENCE OF RESEARCHER DISTURBANCE ON
RACCOON PREDATION OF AMERICAN ALLIGATOR NESTS

By

Clarissa Tuten

A Thesis submitted
in Partial Fulfillment of the Requirements for the
Degree of Master of Science in
Coastal Marine and Wetland Studies

Gupta College of Science
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Dedication

First and foremost, I dedicate this thesis in loving memory of my mother and sole parent, Blakely Canfield, who fought with unwavering courage against breast cancer for six years. You embodied the meaning of strength, resilience, and enduring love and your memory continues to inspire me every day. This thesis is most importantly dedicated to you, my guardian angel.

Secondly, I dedicate my thesis to my children, both furry and scaly. To my fur child Ruger, your wagging tail, love of socks, and wild behavior have provided me with constant entertainment and joy. To my scaly children, Chief and Syrax, you further fostered my love and knowledge of Herpetology and gave me a reason to slow down and enjoy the finer things.

Thirdly, I dedicate my thesis to my fiancé, Connelly, my shoulder to cry on and my biggest supporter. You listened to me talk for hours, sat through hundreds of presentations and rants about alligators, and brought me snacks whenever I needed them. Thank you for always believing in me and pushing me to be the best version of myself.

Fourth, I dedicate my thesis to my nana and papa. Papa, you were never able to pursue your dream of Wildlife Biology and instead chose to live vicariously through me, reading through my drafts, listening to my presentations, and joining me in the field. Above all, you were always there to answer the phone whenever I needed “a word” for my thesis, and without fail, your immediate response of “four” never failed to bring a smile to my face. Nana, you are the finest, unpaid version of Grammarly I have ever had.

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Abstract

Raccoons (*Procyon lotor*) are a dominant predator of American alligator (*Alligator mississippiensis*) nests in the southeastern United States, using a combination of olfactory, visual, and tactile cues to identify nest locations. Studies on alligator nesting ecology typically require researchers to create paths through marsh habitat, potentially introducing visual and olfactory cues raccoons may use to locate nests. The purpose of this study was to evaluate the effect of human visitation to alligator nests on the frequency of raccoon nest predation at two sites in coastal South Carolina, Tom Yawkey Wildlife Center (TYWC) and Santee Coastal Reserve (SCR). We hypothesized that human foot traffic associated with nest monitoring increases the frequency of nest predation by raccoons. We observed a non-significant trend toward higher predation of foot-visited nests compared to drone and non-visited nests independent of study site (Mehta and Patel, $p=0.261$). This trend was similar at both study sites (Mehta and Patel, TYWC $p=0.106$, SCR $p=1$). When comparing predation by nest access method (i.e. boat, drone, foot, no-access), there was an overall non-significant trend towards higher predation of foot-visited nests independent of study site (Mehta and Patel, $p=0.255$). TYWC experienced higher predation of foot-accessed nests (Mehta and Patel, $p=0.031$) while SCR experienced lower predation of foot-accessed nests (Mehta and Patel, $p=1$). These results suggest that at sites of long-term nesting research (e.g., TYWC), alternative access methods may be useful in mitigating olfactory or visual cues left by researchers. *Post-hoc* power analyses, however, indicate low statistical power for our comparisons (Nest Predation by Treatment Group: 33.15%, vs Nest Predation by access method: 29.62% predation). Overall, the results of this study suggest raccoons may use human

cues to locate alligator nests; however, replication of the study across multiple seasons to increase sample size would help to further examine this hypothesis.

Table of Contents

Title page	i
Copyright	ii
Dedication	iii
Acknowledgements	iv
Abstract	v
Table of Contents	vii
List of Figures	viii
List of Symbols and Abbreviations	x
Introduction	1
Methods	4
Study sites	4
Nest location	5
Establishment of treatment groups	5
Nest visitation	6
Statistical analyses	8
Results	10
Comparative analysis between opened and closed cavity nests	10
Effects of human visitation methods on raccoon nest predation	10
Effects of nest access method on raccoon nest predation	13
Discussion	13
Effects of human visitation methods on raccoon nest predation	13
Impact of nest monitoring on predation of crocodilian nests	15
Limitations	15
Use of drones to survey nests	17
Implications for alligator management	19
Literature Cited	20
Figures	27
Tables	33

List of Figures

- Figure 1.** Location of study sites in coastal South Carolina. Tom Yawkey Wildlife Center Georgetown County (red outline), and Santee Coastal Reserve Charleston County, (yellow outline) SC.....27
- Figure 2.** Flow chart of the experimental design used in the study. A total of 54 American Alligator (*Alligator mississippiensis*) nests obtained from Tom Yawkey Wildlife center (TYWC) and Santee Coastal Reserve (SCR) were randomly assigned to each of three treatment groups: foot-visited nests, aerially (drone) visited nests, or never visited nests. At each site, nests within the Foot-visited treatment group were further randomly assigned to two subgroups: nest cavity opened, nest cavity unopened, to evaluate whether opening the nest cavity influenced nest predation; Ra= “Random Assignment”.....28
- Figure 3.** Predation frequency of foot-visited opened versus unopened American Alligator (*Alligator mississippiensis*) nests at Tom Yawkey Wildlife Center (TYWC), Georgetown County, and Santee Coastal Reserve (SCR), Charleston County, South Carolina, May – September, 2024. Pooled predation frequency of opened and unopened nests for TYWC and SCR combined, nest predation frequency of opened and unopened nests at TYWC, and SCR.....29
- Figure 4.** Predation frequency of foot-visited, drone-visited, and non-visited American Alligator (*Alligator mississippiensis*) nests at Tom Yawkey Wildlife Center (TYWC), Georgetown County, and Santee Coastal Reserve (SCR), Charleston County, South

Carolina, May – September, 2024. Pooled nest predation frequency of foot-visited, drone-visited, and non-visited nests for TYWC and SCR combined, nest predation frequency of foot-visited, drone-visited, and non-visited nests at TYWC, and SCR.....30

Figure 5. Predation frequency of physically accessed, and non-physically accessed American Alligator (*Alligator mississippiensis*) nests at Tom Yawkey Wildlife Center (TYWC), Georgetown County, and Santee Coastal Reserve (SCR), Charleston County, South Carolina, May – September, 2024. Pooled nest predation frequency of foot-visited, drone-visited, and non-visited nests for TYWC and SCR combined, nest predation frequency of foot-visited, drone-visited, and non-visited nests at TYWC, and SCR.....31

Figure 6. Predation frequency of boat-accessed, drone-accessed, foot-accessed, and non-accessed American Alligator (*Alligator mississippiensis*) nests at Tom Yawkey Wildlife Center (TYWC), Georgetown County, and Santee Coastal Reserve (SCR), Charleston County, South Carolina, May – September, 2024. Pooled nest predation frequency of boat-accessed, drone-accessed, foot-accessed, and non-accessed nests for TYWC and SCR combined, nest predation frequency of boat-accessed, drone-accessed, foot-accessed, and non-accessed nests at TYWC, and SCR.....32

List of Symbols and Abbreviations

AVN	Aerially/drone-visited nests
FVN	Foot-visited nests
NVN	Non-visited nests
SCR	Santee Coastal Reserve
TYWC	Tom Yawkey Wildlife Center

Introduction

Raccoons (*Procyon lotor*) are considered “omnivorous ecological generalists”, exploiting the most readily abundant food source (Ruilson et al., 2012). Raccoons are recognized as one of the primary predators of many ground-nesting birds and reptiles in the southeastern United States (Deitz and Hines, 1980) including waterfowl (Urban, 1970), turtles (Geller and Parker, 2022), ground nesting game birds (Hernandez et al., 1997), and American alligators (*Alligator mississippiensis*)(hereafter, alligators) (Flemming et al., 1976; Goodwin and Marion, 1978; Joanen, 1969; Joanen, 1989; Rainwater et al., 2024; Saalfeld et al., 2012; Wilkinson, 1983). Raccoons are highly effective nest predators due to their use of a combination of visual and olfactory cues to detect and locate nests (Buzuleciu et al., 2016, Strickland et al., 2010). Raccoons are capable of responding to seasonal changes in food availability, and often take advantage of nutrient-rich eggs during nesting periods (Ruilson et al., 2012). Raccoons exhibit the ability to quickly form lasting associations, particularly pertaining to cues indicating food sources (Davis, 1907). These connections have been found to be “permanently fixed”, with individuals demonstrating the capacity to recall the course of action needed to obtain food based on a specific cue, even following an extended lapse in time (Davis, 1907).

In South Carolina, gravid female alligators construct large nests by heaping surrounding vegetation and soil into mounds from late May to early July (Wilkinson,

1983). Alligator nests are typically located in natural river, swamp, and marsh ecosystems or in modified habitats such as dikes or managed impounded wetlands (e.g., old rice fields) (Goodwin and Marion, 1978; Wilkinson 1983). Gravid female alligators excavate a cavity in the top of the nest, deposit a clutch of eggs, and cover the cavity with additional vegetation. Heat from the sun and decomposing vegetation within the nest support development of embryos during the incubation period (Joanen, 1989). During this time, females will often visit the nest site, a behavior called “nest attendance”, to maintain nest integrity and protect eggs from predators (Merchant et al., 2018, Murray et al., 2019). The incubation period lasts approximately nine weeks, after which the maternal female typically excavates the young from the nest, transports them to nearby water, and continues to protect them for next 1-2 years (Joanen, 1969). Duration of female nest attendance varies throughout the incubation period; females usually exhibit higher rates of nest attendance towards the beginning and end of the incubation period. Additional factors such as time of day, rainfall, and distance of the nest to water have been found to impact female nest attendance (Rainwater et al., in review).

Few studies have examined dynamics of raccoon predation on alligator nests, and to our knowledge, no studies have examined whether human nest monitoring activities increase the frequency of raccoon nest predation on alligator nests. This is relevant because studies on alligator nesting ecology typically require researchers to create paths through dense marsh vegetation to access nesting sites. In addition, researchers may open nests to examine clutch size and egg viability (Wilkinson, 1983), and the exposed nest cavity may emit scents contained within the nest, providing nest or egg-specific olfactory cues (Buzuleciu et al., 2016). As a result of these activities, raccoons may be able to use

olfactory and/or visual cues left by researchers to help locate alligator nests. For example, alligator nests from Florida experienced increased predation after manipulation by researchers, suggesting raccoons may detect olfactory or visual cues introduced by researcher disturbance (Deitz and Hines, 1980). If so, standard nest survey protocols routinely used by researchers may increase the likelihood of nest predation by raccoons.

The purpose of this study was to determine whether human visitation (e.g., foot traffic, drone surveillance) associated with nest monitoring increases the frequency of raccoon predation on alligator nests in coastal South Carolina. We examined alligator nests at two sites, one where active alligator research (including annual nest monitoring) has been ongoing for the previous 15 years, and the other where little alligator nesting research has occurred over the last 20 years. To evaluate the influence of foot traffic on raccoon predation of alligator nests, we created access trails from roads to nests through marsh vegetation and documented the frequency of nest predation for approximately 16 weeks. To assess frequency of raccoon predation on nests in absence of physical access by humans, we used a remotely piloted drone to monitor nests for predation events. We predicted that overall predation on alligator nests accessed on foot would be higher than nests experiencing no physical access. Because raccoons may be able to associate human nest monitoring activities with a food source (eggs), we predicted that human foot traffic associated with nest monitoring would increase the frequency of raccoon predation compared to nest sites monitored remotely (drone) or not monitored at all, and these results will be consistent at both sites, regardless of nest research history (proxy for human disturbance).

Methods

Study sites

This study was conducted at the Tom Yawkey Wildlife Center (TYWC; 33.23°N, 79.22°W), Georgetown County and Santee Coastal Reserve (SCR; 33.15°N, 79.36°W), Charleston County, both in South Carolina, from June-September 2023. TYWC and SCR both consist of approximately 9,700 hectares of uplands, beaches, forests, and both natural and managed impounded wetlands (Nelson and Rayner, 2014; Figure 1). Both sites are wildlife management areas operated by the South Carolina Department of Natural Resources and are situated adjacent to one another along the north-central South Carolina coast. Studies on alligator nesting ecology have been conducted at both sites over the last 40 years but have been much more intensive at TYWC, particularly over the last 15 years. Accordingly, regular presence of researchers in nesting habitat at TYWC may have influenced behavior of both alligators and raccoons at this site (e.g., increased, or decreased wariness by alligators; association of human-related cues with nest locations by raccoons). Therefore, to control for the potential bias of human disturbance-related alterations in alligator and raccoon behavior, we conducted our field experiments at both TYWC (history of intensive alligator nesting research) and SCR Reserve (history of minimal alligator nesting research).

Nest location

Helicopter surveys were conducted in mid-late June 2023 to visually identify alligator nests at both study sites (Wilkinson, 1983). TYWC and SCR were both surveyed on 16 June, and again on 28 June. GPS coordinates (longitude and latitude [± 10 m]) were recorded while hovering over each nest, and each nest was assigned a unique identification number.

Establishment of treatment groups

To test the hypothesis that human foot traffic to-and-from alligator nests increases raccoon predation of nests, we allocated nests to three treatment groups: Foot-visited nests (FVN), aerially (drone) visited nests (AVN), and non-visited nests (NVN). Foot-visited nests consisted of nests that were accessed and manipulated using historical research methods (e.g., cutting paths through marsh from roads to nests, excavating nests to examine eggs, etc.). Drone-visited nests consisted of nests visited by a remotely piloted drone. This allowed us to monitor nests without creating pathways that could potentially be used by raccoons to locate and access nests. Non-visited nests consisted of nests that were not accessed by foot or drone until after the conclusion of the egg hatching period in South Carolina (21 September; Wilkinson, 1983). Accordingly, non-visited nests served as a control. Because raccoons may use scent of disinterred nesting substrate as an olfactory cue to locate reptile nests (Buzuleciu et al., 2016), approximately half of nests in the Foot-visited Nests treatment group were opened to potentially expose scent cues from the egg chamber or unopened. A larger number of opened nests were allocated at TYWC due to the requirements of a separate, but parallel alligator egg incubation study.

AI software (ChatGPT 3.5) was used to randomly assign nests at each site into the three treatment groups (FVN, AVN, NVN). The terminology given to the AI software to generate treatment groups at both sites were as follows: “We have [n] nests at [study site] with ID codes ranging from [first ID code] to [last ID code]. Randomly assign these nests into three treatment groups with the first group consisting of [n] total nests, the second group consisting of [n] total nests, and the third group consisting of [n] total nests”. We conducted three iterations of each random assignment to ensure randomization of nest sites. When establishing the foot-visited nest subgroups for each site (opened egg cavity, unopened egg cavity) the terminology given to the AI software was as follows: “Randomly assign the nests in group 1 into two subgroups, the first group consisting of [n] total nests, the second group consisting of [n] total nests”. We also conducted three iterations of these random assignments to ensure randomization.

Nest visitation

FVN were visited once every two weeks (late June-late September) by 2-4 researchers. We accessed FVN by creating trails through marsh vegetation using a machete. On the initial visit for each FVN, eggs from the “cavity opened” subgroup were counted, fertility (presence or absence of banding; Ferguson 1985) recorded, and then returned to the nest cavity and covered with nest material. Two nests at TYWC (previously assigned to the FVN treatment group) and two nests at SCR (previously assigned to the FVN treatment group) were found predated on the initial visit and were swapped with nests from each site’s “Non-Visited Nest” category as they did not experience any researcher disturbance before a predation event. For all FVN, nest

dimensions (length, width, height, depth of nest cavity) were recorded within seven days of construction. Apart from the initial visit, nests in the opened egg cavity subgroup were not opened for the remainder of the study. The nests in the unopened egg cavity subgroup were not opened but otherwise treated identically to opened nests described above. Nests in both subgroups were equipped with a game camera (Reconyx XR6 Ultrafire, Holmen, WI,) to monitor alligator presence and nest predator activity (Rainwater et al., 2024). This allowed us to examine differences in predatory activity between open (potentially more olfactory cues) and unopened (potentially fewer or no olfactory cues) nests and further explore the possibility that raccoons identify the location of alligator nests primarily from olfactory cues released from opened nests versus olfactory and/or visual cues left by researchers. During each bi-monthly visit, we recorded predation status of each nest and replaced SD cards and batteries in game cameras. Bi-weekly nest visits continued until a given nest successfully hatched or was predated. Nest predation by raccoons was clearly identifiable by an access hole dug from the top of the nest into the egg chamber and eggshells scattered around the outside of the nest (Platt et al., 1995). For FVN, we confirmed predation and hatching events using images from game cameras.

To assess the effect of raccoon predation on alligator nests in absence of human disturbance, we used a remotely controlled drone (DJI Mavic Air 2) equipped with cameras to monitor nests for signs of predation. Using a drone to monitor nests may cause less disturbance to nest guarding females than the presence of humans and eliminates the need to create paths and other disturbance which could alert raccoons to nest locations (Elsy and Trosclair III, 2016). AVN were surveyed once every two weeks (bi-weekly) by flying the drone over each AVN nest site and searching for indicators of

nest predation or hatching events. During each visit, we took photographs from multiple vantage points including images from varying heights (depending on how low the drone could safely hover) as well as different quadrants of the nest mound and the surrounding vegetation. Drone nest monitoring continued until a given nest successfully hatched or was predated, at which time a subsequent foot visit was made to confirm nest fate.

Predation and hatching events were easily distinguishable when viewed remotely with the drone due to the conspicuous difference in nest excavation techniques used by maternal alligators and raccoons. Predatory excavation by raccoons typically involves digging into the top of the nest, leaving one or more holes/tunnels leading to the egg chamber and often eggshells scattered on and around the nest mound. Conversely, during hatching, attending female alligators excavate the nest from the side of the mound, removing large amounts of nest material using the mouth and forelimbs, and eggshells are less commonly observed or may be moved close to the water's edge (Hernandez et al., 1997).

Additionally, presence of hatchlings and the attending female were frequently discernable at recently hatched nests.

Non-visited nests (NVN) were not visited by foot or drone during the egg incubation period and served as a disturbance-free control. These nests were visited in late September, only after all nests in the FVN and AVN treatment groups were either hatched or predated.

Statistical analyses

All statistical analyses were completed in R (Version 4.3.2) using PositCloud (Version 4.3.2). We tested for significant differences in predation frequencies between

groups of interest including (1) the three nest treatments (FVN, AVN, NVN), (2) access method (boat, drone, foot, none), and (3) visitation method (physical access or no physical access). Comparisons were considered at each site individually as well as with sites combined. Chi-squared tests were used when at least 80% of the expected counts were > 5 ; otherwise, the nonparametric alternatives Fisher and Mehta and Patel were used. Post hoc power analyses were conducted to determine statistical power based on the smaller sample sizes in the study.

We conducted a comparative analysis between FVN nests where the cavity was excavated (opened) and those where the cavity was left intact (unopened) to ascertain whether any observed increase of predation frequency was attributed to researcher disturbance/presence rather than egg or cavity-specific olfactory cues, as reported for diamondback terrapin (*Malaclemys terrapin*) nest sites (Buzuleciu et al., 2016).

Results

A total of 81 nests were identified via helicopter flights; 49 nests were located at TYWC, and 32 nests were located at SCR. Of these, 27 nests (15 from TYWC, 12 from SCR) were found to be false or misidentified nests, and were removed from the study (Figure 2, Table 1). Eight nests were NVN, four nests were AVN, and 15 nests were FVN. Following the determination of nest fate, the sizes of our treatment groups were as indicated in Figure 2.

Comparative analysis between opened and closed cavity nests

No statistically significant difference in predation frequency was observed between foot-visited nests with opened and unopened cavities (egg chambers) (Figure 3). Therefore, all subsequent analyses combined these two groups into the broader category labeled as “foot-visited nests”.

Effects of human visitation methods on raccoon nest predation

Overall, there was a non-significant trend towards a higher frequency of predation of foot-visited nests compared to drone-visited and non-visited nests, independent of study site (Mehta and Patel, $p=0.261$). On average, foot-visited nests experienced a predation frequency approximately 4.5 times higher than drone-visited and approximately

twice as high as non-visited nests. The trend towards increased nest predation frequency of foot-visited nests compared to drone and non-visited nests was consistent at both TYWC and SCR; however, there was no significant variation among treatment groups at either study location (Mehta and Patel, TYWC $p=0.106$, SCR $p=1$). At TYWC, predation of foot-visited nests was 3 times higher than at non-visited nests, and none of the nests in the drone-visited treatment group were predated. In contrast, nest predation of foot-visited nests at SCR was about 1.5 times higher than that of both drone and non-visited nests (Figure 4).

When drone-visited and non-visited nests at both study locations were combined into one “non-physically accessed” group and compared to the frequency of predation of physically accessed nests (FVN), there was a non-significant trend towards increased predation of physically accessed nest sites compared to non-physically accessed nest sites, independent of study site (Score Test, $p=.4616$) (Figure 5). On average, physically accessed nest sites experienced a predation frequency 1.5 times higher than non-physically accessed nest sites. The trend towards increased nest predation at physically accessed nest sites was consistent at TYWC; however, there was no significant variation among treatment groups at this location (Score Test, $p=0.2949$). At TYWC specifically, physically accessed nests experienced a predation frequency 3x higher than that of non-physically accessed nests. This trend was again different at SCR, although not significant (Score Test, $p=0.852$), with physically accessed nests showing a predation frequency 1.3x lower than that of non-physically accessed nests (Figure 5).

Effects of nest access method on raccoon nest predation

Overall, when comparing method of access, there was a non-significant trend towards a higher frequency of predation of foot-visited nests compared to nests access by boat, drone, and non-visited nests, independent of study site (Mehta and Patel, $p=0.255$). On average, foot-visited nests experienced a predation frequency about 3.5 times higher than boat-accessed and drone-accessed nests, and nearly twice as high as non-accessed nest sites. Foot-visited nests at TYWC specifically showed a higher predation frequency than boat, drone, and non-visited nests (Mehta and Patel, $p=0.031$). At TYWC, foot-visited nests experienced a predation frequency about 3 times higher than non-visited nests while both boat and drone-visited nests experienced no predation. Conversely, boat, drone, and never-accessed nests at SCR all showed a predation frequency about twice as high as that of foot-accessed nests (Mehta and Patel, $p=1$) (Figure 6).

Discussion

Effects of human visitation methods on raccoon nest predation

Overall, our hypothesis that alligator nests monitored by physical access on foot would experience a higher frequency of nest predation than nests without physical access was not supported. Combined data show a slightly higher, but not statistically significant, overall predation frequency of physically accessed nests. When research sites were analyzed separately, however, physically accessed nests at TYWC experienced predation about three times higher than that of non-physically accessed nests while SCR experienced higher predation of the non-physically accessed nest groups. Again, none of these differences were statistically different.

One possible explanation for the difference in alligator nest predation related to the different methods of human access to nests is that following long-term (15 consecutive years) annual nest monitoring at TYWC raccoons may have become habituated to anthropogenic cues associated with foot visits and may further associate these with the presence of an energy dense food source (nests containing a clutch of eggs) (Edmunds et al., 2018). At SCR, on the other hand, no such anthropogenic cues have been available to raccoons for more than 15 years. In addition, introduction of potential anthropogenic cues detected by raccoons may initially reduce raccoon predatory behavior. However, over time raccoons may become habituated to anthropogenic cues,

presumably learning there is no danger (Naeger et al., 2021). While there are many known nest predators for alligators (Deitz and Heins, 1980; Elsey et al., 2012; Hunt and Ogden, 1991; Rainwater et al., 2024), raccoons are the primary nest predator in coastal South Carolina (Rainwater et al., 2024) and the only predator of focus for this study.

Raccoons exhibit adaptive foraging behaviors, adjusting their strategies and food choices based on seasonal variations in food availability or in response to various environmental or anthropogenic cues (Ruilson et al., 2012; Edmunds et al., 2018). Furthermore, raccoons demonstrate remarkable cognition, including the ability to discriminate between familiar and unfamiliar urinary scents and distinguish differences in other chemosensory cues (Kent and Tang-Martínez, 2014). Additionally, raccoons can learn quickly and associate visual and olfactory cues with food sources (Burke et al., 2005; Dalgish and Anderson, 1979). This associative learning may result in specific behaviors or reactions by raccoons when encountering these cues (e.g., researcher scents or paths to nests) that enable them to locate a food source (Edmunds et al., 2018). Negative cues or associations, such as the presence of humans or predators signaling danger, may eventually become overridden by cues indicating the presence of food (Davis, 1907; Naeger et al., 2021). While associations between olfactory/visual cues and food sources often persist through repeated exposure, they can gradually fade without reinforcement over time or when a cue is removed (Dalgish and Anderson, 1979; Davis, 1907).

Impact of nest monitoring on predation of crocodilian nests

Olfactory, visual, or auditory cues left by researchers monitoring crocodilian nests may attract or repel nest predators. For example, in Spectacled Caiman (*Caiman crocodilus*) nests that experienced researcher disturbance showed a non-significant trend of increased predation by Brown Capuchin Monkeys (*Cebus apella*) compared to nests that did not experience researcher disturbance (Barão-Nóbrega et al., 2014). In contrast, in a long-term study of Diamondback Terrapin nesting ecology, raccoons were initially repelled by human scent at artificial terrapin nests, but after 12 years of researcher activity in the nesting area human scent increased raccoon predation (Burke et al., 2005; Edmunds et al., 2018). These observations suggest that raccoons may initially be deterred by human disturbance, but after repeated exposure, may become complacent or indifferent towards human disturbance after experiencing no negative stimuli. In our study, we observed a trend of lower predation of alligator nests at SCR where there has been little historical human visitation to nest sites compared to increased predation of nests at TYWC where annual nest monitoring has occurred for 15 consecutive years.

Limitations

Although we observed a trend favoring predation at nests that experienced researcher disturbance, our conclusions are limited by sample size. One reason for our small sample sizes were the initial (and unintentional) inclusion of false and misidentified nests when creating the treatment groups. This issue was particularly pronounced in the non-visited treatment group (NVN), where 8 of the 18 nests (44.45%) identified at both sites from the air were later found to be false nests, old nests, or not nests at all. This

resulted in unequal distribution of samples among treatment groups, reducing the sample size for non-visited nests at both study sites. Additionally, 3 nests within the assigned FVN treatment group (2 located at TYWC, 1 located at SCR) were found to be predated on the initial nest visit. Due to the lack of researcher disturbance prior to predation, these three nests were exchanged with three nests from the NVN group at their respective sites. This exchange, coupled with high false nesting of the NVN group may have contributed to the elevated number of predated nests within the NVN group.

We conducted power analyses for our comparisons of predation frequency by treatment group, access method, and physically vs non-physically accessed nests to provide insight into the ability of our data to draw robust conclusions. For our test on predation frequency by treatment groups, post-hoc power calculations revealed a moderate level of power for combined data (30.92%), a slightly higher power for TYWC data (41.3%), and low power for SCR data (6.56%). Similarly, examinations of predation by access method showed a moderate level of power for combined data (41.68%), a slightly higher power for TYWC data (67.06%), and low power for SCR data (9.82%). Lastly, analysis of power for physically vs. non-physically accessed nests demonstrated low post-hoc power for combined data (18.45%), TYWC data (29.36%) and SCR data (7.29%). All post-hoc power analyses demonstrate a need for additional data collection to ensure robust data sets and statistical integrity of our conclusions.

We recommend future studies strive for a larger sample size of nests in each treatment group, as well as continuing the work over multiple years to better examine the relationship between raccoon predation and researcher disturbance over time. In addition, if possible, future studies should incorporate habitat data (e.g., distance of nest to

roadway/tree line, distance of nest to water, salinity or nearest water, canopy cover, etc.) into the analysis to help distinguish between the influence of natural (environmental) and anthropogenic factors on the frequency of raccoon predation of alligator nests. Moreover, employing game cameras to monitor raccoon activity along researcher pathways would be helpful in quantifying how these predators may exploit the presence of humans to locate and access nests. Last, measuring and testing differences in raccoon behavior as it pertains to visual, olfactory, and auditory cues used to locate alligator nests may be beneficial.

Use of drones to survey nests

Helicopter surveys have historically been considered the most efficient and cost-effective method for locating alligator nests in many parts of the southeastern United States (Deitz and Hines, 1980; Wilkinson, 1983); however, recent advancements with drones (unmanned aerial vehicles or UAVs) may provide a relatively inexpensive, alternative method for nest site identification and monitoring. In our study, this approach allowed for efficient coverage of large areas of alligator habitat and provided detailed views of nest sites, similar to other recent studies in crocodilians. For example, drones have been used to detect nests, gain information on nest attendance, and locate critical nesting habitat of various crocodile species (Platt et al., 2023; Evans et al., 2015; Evans et al., 2016), caiman species (Scarpa and Piña, 2019) and alligators (Elsey and Trosclair III, 2016). They have also been used to estimate total length of submerged crocodilians (Aubert, et al., 2024), assess behavioral changes in crocodilians and turtles due to drone presence (Bevan et al., 2018), capture crocodiles (Brien et al., 2020), and survey various

crocodilian species and their population sizes (Aubert et al., 2022; Ezat et al., 2018; Sawan et al., 2023; Thapa et al., 2018).

Limitations associated with drone surveys include limited battery life, weather impacts (high winds, rain, fog), control range, and visibility issues (Elsy and Trosclair III, 2016). Additionally, using drones to locate nest mounds have proven to be challenging, as the need to fly low is often compromised by height of vegetation and canopy cover (Platt et al., 2023). Zoom lenses have been offered as a recommendation to addressing the challenge of flying low. Additionally, using a quadcopter rather than a fixed-wing drone allows for more maneuverability and closer inspection of nest sites (Elsy and Trosclair III, 2016; Platt et al., 2023). Last, our study and others (e.g., Bevan et al, 2018) have shown that crocodilians exhibit varying behavioral response to drones, and these must be considered accordingly depending on the specific research question addressed or management strategy employed.

In our study, attending female alligators exhibited three primary behaviors in response to drones flying over nests, defense, avoidance, and no change. Of the 10 total females observed at nests from the drone, two were observed defending the nest against the drone, a behavior also documented in Estuarine Crocodiles (*Crocodylus porosus*) (Bevan et al. 2018). The behavior of these females typically included leaving their guard hole, crawling towards or onto the nest, and opening their mouth, presumably hissing (our drone did not have audio capabilities). Conversely three females exhibited a tendency to avoid the drone, while five females exhibited no change in behavior. Some females were encountered while basking beside the nest, promptly retreating towards the nearest water body or their guard hole and submerging themselves. Others were

discovered within their guard hole, where they also submerged themselves, only reemerging once the drone was lifted to a higher altitude.

Implications for alligator management

When conducting nesting studies on threatened or endangered crocodylian species, remote monitoring may increase the chance of nest survival by mitigating potential anthropogenic disturbances, thereby reducing the risk of increased predation associated with researcher interference. While we detected no statistically significant differences in nest predation frequency between physically (FVN) and non-physically (AVN, NVN) visited nests, our study suggests that prolonged human visitation (disturbance) to alligator nest sites could potentially increase raccoon predation on alligator nests. In cases such as TYWC, where nesting studies are a main research focus, monitoring nests with drones rather than foot visits, when possible, may be useful in reducing the potential for increased predation caused by researcher disturbance. Our results at TYWC further suggest that minimally invasive methods for accessing alligator nests such as by drones or boats rather than on foot may help to reduce nest predation.

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Figures



Figure 1. Location of study sites in coastal South Carolina. Tom Yawkey Wildlife Center Georgetown County (red outline), and Santee Coastal Reserve Charleston County, (yellow outline) SC.

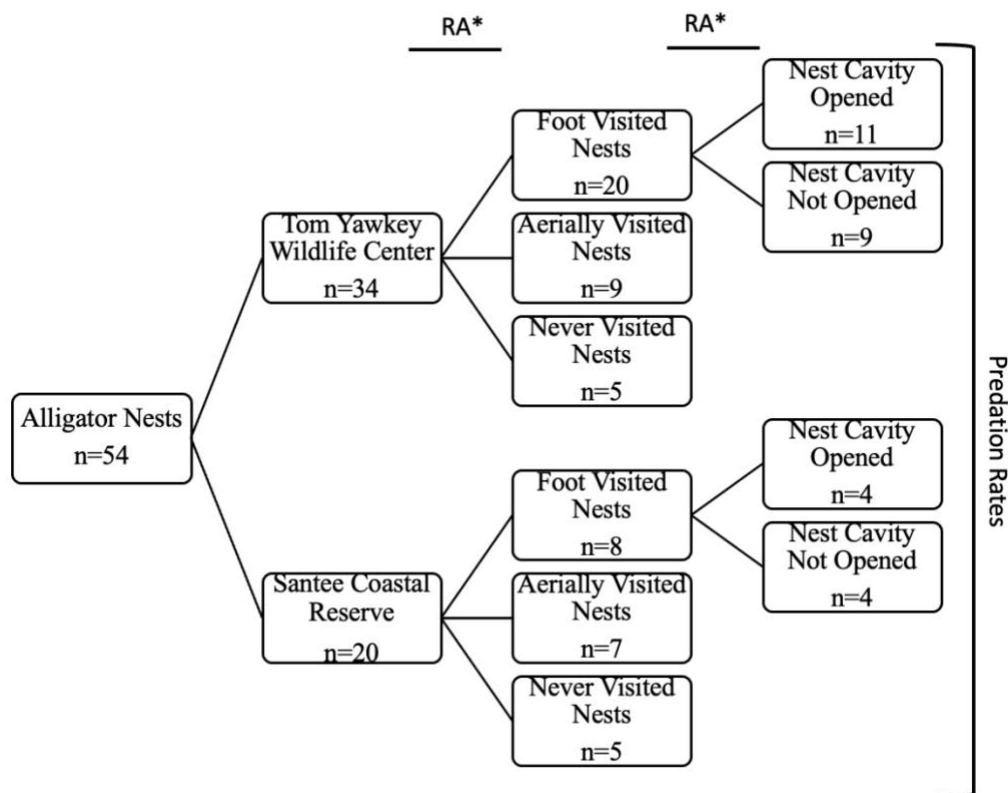


Figure 2. Flow chart of the experimental design used in the study. A total of 54 American Alligator (*Alligator mississippiensis*) nests obtained from Tom Yawkey Wildlife center (TYWC) and Santee Coastal Reserve (SCR) were randomly assigned to each of three treatment groups: foot-visited nests, aerially (drone) visited nests, or never visited nests. At each site, nests within the Foot-visited treatment group were further randomly assigned to two subgroups: nest cavity opened, nest cavity unopened, to evaluate whether opening the nest cavity influenced nest predation; Ra= “Random Assignment”.

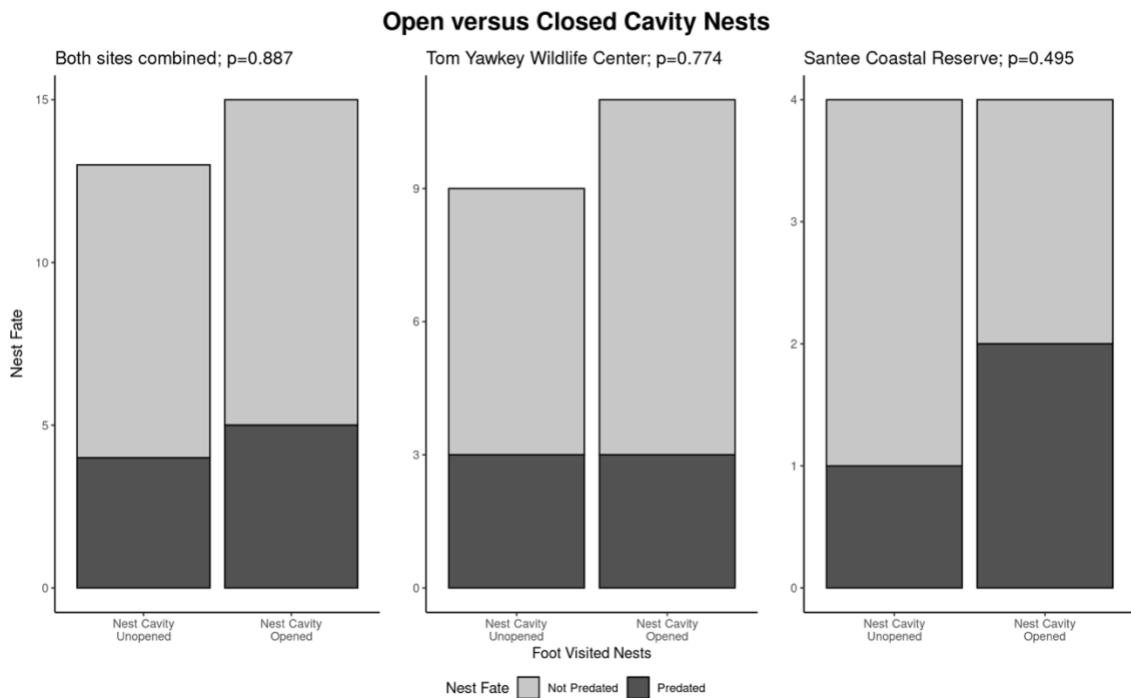


Figure 3. Predation frequency of foot-visited opened versus unopened American Alligator (*Alligator mississippiensis*) nests at Tom Yawkey Wildlife Center (TYWC), Georgetown County, and Santee Coastal Reserve (SCR), Charleston County, South Carolina, May – September, 2024. Pooled predation frequency of opened and unopened nests for TYWC and SCR combined, nest predation frequency of opened and unopened nests at TYWC, and SCR.

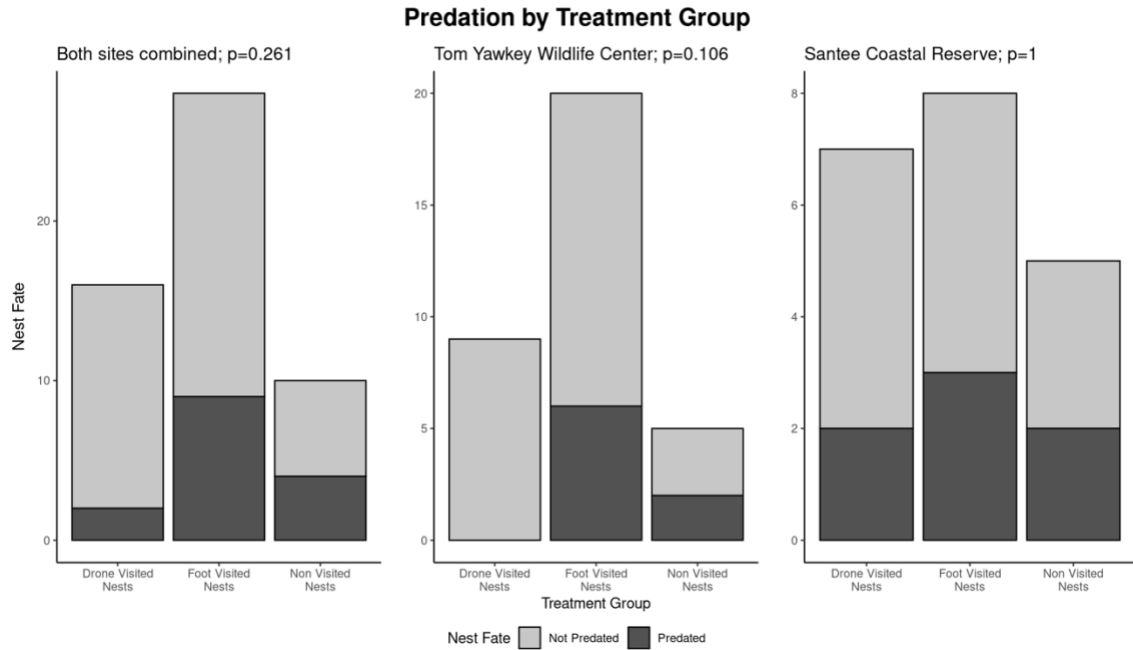


Figure 4. Predation frequency of foot-visited, drone-visited, and non-visited American Alligator (*Alligator mississippiensis*) nests at Tom Yawkey Wildlife Center (TYWC), Georgetown County, and Santee Coastal Reserve (SCR), Charleston County, South Carolina, May – September, 2024. Pooled nest predation frequency of foot-visited, drone-visited, and non-visited nests for TYWC and SCR combined, nest predation frequency of foot-visited, drone-visited, and non-visited nests at TYWC, and SCR.

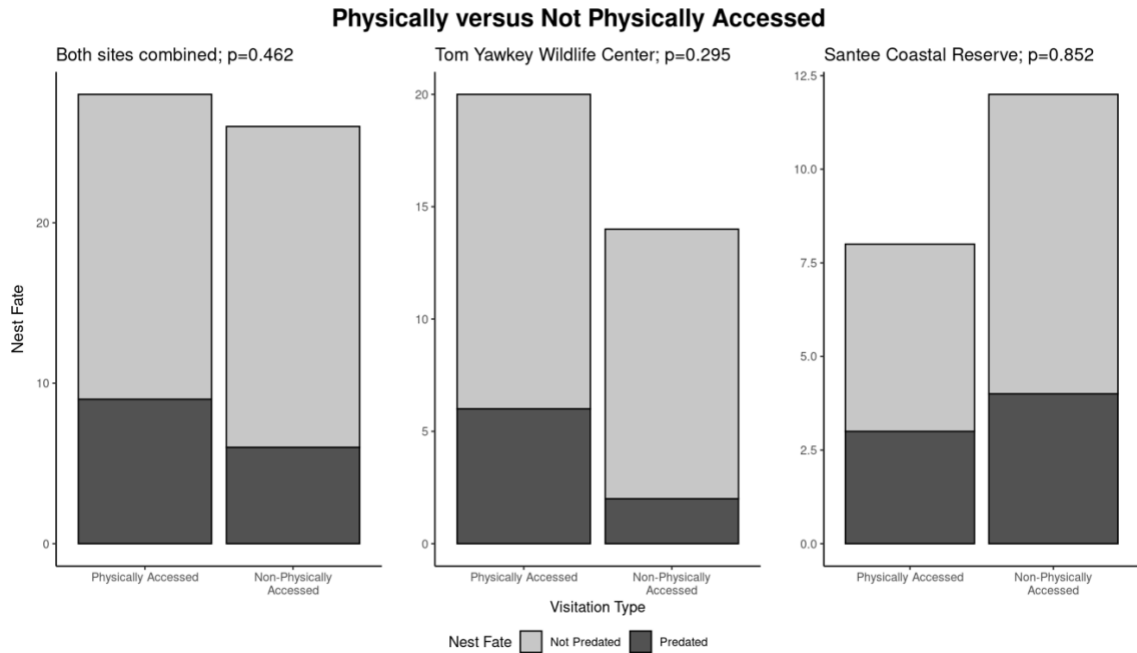


Figure 5. Predation frequency of physically accessed, and non-physically accessed American Alligator (*Alligator mississippiensis*) nests at Tom Yawkey Wildlife Center (TYWC), Georgetown County, and Santee Coastal Reserve (SCR), Charleston County, South Carolina, May – September, 2024. Pooled nest predation frequency of foot-visited, drone-visited, and non-visited nests for TYWC and SCR combined, nest predation frequency of foot-visited, drone-visited, and non-visited nests at TYWC, and SCR.

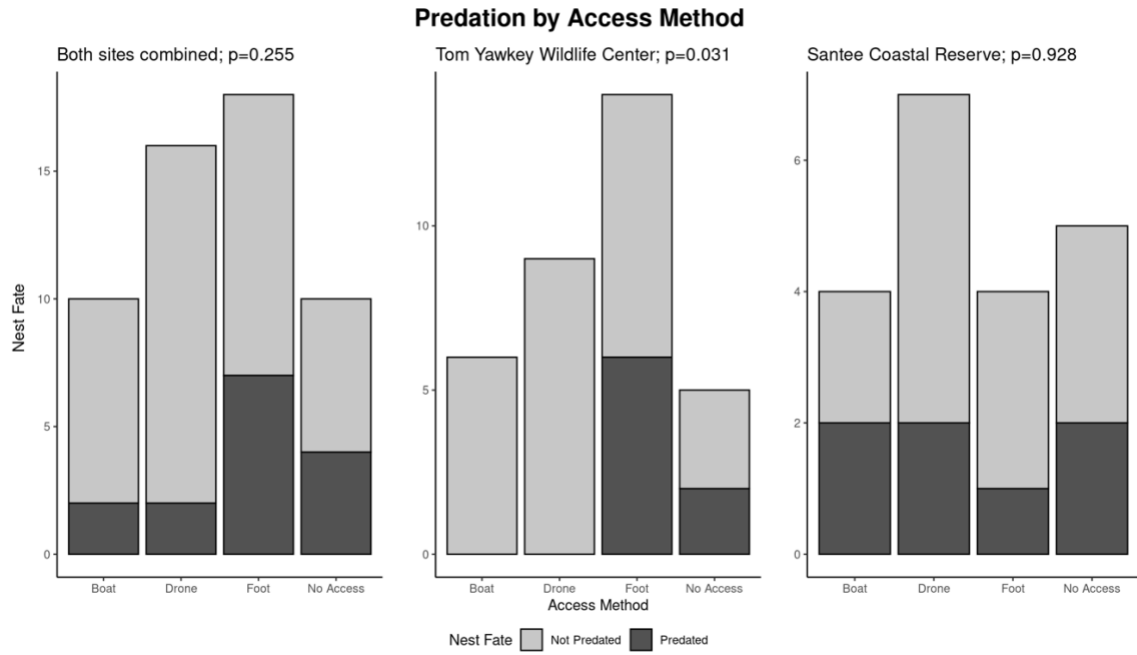


Figure 6. Predation frequency of boat-accessed, drone-accessed, foot-accessed, and non-accessed American Alligator (*Alligator mississippiensis*) nests at Tom Yawkey Wildlife Center (TYWC), Georgetown County, and Santee Coastal Reserve (SCR), Charleston County, South Carolina, May – September, 2024. Pooled nest predation frequency of boat-accessed, drone-accessed, foot-accessed, and non-accessed nests for TYWC and SCR combined, nest predation frequency of boat-accessed, drone-accessed, foot-accessed, and non-accessed nests at TYWC, and SCR.

Tables

	Tom Yawkey Wildlife Center			Santee Coastal Reserve			Total # of Nests	Total false/misidentified		
	Predated	Not Predated	# nests	Predated	Not Predated	# nests		TYWC	SCR	
Foot-Visited Nests	6	14	20	3	5	8	28	7	8	
Open Cavity	2	8	10	2	2	4	14	6	5	
Closed Cavity	3	6	9	1	3	4	13	1	3	
Drone Visited Nests	0	9	9	2	5	7	16	2	2	
Non-visited nests	2	3	5	2	3	5	10	6	2	
Total # of nests		34			20					
Total false/misidentified		15			12					

Table 1. Summary of number of American alligator (*Alligator mississippiensis*) nests allocated to each treatment group (foot-visited, drone-visited, and non-visited nests) nest fate, and false or misidentified nests at Tom Yawkey Wildlife Center (TYWC) Georgetown County, SC, and Santee Coastal Reserve (SCR), Charleston County, SC.

