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American crocodile nesting in sediment-nourished habitats on Crocodile Lake National Wildlife Refuge, Florida, USA

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Abstract. The American crocodile (*Crocodylus acutus*) is the most widely distributed New World crocodile. However, the species has experienced severe declines due to overexploitation. In South Florida, loss of nesting habitat, with coastal degradation from sea level rise and urban development has prompted American crocodiles to nest in novel habitats. Crocodile Lake National Wildlife Refuge (CLNWR) serves as an important nesting site for the South Florida population of American crocodiles and more recently nesting habitat has been supplemented to manage coastal erosion. The goal of this study was to investigate the internal nest biology of American crocodile nests laid in sediment nourished sand mounds on CLNWR. We monitored internal nest temperature and volumetric water content of five live and two control sand nests in 2021 (N = 73 eggs) and 2022 (N = 84 eggs). The metabolic heat generated by incubating eggs in internal nest temperatures ranged from 0.8°C to 2.0°C warmer and more stable than ambient temperatures and reflecting a seasonal pattern. Average clutch size was 31.4 ± 7.09 eggs and incubation period ranged from 78 to 114 days until hatching. These data provide the first insight into thermal regimes of nests laid in novel/supplemented nesting habitat as is the case for a significant proportion of nests in the South Florida population of American crocodiles. Here we provide an opportunity to evaluate the importance of creating artificial nesting habitat for American crocodiles where habitat degradation from climate change threatens species survival.

Keywords: *Crocodylus acutus*, hatching success, incubation, nest environment, thermal biology.

Introduction

The American crocodile (*Crocodylus acutus*, Cuvier, 1807) is the most widely distributed New World crocodile and occurs from South Florida and coastal Mexico down into South America on both Atlantic and Pacific coasts and on islands in the Caribbean (Rainwater et al., 2021). The species has experienced severe declines due to overexploitation and loss of habitat for nesting throughout its historical range; and is presently classified as Vulnerable on the International Union for Conservation of Nature (IUCN) Red List (Rainwater et al., 2021). *Crocodylus acutus* is also listed on Appendix I of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) for all countries except Mexico, Cuba, and Colombia (Cispata Bay) where the species is listed on Appendix II (CITES 2021) as regional threats in these countries have been reduced. The American crocodile is at its northernmost range in South Florida and is likely at the limit of certain ecological tolerances (Kushlan, 1982). In South Florida, nesting of American crocodiles was restricted to a small area of Northeastern Florida Bay (NEFB) in Everglades National Park (ENP) and Northern Key Largo

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by the early 1970s (Kushlan and Mazzotti, 1989). In 1975, the species was placed on the Federal Endangered Species List (Federal Register 40), and in 1980 Crocodile Lake National Wildlife Refuge (CLNWR) was established to protect important nesting habitat. With critical monitoring and management efforts (Brandt et al., 1995; Mazzotti et al., 2007), the Florida population of American crocodiles was reclassified from endangered to threatened in 2007 (Federal Register 72, USFWS 2007). Since then, the American crocodile has been federally protected in South Florida and serves as an ecological indicator species in the Florida Everglades in response to ecosystem restoration (Doren et al., 2009; Mazzotti et al., 2009; Briggs-Gonzalez et al., 2021).

American crocodiles in Florida typically nest on sandy beaches creating a mound and depositing eggs on top of the mound or along marl creek banks as a hole nest where eggs are deposited in a chamber below (Mazzotti, 1989). Continued loss of nesting habitat, particularly with coastal degradation from sea level rise and urban development in South Florida has prompted American crocodiles to nest in novel habitats that include earthen berms within the Turkey Point Power Plant cooling canal system (TP; Gaby et al., 1985), marl canal plugs meant to slow saltwater intrusion at Buttonwood and East Cape canals in ENP (Mazzotti et al., 2007a), and peat berms that were a result of dredging on CLNWR (Kushlan and Mazzotti, 1989; Mazzotti et al., 2022). Whether these novel nesting habitats have an effect on American crocodile nesting, hatching, and hatchling survival is unknown. However, novel substrates have provided new areas for American crocodiles to nest and might be a factor in the shift of crocodile abundance and nesting activity from historical areas such as in NEFB where hypersalinity regimes have altered nesting conditions and affected survival (Mazzotti et al., 2019; Briggs-Gonzalez et al., 2021; Mazzotti et al., 2022).

In crocodylians, thermal biology provides important life history cues that stimulate courtship and timing of nesting (Joanen and McNease, 1989; Lance, 2003). A suite of environmental factors affects nest temperature and include solar radiation, ambient temperature, rainfall, canopy cover, nest material, and proximity to water (Charruau, 2012; Balaguera-Reina et al., 2015; Murray et al., 2016). Nest temperature is also affected by how deep the clutch is laid, by surrounding soil moisture content (Ferguson, 1985), and the size of clutch that generates metabolic heat (Charruau, 2012). Metabolic temperature is defined as the difference between soil and clutch temperature generated by metabolic heat (Charruau, 2012). All crocodilian species exhibit temperature-dependent sex determination (González et al., 2019), and nest temperature influences the incubation period and determines not only sex of hatchlings (Webb and Cooper Preston, 1989; Shine, 2005), but also includes embryonic development (Charruau, 2012), hatchling survival, growth rates (Balaguera-Reina et al., 2015), and the long-term effects of shifting temperatures on thermal regimes (Murry et al., 2016; Bock et al., 2020; Cherkiss et al., 2020).

Nest temperature patterns fluctuate over the incubation period, and in crocodiles, it is universally established that low (<31°C) and high (>33°C) incubation temperatures produce more females and intermediate temperatures produce more males (Lang and Andrews, 1994; González et al., 2019). A relative even sex ratio is dependent on a threshold temperature that produces 50% of each sex (Valenzuela, 2004), which sustains healthy population dynamics. Given rapidly changing climatic conditions, with increasing global temperatures and extreme weather events, nest incubation temperatures are expected to be affected (Girondot et al., 2004) and to influence sex ratios and population structure (i.e., see Charruau (2012) for a male-biased population in Banco Chinchorro, Mexico, and Murray et al. (2015) for high male-bias in Tempisque Basin, Costa Rica). Similarly, extreme nest temperatures have reduced hatchling crocodile survival in several species (Ferguson and Joanen, 1982; Charruau, 2012; Murray et al., 2016; Liu et al., 2023). Cherkiss et al. (2020) further document earlier hatching in *C. acutus* with increasing temperatures in South Florida.

What we know of thermal biology and the effect on American crocodile biology is on wild nests in coastal areas usually from sandy material (Deeming, 2004; Charruau, 2012; Balaguera-Reina et al., 2015; Murray et al., 2015, 2016) that make up either a hole or a mound nest (and there is variation within a species and by locality; Murray et al., 2020). We do not yet know of thermal regimes for nests laid in novel/supplemented nesting habitat as is the case for a significant proportion of nests in the South Florida population of American crocodiles (Mazzotti et al., 2022). The goal of this study was to investigate the environmental characteristics of American crocodile nests laid on CLNWR which included supplemented nesting habitat sand mounds. We assessed variation in incubation temperature within the clutch and evaluated potential effects of environmental conditions of ambient air temperature and volumetric water content on hatching success. These data provide an opportunity to evaluate the importance of creating artificial nesting habitat for American crocodiles where habitat degradation from climate change threaten species survival.

Materials and methods

Study site

Crocodile Lake National Wildlife Refuge (CLNWR) (fig. 1) was established in 1980 as a part of the United States National Wildlife Refuge System under the Land and Water Conservation Fund Act of 1965 prompted by the Endangered Species Act of 1973. The Refuge was originally slated for residential development, but dredge-spoils accumulated on the bayside of North Key Largo and became an important nesting area for the American crocodile (Ogden, 1972). The 6686 acre (27.1 km^2) refuge located in North Key Largo in Monroe County, just off Card Sound Road was established to protect this converted area into critical breeding and nesting habitat for the American crocodile (*C. acutus*; Mazzotti et al., 2007). Recently, nesting habitat has been supplemented by CLNWR staff with sand mounds because the original peat dredge-spoils continue to be lost to sea level rise and coastal erosion (J. Dixon, pers. obs.). CLNWR has consistently contributed to the breeding population of American crocodiles in South Florida since the early 1970's (Ogden, 1978; Mazzotti et al., 2022). A higher percentage of hatchlings successfully disperse from the Refuge than other conservation areas (Mazzotti et al., 2003) and the relatively high survival of hatchlings and high growth rates make CLNWR a critical location for hatchling crocodiles in Florida (Mazzotti et al., 2007).

Study design

In early spring of 2021, sand was brought in and CLNWR staff constructed mounds approximately 1 m in height and 3 m in width (fig. 2a). Infrared-red, motion-detector camera traps (HyperFire 2 Professional HP2X and Reconyx HC500 Hyperfire Semi-Covert IR camera models) were placed approximately 3 m from the sand mound to capture nesting activity by female American crocodiles that included digging and possible laying (nesting period April/May), and nest excavation (hatching July/August) (fig. 2b, d). Staff regularly performed walking surveys and monitored nests for activity throughout the nesting season. Once nests were first detected (in late April), we carefully excavated them to determine nest and clutch size and outfitted each nest with temperature and soil moisture sensors. We marked the top of each egg with the orientation it was found in, removed eggs, and placed beside nest cavity. We counted total number of eggs and the number of visibly damaged eggs (fig. 2c). We noted presence of developmental banding (see visible banding in excavated eggs, fig. 2c, to estimate egg viability (Ferguson, 1985) and measured width of development band per egg in each clutch row. We measured the width and depth of nest cavity and placed loggers at three strata positions (top of clutch, middle and bottom, fig. 3) within the clutch to determine temperature and humidity parameters of the nest environment during incubation. Three temperature loggers were deployed in each nest (HOBO Tidbit MX Temperature 5000' data logger model MX 204, Boston, MA) and a separate temperature logger was attached to a PVC pipe outside of the nest to measure ambient air temperature at the nest surface. Loggers recorded data at 30-minute intervals (fig. 3). We selected a sand mound with no nesting activity and nearest to our target nests to excavate and outfit with temperature loggers to define the metabolic temperature of the internal nest environment relative to live nests deposited in sand mounds. Control sand nests shared similar external characteristics of vegetation shading, and proximity to water as live sand nests. Limited peat substrate did not provide an opportunity for a control peat nest for either study year. A soil moisture kit (HOBOnet Soil Moisture EC-5 Sensor model RXW-SMC-900) was placed inside each nest, including the control sand nest, to measure volumetric water content in the middle strata of the clutch (fig. 3). We noted vegetation shading, distance to nearest water source, and distance to nearest nest outfitted with loggers. Temperature

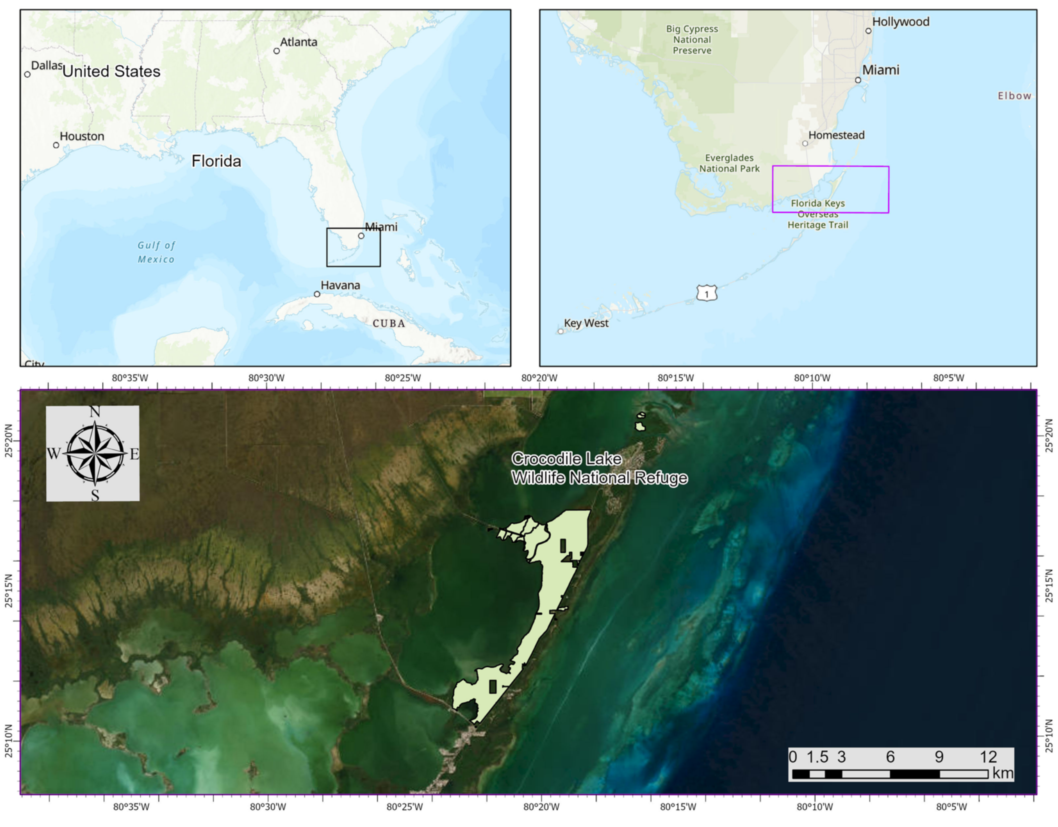

Figure 1. Map of instrumented nests of American crocodiles (*Crocodylus acutus*) at Crocodile Lake National Wildlife Refuge, Monroe County, Florida in 2021 and 2022.

loggers were Bluetooth enabled and CLNWR staff downloaded data using HOBO mobile software application once per week then three times per week when nesting activity increased. The soil moisture probe was connected wirelessly to a monitoring station (RX3000 Remote Monitoring Station, see fig. 3) and uploaded data to the Cloud at 30-minute intervals. Temperature and humidity loggers monitored conditions in the nest cavity and were removed at the end of the season once nests were naturally opened by the female crocodile.

Data analysis

We measured incubation temperature throughout the incubation period and denoted seasonal temperature shifts within the nest cavity strata (top, middle and bottom of clutch), among nests and between nesting years. Temperature and moisture data were assessed for each nest for temporal autocorrelation via *ggAcf*() function from the "forecast" package (Hyndman et al., 2023) in R version 4.2.2 (R Core Team, 2022). We used a maximum lag value of 168 units (1 week) to explore the degree in which data were temporally autocorrelated based on the Pearson correlation coefficient. Once we defined the window in which temporal correlation was weak or not present ($|<5|$ Pearson correlation value) data were averaged (daily; see results) and statistically analyzed. Data were assessed for normality (Shapiro-Wilk test) and homoscedasticity (Fligner-Killeen test) to define the most appropriate statistical approach. We performed analysis of variance (Kruskal-Wallis tests and Dunn's pairwise test) with Bonferroni correction to reveal any effect on temperature and moisture caused by nest cavity strata (ambient, top, middle, and bottom) and by nest. We defined statistical evidence as very strong (p-value $\leqslant$ 0.001), strong (p-value $\leqslant$ 0.01), moderate (p-value $\leqslant$ 0.05), weak (p-value $\leqslant$ 0.10), or little-to-no evidence (p-value $>$ 0.10) as suggested by Muff et al. (2021).

Results

Nest characteristics

In May of 2021, two American crocodile nests were located on CLNWR (fig. 1), one was deposited in original peat dredge spoils alongside a canal (see female digging nest, fig. 2b) and another was deposited in a supplemented

Figure 2. (a) Top left: American crocodile (*Crocodylus acutus*) nest on sand mound constructed by Crocodile Lake National Wildlife Refuge staff prior to the 2021 nesting season. Flag placed at the nest dug by female American crocodile. (b) Top right: Female American crocodile digging activity caught on infra-red camera traps during laying. (c) Bottom left: Egg clutch excavated during instrumenting nests. Developmental banding visible in egg. (d) Bottom right: Female crocodile excavating nest during hatching and transporting hatchlings to the water.

sand mound (fig. 2a). A control sand nest was identified in a supplemented sand mound nearest to the live sand nest being monitored and shared similar external characteristics. In 2022, three nests were identified in sand mounds (no nests were discovered in eroding peat dredge spoils) (fig. 1). A total of five nests and two control sand nests were outfitted with data loggers over the two years (fig. 1). Nests were laid under partial or no shade cover and a range of surrounding vegetation (table 1a). A canal leading out into Barnes Sound provided the nearest source of water with a distance ranging from 4.76-25.9 m with a mean of 12.85 $\pm$ 6.76 m SD (fig. 1). Nest cavity depth ranged from 31 to 43 cm with an average of 35.9 $\pm$ 4.12 cm. The average nest was 17.88 $\pm$ 5.14 cm from the top of the egg clutch to the surface and 35.93 $\pm$ 4.12 cm to the bottom of the clutch to surface, with a nest width of 27.21 $\pm$ 7.58 cm (table 1b).

Of the two nests laid in 2021, the peat dredge nest had 41 eggs from which 11 hatchlings were captured, 20 hatched shells were found (some of these may be of captured hatchlings), 5 eggs failed, 4 hatchlings were found dead within the nest, and 2 unhatched eggs were found near the mound on May 5. The sand mound nest had 32 eggs from which 6 eggs hatched, 21 eggs failed to hatch (no embryonic development in 14 eggs), and 1 dead hatchling was found inside the nest, remaining eggs were not found (table 1a). In 2022, sand mound nest 1 contained 31 eggs, sand mound nest 2 had 21 eggs and sand mound nest 3 had 32 eggs with a lower proportion of surviving hatchlings and two eggs were predated (table 1a, b). Over the two-year

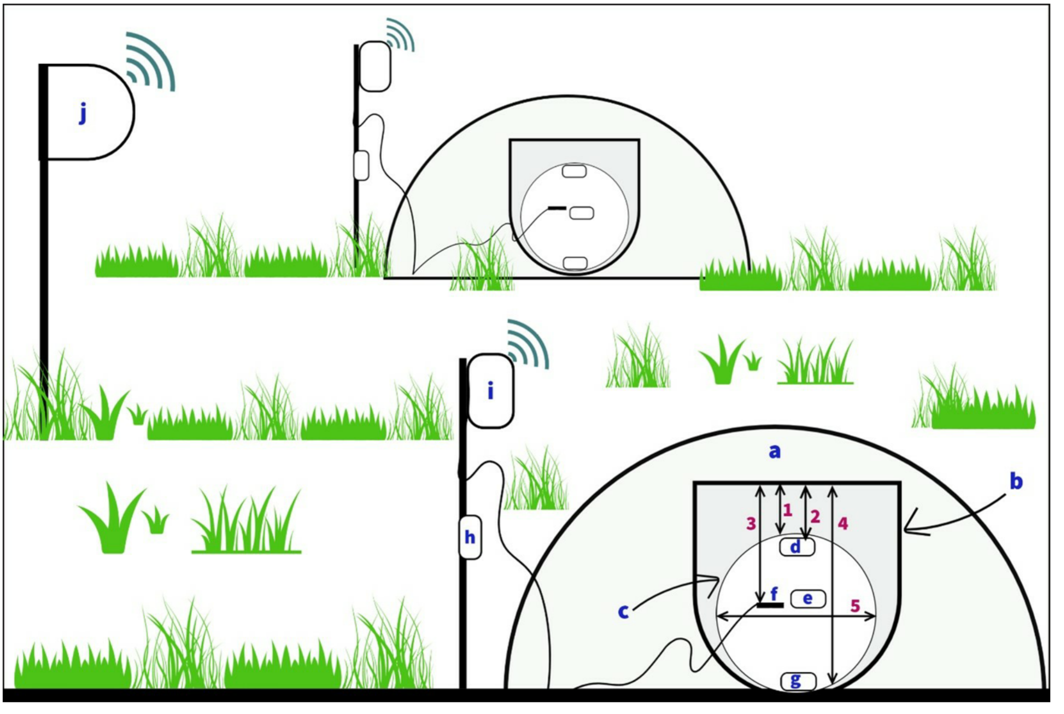

Figure 3. Datalogger instrumentation set up and position of temperature and soil moisture loggers within nests of American crocodiles (*Crocodylus acutus*) and ambient air temperature at nest vicinity at Crocodile Lake National Wildlife Refuge, Monroe County, Florida in May of 2021 and 2022. Soil moisture probe measured nest humidity. (a) Artificial sand mound, (b) nest cavity, (c) egg clutch, (d) temperature datalogger at top of clutch, (e) temperature datalogger in the middle of clutch, (f) soil moisture kit probe, (g) temperature datalogger at the bottom clutch, (h) ambient air temperature datalogger, (i) soil moisture kit mount,(j) monitoring station (soil moisture kits are connected wirelessly to this), measurement (cm) from (1) top-most egg of the clutch to nest cavity opening, (2) top datalogger to nest cavity opening, (3) middle temperature logger and moisture probe to nest cavity opening, (4) nest cavity opening to the bottom of the cavity, (5) width of the emptied egg chamber/nest cavity.

Table 1a. Nesting data for American crocodile (*Crocodylus acutus*) nests.

Nest ID	Nest substrate	Lay date	Hatch date	Incubation time (days)	Total eggs	Banded eggs	Partially banded eggs	Hatched shells	Failed eggs	Dead Hatchlings
2021-1	Peat/ Sand/Rock	22 Apr	10 July	79 d	41	39	1	20	5	4
2021-2	Sand	27 Apr	19 Aug	114 d	32	30	0	6	21	1
2021-CS	Sand	5 May	NA	NA	NA	NA	NA	NA	NA	NA
2022-1	Sand	22 Apr	9 July	78 d	31	28	0	2	15	1
2022-2	Sand	27 Apr	26 July	90 d	21	2	18	5	0	0
2022-3	Sand	2 May	21 July	80 d	32	18	1	16	6	0
2022-CS	Sand	5 May	NA	NA	NA	NA	NA	NA	NA	NA

Table 1b. Physical characteristics of nests laid in 2021 and 2022 on Crocodile Lake National Wildlife Refuge, Florida. Control sand nests are denoted (i.e., 2021-CS, 2022-CS).

Nest ID	Depth of nest cavity (cm)	Width of nest cavity (cm)	Distance to nearest water source (m)	Shaded	Vegetation growth (%)	Distance to nearest outfitted nest (m)	Distance to control nest (m)
2021-1	39.5	41	10.6	No	25%	24.1	13.3
2021-2	34.5	33.5	7.85	No	None	24.1	16.4
2021-CS	36.5	21	4.76	Partially	50%	13.3	NA
2022-1	31	21	15.7	Partially	25%	36.1	55.2
2022-2	33	22	12.5	Partially	None	1	24
2022-3	43	28	12.5	Partially	None	1	24
2022-CS	34	24	25.9	No	None	24	NA

study, average clutch size was 31.4 ± 7.09 eggs ranging from 21 to 41 total eggs. Days until hatching (incubation period) ranged from 78-114 days, with an average of 88 days (table 1a). Average developmental banding was 3-3.5 mm within each clutch. A total of 73 eggs were laid in 2021 and 84 eggs in 2022. Of a total of 157 eggs laid in both years, 49 hatched shells were found at the end of the hatching seasons and 11 hatchlings were caught by hand. It is likely that there were more hatchlings than were caught by hand.

Nest environment

Soil moisture was measured as volumetric water content (m^3/m^3) and was more variable in 2021 than in 2022 (fig. 4) and both control sand and live nests showed similar trends over the season (fig. 5). There was a detectable metabolic heat difference between nests with incubating eggs and control sand nests without eggs such that the temperature in the middle of the clutch of eggs (31.9°C) was 1.9°C warmer than the control sand nest with no eggs (30.0°C) in 2021 (table 2). In 2022, incubating nests ranged from 0.8°C to 2°C warmer than the control sand nest (at 30.8°C; table 2). A sand mound nest of 2021 did not have a noticeable difference (29.9°C) when compared with the control sand nest and 21 of 32 deposited eggs failed to develop.

The internal nest temperature of all nests was both warmer and more stable than ambient (surface) temperatures (table 2) and the topmost layer of eggs that were closer to the surface resembled daily ambient fluctuations (fig. 6, table 2). Temperature data were seasonal showing high levels of temporal autocorrelation during the day with peak values every 12 hours, gradually reducing with time (fig. 4). This tendency was found in both control and live nests in both years (supplementary fig. S1). As expected, ambient day temperatures were positively correlated between each other and negatively correlated with night temperatures. However, this trend was less evident in the top strata of nests and was not present in the middle and bottom strata of nests. Temporal autocorrelation was slightly reduced when data were averaged every 6 and 12 hours, showing weak to no correlation when using a 24-hour window (fig. 4). This pattern was observed across all nests and strata although 2022 data presented slightly higher autocorrelation values than 2021 data (supplementary fig. S2). Moisture data were also highly correlated gradually reducing with time but did not show a seasonal pattern. This tendency was found in both control sand and live nests in both years. Moisture autocorrelation was drastically reduced when data were averaged every 6, 12, and 24 hours, showing no correlation ($|<2|$) across all time windows (fig. 4).

Temperature and moisture data of each nest and within the nest cavity strata did not fit assumptions of samples coming from a normally distributed population. Temperatures were on average higher within the clutch (top, middle, and bottom ~ 31.2°C) with no evidence

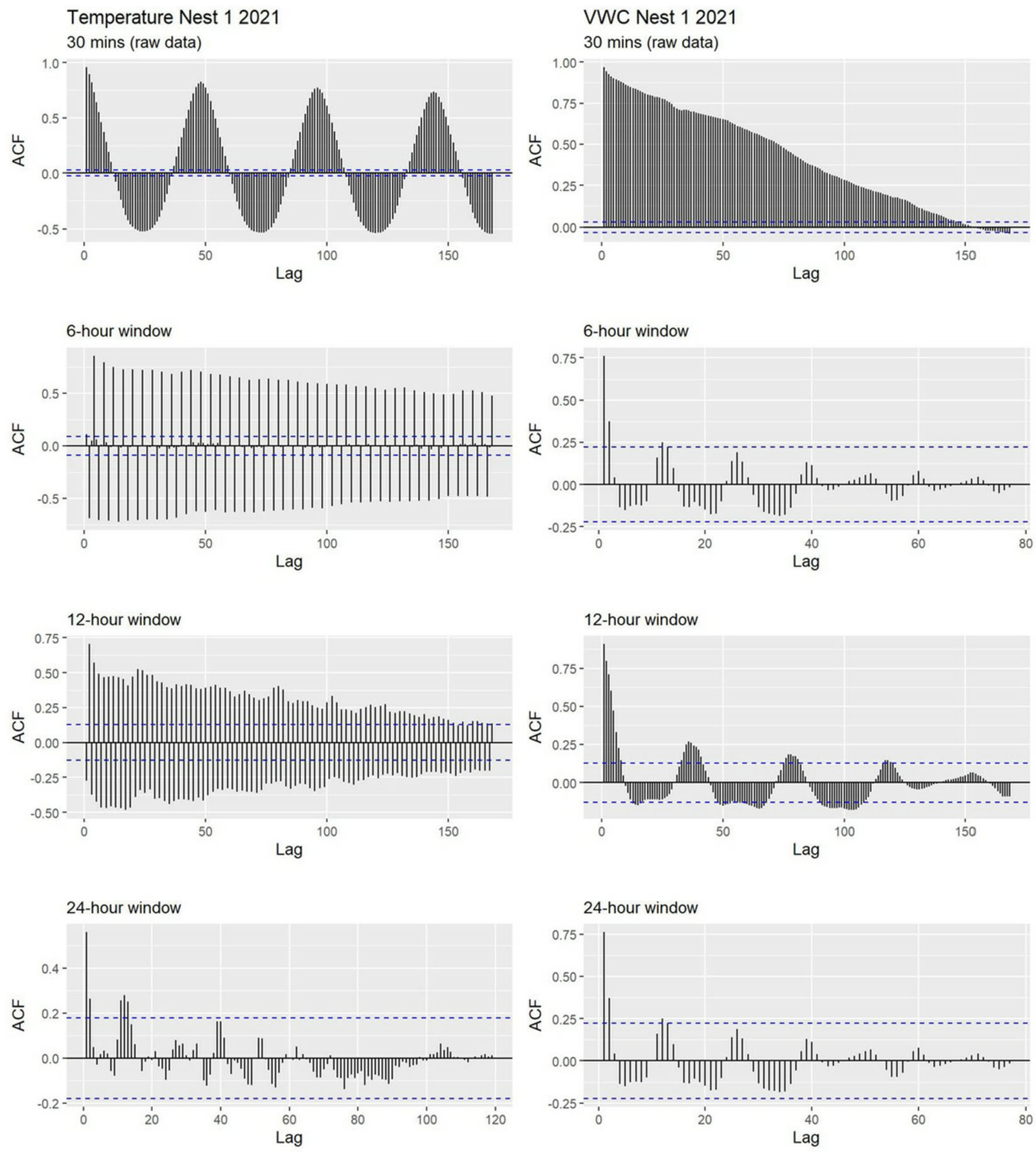

Figure 4. Temperature and moisture (volumetric water content (VWC)) autocorrelation function (acf) analysis using raw ambient data from American crocodile (*Crocodylus acutus*) nests (in the case of temperature) every 30 mins and at 6-, 12-, and 24-hour average windows for Nest 1 collected in 2021 at Crocodile Lake National Wildlife Refuge, Florida. ACF plots per nest using raw data per strata (ambient, top, mid, and bottom) in the case of temperature and volumetric water content from the middle of the clutch for moisture can be found in supplementary figs. S1 and S2.

of internal variation (Dunn's test $P =$ bottom-top $= 0.26$, top-middle $= 0.48$, and mid-bottom $= 1.0$) comparing to ambient temperature (~ 29.6°C) for which we found very strong evidence of temperature variation (Dunn's test $p < 0.001$) across nests. Interestingly, the inverse pattern was observed when analyzing temperature ranges across strata, with a larger range of variation detected in ambient temperature (25.5 ± 3.8°C) compared to the top (21.9 ± 6.5°C), middle (18.1 ± 6.0°C), and bottom (15.5 ± 7.2°C) strata temperatures within

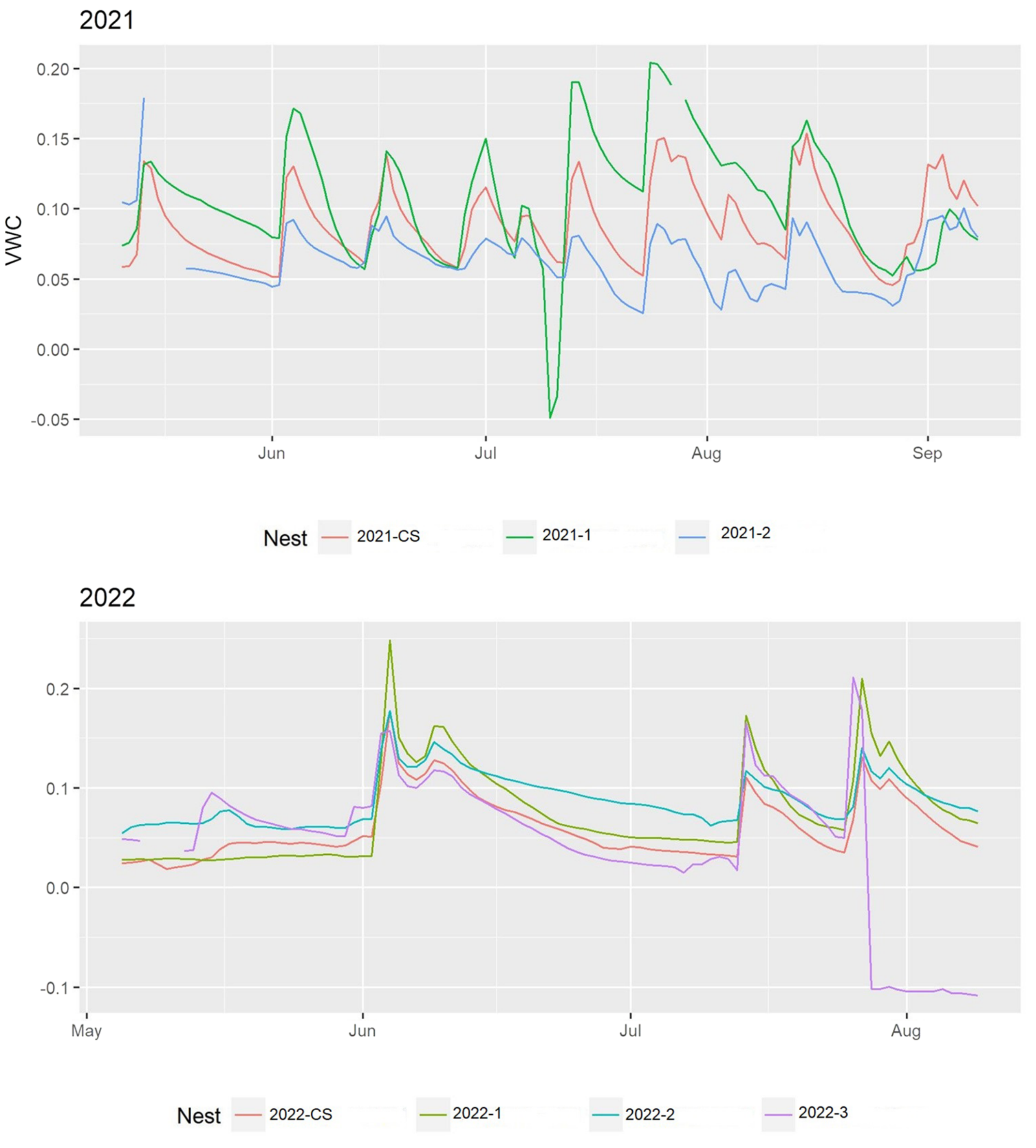

Figure 5. Average moisture measured as volumetric water content (within a 24 h window) of American crocodile (*Crocodylus acutus*) nests laid at Crocodile Lake National Wildlife Refuge, Florida in 2021 and 2022 for control sand (CS) and live nests.

the nest. In this case, we found a significant difference between all pairwise strata (Dunn's test $p < 0.001$). We also found strong evidence of pairwise differences in internal temperatures (top, middle, and bottom) and external (ambient) nest temperatures (Dunn's test $p < 0.001$) except between control nest 2021 and nest 2 2022, nest 1 2021 and 2022, nest 1 2021 and nest 2 2022, and nest 1 and 2 2022 (Dunn's test $p < 1.0$).

Moisture data differed between nests. Moisture data were on average higher in the peat nest (nest 1) of 2021 (0.11 ± 0.05 m^3/m^3) followed by control sand nest 2021 and nest 2 laid in a sand mound of 2022 (0.09 ± 0.03 m^3/m^3, each), sand nest 1 2022 (0.07 ± 0.05 m^3/m^3),

Table 2. 24H Thermal profile of strata layers in American crocodile (*Crocodylus acutus*) nests laid in 2021 and 2022 on Crocodile Lake National Wildlife Refuge, Florida. Control sand nests are denoted (i.e., 2021-CS, 2022-CS).

Year	Nest	Logger position	Mean temp (°C)	SD temp (°C)	Median temp (°C)	Min temp (°C)	Max temp (°C)
2021	2021-CS	Ambient	29.3	1.6	29.5	26.0	32.7
2021	2021-CS	Top	29.8	1.8	29.7	26.4	35.4
2021	2021-CS	Mid	30.0	1.4	29.9	27.2	33.2
2021	2021-CS	Bottom	30.2	1.2	30.1	27.6	33.0
2021	2021-1	Ambient	29.3	1.4	29.4	26.1	32.0
2021	2021-1	Top	31.4	2.0	31.1	27.7	36.2
2021	2021-1	Mid	31.9	1.5	32.0	27.4	35.0
2021	2021-1	Bottom	31.8	1.3	31.9	27.5	34.6
2021	2021-2	Ambient	29.8	1.6	29.8	25.8	32.9
2021	2021-2	Top	29.7	1.6	29.6	26.2	33.9
2021	2021-2	Mid	29.9	1.5	29.9	26.8	34.0
2021	2021-2	Bottom	30.0	1.4	30.0	27.1	33.7
2022	2022-CS	Ambient	29.6	1.7	30.0	23.7	32.1
2022	2022-CS	Top	30.7	2.0	31.0	25.3	34.3
2022	2022-CS	Mid	30.8	1.9	31.0	25.6	34.2
2022	2022-CS	Bottom	30.7	1.8	30.8	25.5	33.9
2022	2022-1	Ambient	29.5	1.9	29.9	23.6	32.2
2022	2022-1	Top	32.0	2.4	32.3	25.3	36.3
2022	2022-1	Mid	31.6	2.1	31.8	25.1	34.9
2022	2022-1	Bottom	31.5	2.0	31.5	25.2	34.5
2022	2022-2	Ambient	29.2	1.7	29.8	23.5	31.4
2022	2022-2	Top	31.4	2.5	31.8	24.1	35.2
2022	2022-2	Mid	31.6	2.6	31.7	24.9	35.8
2022	2022-2	Bottom	31.6	2.4	31.9	24.8	35.4
2022	2022-3	Ambient	30.3	2.5	30.2	23.6	34.5
2022	2022-3	Top	32.7	2.6	33.2	25.2	36.2
2022	2022-3	Mid	32.7	2.5	33.1	25.2	36.1
2022	2022-3	Bottom	32.7	2.4	33.1	25.0	36.3

sand nest 2 2021 and control sand nest 2022 (0.06 ± 0.03 m^3/m^3 each) and sand nest 3 2022 (0.05 ± 0.07 m^3/m^3). Nests with the highest and lowest average moisture had the largest range differences (0.68 and 0.54, respectively). We found evidence of pairwise moisture differences among nests except between control sand nest 2021 and sand nest 2 2022, control sand nest 2022 and sand nest 1 2022, sand nest 2 and sand nest 3 2022, sand nest 1 and sand nest 2 2022, and sand nest 2 2021 and sand nest 3 2022 (Dunn's test $p > 0.1$).

Discussion

CLNWR has been an important contributor to the South Florida population of American crocodiles since the first four nests were reported in the early 1970's (Ogden, 1978; Mazzotti et al., 2022). Since then, nests have been identified and monitored every year and produce on average between 5 and 8 nests per year in a small fraction of the 27 km^2 refuge. This location provided a unique opportunity to monitor nest temperatures and volumetric water content in addition to documenting potential variation within nests deposited by female American crocodiles that included artificially created nesting substrate (sand mounds). To quantify the artificial nest environment used by wild crocodylians, this study is the first step toward providing supplementary nesting habitat in areas of declining nesting habitat for the American crocodile. Here we highlight the stability of the internal nest environment despite fluctuating external conditions and confirm that these supplemented nest mounds have

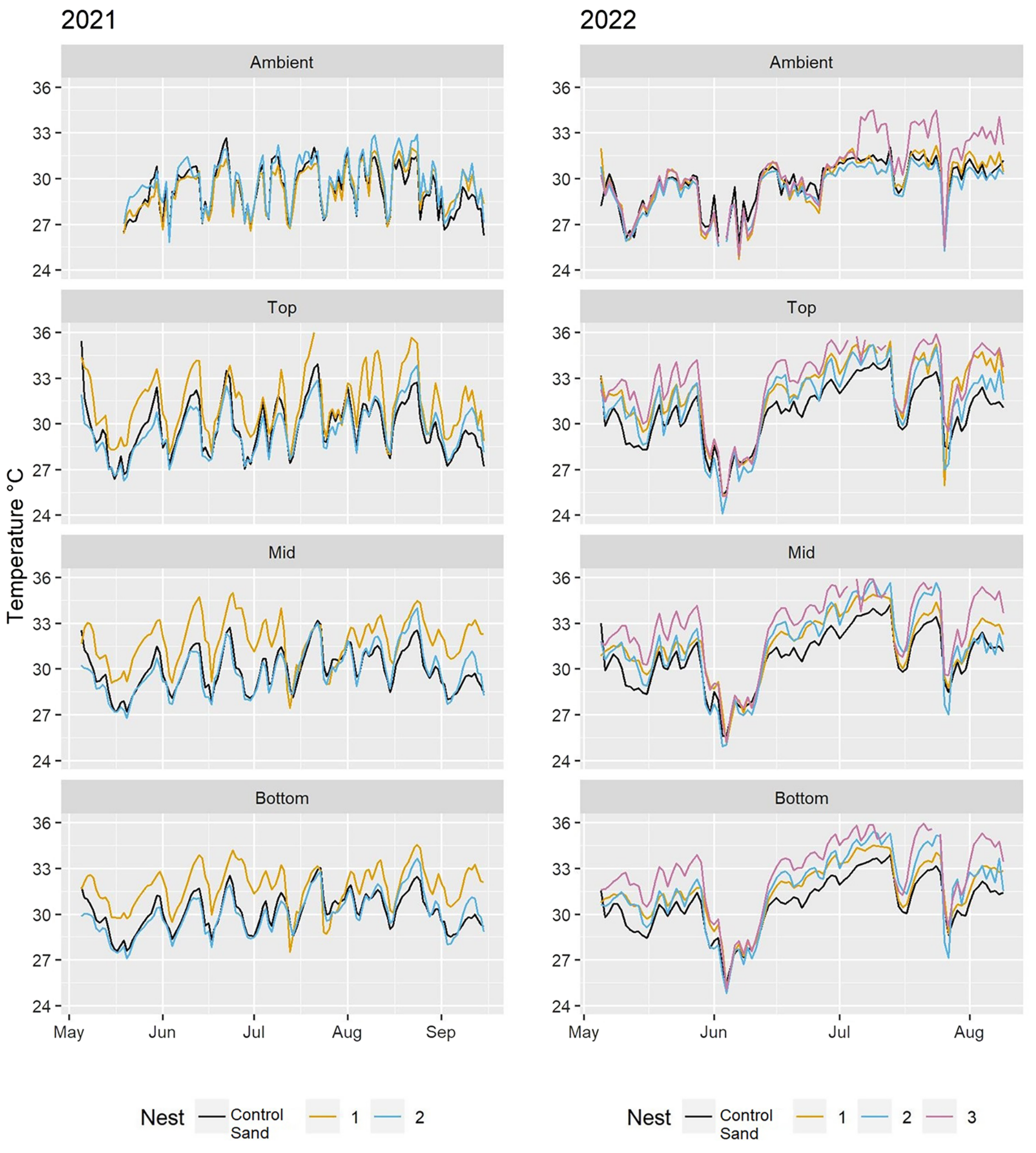

Figure 6. Average thermal regime (within a 24 h window) of American crocodile (*Crocodylus acutus*) nests laid at Crocodile Lake National Wildlife Refuge, Florida in 2021 and 2022 at different strata layers: ambient = surface, top of the egg clutch, middle of clutch, bottom of clutch. Control sand nests and live nests included.

nest success producing viable eggs and hatchling crocodiles on CLNWR.

Global temperatures are shifting, and ectothermic animals employ a suite of responses to maintain homeostasis and regulate functional body temperatures (Parmesan, 2006). Included in this is a female's ability to select suitable nesting sites that ensure nest success and hatchling survival. A nest ought to be far enough from the water's edge and at a higher elevation to prevent clutch inundation since it is a known mortality of eggs and in substrates that prevent 100% desiccation (Joanen et al., 1977; Mazzotti et al., 1988; Murray et al., 2015). Crocodylians display both hole and ancestral mound nesting and varies within a species and by location (see Murray et al., 2020). In South Florida, American crocodiles are both hole and mound nesters (Ogden, 1978) and nesting substrate along coastal beach and coastal berm nests are composed of marl and sand/shell material with sandy/shell substrate being more porous than the higher organic content of marl substrate (Lutz and Dunbar-Cooper, 1984; Mazzotti et al., 2021). American crocodile nests are found in both substrates and produce viable offspring yearly (Ogden, 1978; Cherkiss et al., 2020; Mazzotti et al., 2021).

In this study, we document a mean clutch size across 2021 and 2022 of 31.4 ± 7.1 eggs in CLNWR which is similar to what Kushlan and Mazzotti (1989) found in NE Florida Bay with 38 ± 0.4 eggs and is more than has been found in Mexico (16.2 ± 4.6 eggs; Charruau et al., 2010), Belize (22.3 ± 6.0 eggs; Platt and Thorbjarnarson, 2000b), Haiti (22.5 ± 2.7 eggs; Thorbjarnarson, 1989), and Coiba Island, Panama (25.2 ± 9.5 eggs; Balaguera-Reina et al., 2015), and is less than clutches from mainland Panama (46 eggs, Breder, 1946), clutches found in Florida Bay (45.2 ± 17.2 eggs; Ogden, 1978) and two separate Colombian populations (40-60 eggs; Medem, 1981). Across both nesting years, four of the five clutches contained eggs that showed embryonic development, visible as developmental banding, in most eggs, except for the smallest clutch of 21 eggs. Of these, only 2 eggs were fully banded and produced 5 hatched shells which may reflect the inexperience of a young female during copulation where only a subset of eggs may have been fertilized by the male. Though it is not equivalent to counting hatchlings, hatched shells at the nest site are an indication of nest success (but see Ogden, 1978) and often is the only measure available under field conditions.

Here, incubation time or time until hatching was on average 88.2 days across the two nesting periods (similar to other locales, Kushlan and Mazzotti, 1989; Charruau, 2012; Balaguera-Reina et al., 2015), however a nest deposited in a supplemented sand mound in 2021 went as long as 114 days before hatching. This may be a combination of a longer incubation period and more subtle hatching signs not detected during walking surveys, as well as potential substrate effects of supplemented sand material. Infra-red cameras documented female visits to the nests to assist with hatching and carry hatchlings to the water (see fig. 2d). Female presence was detected with infra-red cameras at all nests and likely contributed to hatching success (Thorbjarnarson, 2010; Balaguera-Reina et al., 2015), since female scratching/digging serves to help loosen up the nest substrate to allow for hatchlings to emerge but also making it easier for predators such as raccoons to unearth eggs (Ogden, 1978). The finer, more porous sand substrates are easier to unearth and open than nests made of marl/peat substrate.

In our study, nest temperatures varied by strata with internal temperatures reflecting a more stable environment than ambient temperatures subject to daily fluctuations. A more stable internal nest temperature promotes a balanced environment for temperature-sex-determination to function (Lang and Andrews, 1994). The internal nest environment, however, is dynamic and undergoes temperature variation on a daily cycle and throughout phases of incubation. Here metabolic heat was generated by viable, incubating eggs at 0.8°C to 1.9°C warmer than control sand nests and is within the range of temperature variation found in *C. acutus* nests in parts of their range (Charruau, 2012; Murray et al., 2016); low or no metabolic heat is an indication of higher mortality rates as was seen in one clutch of 2021. Nest temperatures reflected a bi-monthly fluctuation that ranged from 34°C at the beginning of the month and decreasing to 27°C by the middle of the month only in the 2021 nesting season. In 2022, nest (and ambient temperatures) followed a more normal pattern of increasingly warmer temperatures in the later summer months, however there were three noticeable rainfall events that occurred in early June, and middle and late July that reduced nest temperatures by several degrees (fig. 6) potentially affecting incubation period and hatching success.

Historically, American crocodiles nested on high, well-drained beaches, creek banks or canal levees that are not exposed to wind action and human disturbance throughout Florida Bay (Ogden, 1978; Mazzotti, 1983). Nesting on northern Key Largo, now CLNWR, would not likely have been prime nesting habitat according to Ogden (1978) as the area is "low and swampy with few beaches or elevated creek banks". With the attempted development during the 1920's that left dredge spoils surrounded by mangroves; this may have provided suitable nesting habitat. The remaining dredge spoils have continued to oxidize and erode in recent years and have not been naturally replenished which prompted USFWS staff to augment the Refuge's nesting sites with artificially constructed sand mounds. These sand mounds have provided nesting habitat for American crocodiles but there is more to be understood of the nesting conditions that would provide optimal hatching success. We provide the baseline nesting conditions and internal thermal regime of anthropogenically supplemented nesting habitat in South Florida that will be useful for managers working toward American crocodile conservation, particularly in areas where nesting habitat is declining.

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