

The Effect of Australian Pine (*Casuarina equisetifolia*) Removal on Loggerhead Sea Turtle (*Caretta caretta*) Incubation Temperatures on Keewaydin Island, Florida

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ABSTRACT



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The spread of invasive exotic vegetation is a serious threat to native habitats in South Florida. The exotic Australian pine proliferates in coastal areas forming monocultures. They fall over easily during strong winds, making nesting habitat inaccessible to sea turtles. The objective of this study was to determine if removing the standing pines would alter hatchling sex ratios of sea turtles because nest temperatures could increase in the absence of shade provided by the exotic pine species. A total of 274 Hobo temperature data loggers were deployed in sea turtle nests on Keewaydin Island, Collier County, Florida, during the 2001, 2002, 2004, 2005, and 2006 nesting seasons to monitor the effect of Australian pine removal on incubation temperatures. The results indicated that shading from the pines did not affect incubation temperatures differently than native vegetation. Therefore, removing the pines did not alter hatchling sex ratios. During the study, southwest Florida was affected by several storm events and hurricanes washing out 57 data loggers and causing an additional 15 data loggers to malfunction. Nest temperatures during the thermosensitive period ranged from 23.24°C to 34.85°C with mean temperatures ranging from 26.61°C to 31.51°C. Hatchling sex ratios were predicted based on mean incubation temperatures during the thermosensitive period. The predictions indicated that nests on Keewaydin Island were producing predominately mixed ratio and male-biased clutches. Dead hatchlings were collected for histological examination to substantiate sex ratio predictions. Histology samples were female-biased in 2002 and male-biased in 2001 and 2004.

ADDITIONAL INDEX WORDS: *Habitat restoration, temperature-dependent sex determination (TSD).*

INTRODUCTION

Sea Turtle Nesting

Sea turtles have temperature-dependent sex determination (TSD) in which the temperature of the sand influences the sex of the hatchlings (YNTEMA and MROSOVSKY, 1980). Sex is determined during the middle third of incubation (YNTEMA and MROSOVSKY, 1982), which is referred to as the thermosensitive period (MROSOVSKY and PIEAU, 1991). Incubation duration of natural nests in Florida averages 53–55 days (DODD, 1988). MROSOVSKY (1988) demonstrated that the pivotal temperature in which equal numbers of males and females are produced is 29°C for North American loggerheads (*Caretta caretta*). In a controlled laboratory experiment, YNTEMA and MROSOVSKY (1982) found incubation temperatures greater than or equal to 32°C produced all females and temperatures less than or equal to 28°C produced all males.

Nest temperature has been used to predict loggerhead hatchling sex ratios in several Florida studies. HANSON *et al.* (1998) predicted that 92.5% of the nests examined were 100% female on Hutchinson Island, Florida. MROSOVSKY and PROVANCHA (1992) examined nest temperatures and collected hatchlings along Cape Canaveral, Florida. They estimated that hatchlings during 1986, 1987, and 1988 were 92.6%–96.7%, 94.7%–99.9%, and 87.0%–89.0% females, respectively. MROSOVSKY *et al.* (1995) found that nests shaded by condominiums in Boca Raton, Florida, were 1°C–2°C cooler than nests that were not shaded. However, mean sand temperatures were above the pivotal temperature, ranging from 30°C to 31°C, so the authors believed that mostly female hatchlings were produced regardless of the shade. FOLEY *et al.* (2000) examined nests in the Ten Thousand Islands on the southwest coast of Florida during 1992–1994 and estimated 1:1 sex ratios. FOLEY *et al.* (2000) also found that sand temperatures in the shade or close to the water were lowest and

therefore more likely to produce males. These studies illustrate that nest site selection can influence the thermal environment and consequently hatchling sex ratios.

Invasive Exotic Vegetation

During the early 1900s, Australian pines (*Casuarina equisetifolia*) were planted along beaches and near homes in southern Florida for protection against hurricanes and strong winds (CRAIGHEAD, 1971; NELSON, 1994). Ironically, the shallow root system makes the trees susceptible to falling under strong wind and beach erosion. Since their introduction, Australian pines have become one of the greatest threats to native beach vegetation (CRAIGHEAD, 1971; JOHNSON and BARBOUR, 1990). The pines invade disturbed regions such as accretion areas and spoil islands. Once established, the shade and the thick litter layer under the Australian pines prevent the germination and growth of native vegetation (e.g., sea oats [*Uniola paniculata*], inkberry [*Scaevola plumier*], and sea grape [*Coccoloba uvifera*]) in coastal areas (JOHNSON and BARBOUR, 1990; NELSON, 1994).

Nests shaded by the pines may produce a higher percentage of male hatchlings than nests receiving more sun on the open beach because the sex of a sea turtle is temperature dependent. During 1986 and 1987, the nests on Keewaydin Island were relocated to a hatchery, which was shaded by Australian pines. A preliminary study by SCHMELZ and MEZICH (1988) found that the mean sand temperatures in the shaded hatchery partially shaded by pines outside the hatchery and open beach were 26.4°C, 27.0°C, and 27.9°C, respectively. Over time, this alteration of sex ratio could have implications on the operational sex ratio of loggerhead populations. Restoration projects to reduce or eradicate Australian pines and other invasive exotic plants have been implemented to restore native coastal Florida vegetation and reclaim sea turtle nesting beaches in the Dry Tortugas National Park (KLUKAS, 1967; REARDON, 1998), Blowing Rocks Preserve (RENDA and RODGERS, 1995), and Keewaydin Island (SCHMID, 2003).

The objective of this study was to compare nest incubation temperatures in areas where Australian pines were present, where Australian pines were removed, and where native vegetation was present. It was conducted to determine whether native habitat restoration efforts have the potential to alter loggerhead hatchling sex ratios.

MATERIALS AND METHODS

Study Area

Keewaydin Island is an unbridged, 12-km-long primary barrier island located south of Naples, Collier County, Florida (Figure 1). Approximately 85% of the island is owned and managed by the Florida Department of Environmental Protection (FDEP) and is within the Rookery Bay National Estuarine Research Reserve (RBNERR). The beach is backed by native vegetation (e.g., sea oats, sea grape, inkberry, bay cedar [*Suriana maritima*], beach elder [*Iva imbricate*], railroad vine [*Ipomoea pes-caprae*], and sea purslane [*Sesuvium portulacastrum*]) and invasive exotic vegetation (e.g., beach

naupaka [*Scaevola taccada sericea*], Brazilian pepper [*Schinus terebinthifolius*], latherleaf [*Colubrina asiatica*], and Australian pine).

The RBNERR staff began a large-scale restoration project on Keewaydin Island in March, 1998. Australian pines were removed from approximately 142 ha of beaches, state lands, and some privately owned land. Native vegetation was planted in selected areas to increase the native seed source and to help stabilize heavily disturbed areas. Exotic removal maintenance is an ongoing effort and is contracted as funding becomes available.

Sea Turtle Monitoring

The Conservancy of Southwest Florida, a local nonprofit environmental agency, has been monitoring sea turtle activity on Keewaydin Island since 1982. Keewaydin Island is located within the South Florida Nesting Subpopulation (TURTLE EXPERT WORKING GROUP, 2000) and is part of the Florida Index Nesting Beach Survey Program (INBS). There is an average of 195.7 nests annually with a range of 112–284 nests (FUNCK and ADDISON, 2008). Keewaydin Island is monitored nightly during the nesting season from May until the beginning of August and subsequently is patrolled daily for hatching activity until October. Monitoring efforts include collecting turtle morphometric data (carapace straight length and width, carapace curved length, and width and head width), tagging nesting females with flipper tags, recording nest and false crawl location data, and caging nests *in situ* to prevent raccoon depredation (FUNCK and ADDISON, 2008). According to the Marine Turtle Conservation Guidelines (FLORIDA FISH AND WILDLIFE CONSERVATION COMMISSION, 2007), contents of the nest are excavated and hatching success is evaluated 3 days after hatchling emergence or 70 days after the nest was deposited (80 days if the nest was inundated), whichever occurs first.

Incubation Temperature

During the 2001, 2002, 2004, 2005, and 2006 sea turtle nesting seasons, Hobo temperature data loggers (Onset Computer Corporation, Pocasset, MA) were deployed in sea turtle nests to record hourly incubation temperature. Data loggers were calibrated and vacuum sealed in plastic with desiccant prior to deployment. Throughout each nesting season, the data loggers were opportunistically deployed in the middle of the egg chamber during egg deposition.

To determine whether removing the Australian pines influenced incubation temperatures, we categorized the vegetation along the dune as Australian pine present, Australian pine removed, and native vegetation present. The Australian pine present areas were dominated by pines, and in some areas, beach naupaka or sea grape were also present. The Australian pine removed areas were colonized by low ground cover (e.g., grasses, sea purslane, and railroad vine) and shrubs (e.g., sea grape, inkberry, and bay cedar). The native vegetation areas occurred where pines had never been present and were predominantly sea oats with interspersed beach elder.

The loggerhead nesting season was divided into early, mid-

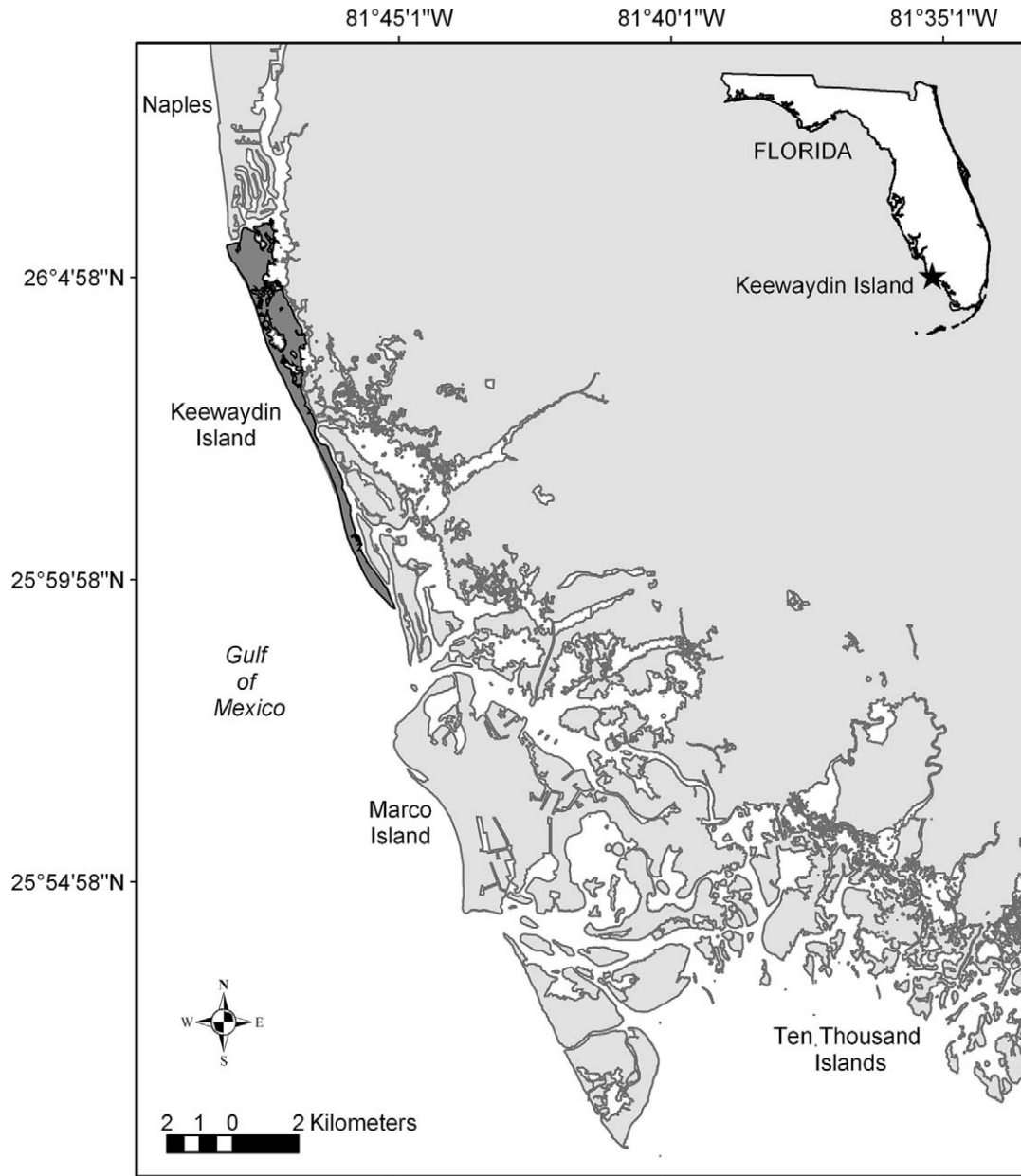


Figure 1. Location of Keewaydin Island, Collier County, Florida.

dle, and late season to examine seasonal variation. Individual nests were categorized by the date they were deposited: early (1 May–31 May), middle (1 June–30 June), or late (1 July–15 August). After hatchling emergence, each nest was excavated and the contents evaluated to calculate hatching success. Data loggers were recovered and temperature data were downloaded using BoxCar Pro 4.0 software (Onset Computer Corporation, Pocasset, MA). During 2001, 2002, and 2004, dead hatchlings found in the nest were preserved in formalin and sex was determined histologically.

The Kruskal-Wallis nonparametric test was used in the analyses because data lacked normality and homogeneity of

variance. To test for differences in annual nest incubation temperatures, we compared thermosensitive temperatures by year. To test for seasonal differences, we compared thermosensitive temperatures among nests laid early, middle, and late for each year of the study. To test for differences by vegetation category, we compared thermosensitive temperatures among nests laid in areas where Australian pines were present, Australian pines were removed, and native vegetation was present for each year of the study. Seasonal effects were removed from the vegetation analysis by only using nests deposited during the middle of the season. In the event of significant differences, pairwise comparisons were performed

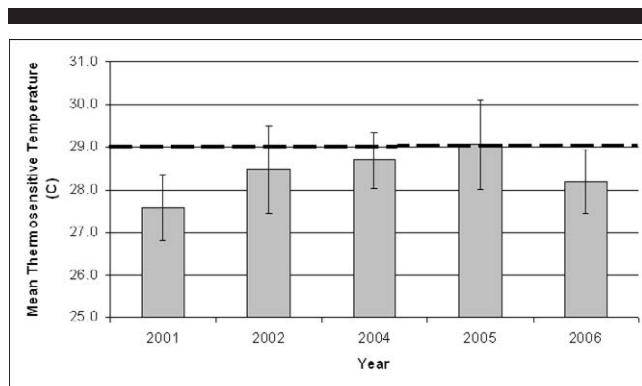


Figure 2. Mean and standard deviation of thermosensitive temperatures by year. The dashed line represents the pivotal incubation temperature of 29°C. Incubation temperatures during 2001 were significantly cooler than 2002, 2004, 2005, and 2006, and temperatures during 2006 were significantly cooler than 2004 and 2005.

using the Kruskal-Wallis test with Bonferroni corrected significance levels.

Storm Events

Loggerhead nesting activity in Florida occurs from May to August with hatching continuing until October. The nesting season overlaps with hurricane season, which extends from June to November and peaks during September. Most nests are either hatched or fairly advanced in development by the time catastrophic storms affect the coast. Several hurricanes and storm events affected Keewaydin Island during the present study. Observations on incubation temperature and on nest loss due to the storms were documented during the course of the study.

RESULTS

During the 2001 nesting season, 54 temperature data loggers were deployed on Keewaydin Island, but only 21 were retrieved. A July storm event washed away 33 data loggers. During 2002, 44 data loggers were deployed, and 37 were successfully retrieved and downloaded (7 data loggers malfunctioned). During 2004, 51 data loggers were deployed, and 32 were retrieved. Nineteen data loggers were washed away during Hurricanes Charley, Frances, and Ivan. During 2005, 64 data loggers were deployed, and 60 were retrieved. Four loggers were lost during Hurricane Dennis and Tropical Storm Katrina. During 2006, 79 data loggers were deployed, and 77 were retrieved. Tropical Storms Alberto and Ernesto minimally affected Keewaydin Island, washing out only two data loggers. Of the 2001, 2002, 2004, 2005, and 2006 successfully downloaded data loggers, 2 were in green turtle nests, and 201 were in loggerhead nests. Logger data exhibited sharp decreases in temperature following heavy rainfall and tidal inundation associated with storm events and hurricanes. Temperatures decreased an average of 2.62°C and decreases ranged from 0.78°C to 7.53°C. During the thermosensitive period, mean nest temperatures during 2001, 2002, 2004, 2005, and 2006 ranged from 26.61°C to 31.51°C.

Table 1. Annual mean thermosensitive temperatures by season. Chi-square and p-value results from the Kruskal-Wallis test. Asterisk denotes a significant difference among seasons within the same year. Data loggers from late season nests in 2004 were washed out by a hurricane.

Year	Season			χ ²	p
	Early	Middle	Late		
2001	26.8	27.59	28.28	7.2857	0.0262*
2002	27.36	29.07	28.74	14.3282	0.0008*
2004	28.31	28.89	—	4.7742	0.0289*
2005	27.60	29.25	29.60	17.8237	0.0001*
2006	28.33	27.88	28.66	13.4561	0.0012*

There was a significant difference in thermosensitive incubation temperatures among years (χ² = 42.4709; p < 0.0001; Figure 2). Results from the pairwise multiple comparison tests indicated that incubation temperatures during 2001 were significantly cooler than in 2002, 2004, 2005, and 2006, and temperatures during 2006 were significantly cooler than in 2004 and 2005.

There was a significant difference in annual thermosensitive incubation temperatures among early, middle, and late season nests (Table 1). In 2001, early nest thermosensitive temperatures were significantly cooler than late season nests. In 2002 and 2005, early nests were significantly cooler than middle and late season nests. In 2004, early nests were significantly cooler than middle season nests. Late season nests were missing from the analysis because all the late data loggers were lost in a hurricane. In 2006, middle nests were significantly cooler than early and late season nests. There was not a significant difference in annual thermosensitive incubation temperatures for middle season nests among native vegetation present, Australian pine present, and Australian pine removed (Table 2).

Sex ratios were predicted for each nest based on incubation temperatures during the thermosensitive period. Nests were predicted to produce mostly mixed ratio or male-biased clutches (Table 3). Histology was conducted to determine the sex of 43 hatchlings from 11 nests in 2001, 26 hatchlings from 4 nests in 2002, and 47 hatchlings from 3 nests in 2004. In 2001, of the 43 hatchlings there were 18 females, 24 males, and 1 undetermined. In 2002, of the 26 hatchlings there were 22 females, 0 males, and 4 undetermined. In 2004, of the 47 hatchlings there were 14 females and 33 males (Table 4).

Table 2. Annual mean thermosensitive temperatures by vegetation category. (AP = Australian pine present, AP Removed = Australian pine removed, Native = Native vegetation present). Chi-square and p-value results of the Kruskal-Wallis test.

Year	Vegetation Category			χ ²	p
	AP	AP Removed	Native		
2001	27.39	27.55	28.12	1.7436	0.4182
2002	29.03	29.01	29.19	0.6303	0.7297
2004	28.92	28.99	28.60	2.1837	0.3356
2005	29.01	29.28	29.21	0.4953	0.7806
2006	27.31	28.0	27.86	4.3429	0.1140

Table 3. Predicted sex ratios of nests based on mean incubation temperatures during the thermosensitive period.

Year	Predicted Sex Ratios		
	% Female-Biased	% Mix	% Male-Biased
2001	0.0	25.0	75.0
2002	3.0	70.5	26.5
2004	3.8	84.6	11.5
2005	16.7	68.8	14.6
2006	1.4	50.7	47.9

DISCUSSION

The results from our study demonstrated annual variation in incubation temperatures that can most likely be attributed to rainfall and tidal inundations. Most nests exhibited sharp decreases in temperature corresponding to dates of storms and hurricanes. The amount of temperature decrease varied widely with a range from 0.78°C to 7.53°C. Nests with large temperature declines may have been tidally inundated, whereas nests with small declines may have been a result of rainfall events. Studies of green turtle nests in Heron Island, Great Barrier Reef (BOOTH and ASTILL, 2001), leatherback and green turtle nests in Suriname (GODFREY, BARRETO, and MROSOVSKY, 1996), and green turtle nests in Tortuguero, Costa Rica (SPOTILA *et al.*, 1987) have reported decreases in incubation temperature associated with substantial rain events. Heavy rainfall may influence sex ratios if the decrease in temperature occurs during the thermosensitive period, thus producing a high percentage of males. However, there may be a trade-off between producing male hatchlings

and risking nest loss from heavy rain and tidal inundation. The 2001 storms washed out 50.8% of the nests and 61.1% of the temperature data loggers that were deployed. The 2004 storms washed out 59.4% of the nests and 42.2% of the data loggers. Storm events may periodically contribute higher percentages of male hatchlings to the female-biased nesting beaches on the east coast of Florida, whereas west coast beaches such as Keewaydin Island appear to be regularly producing male hatchlings.

Seasonal differences in incubation temperatures were detected among years, but the seasons that differed varied depending on the year. In 2002 and 2005, early season nests were cooler than middle and late season nests. Because these were relatively uneventful storm years, the difference can most likely be attributed to air temperature being cooler in May at the start of the nesting season and warmer in July and August, the middle and end of the nesting season. However, in 2006 middle season nests were cooler than early and late season nests because the middle of the nesting season was particularly rainy. Seasonal affects on incubation temperatures have been documented in several studies (GODFREY, BARRETO, and MROSOVSKY, 1996; MATSUZAMA *et al.*, 2002; MROSOVSKY HOPKINS-MURPHY and RICHARDSON, 1984). Sea turtles may nest several times throughout the season to not only improve the chances of producing viable offspring through temporal spreading of reproductive effort, but also to produce both male and female offspring in a given season.

Mean incubation temperatures did not differ among vegetation areas (Australian pines present, Australian pines removed, or native vegetation). Therefore, shading from the

Table 4. Summary of the annual histology results. The nest number, number of hatchlings collected, number of female hatchlings, number of male hatchlings, number of hatchlings whose sex could not be determined, the mean temperature during the thermosensitive period, and the standard deviation during the thermosensitive period.

Nest	Hatchlings Examined	Female	Male	Undetermined	Thermosensitive Mean (°C)	Standard Deviation
2001 histology results						
4	3	0	3	0	26.61	0.7017
18	5	3	1	1	26.86	0.9195
21	1	1	0	0		Logger washed out
22	1	1	0	0		Logger washed out
23	1	0	1	0	26.77	0.7626
51	14	9	5	0	27.55	1.2192
52	1	0	1	0	26.74	1.2989
55	2	0	2	0	27.26	1.0590
71	1	0	1	0		Logger washed out
81	2	2	0	0	27.85	1.3893
105	12	2	10	0	27.68	2.0759
Total	43	18	24	1		
2002 histology results						
D	10	7	0	3	27.22	0.9433
3	5	4	0	1	26.61	0.5391
9	8	8	0	0		Logger washed out
49	3	3	0	0	29.19	0.7967
Total	26	22	0	4		
2004 histology results						
10	18	1	17	0	28.56	1.0184
13	15	7	8	0	29.04	1.3487
15	14	6	8	0	28.28	1.3679
Total	47	14	33	0		

Australian pines does not appear to affect incubation temperatures differently than shading from native vegetation. This contradicts studies by SCHMELZ and MEZICH (1988), MROSOVSKY, LAVIN, and GODFREY (1995), and FOLEY *et al.* (2000) who found that nests shaded by Australian pines, non-specific vegetation, and condominiums, respectively, influenced incubation temperatures. The aforementioned studies mainly used sand temperature or small sample sizes of nest temperatures, whereas the current study used large sample sizes of *in situ* nest temperatures. Metabolic heating from within the nest may offset the cooling effect of shade which would be omitted if only sand temperatures were being recorded. Future studies should measure the amount of shading by vegetation in addition to categorizing the nest by vegetation type. Research on sea turtle nest temperatures should be continued on Keewaydin Island and other west coast beaches to verify the results of our study and to examine long-term trends and population-level effects of nest temperature variation.

Sex ratios were predicted for Keewaydin Island using pivotal incubation temperature. Based on these predictions, Keewaydin Island appears to be producing mostly male-biased or mixed ratio clutches (Table 3), although the histology samples from these nests indicated that females were also being produced (Table 4). Nests with similar temperature means and standard deviations had opposing histology results in which females were found in one nest but males in the other (Table 4, Nests: 3 in 2002 and 4 in 2001; D in 2002; and 55 in 2001). The 2004 histology results had twice as many males as females even though the nest temperatures were slightly higher than the sample nests in 2001 and 2002. There are several possibilities for these discrepancies: (1) metabolic heating within the nest increased the temperatures enough to produce females depending on where the egg was located in the clutch; (2) large variations of sexes are produced when incubation temperatures are at or near the pivotal temperature; and (3) decreases in incubation temperatures during rain events were enough to affect sex determination. Extremely small sample sizes were used in the histological assessment because of the rarity of finding freshly dead hatchlings, which makes it difficult to predict the sex ratio of an entire clutch. Some histological results differed from temperature-based sex ratio predictions. This discrepancy emphasizes the importance of using both methods collectively to predict hatchling sex ratios.

Keewaydin Island is a very important nesting ground for the south Florida nesting subpopulation. Based on predicted sex ratios and histology results, male hatchlings are being produced regularly regardless of the pine removal. The male hatchlings being produced on Keewaydin Island may help balance the female-biased nests regularly produced on the east coast of Florida, such as at Cape Canaveral (MROSOVSKY and PROVANCHA, 1992) and Hutchinson Island (HANSON, WIBBELS, and MARTIN, 1998).

CONCLUSIONS

The results of this study illustrate the importance of multiyear data sets to examine incubation temperatures. There

appears to be annual and seasonal variability that may not be observed during shorter term studies. Examining longer term trends would provide even greater insight into the role that various environmental factors have in influencing hatchling sex ratios.

Restoration of native vegetation on sea turtle nesting beaches should still be encouraged because removing the Australian pines did not appear to affect incubation temperatures and consequently hatchling sex ratios. It is important to remove the pines before they are blown over and restrict access to nesting sea turtles. This is a proactive measure that can be taken to prevent the loss of valuable nesting beach habitat.

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