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THE EFFECT OF AUSTRALIAN PINE REMOVAL ON LOGGERHEAD SEA
TURTLE NESTING PATTERNS, KEEWAYDIN ISLAND, FLORIDA

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DEDICATION

This is dedicated in loving memory of my Grandparents, Eleanor and “Fritz” Sullivan for teaching me some of life’s greatest lessons.

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ABSTRACT OF THE THESIS

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Historic nesting data, incubation temperatures, and spatial nesting patterns of the loggerhead sea turtle, *Caretta caretta*, were examined on Keewaydin Island, Florida. Thirteen years of historic data were compiled to compare hatching success, clutch size and incubation duration before and after the removal of exotic Australian pine, *Casuarina equisetifolia*. Temperature data loggers were used to record nest incubation temperatures among areas where Australian pines were removed, pines present, and native vegetation present. Geographic Information System (GIS) was used to compare emergence densities between areas where fallen pines were removed and natural areas without fallen pines. The results of the study indicated that removing the pines was successful in restoring nesting beach habitat without altering beach dynamics, hatching success, clutch size, incubation duration or incubation temperatures. Incubation temperatures indicated that Keewaydin Island is producing 1:1 and male-biased sex ratios, which may help balance the female-biased nests produced on the southeast coast of Florida

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Introduction

Florida has the second largest loggerhead sea turtle (*Caretta caretta*) nesting aggregation in the world (Magnuson et al., 1990). Ninety percent of all loggerhead nesting in the United States occurs in Florida (Shoop et al., 1985; Conley and Hoffman, 1987; Meylan et al., 1995). Increased human development, predation, beach erosion, and exotic vegetation have all had a negative impact on loggerhead nesting, resulting in degradation of nesting beach habitat and an overall decline in the size of the population (Bustard, 1972; Fletemeyer, 1991). In 1978, the loggerhead was listed as a threatened species under the Endangered Species Act (ESA) of 1973 (National Marine Fisheries Service and U.S. Fish and Wildlife Service, 1991). The ESA provides protection to listed species and their habitat through federal law and international treaties. In 1977, Florida enacted the Florida Endangered and Threatened Species Act (FETSA) to protect the five species of sea turtles found in state waters, from being sold, poached or killed by incidental catch. Worldwide, all seven species of sea turtles are listed in Appendix I of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), which bans commercial trade among the 150 nations who have signed the convention (www.cites.org).

To prevent further decline of sea turtle populations, many actions have been taken to protect turtles and their habitat. Most of these conservation efforts have focused on nesting beaches, since suitable nesting sites are critical to sea turtle reproduction and are central to the survival of the population (Witherington, 1999). The purpose of my study is to examine the effect of Australian pine (*Casuarina equisetifolia*) removal on loggerhead nesting and incubation temperatures in southwest Florida, and to determine

whether exotic vegetation removal is an effective solution to the growing problem of nesting habitat loss.

Background on Sea Turtles

There are seven species of sea turtles: flatback (*Natator depressa*), green (*Chelonia mydas*), hawksbill (*Eretmochelys imbricata*), Kemp's ridley (*Lepidochelys kempfi*), leatherback (*Dermochelys coriacea*), loggerhead (*Caretta caretta*), and olive ridley (*Lepidochelys olivacea*). Sea turtles are only terrestrial when females come ashore to lay their eggs, during incubation of the nest and when hatchlings emerge from their nest. With the onset of the nesting season, adult sea turtles leave their foraging area and migrate towards their nesting beach to mate (Musick and Limpus, 1997). Five sea turtle species (green, Kemp's ridley, leatherback, hawksbill and loggerhead) feed in waters off Florida (Meylan et al., 1995). Three sea turtle species (leatherback, green and loggerhead) commonly nest along the east coast of Florida. Nesting on the west coast of Florida is predominately by loggerhead and occasionally green sea turtles (Foley, 1997; LeBuff, 1990).

The distribution of loggerheads includes continental shelves, bays, and estuaries in temperate, subtropical and tropical waters, with major nesting beaches in Oman, southeastern United States, Mexico, Australia, South Africa, Mediterranean and Japan (Dodd, 1992; Magnuson et al., 1990). Loggerheads are omnivores, feeding on mollusks, crustaceans, shellfish, sponges, jellyfish, fish and occasionally algae (Bustard, 1972; Dodd, 1992). The loggerhead is a medium-sized turtle with a large head compared to the other sea turtle species. Their carapace and skin are reddish-brown and the plastron is cream colored (Dodd, 1992). The loggerhead carapace supports an epibiotic community

composed of barnacles, algae and other benthic invertebrates (LeBuff, 1990). An adult loggerhead weighs 70 – 180 kg with a carapace length of approximately 70 –125 cm (Dodd, 1992). Loggerhead hatchlings weigh approximately 21 g with an average carapace length of 45 mm (Ernst and Barbour, 1972). Hatchlings vary in color from light to dark brown (National Marine Fisheries Service and U.S. Fish and Wildlife Service, 1991).

The four developmental stages in the lifecycle of loggerheads are hatchling, pelagic or juvenile, sub-adult, and adult. The hatchling stage consists of hatching from the egg and swimming offshore, dependent on the absorbed yolk sac for energy (Musick and Limpus, 1997). Once the turtle begins feeding, it enters the pelagic stage.

Loggerheads spend their pelagic stage drifting in sargassum mats which provide camouflage and a food resource (Carr, 1986). Several years later, juveniles actively swim towards developmental habitats in the tropical and temperate zones (Musick and Limpus, 1997). As turtles mature they migrate towards adult foraging habitats and nesting beaches. Loggerheads reach maturity at 12 – 30 years of age (National Marine Fisheries and U.S. Fish and Wildlife Service, 1991; Dodd, 1992) and their longevity in the wild is unknown.

Estimates of hatchlings surviving to maturity range from 1 in 100 to 1 in 1000. Predators of sea turtles vary by life stage. Eggs are depredated by raccoons, ghost crabs, fire ants, hogs, and humans (Magnuson et al., 1990). Hatchlings emerging from the nest are prey for birds, raccoons, and ghost crabs. Disorientation caused by artificial lighting is another cause of hatchling mortality (Ehrhart and Witherington, 1987). Once they enter the ocean, juvenile turtles are prey for birds, sharks, and fish. Adult turtles are prey for

sharks and humans. Incidental capture in shrimp trawls has been identified as a major source of mortality, although mortality has decreased with the use of Turtle Excluder Devices (TEDs) (Magnuson et al., 1990). There are several types of TEDs that function similarly to channel the turtle toward an opening in the net that releases them from the trawl. Turtle Excluder Devices have been shown to reduce the capture of sea turtles by 97% (Magnuson et al., 1990).

Most of our knowledge regarding sea turtles is connected with nesting females and their incubating eggs since they are the most accessible and easily observed life stages. However, with increasing technology of time-depth recorders (TDR), radio, sonic and satellite telemetry additional knowledge is being gained regarding dive behavior, habitat utilization, and foraging and nesting migrations (Addison et al., 2002; Coles and Musick, 2000; Dellinger and Freitas, 2000; Hickerson and Peccini, 2000).

Sea Turtle Nesting

Sea turtles have demonstrated nest site fidelity and for this reason, efforts to maintain established nesting beaches have been a major emphasis of turtle conservation (Talbert et al., 1980). Tagging data have indicated that loggerhead turtles return to the same beach every 2 – 4 years (Bustard, 1979), and may nest up to seven times during a season (Caldwell et al., 1959a; Addison, 1996). Each nest contains on average 112 eggs that are approximately 4.0 cm in diameter (Miller, 1997). Sea turtles must find alternate nesting sites if a nesting beach becomes unsuitable. Humans have degraded beaches with development and altered beach dynamics with sand renourishment activities. Consequently, alternate beaches may not be available and those that are available may not be conducive to successful nesting or embryonic development.

Genetic research has confirmed nest site fidelity and indicated that independent loggerhead populations exist with limited gene flow between subpopulations (Bowen et al., 1993, 1994; Enclada et al., 1998; Meylan and Ehrenfeld, 2000). A genetic study on loggerheads by Enclada et al. (1998) found six independent nesting aggregates around the world and three of these are in Florida (Figure 1). Protecting individual subpopulations is vital to conserving this threatened species and maintaining the genetic diversity of the species as a whole (Turtle Expert Working Group, 2000).

Once a female turtle reaches the nesting beach, several factors may influence the selection of a nesting site. Caldwell et al. (1959b) suggested that loggerheads prefer to nest on wide beaches above the high tide line and close to low dunes. Nesting females also appear to prefer unlit, obstacle free beaches with an open, offshore approach (Mortimer, 1981). Obstacles may include pilings, sea walls, fallen trees or beach debris. When ascending a beach, nesting females are easily disturbed by lights and movement (Caldwell et al., 1959b; Dodd, 1988). If a female is startled she may return to the ocean without nesting. These instances are known as non-nesting emergences or false crawls. Turtles have been observed false crawling several times prior to nesting, sometimes on the same night (D. Addison, Conservancy of Southwest Florida, unpubl. data).

The physical properties of the sand and beach, such as texture, moisture, porosity, temperature, beach slope and width have been identified as factors influencing nest site selection (Stoneburner and Richardson, 1981; Mortimer, 1990; Ackerman, 1997; Garmestani et. al., 2000; Wood and Bjorndal, 2000). Sand texture and moisture must be such that females are able to dig an egg chamber without the walls of the chamber collapsing, and the hatchlings must be able to dig their way out of the nest (Mortimer,

1981). The sand must have a porosity that allows for respiratory gas exchange and provides a suitable thermal environment for the developing embryos (Bustard and Greenham, 1968; Mortimer, 1990; Salmon et al., 1995).

Sea turtles have temperature-dependent sex determination (TSD). The temperature of the sand influences incubation duration and the sex of the hatchlings (Yntema and Mrosovsky, 1980; Morreale et al., 1982; Yntema and Mrosovsky, 1982; Standora and Spotila, 1985; Godley et al., 2001). Incubation duration is the number of days between nest deposition and hatchling emergence. 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). Mrosovsky (1988) demonstrated that the pivotal temperature, in which equal numbers of males and females are produced is 29°C for North American loggerheads. 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. Studies by Godfrey and Mrosovsky (1997) and by Godley et al. (2001) have indicated that the pivotal incubation duration, in which equal sex ratios are produced, for loggerhead nests in the southeast United States is 60.2 and 61.7 days respectively. Incubation periods longer than the pivotal incubation duration produce male biased clutches and incubation periods shorter than the pivotal incubation duration produce female biased clutches (Godfrey and Mrosovsky, 1997; Godley et al., 2001). Hatchling sex ratios have been predicted based on nest temperature in studies along the east coast of Florida. Hanson et al. (1998) predicted that 92.5% of the nests examined were 100% female on Hutchinson Island, Florida. Mrosovsky and Provanha (1992) examined nest

temperatures and collected hatchlings along Cape Canaveral, Florida. They estimated that loggerhead nests during 1986, 1987, and 1988 produced hatchlings that 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 - 2°C cooler than nests that were not shaded. 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. Nest site selection can influence the thermal environment and affect incubation duration and 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). 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 trees prevent the germination and growth of native vegetation (Johnson and Barbour, 1990). Exotics, such as Australian pines, can exclude native species [(e.g., sea oats (*Uniola paniculata*), inkberry (*Scaevola plumieri*), and sea grape (*Coccoloba uvifera*)] in coastal areas (Nelson, 1994). Exotic vegetation may also form impenetrable root mats, which could impede nest construction. Ironically, the shallow root system of the Australian pine makes them more susceptible to the effects of storm winds and erosion. When Australian pines fall, they create a barrier to sea turtles searching for nest sites on beaches (Schmelz

and Mezich, 1988; LeBuff, 1990; Nelson, 1994; Reardon, 1998). Fallen Australian pine snags have reduced or interfered with sea turtle nesting on several Florida beaches, including: Dry Tortugas National Park (Reardon, 1998), Everglades National Park (Klukas, 1967; Davis and Whiting, 1977), Sanibel Island (LeBuff, 1990), and Keewaydin Island (Schmelz and Mezich, 1988; Ryder et al., 2000).

Since the sex of sea turtles is temperature dependent, nests shaded by the pines could produce a higher percentage of male hatchlings than nests in the open beach. During 1986 and 1987, the nests on Keewaydin Island were relocated to a hatchery, which was shaded by Australian pines. Schmelz and Mezich (1988) recorded temperatures four times a day at ten sand locations and in two nests within the hatchery and at two locations outside the hatchery. They found 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 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 (Ryder et al., 2000). The purpose of my study is to gain a better understanding of how Australian pines impact loggerhead nesting and incubation temperatures, and determine whether the removal of the pines has any effect on sand temperature and nesting success.

Study Objectives

The first objective of the study was to compile and analyze thirteen years of historic sea turtle nesting data for Keewaydin Island collected by the Conservancy of Southwest Florida. I used these data to compare clutch size, hatching success, and incubation duration for the years before and after Australian pine removal. An increase in clutch size and hatching success after Australian pine removal may indicate that the beach is more favorable for nesting than it was prior to exotic removal. A decrease in incubation duration after the pine removal would indicate that the shade from the Australian pines had been maintaining cooler sand temperature prior to their eradication.

The second objective was to examine nest incubation temperatures in areas where Australian pines are present, in areas where Australian pines were removed, and in areas with native vegetation. Incubation temperatures that are lower in nests where Australian pines are present than in nests where Australian pines were removed or native vegetation may suggest the shade from the pines is maintaining cool temperatures that could result in male-biased clutches. Incubation temperatures that are higher in nests where Australian pines were removed and native vegetation than in nests where Australian pines are present may suggest there is less shading and could result in female-biased clutches. Equal incubation temperatures among the three vegetation types would indicate that the shade from the Australian pines is no different than the shade from native vegetation.

The third objective was to determine whether sea turtles are using portions of the beach where fallen Australian pine snags were removed. If the density of nests and false crawls are approximately equal between areas where fallen Australian pine snags were removed and natural areas, this would indicate that the fallen Australian pine removal

was successful in restoring nesting habitat. If the amount of nesting is lower where the Australian pines were removed than in native vegetation, this would indicate that the Australian pine removal was not completely successful in restoring suitable nesting habitat.

Materials and Methods

Study Area

Keewaydin Island, also known as Key Island, is an unbridged, primary barrier island, located south of Naples, Collier County, Florida (Figure 2). The area of the island is 560 hectares with 12 km of coastline. Approximately 85% of the island is owned and managed by Florida Department of Environmental Protection (FDEP) through Rookery Bay National Estuarine Research Reserve (RBNERR). The remaining 15% is owned privately. The beach is popular among recreational boaters and campers.

The beach consists of natural quartz sand and is constantly undergoing erosion and accretion (Addison and Shelby, 2003). The beach is backed by native vegetation [e.g., sea oats (*Uniola paniculata*), sea grape (*Coccoloba uvifera*), railroad vine (*Ipomoea pes-caprae*), sea purslane (*Sesuvium portulacastrum*), and inkberry (*Scaevola plumieri*)] and invasive exotic vegetation [such as, beach naupaka (*Scaevola taccada sericea*), Brazilian pepper (*Schinus terebinthifolius*), lather leaf (*Colubrina asiatica*) and Australian pine (*Casuarina equisetifolia*)]. Several threatened or endangered species have been observed inhabiting the island, such as gopher tortoises (*Gopherus polyphemus*), bald eagles (*Haliaeetus leucocephalus*), burrowing owls (*Athene cunicularia*). The beach is also a nesting site for loggerhead sea turtles (*Caretta caretta*), least terns (*Sterna antillarum*), and piping plovers (*Charadrius melodus*).

Rookery Bay National Estuarine Research Reserve (RBNERR) began a large-scale restoration project on Keewaydin Island in March of 1998. Australian pines were removed from approximately 142 hectares of beaches, state lands and some privately owned land. Live pines and fallen snags were cut at their base, piled and burned. The stumps were left in place and treated with the herbicide Garlon. Native vegetation was planted in selected areas to increase the native seed source and to help stabilize disturbed areas. During 2000, contractors made another sweep of the island to clear any re-growth of invasive exotic vegetation, including Brazilian pepper, beach naupaka and Australian pine on state land. Rookery Bay NERR staff and volunteers and Collier County community service workers have cut and treated re-growth on a continual basis. Overall the exotic removal project cost approximately one million dollars in federal and state grants and private funds.

Rookery Bay NERR staff have been monitoring shoreline change annually along Keewaydin Island since 1998. This effort was initiated in response to public concerns that removing the fallen Australian pine snags would increase erosion. Every year in February or March, Global Positioning System (GPS) points are collected systematically along the dune line while riding an All-Terrain Vehicle (ATV). The shoreline is also mapped opportunistically before and after hurricanes or major storm events.

Sea Turtle Monitoring

The Conservancy of Southwest Florida, a local non-profit agency, has been monitoring Keewaydin Island for sea turtle activity since 1982. The island is part of the Florida Index Nesting Beach Survey Program (INBS). The INBS was initiated in 1989 to standardize monitoring efforts and methodology on select Florida nesting beaches to

allow state-wide comparison among beaches (Schroeder, 1994). Over the years, the Conservancy sea turtle monitoring project has adapted as knowledge on sea turtle biology and predator prevention measures have become better understood. The following is a description of the sea turtle monitoring program on Keewaydin Island.

Prior to the loggerhead nesting season, the beach is divided into 152.4 m (500 ft) increments and each increment is marked with a PVC pole. Each reference marker is labeled with the numerical location in feet. The beach is measured with a surveyor's wheel while either riding an ATV or walking. The north tip of the island is designated as the starting point. The location of the southern tip changes yearly due to accretion and erosion, but measures approximately 12,040 m (39,500 ft) from the north tip. The accessibility of the beach varied over the years due to the density of fallen pines, erosion, accretion, and daily tidal stages. The inaccessible areas associated with fallen Australian pines were documented yearly in the Sea Turtle Monitoring Project final reports.

Beginning in 1985, Conservancy interns monitored the south end (5,182 to 12,040 m; 17,000 to 39,500 ft) of Keewaydin Island for sea turtle nesting activity nightly from 2000 h until 0600 h. Monitoring efforts include collecting turtle morphometric data (straight length and width, curved length and width and head width), tagging nesting females with flipper tags, recording nest and false crawl location data, and caging the nests *in situ* to prevent raccoon depredation (Addison and Shelby, 2003). Contents of the nest are excavated and evaluated three days after hatchling emergence or 75 days after the nest was deposited, whichever occurs first, according to the Marine Turtle Conservation Guidelines (Florida Fish and Wildlife Conservation Commission, 2002). Nest evaluation includes counting the number of hatched eggshells, unhatched eggs

(embryo or undifferentiated), pipped eggs with live hatchlings, pipped eggs with dead hatchlings, and live and dead hatchlings. Hatching success is calculated for each nest by dividing the number of hatched eggs by the total number of eggs. The location of nests and false crawls were recorded by pacing to the nearest PVC reference marker to obtain a numerical location in feet. The north portion of the island (0 to approx. 3960 m; 0 to approx. 13,000 ft) was added to the monitoring project in 1993, but this section was only monitored in the morning. The only portions of the island not monitored were areas inaccessible due to the fallen Australian pines. Nest and false crawl location data were recorded and nests were caged using the aforementioned procedures for the south end. The Australian pine removal in 1998 enabled access to the entire beach and allowed complete monitoring for the first time since the initiation of the project. In 1998, RBNERR and the Conservancy of Southwest Florida collaborated to analyze the location data using Geographic Information System (GIS) software. The location of each nest and false crawl, were recorded using Differential Global Positioning System (DGPS; Trimble, model TSC1, Sunnyvale, CA).

Historic Nesting Data

Thirteen years of sea turtle monitoring data from the Conservancy of Southwest Florida were compiled to compare clutch size, hatching success and incubation duration for the years before and after Australian pine removal. The computer database was proofed against the original field datasheets and screened for errors. Annual nesting data were pooled as before pine removal (1990 – 1997) and after pine removal (1998 – 2002). Analysis of variance (ANOVA) was used to test the null hypotheses that mean clutch

size, hatching success, and incubation duration did not differ between pre-removal and post-removal treatments.

Monthly rainfall totals for 1990 – 2002 were obtained from the Southwest Florida Water Management District for the Marco Island station (www.sfwmd.gov). Rainfall totals were summed per sea turtle nesting season (May to October) each year. Monthly mean air temperatures for 1990 - 2002 were obtained from the Southeast Regional Climate Center for the Naples station (<http://www.dnr.state.sc.us/climate/sercc>). These data were used to calculate the mean annual air temperature during the sea turtle nesting season (May – October). To determine if hatching success was related to environmental factors (rainfall, air temperature and incubation duration) I used Pearson correlation coefficients.

Incubation Temperature Analysis

During the summers of 2001 and 2002, six Hobo[®] temperature data loggers (Onset Computer Corporation, Pocasset, MA) were deployed along the dune line and in sea turtle nests to record hourly sand temperature. Data loggers were calibrated and encased in sealed plastic bags with desiccant prior to deployment. Vegetation along the dune line was categorized as Australian pine present, Australian pine removed, and native vegetation present. A control temperature data logger was deployed at a depth of 30 cm in each of the beach categories. These control loggers were deployed at the start of sea turtle nesting season (15 May 2001; 10 May 2002) and remained in the sand until the last nest hatched (18 September 2001; 26 September 2002). Throughout each nesting season, temperature data loggers were opportunistically deployed in egg chambers during egg deposition. Temperature loggers were placed in approximately the middle of the egg

chamber. In 2001, two 4-probe loggers were also deployed to record temperatures of the bottom, middle, top, and outside the nest to provide information regarding intra-nest temperature variation. After hatchling emergence, each nest was excavated and the contents evaluated to calculate hatching success. Dead hatchlings found in the nest were preserved in formalin and sex was determined histologically (T. Wibbels, University of Alabama at Birmingham, pers. comm.). Data loggers were recovered and temperature data were downloaded using BoxCar[®] Pro 4.0 software (Onset Computer Corporation, Pocasset, MA).

For the 4-probe loggers, ANOVA was used to test the null hypothesis that mean daily temperatures throughout incubation did not differ among the bottom, middle, top and outside the nest. The loggerhead nesting season was divided into early, middle, and late season, and nests were categorized by the date they were deposited: early (1 May – 31 May), middle (1 Jun – 30 Jun), and late (1 Jul – 1 Aug). I used ANOVA to test the null hypothesis that mean temperature during the thermosensitive period did not differ among nests deposited early, middle and late in the season. I used ANOVA to test the null hypothesis that mean incubation temperatures during the thermosensitive period did not differ among nests deposited in native vegetation present, Australian pine present and Australian pine removed. To test the null hypothesis that mean hourly temperature during the thermosensitive period did not differ by time of day I used ANOVA. Seasonal effects were removed by using only nests deposited during the middle of the season in these analyses. Pearson correlation coefficient was used to test for correlation between mean temperature throughout incubation and incubation duration.

Geographic Information System (GIS)

Locations of all nests and false crawls on Keewaydin Island were recorded using a DGPS during 1998 - 2002. The beach was divided into 5 sections, 2 areas where fallen Australian pine snags were removed from the beach and 3 natural areas that lacked snags (Figure 3). Nest and false crawl locations were mapped along with the Australian pine snag removal and no snag areas using GIS software ArcView version 3.2a (ESRI, Redlands, CA). Numbers of nests and false crawls were summed in each treatment section for each year and crawl density (number of crawls/km) was calculated for each treatment and year. Two-way ANOVA was used to test the null hypothesis that mean crawl density did not differ in snag removal versus snag areas across years.

Annual shoreline data from April 1998 to February 2003 were mapped in ArcView along with the Australian pine snag removed and no snag areas. Three random samples were taken in each section and a line was drawn across the shorelines at each sample point. Shoreline change was measured across the line between consecutive years. Repeated measures ANOVA was used to test the null hypothesis that mean shoreline change did not differ in Australian pine snag removal and no snag areas among years (1998 – 2003). All statistical tests were conducted using Number Cruncher Statistical System (NCSS; Hintze, 2001).

Results

Historic Nesting Data

During 1990 – 2002 there were a total of 2443 nests, 3404 false crawls and an estimated 119,455 hatchlings produced on Keewaydin Island. Six of the nests were documented as green turtles and the remaining nests were believed to be loggerhead turtles. According to the Conservancy's tagging data, one green nested once in 1994 and

a different green nested 5 times in 2002, temperature data loggers were deployed in two of these nests. The highest nest and false crawl densities (number of crawls/ km surveyed) occurred in 1991 with 25.89 nests/km and 40.55 false crawls/km (Figure 4).

The mean clutch size during 1990 – 2002 was 101.39 eggs with yearly means ranging from 93.91 to 105.14 eggs (Figure 5). The smallest clutch was deposited in 1995 with only 11 eggs and the largest clutch was deposited in 1991 with 182 eggs. There was no significant difference in mean clutch size between pre-removal and post-removal years ($F_{1, 1811} = 0.59$; $P = 0.4422$).

The mean hatching success during 1990 – 2002 was 68.23 % with yearly means ranging from 50.46 to 79.79 % (Figure 6a). There was no significant difference in mean hatching success between pre-removal and post-removal years ($F_{1, 1516} = 0.42$; $P = 0.5151$). Nests that were depredated by raccoons were excluded from the analysis.

The mean incubation duration during 1990 – 2002 was 62.06 days with yearly averages ranging from 58.96 to 67.64 days (Figure 6b). The shortest incubation duration of a nest was 46 days in 1992 and the longest incubation duration was 78 days in 1993 and 1999. There was no significant difference in mean incubation duration between pre-removal and post-removal years ($F_{1, 1522} = 0.01$; $P = 0.9269$).

The highest and lowest rainfall amounts during turtle nesting season, May to October, occurred in 1995 with 195.47 cm and in 1997 with 75.92 cm, respectively (Figure 6c). Rainfall amounts were generally highest in June and July and lowest in May. The highest and lowest mean air temperature during May to October occurred in 1995 with 27.87°C and in 2000 with 26.43°C, respectively (Figure 6d). The mean air temperature during 1990 – 2002 varied less than 1.5°C. Mean air temperature was

generally warmest in July and August and coolest in May and October. Results of the Pearson Correlation among hatching success, rainfall, incubation duration, and air temperature indicated that hatching success significantly decreased as rainfall increased ($r = -0.5743$; $P = 0.0401$; Table 1).

Incubation Temperature

During the 2001 nesting season, 54 temperature data loggers were deployed on Keewaydin Island. Several storm events during the 2001 nesting season reduced the effectiveness of the data loggers. Thirty-three loggers were washed away and only 21 were retrieved. Incubation temperatures decreased sharply during the storm events (Figure 7). During the 2002 nesting season 44 loggers were deployed and 37 were retrieved and successfully downloaded (1 logger filled with water, 4 malfunctioned, and 2 were lost). Two of these loggers were in green nests, 52 were in loggerhead nests and 4 were control loggers. During the thermosensitive period, individual nest temperatures during 2001 and 2002, ranged from 23.24 - 34.01°C with mean temperatures ranging from 26.61 - 31.09°C.

One of the two 4-probe loggers deployed in 2001 was washed away. Data from the remaining logger indicated that mean daily incubation temperatures were lowest outside of the nest and highest at the top of the nest, although the difference was less than 1.0°C (Figure 8). There was a significant difference in mean daily temperatures among bottom, middle, top, and outside the nest ($F_{3,300} = 6.27$; $P = 0.0004$). Results of the Bonferroni Multiple Comparison Test indicated that there were significant differences in temperature among logger positions. (Table 2).

Mean incubation temperature was lowest in nests laid early in the season and highest in nests laid during the middle and late in the season (Figure 9). There was a significant difference in mean incubation temperatures among early, middle and late season nests ($F_{2, 54} = 11.32$; $P < 0.0001$). Results from the Bonferroni Multiple Comparison test indicated that early nests were cooler than middle and late nests. Incubation temperatures in middle and late nests did not differ from each other.

There was not a significant difference in mean incubation temperatures among areas (native vegetation present, Australian pine present and Australian pine removed) during the thermosensitive period ($F_{2, 25} = 1.13$; $P = 0.3444$; Figure 10). Mean incubation temperatures differed significantly during the day ($F_{23, 492} = 2.05$; $P = 0.0030$). Temperatures were highest at 2200h and lowest at 1100h (Figure 11), although the results of the Bonferroni Multiple Comparison test did not identify times of day that differed significantly.

There was a significant negative correlation between incubation duration and mean temperature throughout incubation, however the low R^2 indicates that temperature does not explain much of the variation in incubation duration ($F_{1, 33} = 19.7955$; $P = 0.0001$; $R^2 = 0.3897$). Incubation duration and mean incubation temperature were inversely correlated, as incubation duration increased the mean incubation temperature decreased (Figure 12).

Geographic Information System (GIS)

There was no significant difference in mean crawl density between Australian pine snag removal (SR) and no snag (NS) among the years 1998 – 2002 ($F_{4, 25} = 0.43$; P

= 0.7826), between treatments ($F_{1, 25} = 0.17$; $P = 0.6862$), and there was no significant interaction between the main effects ($F_{4, 25} = 0.19$; $P = 0.9420$; Figure 13).

There was no significant difference in shoreline change between Australian pine snag removed (SR) and no snag (NS) areas ($F_{1, 75} = 1.09$; $P = 0.3148$) and there was no significant interaction between the main effects ($F_{4, 75} = 1.65$; $P = 0.1769$) but there was a significant difference in shoreline change among years ($F_{4, 75} = 8.51$; $P < 0.0001$; Figure 14).

Discussion

Removing the fallen Australian pine snags from the beach appears to have been successful in restoring nesting beach habitat without altering beach dynamics. Mean crawl density did not differ between areas where the fallen pines were removed and areas where snags never occurred, suggesting that removal of Australian pines can restore nesting habitat. Shoreline change did not differ between areas where the fallen Australian pine snags were removed and no snag areas. The significant difference in shoreline change among years is most likely due to storm events and hurricanes in a given year (Pilkey, 1991). Most of Keewaydin Island seems to be eroding but the south tip of the island is mainly accreting. As a result, the island appears to be migrating southward.

Loggerhead nesting data collected on Keewaydin Island from 1990 – 2002 suggest that annual clutch sizes are smaller, hatching success was similar, and incubation durations are longer relative to other loggerhead nesting studies. The mean annual clutch sizes from Keewaydin Island were lower than those reported during 1965 - 1983 on Little Cumberland Island, Georgia (Frazer and Richardson, 1985) and in 1996 on Melbourne Beach, Florida (Tiwari and Bjorndal, 2000). Mean annual clutch sizes ranged from 93.91

– 105.14 eggs on Keewaydin Island, 115.11 – 127.54 eggs on Little Cumberland Island and 109.6 eggs on Melbourne Beach. The range of individual clutch sizes over thirteen years on Keewaydin Island was 11 – 182 eggs, which is similar to a range of 28 – 144 eggs recorded during a nine-year study on Cyprus (Broderick et al., 2003). The range in clutch sizes in each of the studies indicates that there is a great deal of variability in the number of eggs deposited in loggerhead nests. The mean hatching success on Keewaydin Island (71.9%) is comparable to that reported in the neighboring Ten Thousand Islands (68.9%) during 1993 - 1994 (Foley et al., 2000). However, these percentages do not include nests that were completely depredated by raccoons. Raccoons depredated eighty percent of nests in the Ten Thousand Islands compared to the minimal loss on Keewaydin Island because of nest caging efforts. The mean yearly incubation durations on Keewaydin Island (59.19 – 67.64 days) were longer and more variable than those during 1993-1998 in Cyprus (47.7 – 48.7 days; Godley et al., 2001) and during 1997 in Hutchinson Island, Florida (48.31 days; Hanson et al., 1998).

Australian pine removal did not appear to have an effect on clutch size, incubation duration, or hatching success of loggerhead turtles on Keewaydin Island. Mean clutch sizes before and after the pine removal were 101.02 eggs and 101.9 eggs. Mean hatching success before and after the pine removal was 67.9% and 68.64%. Mean incubation duration before and after the pine removal was 62.06 days and 62.08 days. The results indicate that there can be a great deal of annual variation in nesting data (Figures 5, 6a and b) but the long-term means are approximately equal. This illustrates the importance of long-term data collection.

Correlation analyses among incubation duration, air temperature, hatching success and rainfall indicated that as the amount of rainfall increased, the percent of hatching success decreased. Heavy rainfall is usually associated with weather systems that cause high tides that over wash nests. Based on personal observations, rainfall affects hatching success in two ways depending on the stage of nest development. Large amounts of rainfall or tidal inundation during incubation can result in moist conditions that cause the entire clutch to perish. Heavy rainfall and high tides concurrent with hatchling emergence compact the surface sand, making it impossible for the hatchlings to emerge from the nest and they consequently drown. Hurricane Barry and an unnamed storm event in 2001 caused a great deal of erosion on Keewaydin Island washing out nests and temperature data loggers. Total rainfall during October to May in 2001 and 2002 were 188.85 cm and 113.51 cm. The large amount of rainfall in 2001 maintained overall cooler sand temperatures than in 2002 according to the control temperature loggers (Figure 15).

Most of the nests during 2001 exhibited sharp decreases in incubation temperature associated with Hurricane Barry and an unnamed storm event (Figure 7). Studies of green nests in Heron Island, Great Barrier Reef (Booth and Astill, 2001), leatherback and green nests in Suriname (Godfrey et al., 1996), and green nests in Tortuguero, Costa Rica (Spotila et al., 1987) have reported decreases in incubation temperature associated with substantial rain events. Rainfall could affect sex ratios if the decrease in temperature occurs during the thermosensitive period, thus producing a higher 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. Perhaps sea turtles need rainy nesting seasons periodically to contribute males into the population and a few beaches such as Keewaydin Island that regularly produce male hatchlings. For example, the males produced on Keewaydin Island may help balance the female-biased nests regularly produced on Cape Canaveral (Mrosovsky and Provancha, 1992) and Hutchinson Island (Hanson et al., 1998) beaches since all three beaches contribute to the overall sex ratio of the South Florida Nesting subpopulation (Figure 1). Likewise, concerns about global warming altering sex ratios (Janzen, 1994a) may not be detrimental since the warming trend would cause sea turtles to produce more females. Since all species of sea turtles are either threatened or endangered, they would benefit from a strategy to produce more females to increase the size of the population, provided that males continue to be produced during rainy years or on a few beaches.

Several studies have examined intra-nest temperature variation. Studies by Foley et al. (2000), Hanson et al. (1998) and Kaska et al. (1998) examined temperatures at the top, bottom, middle and sand temperatures outside the nest similar to the present study. Booth and Astill (2001) examined only temperatures within the nest, at the top, middle, side and bottom. Average temperatures were highest at the top of the nest, except for the Foley et al. (2000) and Hanson et al. (1998) studies in which, highest temperatures were in the middle. In studies with data loggers outside the nest, including the present study, average temperatures were lowest outside the nest, which may be explained by metabolic heating within the nest. Overall temperature differences within the nests were less than 1.3°C except for the Foley et al. (2000) study, in which the difference was 1.9°C. A one-degree variation within a nest could create different sexes consequently where the logger

is placed in a nest could influence hatchling sex prediction. In the present study, loggers were deployed closest to the middle of the clutch as possible but placement may be a source of error in predicting sex ratios. The intra-nest temperature results in this study were comparable to the aforementioned studies. However, only one 4-probe logger was used in the analysis, additional 4-probe loggers need to be deployed to determine if the trends were valid.

Mean incubation temperatures were cooler in nests laid early than in nests laid in the middle or late in the sea turtle nesting season. This 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.

Mean incubation temperatures did not differ among areas where Australian pines are present, Australian pines were removed or native vegetation. Shading from the Australian pines does not appear to affect incubation temperatures any differently than native vegetation. Therefore, removing the Australian pines does not alter sex ratios. These results are surprising since other studies have documented that shade from condominiums and vegetation does affect nest temperature (Foley et al., 2000; Janzen, 1994b; Mrosovsky et al., 1995; Schmelz and Mezich, 1988). One possibility for the discrepancy could be that the effect of shading on incubation temperature is dependent on the distance of the nest from the vegetation or condominium. Nests in the current study were only categorized by vegetation type and not as shaded or unshaded.

The difference in mean hourly temperature indicated that there were daily temperature cycles in both loggerhead and green nests on Keewaydin Island. The difference in mean hourly temperatures during the thermosensitive period, in middle

season loggerhead nests were less than 1.0°C. The difference in mean hourly temperature during the thermosensitive period, in two late season green nests were 0.54°C and 0.99°C. Studies of loggerhead nests have documented daily fluctuations ranging from 0.3°C – 1.4°C in the Mediterranean (Godley et al., 2001), and mean daily variations of 0.5°C in Bahia, Brazil (Marcovaldi et al., 1997) and 1.5°C in the eastern Mediterranean (Kaska et al., 1998). Mrosovsky et al. (1999) and Georges et al. (1994) argue that daily fluctuations in loggerhead nests are not sufficient to influence sex ratios. Studies of green nests in Bahia, Brazil and Heron Island, Great Barrier Reef did not detect any daily temperature fluctuations (Kaska et al., 1998; Booth and Astill, 2001) and a study in Tortuguero, Costa Rica found fluctuations to be less than 0.5°C (Standora and Spotila, 1985). The nests on Keewaydin Island exhibited a delayed heating and cooling time due to the thermal insulation of the sand. Temperatures in the loggerhead nests were highest at 2200h and lowest at 1100h as opposed to air temperature, which is generally highest in late afternoon and lowest after midnight. The delayed time in the green nests was longer than the loggerhead nests since the green nests were deeper than those of loggerheads.

The correlation between incubation temperature and incubation duration is similar to the results obtained in other studies. Incubation duration can be used as a method to estimate hatchling sex ratios (Godley et al., 2001; Marcovaldi et al., 1997; Mrosovsky et al., 1999). This is useful when examining historic data or nests without temperature data loggers. Sex ratios were predicted for Keewaydin Island using pivotal incubation temperature and incubation duration (Appendix 1). Sex ratio predictions varied between methods, illustrating the need to sex dead hatchlings when they are available.

Conclusions

Once the Australian pines were removed from Keewaydin Island native vegetation quickly re-colonized in most areas. Areas that were highly disturbed from the pine removal especially near the burn piles, benefited from planting native vegetation to help stabilize and to increase the native seed source. There are still Australian pines standing on Keewaydin Island on private land, which supply a constant seed source. Five years after the pine removal the battle against exotic vegetation continues. Due to the constant available seed source and sporadic funding, completely eradicating exotic vegetation may be an overly optimistic goal. A more realistic and cost effective strategy may be to focus on keeping certain areas of the island exotic free and on the remaining areas of the island the goal should be to prevent the exotics from spreading. Overall the Australian pine removal project was successful in restoring suitable beach for sea turtle nesting and native vegetation habitats such as coastal strand and tropical hardwood hammocks on the island. The project provided an excellent opportunity to educate the public on exotic vegetation and to get them involved in native plant restoration. Removing the pines did not appear to have an effect on hatchling sex ratios, hatching success, clutch size, incubation duration or shoreline change. The biggest disadvantage of the project was the high cost for the initial removal project and the commitment to long-term periodic maintenance. The goal of RBNERR is to use “best management practices” to conserve natural biodiversity. Based on this goal the project was considered a success. Whether the benefits of habitat restoration outweigh the cost to other agencies depends on their goals and the habitats and species at risk.

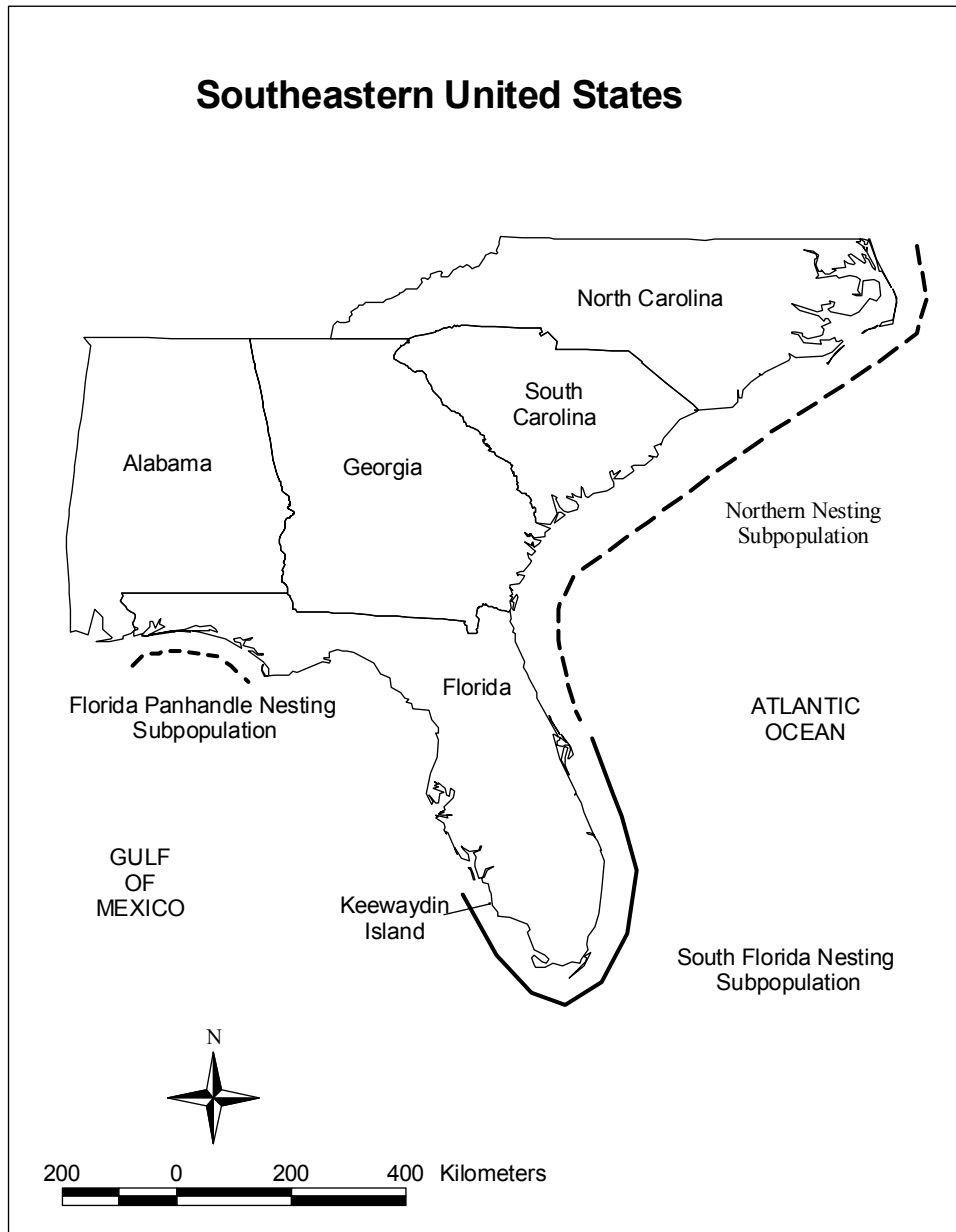


Figure 1. Loggerhead nesting subpopulations in Florida (from Turtle Expert Working Group, 2000). Solid line represents the nesting subpopulation, which includes Keewaydin Island.

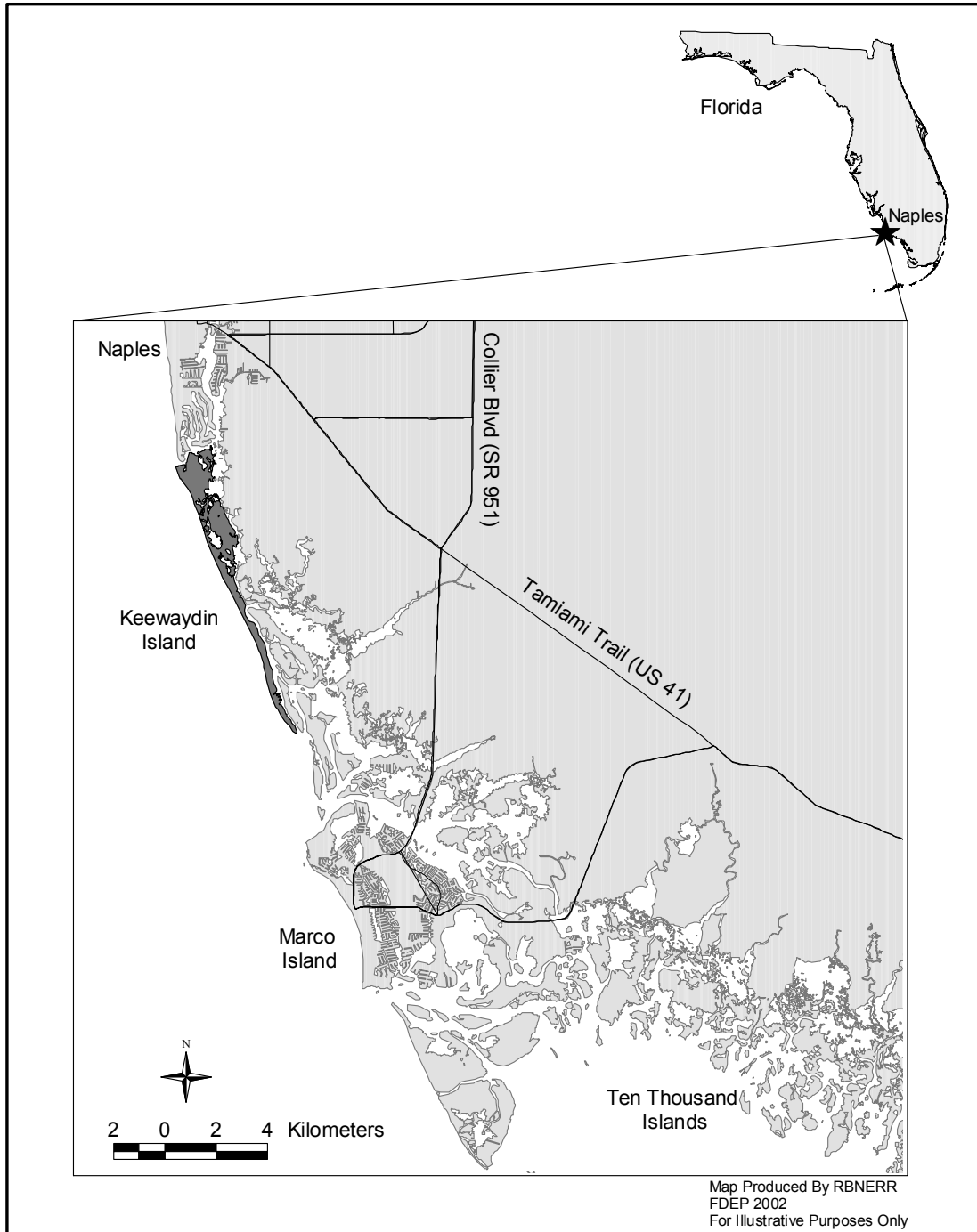


Figure 2. Location of Keewaydin Island, Florida (dark shaded portion of map).



Figure 3. Keewaydin Island divided into treatment areas with their respective distances in kilometers (km): fallen Australian pine snag removal (SR) and no snag (NS).

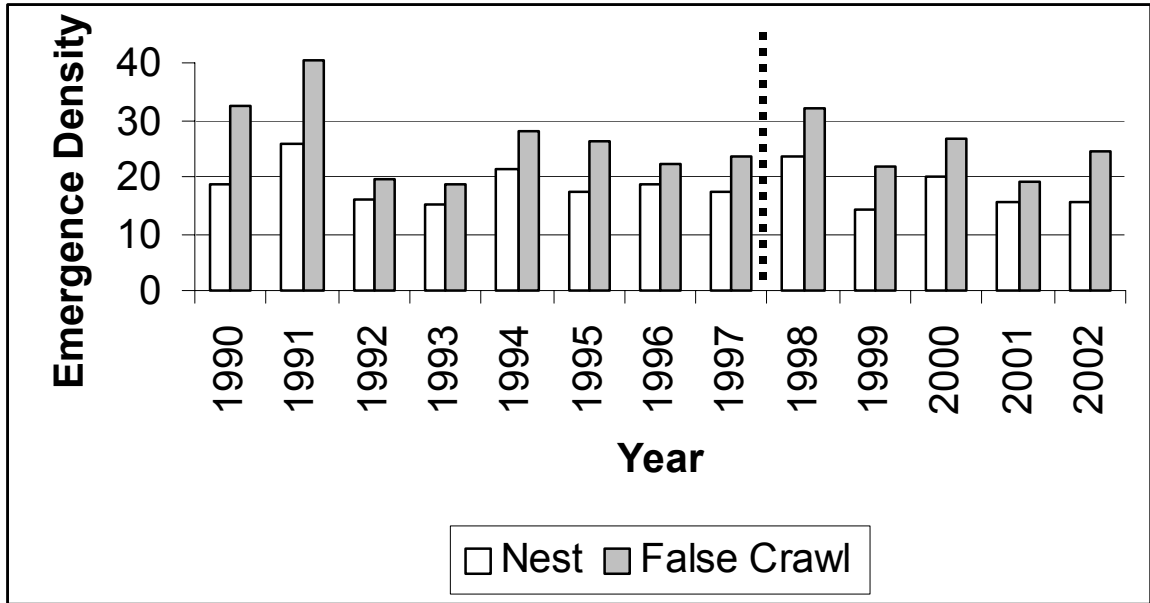


Figure 4. Annual nest and false crawl densities (number of crawls/ km surveyed) on Keewaydin Island. During 1990 – 1992 only the southern portion of Keewaydin Island was monitored. During 1993 – 1997 the entire island was monitored except for portions that were inaccessible due to fallen Australian pine snags. During 1998 – 2002 the entire island was monitored. The vertical dotted line represents the removal of Australian pine.

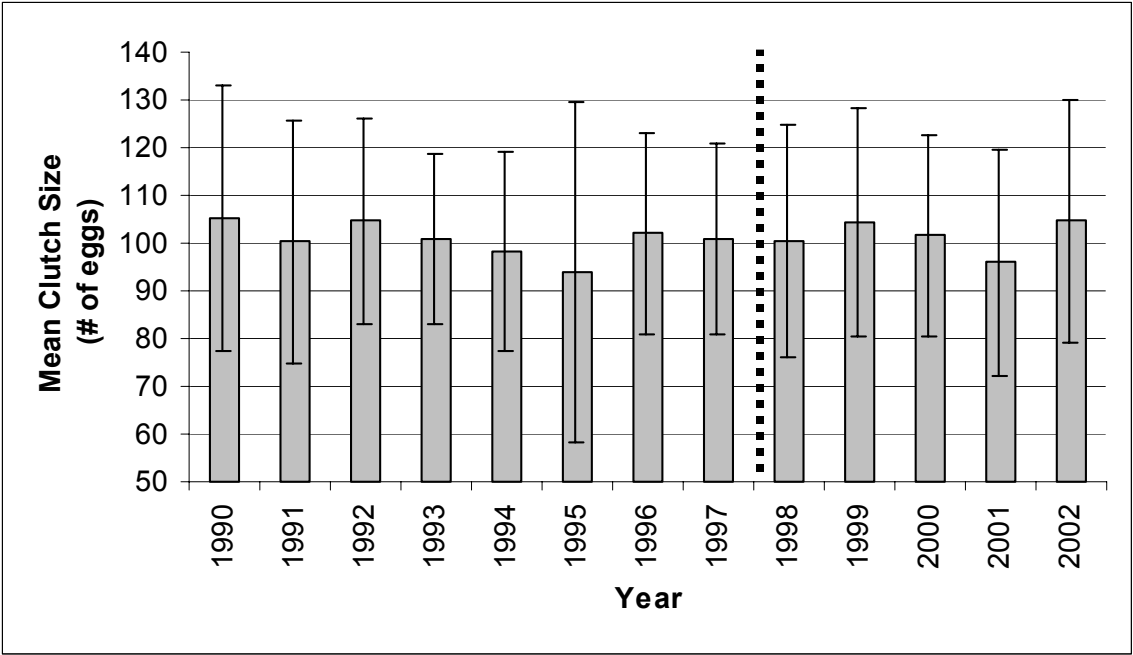


Figure 5. Means and standard deviations of annual clutch size. The vertical dotted line indicates when the Australian pines were removed.

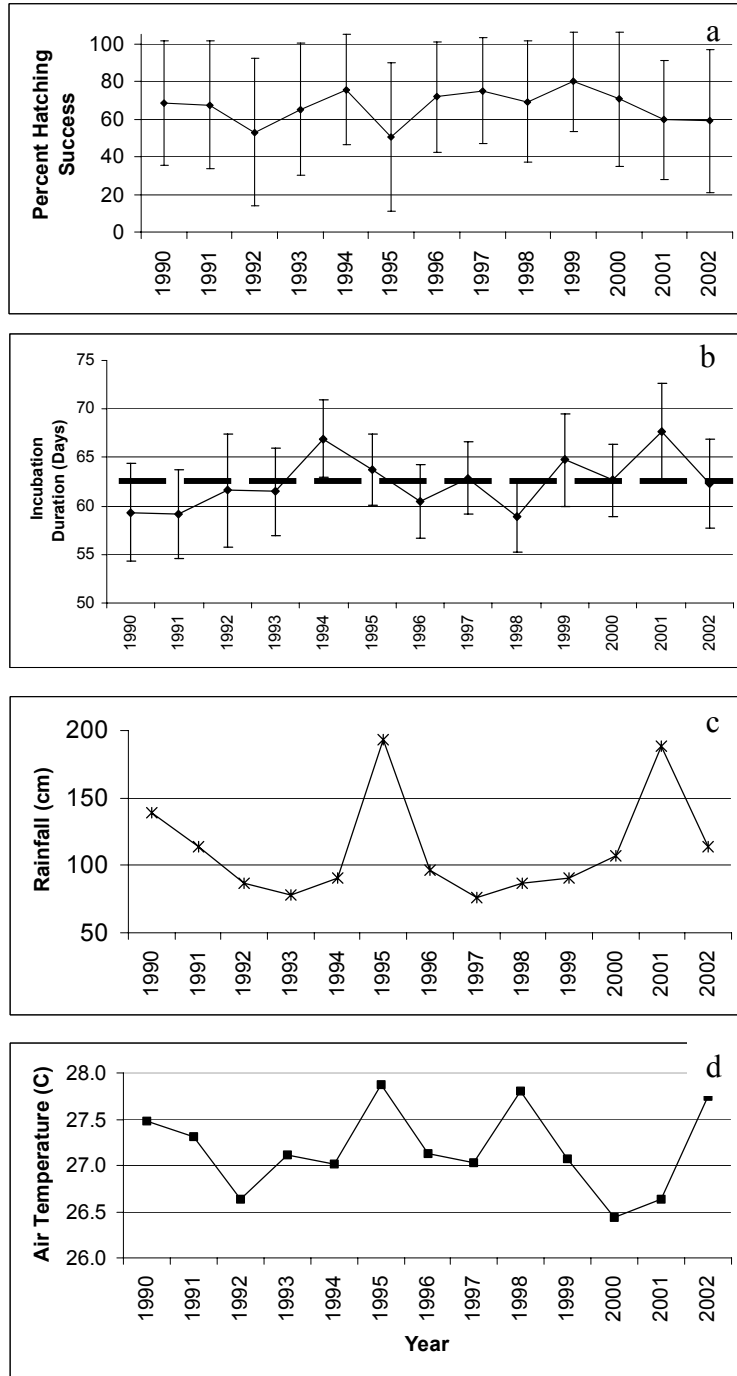


Figure 6. a) Mean annual hatching success (%). b) Mean annual incubation duration. Dashed line indicates the pivotal duration of approximately 62 days. c) Total rainfall (cm) during May – October recorded in Marco Island. d) Mean air temperature (°C) May – October recorded in Naples. Error bars are standard deviations.

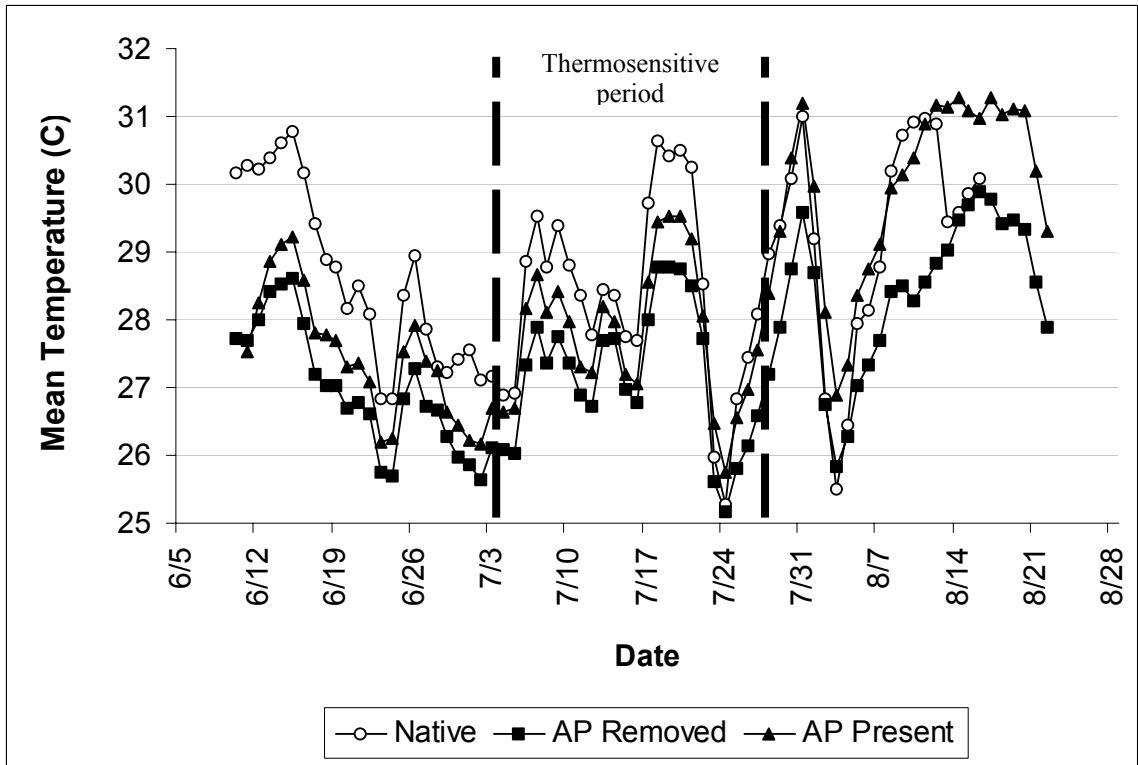


Figure 7. Mean daily temperature in three nests. The sharp decreases in incubation temperature were typical in 2001 logger data during Storm Event (24 July 2001) and Hurricane Barry (5 August 2001). Vertical dashed lines indicate the thermosensitive period of incubation.

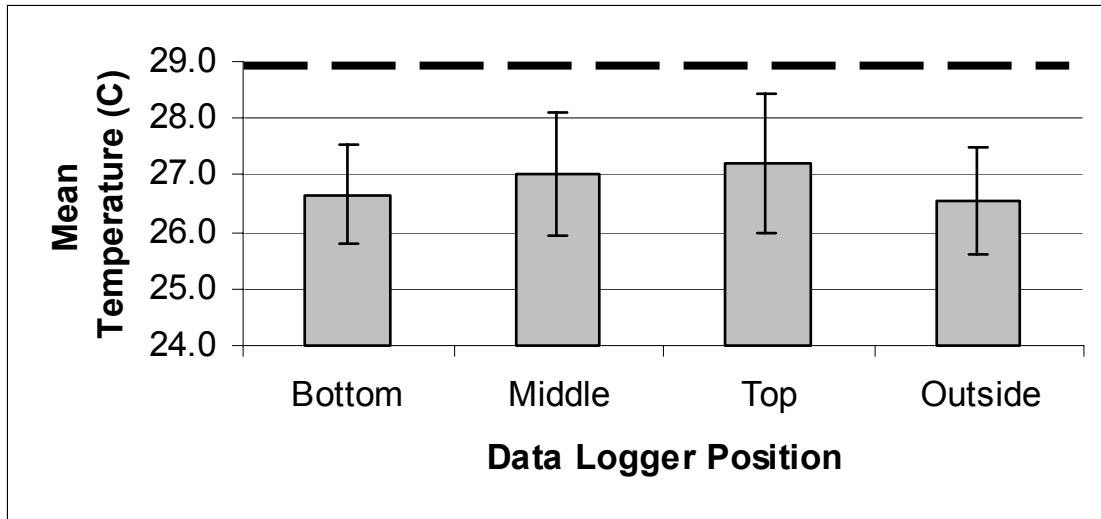


Figure 8. Means and standard deviations of daily temperature (°C) throughout incubation (22 June 2001 – 4 August 2001) for data logger probes placed in the bottom, middle, top and outside the nest. The dashed line indicates the pivotal temperature of 29°C.

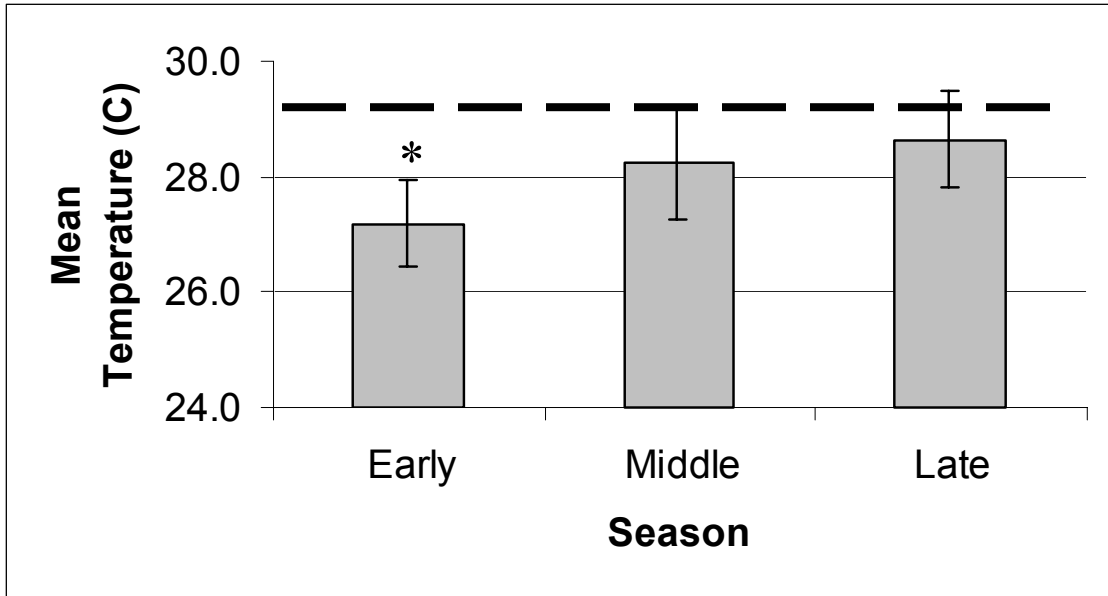


Figure 9. Means and standard deviations of temperature (°C) of nests deposited during the early (1 May – 31 May), middle (1 Jun – 30 Jun) and late (1 Jul – 1 Aug) part of the sea turtle nesting season. Asterisk indicates a significant difference. Dashed line indicates the pivotal temperature of 29°C.

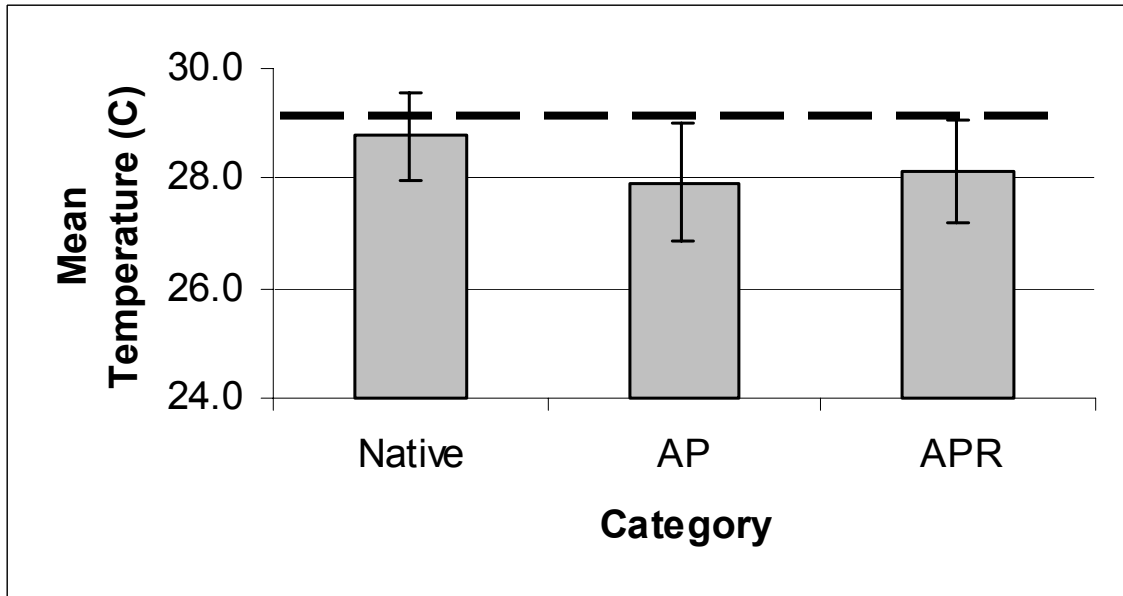


Figure 10. Means and standard deviations of temperature (°C) during the thermosensitive period incubation for middle season nests (1 Jun – 30 Jun) in native vegetation (Native), Australian pine (AP), and Australian pine removed areas (APR). Dashed line indicates pivotal temperature of 29°C.

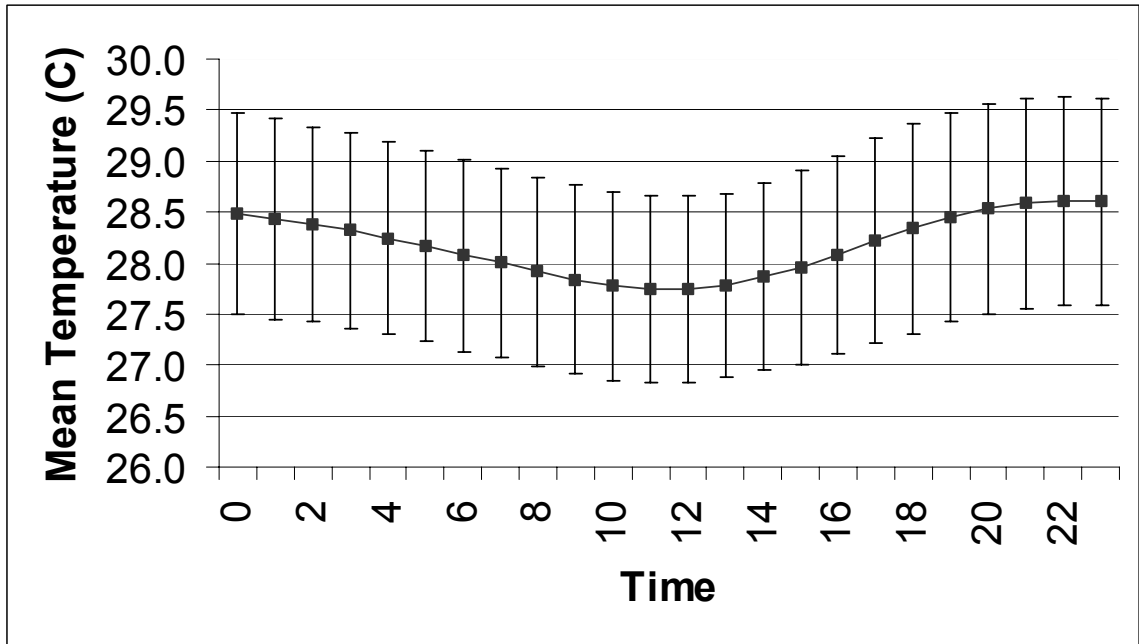


Figure 11. Means and standard deviations of hourly incubation temperature (°C) during the thermosensitive period for midseason nests (1 Jun – 30 Jun).

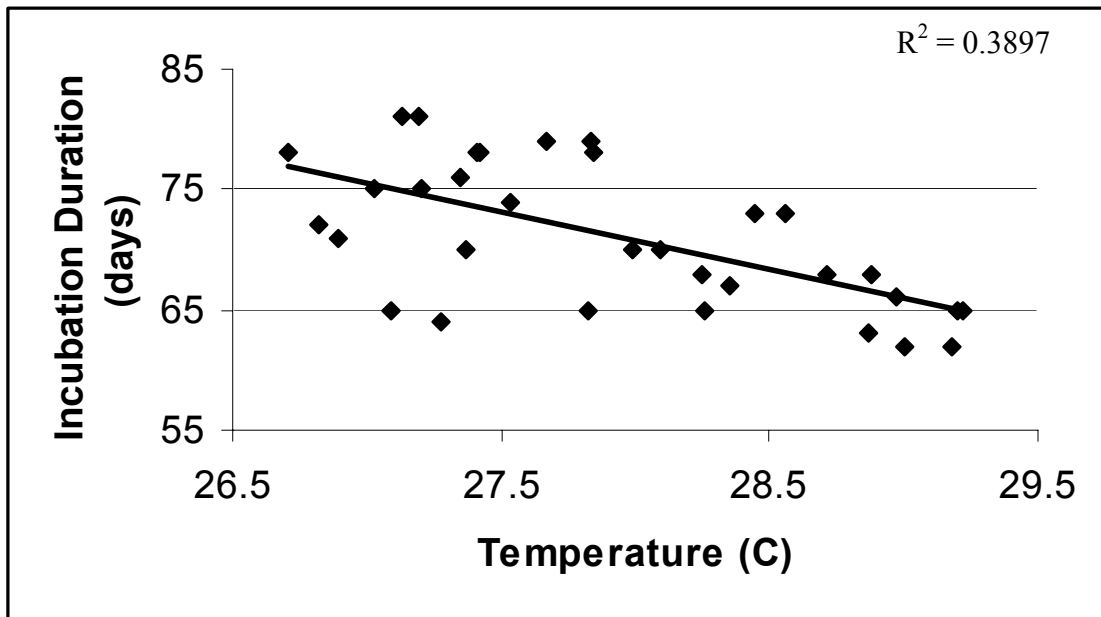


Figure 12. Scatterplot of incubation duration (days) versus mean incubation temperature (°C). Solid line represents fitted line.

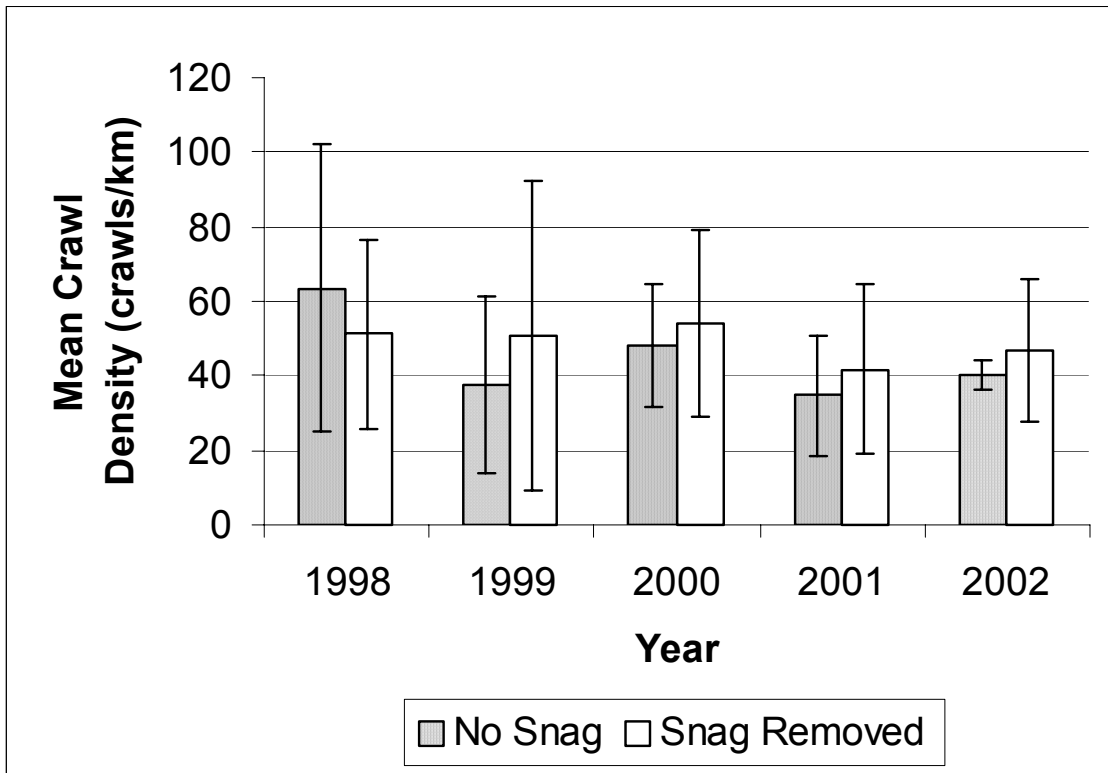


Figure 13. Mean crawl density in areas where snags were removed (Snag Removed) and areas that lacked snags (No Snag). Error bars are standard deviations.

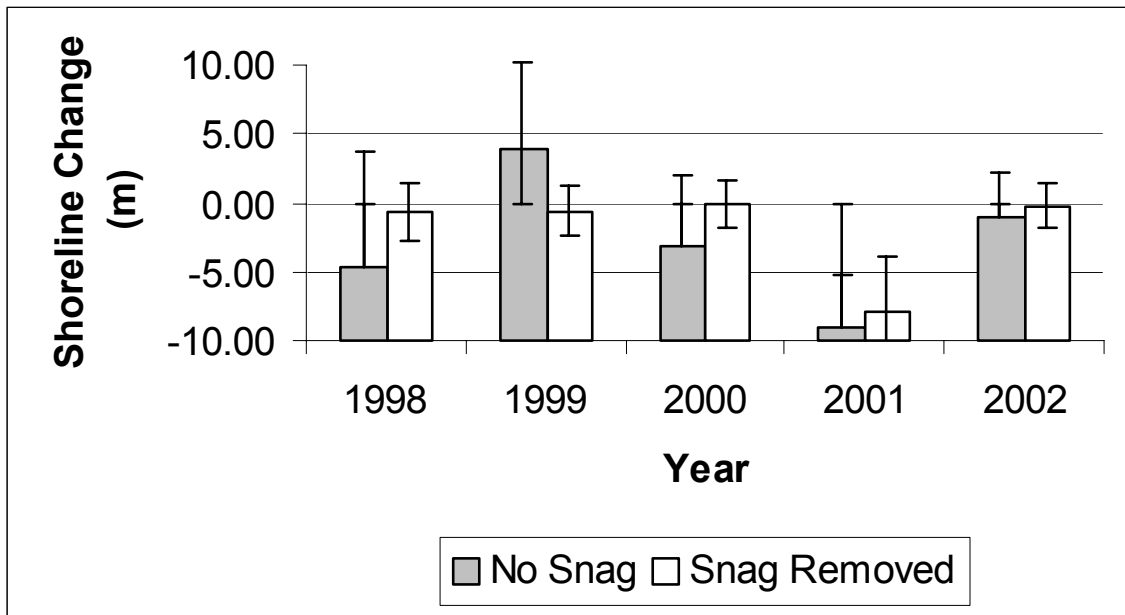


Figure 14. Mean annual shoreline change in areas where snags were removed and areas that lacked snags (No Snag). Error bars are standard deviations.

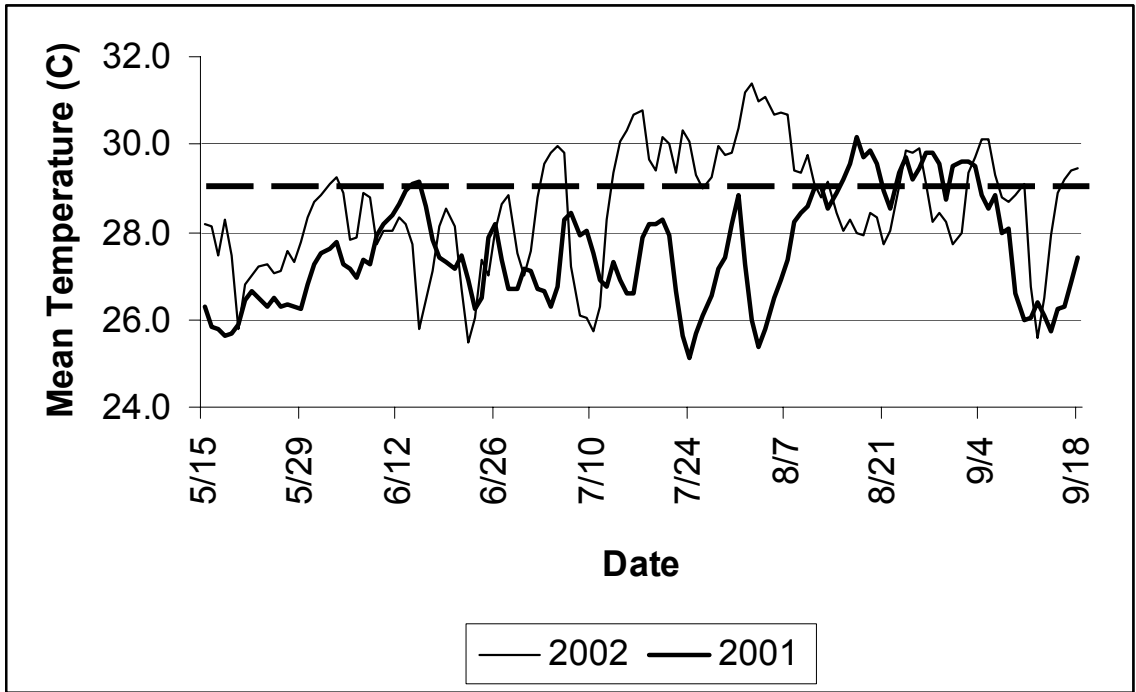


Figure 15. Mean daily sand temperatures from the native control loggers during 2001 and 2002. The sharp decreases in temperature during 2001 were associated with an unnamed Storm Event (24 July 2001) and Hurricane Barry (5 August 2001). Horizontal dashed line indicates the pivotal temperature of 29°C.

Table 1. Pearson Correlation results among average hatching success (%), total rainfall (cm), average incubation duration (days) and air temperature (°C). P-values are in parentheses. Bold font and asterisk indicates correlation.

	Temperature	Rainfall	Incubation
Rainfall	0.1902 (0.5337)		
Incubation Duration	-0.3966 (0.1797)	0.3250 (0.2786)	
Hatching Success	-0.1925 (0.5286)	-0.5743 (0.0401*)	0.0077 (0.9802)

Table 2. Results of the Bonferroni Multiple Comparison Test among the data logger positions within and outside the nest during the thermosensitive period.

<u>Data Logger Position</u>	<u>Mean</u>	<u>Significantly Different (P < 0.05)</u>
Bottom	26.66	top
Middle	27.02	outside
Top	27.20	outside, bottom
Outside	26.54	middle, top

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Appendix 1. Individual temperature logger data.

Category where nest was deposited: AP – Australian pine, APR – Australian Pine Removed, or Native – Native Vegetation. **Season** nest was deposited: Early (1 May – 31 May), Mid (1 Jun – 30 Jun), or Late (1 Jul – 1 Aug). **Incubation Duration** is the number of days between nest deposition and hatchling emergence. **Predicted Sex (Duration)** is based on the pivotal incubation duration of 62 days. **Critical Average** is the mean temperature during the thermosensitive period. **Predicted Sex (Temp)** is based on the pivotal temperature of 29°C. Asterisk indicates loggers that were in green nests.

Logger	Year	Category	Season	Incubation Duration (days)	Predicted Sex (Duration)	Critical Mean (C)	Predicted Sex (Temp)
UAB1	2001	AP	early	75	Male-biased	26.61	Male-biased
KI13	2001	APR	early	76	Male-biased	26.94	Male-biased
KI6	2001	APR	early	70	Male-biased	26.86	Male-biased
KI7	2001	Native	early	72	Male-biased	26.77	Male-biased
UAB245	2001	APR	late	68	Male-biased	29.13	1:1
UAB247	2001	APR	late	70	Male-biased	28.59	Mix
UAB246	2001	APR	late	65	Male-biased	27.68	Male-biased
UAB248	2001	APR	late	70	Male-biased	27.73	Male-biased
KI8	2001	APR	mid	78	Male-biased	26.83	Male-biased
UAB219	2001	APR	mid	64	Mix	27.55	Male-biased
UAB220	2001	AP	mid	75	Male-biased	26.74	Male-biased
UAB224	2001	Native	mid	68	Male-biased	28.38	Mix
UAB225	2001	APR	mid	74	Male-biased	27.26	Male-biased
UAB221	2001	AP	mid	73	Male-biased	27.93	Male-biased
UAB238	2001	AP	mid	78	Male-biased	26.72	Male-biased
UAB236	2001	APR	mid	78	Male-biased	27.09	Male-biased
UAB237	2001	APR	mid	65	Male-biased	28.90	Mix
STOW1	2001	Native	mid	73	Male-biased	27.85	Male-biased
UAB240	2001	APR	mid	67	Male-biased	27.65	Male-biased
STOW2	2001	AP	mid	65	Male-biased	28.17	Mix
KI1	2002	Native	early	81	Male-biased	26.84	Male-biased
KI6	2002	APR	early	81	Male-biased	26.78	Male-biased
KI7	2002	APR	early	65	Male-biased	27.22	Male-biased
KI8	2002	APR	early	71	Male-biased	26.61	Male-biased
N107	2002	APR	early	68	Male-biased	28.50	Mix
UAB124	2002	Native	early	78	Male-biased	26.96	Male-biased
UAB191	2002	APR	early	79	Male-biased	27.09	Male-biased
UAB200	2002	APR	early	79	Male-biased	27.19	Male-biased
UAB125	2002	APR	early	Did not hatch		29.08	1:1
*UAB332	2002	APR	late	64	Mix	29.46	1:1
UAB330	2002	APR	late	63	1:1	29.20	1:1
UAB329	2002	Native	late	65	Mix	28.48	Mix
UAB331	2002	APR	late	73	Male-biased	27.30	Male-biased
KI7	2002	APR	late	62	1:1	28.49	Mix
UAB351	2002	Native	late	60	Mix	29.06	1:1
UAB349	2002	APR	late	61	1:1	28.43	Mix
UAB352	2002	APR	late	63	1:1	28.32	Mix
UAB333	2002	APR	late	78	Male-biased	31.09	Female-biased

UAB350	2002	APR	late	63	1:1	28.51	Mix
UAB353	2002	AP	late	60	Mix	29.11	1:1
UAB32	2002	APR	late	59	Mix	28.74	Mix
UAB33	2002	APR	late	58	Female-biased	29.30	1:1
*UAB37	2002	APR	late	62	1:1	28.70	Mix
UAB64	2002	APR	late	61	1:1	27.45	Male-biased
UAB55	2002	APR	late	74	Male-biased	28.14	Mix
UAB293	2002	APR	mid	63	1:1	29.19	1:1
UAB294	2002	Native	mid	68	Male-biased	28.35	Mix
UAB296	2002	Native	mid	62	1:1	29.51	1:1
UAB295	2002	Native	mid	62	1:1	29.72	1:1
UAB303	2002	APR	mid	71	Male-biased	28.75	Mix
UAB292	2002	AP	mid	65	Male-biased	28.75	Mix
UAB302	2002	APR	mid	65	Male-biased	28.76	Mix
UAB31	2002	AP	mid	69	Male-biased	29.30	1:1
UAB304	2002	APR	mid	80	Male-biased	29.34	1:1