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Temperature-Dependent Sex Determination (TSD): Implications for the Biology and Conservation of the Kemp's Ridley Sea Turtle (*Lepidochelys Kempii*)

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TEMPERATURE-DEPENDENT SEX DETERMINATION (TSD): IMPLICATIONS
FOR THE BIOLOGY AND CONSERVATION OF THE KEMP'S RIDLEY SEA
TURTLE (*LEPIDOCHELYS KEMPII*)

by

KATHERINE PRESZ

THANE WIBBELS, COMMITTEE CHAIR
JENNIFER LAYTON
KEN MARION

A THESIS

Submitted to the graduate faculty of The University of Alabama at Birmingham in partial
fulfilment of the requirements for the degree of
Master of Science

BIRMINGHAM, ALABAMA

2022

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2022

TEMPERATURE-DEPENDENT SEX DETERMINATION (TSD): IMPLICATIONS
FOR THE BIOLOGY AND CONSERVATION OF THE KEMP'S RIDLEY SEA
TURTLE (*LEPIDOCHELYS KEMPII*)

KATHERINE PRESZ

BIOLOGY

ABSTRACT

The Kemp's ridley sea turtle (*Lepidochelys kempii*) has temperature-dependent sex determination (TSD) where the incubation temperature of the egg determines the sex of the hatchling. This has significant implications for the ecology, evolution, and conservation of this species because it can affect sex ratios. The studies presented in this thesis address hatchling sex ratios produced in Kemp's ridley conservation programs using both natural as well as manipulated incubation regimes. First, a histology-based study was utilized to investigate sex ratios produced at the Padre Island National Seashore from 2002-2019. The results indicate that a female bias has been produced each year through the Kemp's Ridley Conservation Program. A female bias could be assisting in the recovery of this species. A second study investigated yearly beach temperatures at Rancho Nuevo, the primary nesting beach for the Kemp's ridley. This study evaluated beach temperatures from 1998-2021. Our results indicate that early in the nesting season males are more likely to be produced. In the middle of the nesting season, the temperatures have reached pivotal temperature, leading to more female production from late May through July when the majority of nesting occurs. A third study evaluated

optimal methodology for predicting hatchling sex ratios in the Kemp's ridley. This study included the development of an Excel model and a comparison of that model to a previously published R package software program. These models utilize beach temperatures and incubation duration to predict hatchling sex ratios. Additionally, the models were used to predict hatchling sex ratios produced during 2020 on the primary nesting beach. Collectively, these studies provide insight on the long-term impact of temperature on hatchling sex ratios of Kemp's ridley sea turtles. Further, these findings can provide insight on the potential impact of global climate change on hatchling sex ratios in the future.

Kemp's ridley - temperature-dependent sex determination - conservation – climate change – Padre Island – Rancho Nuevo

DEDICATION

I would like to dedicate this thesis to my Grandpa, Dr. Walter M. Presz Jr., who inspired me to continue my education and pursue a graduate degree. I would also like to dedicate this thesis to my parents, Walter M. Presz III and Kristin Presz, who supported my decision to pursue this academic journey. I would not have been able to follow my passion for research without their support.

ACKNOWLEDGEMENTS

First, I would like to thank the members of my committee, Thane Wibbels, Ken Marion, and Jennifer Layton. My research greatly benefitted from their guidance and vast knowledge of turtles. I would like to thank the University of Alabama at Birmingham's Department of Biology for funding my research. Additionally, I would like to thank all of my fellow graduate students and friends (specifically Abigail Trammell, Forrest Collins, Robby Brannum, Khalid Freij, Logan Holfelder, and many others) who helped with my research and made my UAB experience incredible. Finally, I would like to thank my boyfriend Mitchell Metzger, who read every paper, poster, and presentation along my journey. Thank you for correcting my grammatical errors and abundant misuse of commas.

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GENERAL INTRODUCTION

A wide variety of reptile species possess temperature-dependent sex determination (TSD), where the incubation temperature of the egg determines the sex of the hatchling. There are several patterns of sex determination in reptiles. In the FMF pattern, females are produced at relatively high or low temperatures, and males are produced at medium or intermediate temperatures. In the MF pattern, males are produced at low temperatures, and females are produced at higher temperatures. In the FM pattern, the males are produced at higher temperatures and females at lower temperatures (Ewert et al., 1994; Mizoguchi and Valenzuela, 2016; Wibbels et al., 2018; Lockley and Eizaguirre, 2021). Regardless of the sex determination pattern, the transitional range of temperature (TRT) affecting sex is relatively narrow (Pieau and Mrosovsky, 1991; Mrosovsky, 1994; Mrosovsky et al., 1999). Temperatures above or below the narrow range have the potential to negatively impact the fitness of the hatchling or even cause embryonic mortality, depending on the magnitude of temperature. Since TSD can produce a variety of sex ratios, it has implications for the ecology, evolution, and conservation of reptiles.

In species with TSD, the hatchling and population sex ratios may not conform to a 1:1 sex ratio, the ratio often occurring in vertebrate groups with genotypic sex determination (GSD) (Fisher, 1930; Shine, 1999). Species that have TSD have the potential to produce a variety of hatchling sex ratios. The production of extreme male or female biases could affect the survival and recovery of endangered populations. Therefore, it is essential to monitor sex ratios produced in natural populations and reptile

conservation programs (Wibbels, 2007). The narrow ranges of temperatures associated with the physiology of TSD and embryonic development make reptiles particularly vulnerable to changes in environmental temperature. Thus, it is imperative to monitor sex ratio production in natural populations and evaluate the impact of current temperatures as well as future changes in environmental temperatures due to global climate change. The future of species with TSD is dependent on the stability of global environmental temperatures as well as the ability of these species to adapt to changing environmental temperatures. However, current predictions by the Intergovernmental Panel on Climate Change (IPCC) suggest rising environmental temperatures of several degrees centigrade over the next century, and such a temperature change could significantly impact various aspects of embryonic development including sex determination, hatchling fitness, and embryonic mortality in reptile populations (IPCC Special Reports SP1.5, 2019).

The current thesis addresses temperature-dependent sex determination (TSD) in the Kemp's ridley sea turtle (*Lepidochelys kempii*). Historically this species was the most endangered sea turtle in the world and was on the brink of extinction in the 1980's (Pritchard and Marquez, 1973; Groombridge, 1982). Due to their endangered status, they have been the focus of intense conservation efforts for over five decades. The comprehensive U.S./Mexico Binational Kemp's Ridley Conservation Program was created in 1978, as the species was nearing extinction. Due to the program, the Kemp's ridley population has shown a gradual recovery over the last several decades (Wibbels and Bevan, 2016). The distribution of the Kemp's ridley is limited to the Gulf of Mexico and the southeast Atlantic coast of the United States. In contrast to other sea turtle

species, this species exhibits a pan-specific migration to the primary nesting beach in the western Gulf of Mexico. Furthermore, following the migration, this species exhibits synchronized nesting in large groups referred to as arribadas. In addition to the Kemp's ridley's primary nesting beach they also have a number of secondary nesting beaches. The great majority (approximately 70% or more) of nesting occurs at the primary nesting beach which is located at Rancho Nuevo, Tamaulipas, Mexico. One of the major secondary nesting beaches is at Padre Island, Texas, United States. Due to their TSD, pan-specific migration, arribada nesting behavior, and limited number of nesting beaches, the Kemp's ridley is particularly susceptible to changes in environmental temperatures.

The purpose of this thesis is to evaluate the impact of current and future temperatures on hatchling sex ratios produced in a species of turtle with TSD: the Kemp's ridley (*Lepidochelys kempii*). This study will evaluate hatchling sex ratios being produced at Rancho Nuevo, Mexico and Padre Island, Texas. Further, it will address the potential impact of global climate change by evaluating current beach temperatures as well as long-term trends of beach temperatures at the primary nesting beach. The thesis includes three studies. The first study is a histology-based analysis of hatchling sex ratios produced in the Kemp's Ridley Recovery Program at the Padre Island National Seashore. The second study examines long-term trends in nesting beach and egg hatchery temperatures from the primary nesting beach for the Kemp's ridley sea turtle at Rancho Nuevo, Mexico. The third study evaluates incubation temperature during the thermosensitive period to predict hatchling sex ratios produced on the primary nesting beach at Rancho Nuevo, Mexico.

EVALUATION OF LONG-TERM HATCHLING SEX RATIO PRODUCTION IN THE
KEMP'S RIDLEY RECOVERY PROGRAM AT PADRE ISLAND NATIONAL
SEASHORE, TEXAS: 2002-2019

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In preparation for Herpetological Conservation and Biology

Format adapted for thesis

Abstract

A variety of reptiles possess temperature-dependent sex determination (TSD), where the incubation temperature of the egg determines the sex of the hatchling. TSD can potentially produce highly biased sex ratios. Therefore, this form of sex determination has significant implications for the ecology, evolution, and conservation of these reptiles. The existence of TSD necessitates the monitoring of sex ratios produced in conservation programs. The current study evaluated long-term hatchling sex ratio production in the Kemp's Ridley Recovery Program at Padre Island National Seashore, Texas (PAIS). This conservation program protects and incubates nests of the critically endangered Kemp's ridley sea turtle by utilizing egg hatcheries. Histological analysis was utilized to determine the sex of hatchlings from nests laid at PAIS from 2002 through 2019. These tissues were obtained from hatchlings that were found dead in the nest after all live hatchlings had emerged. The results indicated a female bias in each year evaluated. It is plausible that the female bias could be enhancing the recovery of this population by increasing the reproductive output of this endangered species. Further, this female bias is an example of a large-scale manipulation of hatchling sex ratios in an endangered species with TSD.

Introduction

In the 1980s, the Kemp's ridley sea turtle (*Lepidochelys kempii*) was on the verge of extinction and was the most endangered sea turtle in the world (Pritchard and Marquez, 1973; Groombridge, 1982, Wibbels and Bevan, 2016). Due to their endangered

status, they have been the focus of intense conservation efforts for over five decades. The program was expanded in 1978 to include a comprehensive U.S./Mexico Binational Kemp's Ridley Conservation Program, as the species was nearing extinction. Due to these intense conservation efforts, the Kemp's ridley population has shown a gradual recovery over the last several decades (Wibbels and Bevan, 2018). The recovery program includes the protection of females and their nests at the primary nesting beach in Mexico. Additionally, nests are protected in Texas, with the majority being laid at Padre Island National Sea Shore (PAIS), Texas. PAIS gets approximately 150-300 nests yearly.

The Kemp's ridley has TSD, where the incubation temperature of the egg determines the sex of the hatchling. In the case of the Kemp's ridley, relatively cool incubation temperatures produce males, whereas warmer temperatures produce females. Specifically, sex determination shifts from all male-producing to all female-producing over a range of approximately 26-31°C, referred to as the transitional range of temperatures (TRT). The Kemp's ridley has a pivotal temperature (temperature of producing an approximate 1:1 sex ratio) of approximately 30.2°C, which is located within the TRT (Pieau and Mrosovsky, 1991; Mrosovsky, 1994; Godfrey and Mrosovsky, 2006; LeBlanc et al., 2012a). The conservation program at PAIS translocates nests to protected egg hatcheries that utilize controlled incubation temperatures that fluctuate between approximately 25.5°C and 31°C daily, with an average of 29.75°C.

The goal of the PAIS conservation program is to increase the recovery of the species by enhancing hatchling production, including the use of a beneficial sex ratio. This program represents a relatively large-scale manipulation of hatchling sex ratios in a

species with TSD. The manipulation of a sex ratio provides a potential avenue for enhancing the recovery of an endangered species; however, the effects of sex ratio manipulation are currently speculative and extreme biases could be detrimental (Belchschmidt et al., 2020; Martins et al., 2020).

Therefore, it is important to monitor hatchling sex ratios and continually evaluate their impact on these conservation programs. Furthermore, the long-term monitoring of hatchling sex ratios is a prerequisite to evaluating the potential impact of global climate change on population sex ratios in sea turtle populations. The goal of the current study is to evaluate the long-term production of hatchling sex ratios in the Kemp's Ridley Conservation Recovery Program at PAIS.

Methods and Materials

In order to enhance the protection of Kemp's Ridley nests, the National Parks Service at PAIS collects and then translocates nests to egg hatcheries with controlled incubation temperatures (LeBlanc et al., 2012a; Shaver and Caillouet, 2015). The protected egg hatcheries utilize incubation temperatures that fluctuate daily between 25.5°C and 31°C daily, to mimic the natural beach temperatures, with a daily average of 29.75°C. Upon hatching all live hatchlings were released and any deceased hatchlings found in the nest were collected and preserved with 10% formalin. These tissues were then transported to UAB (University of Alabama at Birmingham) for histological processing and data analysis. Tissues were obtained yearly and examined from 2002 to 2019.

During histological processing, the adrenal-kidney-gonad complexes were isolated and processed using standard paraffin histology with a hematoxylin/eosin staining regime (Humason, 1979). Following the staining process, the tissues were examined using light microscopy to determine the sex of the hatchling by identifying gonadal and oviduct morphology (Pieau and Mrosovsky, 1991; Mrosovsky, 1994; Wibbels et al., 2003). Tissues were documented using light microscopy and results were collated and analyzed relative to the year, nest, and hatchling.

Results

Tissues utilized in this study were from hatchlings that were found dead in the nest and were in various stages of decomposition. A percentage of the hatchlings were too decomposed to produce useful data (see Table 1). However, we were able to successfully utilize the tissues from a majority of the hatchlings. In those tissues, female Kemp's ridley hatchlings had a relatively thick and distinct outer (cortical) region that stained heavily with hematoxylin (Figure 1). In contrast, the cortex was not developed in males; however, the inner (medullary) region of the gonad in males exhibited distinct organization including partially developed seminiferous tubules (Figure 2). The medullary region of the female gonad did not show the distinct organization exhibited in male gonads. Additionally, an oviduct was obvious in female hatchlings (Figure 3), extending from the mesonephric region of the kidney; however, male hatchlings either lacked an oviduct or exhibited a regressed oviduct. Figures 1, 2, and 3 show an ovary, a testis and an oviduct, respectively. Tissue quality varied among individuals due to

degradation prior to preservation since tissues were not collected until all live hatchlings had emerged from the nest.

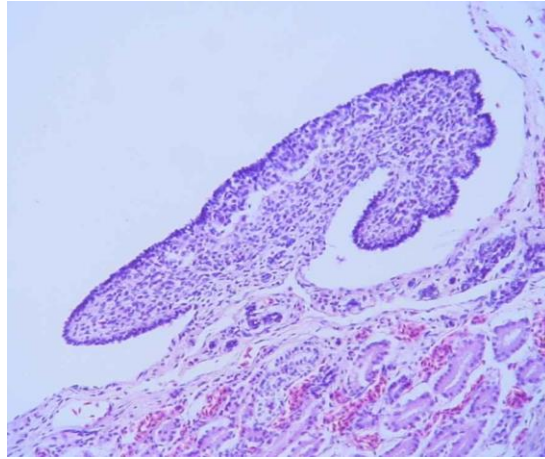


Figure 1. A female hatchling gonad identifiable by the dark pronounced outer cortex and a medulla lacking distinct organization.

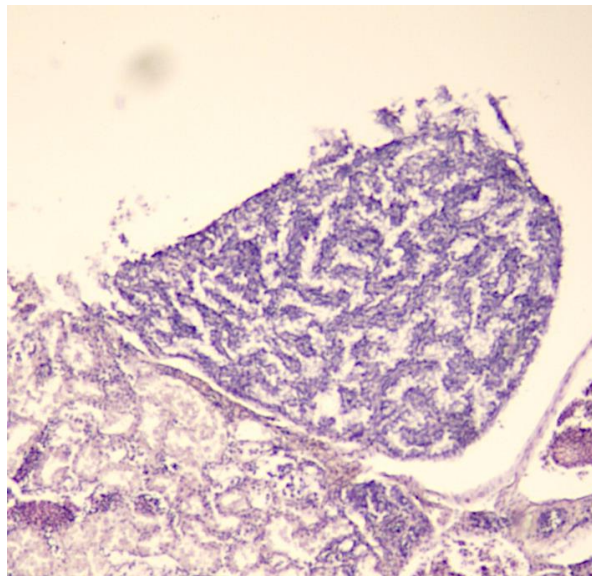


Figure 2. An image of a male hatchling gonad, which lacks a well-developed cortex and has an organized medulla with developing seminiferous tubules.

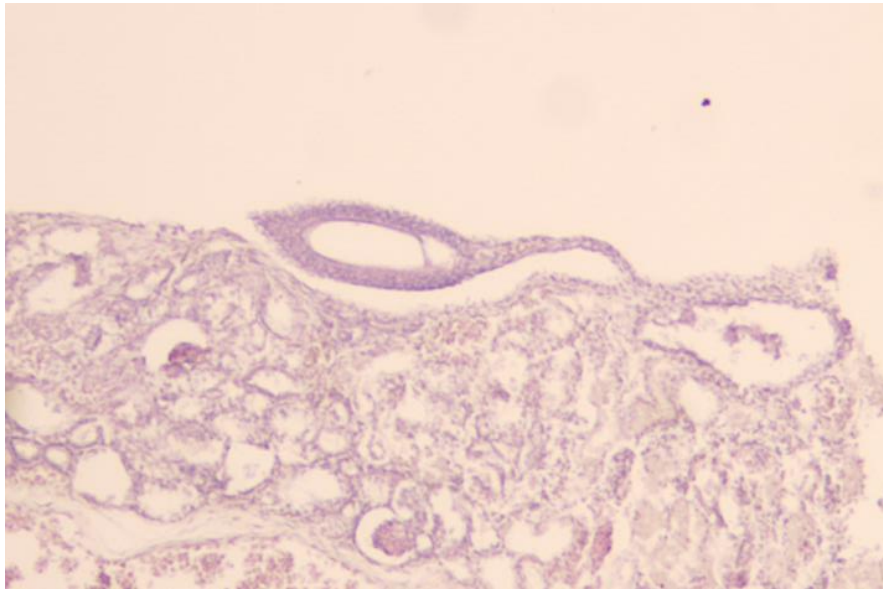


Figure 3. Example of a well-developed oviduct in a female hatchling, whereas in males the oviduct is absent or regressed.

A summary of the results organized by year is shown in Table 1. The number of tissues examined yearly ranged from 63-827, and a total of 5,077 hatchlings were successfully identified during the study. Overall 79.68 percent of these hatchlings were identified as female. Further, a female bias was recorded for each year of the program evaluated, 2002 through 2019. The yearly sex ratios documented in the current study ranged from 62-95%, with an average of the yearly sex ratios of 80.12 percent female. The sex ratio from each year of the study was significantly different from a 1:1 predicted sex ratio (Fisher's Exact Test, $p < 0.001$). Significant differences were detected among the yearly sex ratios (Replicated goodness of fit test, $p > 0.05$).

Table 2 shows an analysis of results organized by nest. The total number of nests per year represented by the data ranged from 11 to 184. The average sample size per year

ranged from 1.14 to 11.03 hatchlings per nest. The average yearly nest sex ratio of the hatchlings examined ranged from 61.43 to 93.88 percent female, with an overall average of 78.08 percent female. Further based on the hatchlings examined, the percentage of nests with a female bias ranged from 58.33 to 94.12 per year.

Table 1. Summary of The Analysis and Results From 2002-2019. The table includes the number of hatchlings examined, number of identified males, females, unknowns, and the overall yearly sex ratio.

Padre Island National Seashore Data Analysis					
Year	Hatchlings Examined	Male	Female	Unknown	Sex Ratio
2002	155	7	139	9	95.21
2003	63	7	51	5	87.93
2004	141	12	111	18	90.24
2005	237	15	136	86	90.07
2006	827	88	532	226	85.81
2007	315	44	161	110	78.54
2008	335	93	189	53	67.02
2009	333	71	193	69	73.11
2010	184	50	102	33	67.11
2011	353	88	160	105	64.52
2012	439	63	237	139	79.00
2013	190	13	137	40	91.33
2014	119	10	88	21	89.80
2015	307	45	182	80	80.18
2016	515	48	148	319	75.51
2017	281	8	77	196	90.59
2018	151	14	40	97	74.07
2019	132	14	23	95	62.16

Table 2. Evaluation of Nest Sex Ratios From 2002-2019. The table lists the number of nests examined each year, average number of hatchlings per nest, number of nests with female bias, overall percentage of nests that were female-biased, and the average sex ratio per nest.

Year	Total Nests Examined	Average Number of Hatchlings per Nest	Average Yearly Sex Ratio per Nest	Percent of Nests with Female Bias
2002	35	4.43	93.88	94.12
2003	11	5.73	82.72	90.91
2004	67	2.10	87.50	85.94
2005	42	5.64	93.35	96.77
2006	75	11.03	84.00	82.19
2007	95	3.32	76.73	77.33
2008	163	2.06	68.48	63.27
2009	91	3.66	66.03	61.36
2010	184	2.24	61.80	58.33
2011	110	3.43	65.25	58.89
2012	169	2.60	75.54	75.00
2013	44	4.32	87.30	88.10
2014	64	1.86	87.18	84.21
2015	134	2.30	77.73	76.99
2016	122	4.25	73.84	71.25
2017	69	4.09	87.71	88.89
2018	133	1.14	75.00	72.00
2019	51	2.62	61.43	60.00

The number of males and females identified each year is shown in Figure 4. The number of nests examined with male, female, or equal bias is shown in Figure 5. The results from 2002 to 2019 indicate that the majority of nests examined each year had a female bias. The female bias is shown individually in Figure 4 and relative to nest in Figure 5.

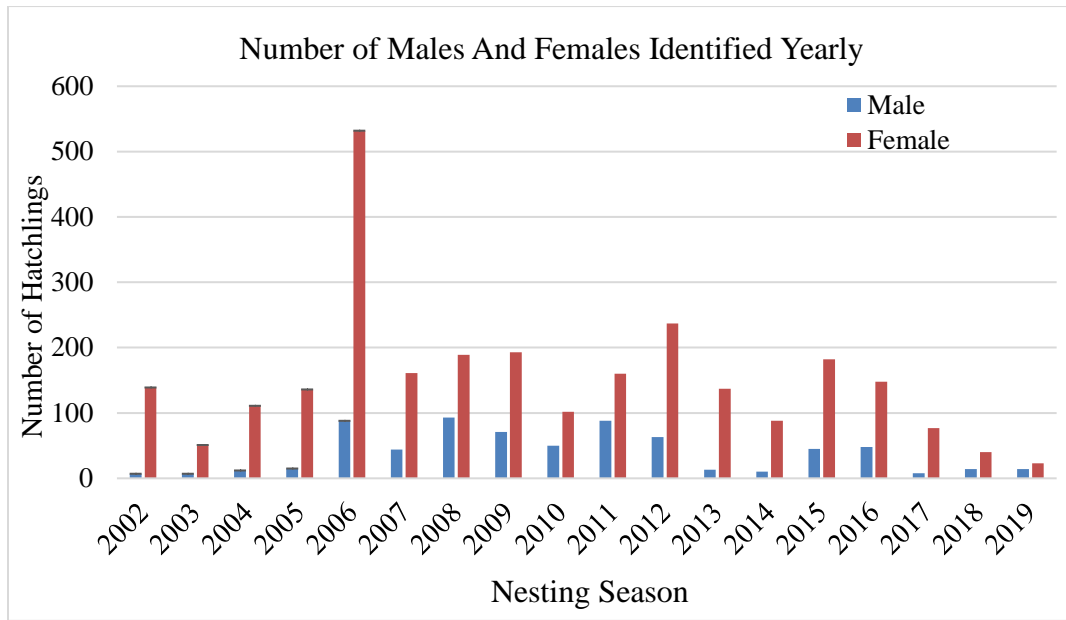


Figure 4. Yearly comparison of male and female hatchlings from 2002-2019. Each year more females than males have been produced in identified hatchlings.

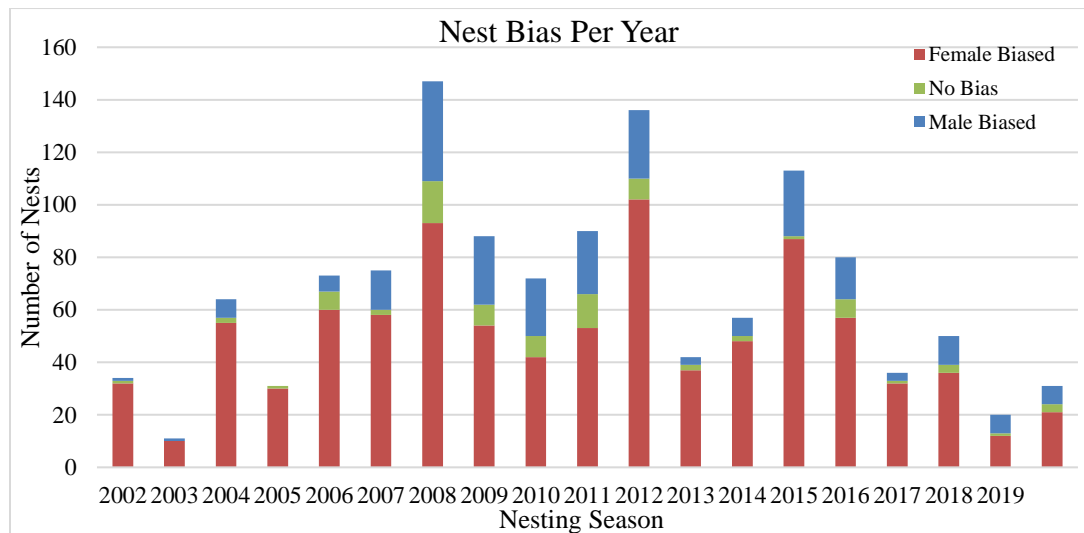


Figure 5. Yearly Nest Bias From 2002-2019. A comparison of the number of female-biased nests in red to male or equally biased nests in blue. Every year shows a higher number of female-biased nests being produced than male or equally biased nests.

Discussion

The critically endangered status of the Kemp's ridley sea turtle has resulted in long-term conservation efforts in both Mexico and the United States. These efforts include the protection and incubation of nests in order to enhance hatchling production. This facet of the conservation program has the potential to impact hatchling sex ratios through their temperature-dependent sex determination. Therefore, it is essential to monitor the sex ratio production and evaluate its impact. The current study represents a long-term (2002 through 2019) evaluation of hatchling sex ratios produced at the Padre Island National Seashore, Texas. The results reveal that female biases predominated in all of the years examined. This included an overall female bias of 79.68% relative to the total number of hatchlings examined for the entire study. The average yearly sex ratio was 80.12% female. Analysis of the data relative to individual nests revealed similar results, averaging 78.08% female per nest in the study.

In evaluating the results of this study, the potential of sampling bias should be considered. The sexes are identified in hatchlings found dead in the nest after all other hatchlings have emerged. Sex specific mortality could bias the sampling. However, a previous study has addressed this subject in natural nests and did not detect sex specific mortality (LeBlanc et al., 2012a). Further as indicated above, these nests were incubated at temperatures within the normal TRT range, thus avoiding extreme temperatures. Therefore, we have no indication that sex specific mortality was occurring in the current study.

The results of this study suggest that the Kemp's Ridley conservation recovery program at Padre Island National Sea Shore has been producing an overall female-biased hatchling sex ratio for almost two decades. The incubation temperatures that have historically been utilized in that program were chosen in an effort to produce mixed sex ratios with a potential female bias (LeBlanc et al., 2012a; Shaver pers. comm.) The results of the current study suggest a female bias was produced each year analyzed, 2002 through 2019.

The number of turtles nesting has been closely monitored over the past several decades at PAIS, and it has significantly increased since the 1990s. However, it is not clear if or how the hatchling female bias has affected this recovery. It has long been suggested that TSD could potentially provide an avenue for enhancing the recovery of an endangered species by sex ratio manipulation (Coyne and Landry, Jr., 2007). The Kemp's ridley program at PAIS represents an example of a relatively large-scale and long-term manipulation of a sex ratio in an endangered species with TSD. It is plausible that a female-biased hatchling sex ratio in this program could be enhancing the recovery of this species. However, the impact of a female bias is currently speculative.

A variety of theoretical studies have reviewed the impact of female biases on sea turtle populations. A female bias in both adult and embryonic sex ratios could potentially increase population size if large numbers of female offspring are produced and recruited into the reproductive population (Boyle et al., 2014; Santidrián Tomillo et al., 2015; Patrício et al., 2019). A previous study by Coyne and Landry, Jr. (2007) indicated that if the number of males is sufficient for mating, a female-biased population sex ratio could

enhance hatchling production and species recovery. Several other studies have suggested that a limited number of males may be sufficient to support reproduction in a female-biased sea turtle population, due to factors related to differences in physiological reproduction costs in males vs females (Laloë et al., 2017; Santidrián Tomillo and Spotila, 2020). As such, a female-biased sex ratios being produced at PAIS could potentially be benefitting the recovery of the Kemp's ridley. Furthermore, data from the primary nesting beach at Rancho Nuevo, Mexico suggest warm incubation temperatures that are indicative of a female bias at this species primary nesting beach over the past several decades (Wibbels, 2007; Eich, 2009; see chapter 2 of this current thesis). Thus, the Kemp's ridley recovery program may exemplify a large-scale manipulation of a hatchling sex ratio that may be enhancing the recovery of an endangered species with TSD.

While a female-biased sex ratio could potentially benefit the recovery of a sea turtle species, it could also become problematic in the future due to global climate change. It has been projected that global temperatures could increase approximately 0.5 to 4°C by 2100 (IPCC Special Reports SP1.5, 2019). An increase of this magnitude would not only increase female production but could result in extreme biases, as well as affecting hatchling fitness and mortality. The ability of sea turtles to adapt their behavior and physiology (including the adaptability of TSD) to global climate change is currently speculative (Santidrián Tomillo and Spotila, 2020). Rising temperatures could increase vulnerability of eggs resulting in higher hatchling mortality rates (Fuentes et al., 2011). However, TSD could also offer some resilience to increasing temperatures by increasing

female offspring production which could compensate for decreased hatchling survival (Santidrián Tomillo et al., 2015). Natural selection and adaptive behaviors may already be starting in some populations where changes in nesting behavior have been seen to enhance hatchling emergence (Patrício et al., 2019). Several studies indicate there may be potential options for sea turtles to adapt to rising temperatures by shifting nesting northward (to cooler climates) or nesting earlier in the season, both allowing for cooler incubation temperatures (Weishampel et al., 2010; Reece et al., 2013; Schwoerer, 2013). It is not clear if changes in sea turtle behavior and physiology will be able to compensate for global climate change. Therefore, it is paramount to continue to monitor the reproductive ecology of sea turtles relative to environmental temperature, including hatchling survival and sex ratios in conservation programs.

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DOCUMENTATION OF CURRENT THERMAL TRENDS AT THE PRIMARY
NESTING BEACH FOR THE KEMP'S RIDLEY SEA TURTLE (*LEPIDOCHELYS*
KEMPII)

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In preparation for Herpetological Conservation and Biology

Format adapted for thesis

Abstract

The current study documents recent temperature data from the main nesting beach for the Kemp's ridley sea turtle (*Lepidochelys kempii*), at Rancho Nuevo, Mexico. The beach temperatures impact sex determination and successful embryonic development in this species. Temperatures were monitored during 2021 in the egg hatcheries (i.e., egg corrals) and on the nesting beach in locations where the majority of nesting occurs. This included a series of temperature transects over approximately 20 km of nesting beach at index locations. All data loggers were buried at mid nest depth. The 2021 nesting season data from the egg corrals were evaluated and compared to the those from the nesting beach transects. The data were also analyzed relative to long-term data from the index transect locations from the nesting beach from the 2013 – 2020 nesting seasons. The dynamic of the seasonal trends was generally similar over the years examined. Temperatures were below the pivotal temperature early in the nesting season (April through mid-May). During the nesting season, temperatures gradually increased above pivotal temperature by approximately mid to late May and remained relatively high through the remainder of the nesting season, with the exception of rain events. In general, beach temperatures during the majority of the nesting season were conducive to the production of female-biased sex ratios, and temperatures periodically neared lethal temperatures. Long-term analysis of the temperature data indicated significant yearly variation with temperatures gradually increasing over the nine-year study period. Collectively, the results indicate that current temperatures are relatively high at Rancho

Nuevo, and that survival of the Kemp's ridley could be significantly impacted by the projected increases in environmental temperature over the next century.

Introduction

As stated previously, the Kemp's ridley sea turtle (*Lepidochelys kempii*) is considered "critically endangered" and came close to extinction in the mid-1980s (Pritchard and Marquez, 1973). For over five decades, the Kemp's ridley has been the study of intense conservation efforts. They have a single primary nesting beach (Rancho Nuevo, Mexico), where approximately 80% of the nesting for the entire species occurs. Since 1978, the U.S./Mexico Binational Kemp's Ridley Conservation Program has focused on the recovery of the species at their primary nesting beach (Frey et al., 2014; Wibbels and Bevan, 2016).

Kemp's ridleys undergo mass nesting events referred to as arribadas, where hundreds to thousands of adult females nest within the same period of time (Márquez, 1994; Wibbels, 2007). A majority of nesting occurs during this small-time frame on a limited stretch of beach. This type of nesting causes a large number of hatchlings to be incubated during the same range of temperatures.

The use of a single primary nesting beach by a species with temperature-dependent sex determination (TSD) makes the Kemp's ridley extremely sensitive to changes in environmental temperatures. As such, this species is particularly vulnerable to the projected global climate change over the next century (IPCC Special Reports SP1.5, 2019). Therefore, it is imperative to document the current and future impact of the

thermal environment on this species' primary nesting beach. The current study documents and evaluates current beach and egg hatchery (corral) temperatures at Rancho Nuevo during the 2021 nesting season, and includes the comparison of temperature trends over the past several nesting seasons (2013-2021). The results of this study will provide insight into the seasonal and yearly variation of beach temperatures at Rancho Nuevo. Obtaining this type of baseline data on the current thermal environment is essential for the accurate evaluation of the impact of future global climate change.

Methods and Materials

Temperature data were collected during the 2021 nesting season at Rancho Nuevo, Mexico. HOBO pendant temperature data loggers were utilized to record temperatures both on the beach and in egg corrals. The data loggers were programmed to record temperatures at one-hour intervals for the duration of the nesting period. In order to monitor beach temperatures, data loggers were used on a series of beach transects that spanned approximately 30 km of the nesting beach. Temperature transects were conducted at approximately 1.5 km intervals for a total of 17 transects. Two data loggers were utilized per transect in the area of beach where the majority of nesting occurs (i.e., at the base of the dune and approximately 10 meters seaward from the dune). All data loggers were buried at 35 cm deep, the average mid-nest depth for a Kemp's ridley nest (Márquez, 1994; Eich, 2009).

Additionally, data loggers were placed into egg hatcheries (i.e., egg corrals) at Rancho Nuevo. Three egg corrals are utilized at Rancho Nuevo (Corral Central, Corral

Norte, and Corral Sur). A relatively large number of nests each year are translocated into these egg corrals in order to enhance egg production by avoiding predation. The main egg corral (Corral Central) is located near the center of the Rancho Nuevo nesting beach and turtle camp. The other egg corrals are located 5 km north (Corral Norte) and 5 km south (Corral Sur) of the turtle camp. Nine data loggers were deployed in the largest egg corral (Corral Central) and 6 data loggers were deployed in Corral Sur and Corral Norte. In the 2021 nesting season, the data loggers were deployed during the middle of May and recovered in mid-August.

Data loggers were deployed at several other nesting beaches in the state of Tamaulipas, Mexico, that are known satellite nesting beaches for the Kemp's ridley. These sites spanned approximately 200 km of beaches to the north and south of Rancho Nuevo, from Tampico to La Pesca, Mexico, and included the following beaches; Miramar, Altimira, Bara del Tordo, Tepehujes, La Pesca. Approximately 2-15 data loggers were deployed per beach in areas where a majority of nesting occurred (e.g., near the base of the dune, similar to the transect locations at Rancho Nuevo).

In order to gain insight on the long-term thermal variability of beach temperatures at Rancho Nuevo, historic data from 2013 through 2020 were analyzed for comparison to the 2021 data. Data logger transect locations and data logger placement (i.e., depth of 35 cm) have been relatively consistent over the past several decades at Rancho Nuevo.

Hourly temperature data from each data logger were downloaded and daily averages were generated. The daily average data were utilized in all of the statistical analyses. Monthly variation in beach temperatures over the nesting season at Rancho

Nuevo was evaluated using ANOVA. Nesting beach temperatures at Rancho Nuevo were compared to egg corral temperatures using ANOVA. Nesting beach temperatures at Rancho Nuevo were compared to those from other satellite nesting beaches in Tamaulipas using ANOVA. Beach temperatures at Rancho Nuevo during 2021 were compared to data from previous years (ranging from 2013-2020) using ANOVA. Regression analysis was also used with these historic data to provide insight on long-term changes in beach temperatures.

Results

In late August, data loggers were retrieved from the Rancho Nuevo nesting beach and egg corrals, and from a series of peripheral nesting beaches located to the north and south of Rancho Nuevo in Tamaulipas, Mexico. The data loggers were downloaded using the HOBO Computer Software (HOBOWare), and all data was exported to Microsoft Excel for archiving and analysis.

Average daily temperature data from Corral Central at Rancho Nuevo are shown in Figure 1. Temperatures in the egg corrals varied significantly over the nesting season (ANOVA; $p < 0.001$). By mid to late-May, corral temperatures were at or above the pivotal temperature of sex determination for the Kemp's ridley (see Figure 1). During June and July, corral temperatures remained above the pivotal temperature with the exception of time periods when weather systems moved through the area. Average temperatures periodically rose to approximately 33°C or greater in June and July. A dichotomy of temperatures can be seen in Figure 1 starting at approximately 7/10/2021.

This dichotomy resulted from shading a portion of the corral to decrease incubation temperatures and embryonic mortality.

The average daily temperatures for Corral Central are shown in Figure 2 together with the two smaller egg corrals (Corral Norte and Corral Sur). Significant variation in temperature was detected between the egg corrals; however, they showed similar trends and similar ranges of temperatures, including maximal values (ANOVA; $p < 0.001$).

Average daily nesting beach temperatures (at mid-nest depth) for Rancho Nuevo (from the temperature transects) during the 2021 nesting season are shown in Figure 3. Significant variation was detected in beach temperatures over the nesting season (ANOVA; $p < 0.001$). Beach temperatures exhibited a general increase during mid to late-May and were at or near the pivotal temperature by the end of May. During June and July, average daily temperatures remained near or above the pivotal temperature with the exception of periodic weather events in the area that cooled the beach. From mid-July through August, temperatures in general were above the pivotal temperature and frequently reached a temperature of 33°C or greater.

Nesting beach average daily temperatures are compared to those of Corral Central in Figure 4. The nesting beach temperatures were significantly different than those in the egg corral (ANOVA; $p < 0.001$). Nesting beach temperatures were consistently cooler than the corral temperatures, but they showed the same general trends in temperature variation throughout the nesting season.

The average daily nesting beach temperatures at Rancho Nuevo are compared to those from a series of secondary nesting beaches in the state of Tamaulipas, Mexico, in

Figure 5. Significant variation was detected between the nesting beaches (ANOVA; $p < 0.05$), however, the secondary nesting beaches showed the same general trends as the primary nesting beach at Rancho Nuevo. Overall, the average daily beach temperatures varied from approximately 27°C early in the nesting season (May) to 33°C or greater in August.

Average monthly (April through August) beach temperatures for the 2021 nesting season are compared to recent years at Rancho Nuevo (from 2013-2020) in Figures 6-10 (April-August, respectively). The monthly temperatures showed a general seasonal trend during all years examined. Average yearly temperatures for April were several degrees below the pivotal temperature (Figure 6). In May, the average yearly temperatures were slightly higher but were still below the pivotal temperature (Figure 7). By June, the average yearly temperatures rose above the pivotal temperature (Figure 8). In July and August average yearly temperatures remained above the pivotal temperature, reaching a maximum monthly average of approximately 32°C during those months (Figures 9 and 10, respectively).

A significant correlation was detected between years (2013-2021) and average daily temperatures (May 15-August 15) (Pearson Correlation; $p < 0.05$). Regression analysis indicated there was a positive relationship between years and average daily temperatures ($R^2 = 0.007$; $p < 0.05$). Average monthly temperatures varied significantly between the years (2013-2021) (ANOVA; $p < 0.05$). Significant correlations were not detected between the year and average monthly temperatures during June and August. However, a significant correlation was detected between year and average monthly

temperature during May and July with higher temperatures in more recent years (Pearson Correlation; $p < 0.05$).

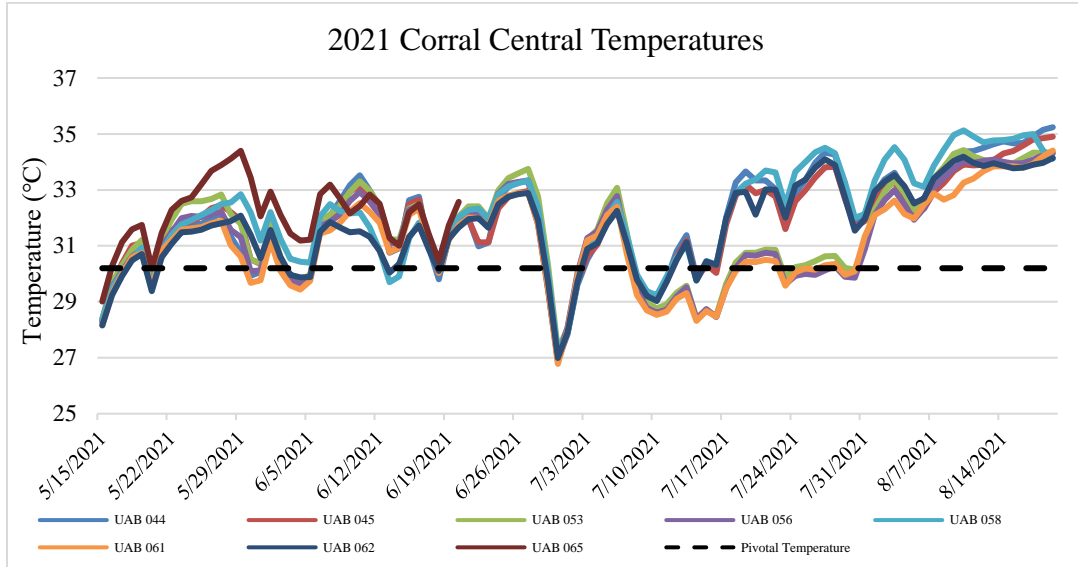


Figure 1. 2021 Average Daily Corral Central Temperatures. Each line represents a different data logger. Significant data points include a rain event starting on 6/30/2021.

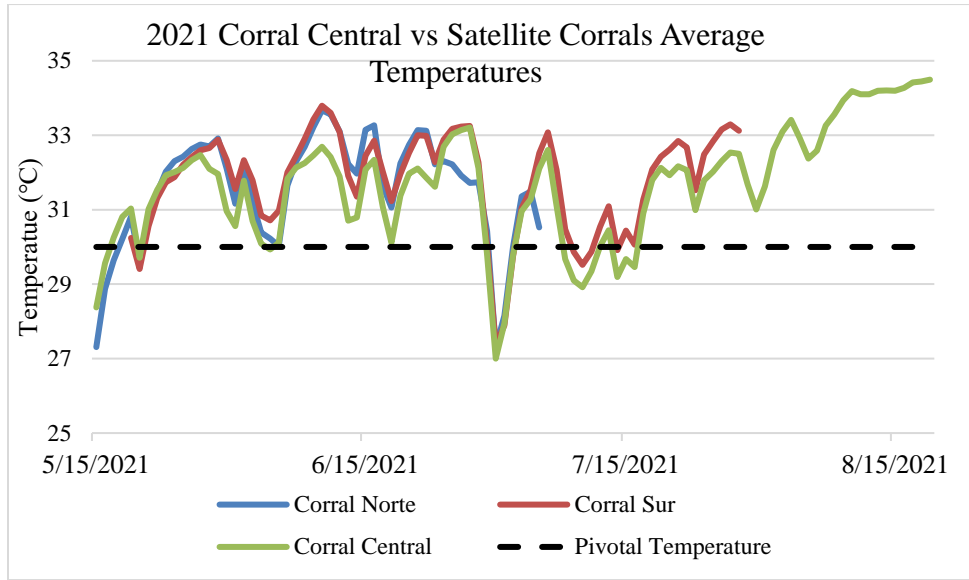


Figure 2. 2021 Corral Central vs Satellite Corral Average Temperatures at Rancho Nuevo Mexico. Each line represents the average of all data loggers in each of the 3 corrals (Norte, Sur, and Central).

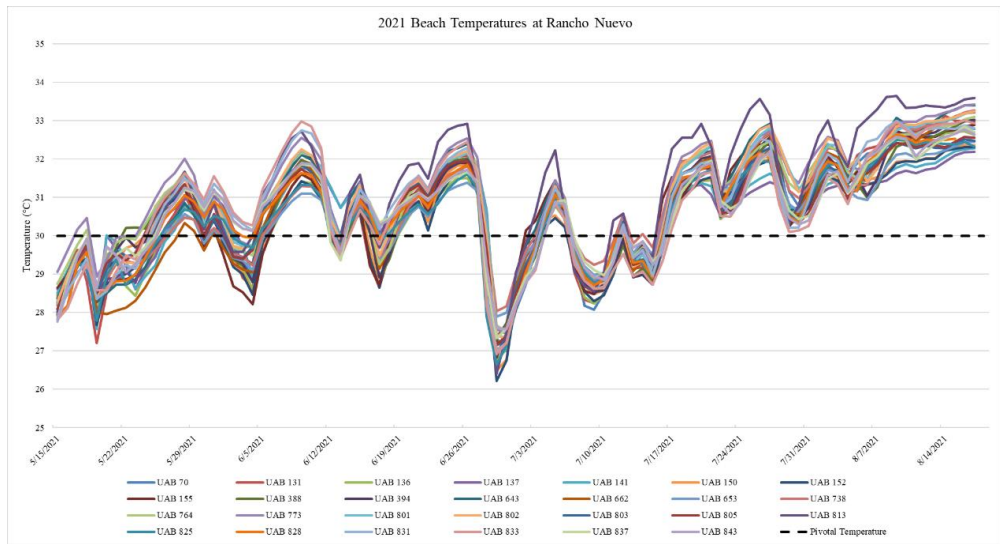


Figure 3. 2021 Beach Temperatures at Rancho Nuevo, Mexico from various locations across Rancho Nuevo, Mexico in the 2021 nesting season.

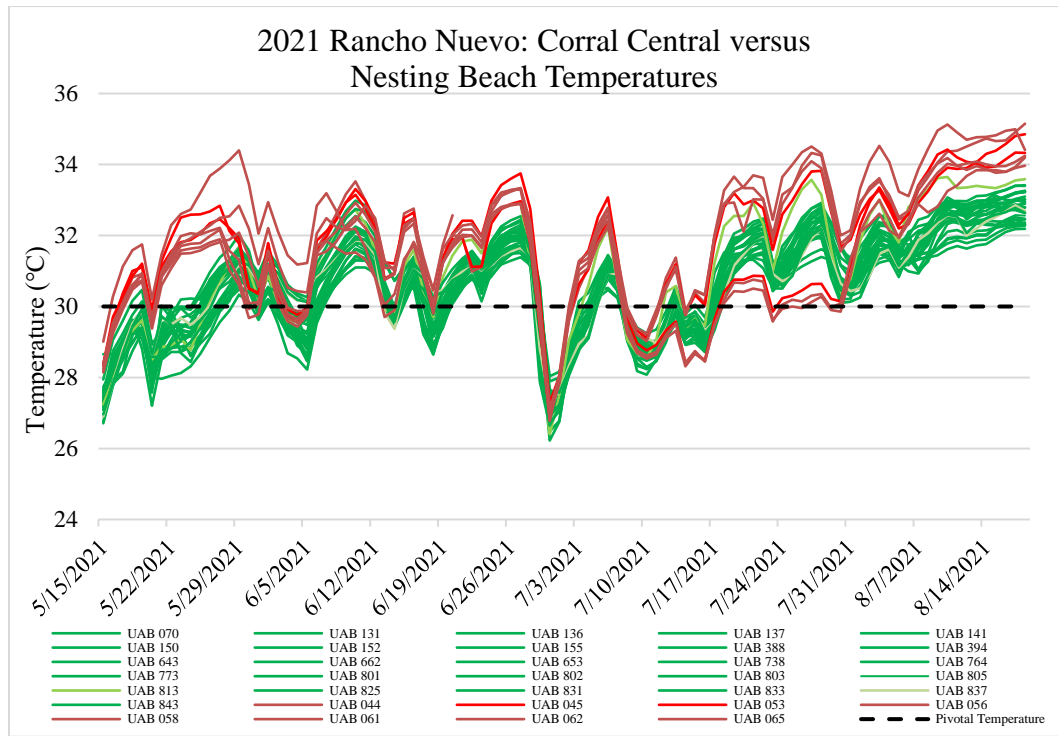


Figure 4. 2021 Corral Central vs Beach Temperature Averages. Corral temperatures are shown in red and beach temperatures are in green.

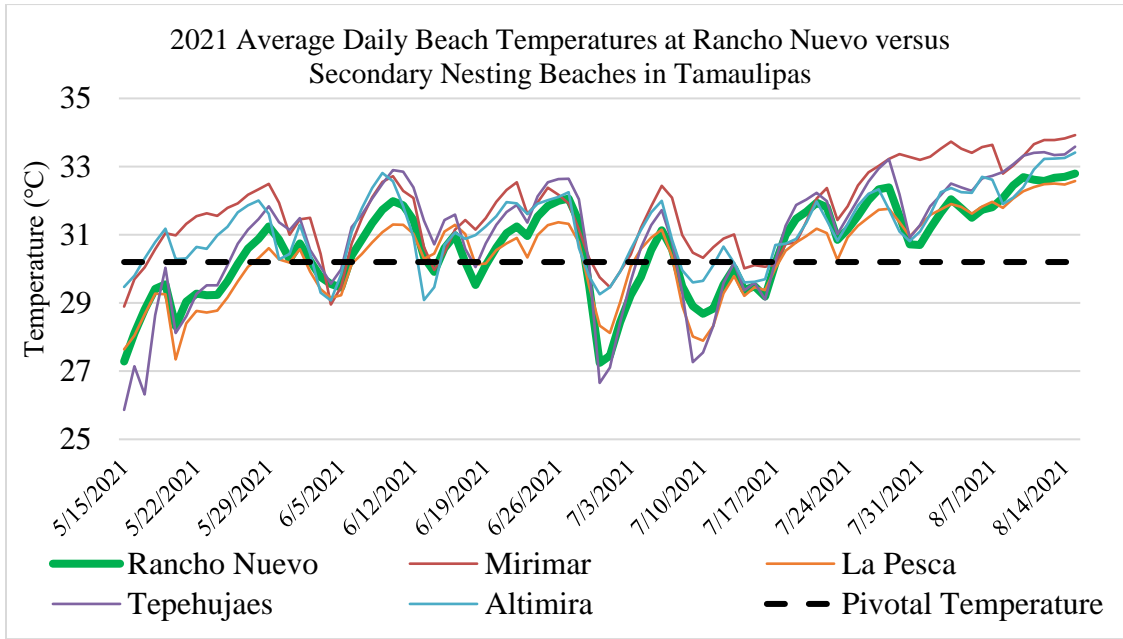


Figure 5. 2021 Average Daily Beach Temperatures at Rancho Nuevo versus Secondary Nesting Beaches in Tamaulipas.

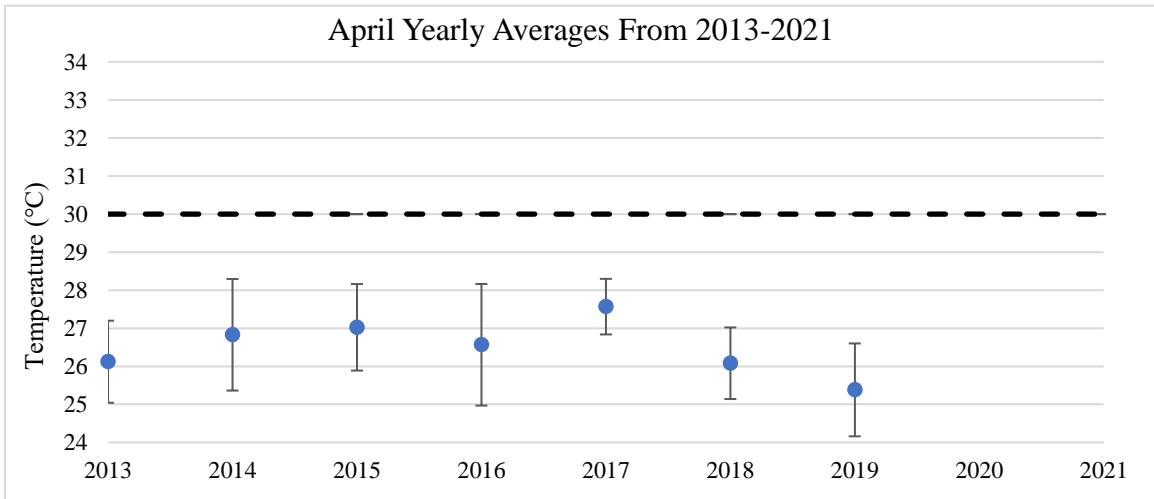


Figure 6. April Yearly Averages From 2013-2021. Values for April 2020 and 2021 are absent (because data loggers were deployed in early May).

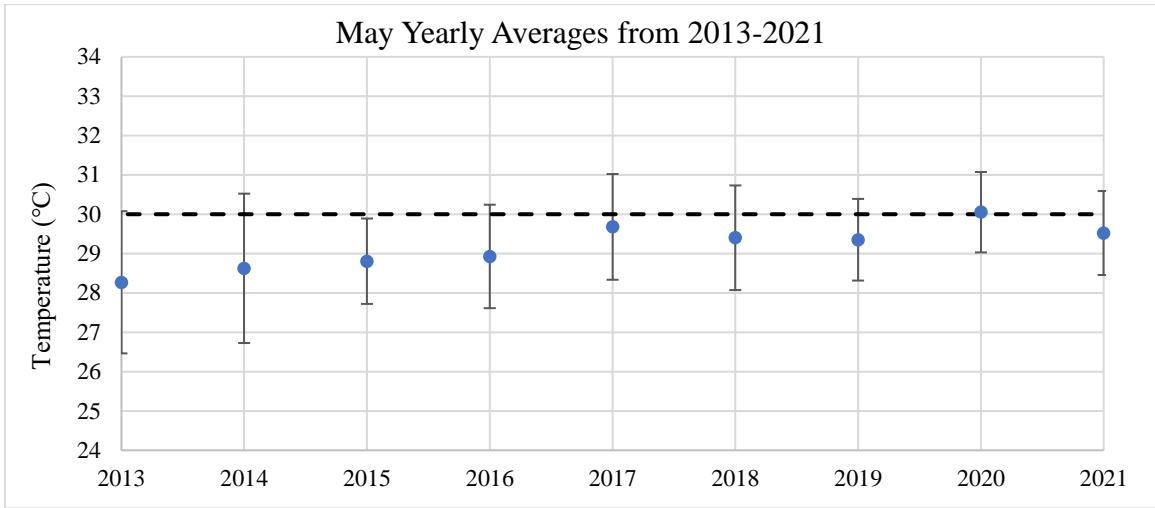


Figure 7. May Yearly Averages From 2013-2021.

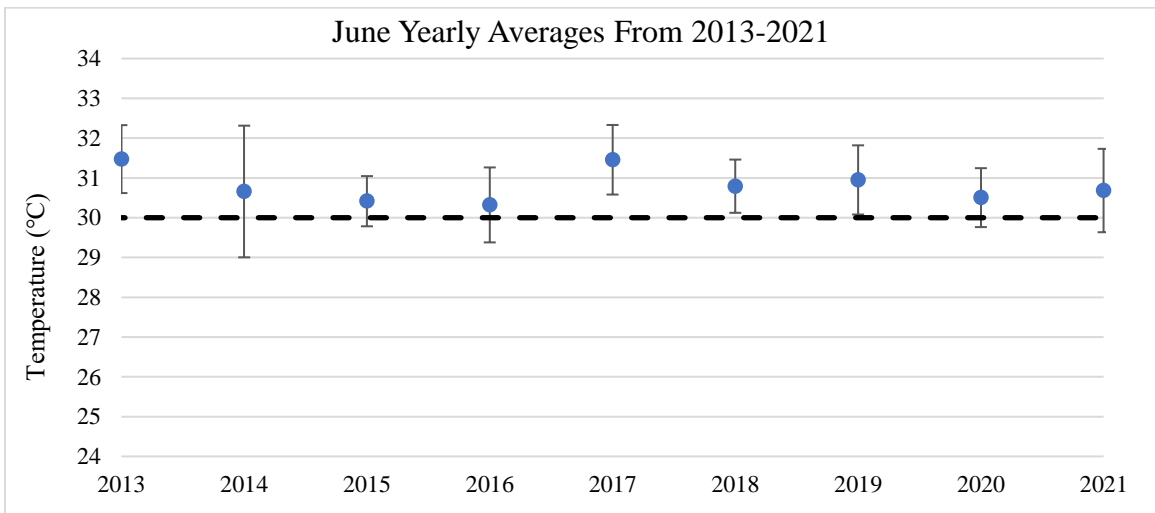


Figure 8. June Yearly Averages From 2013-2021.

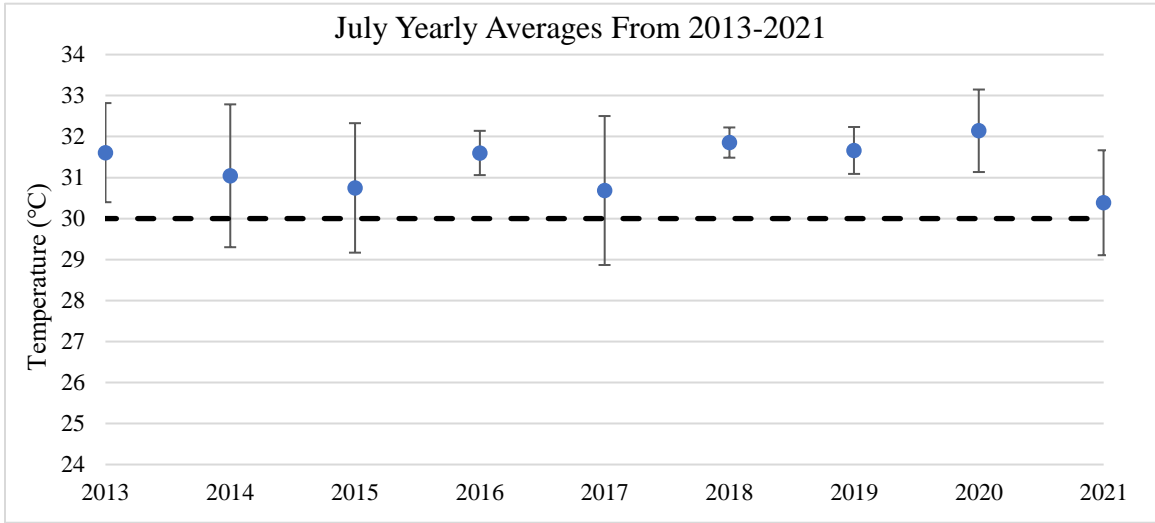


Figure 9. July Yearly Averages From 2013-2021.

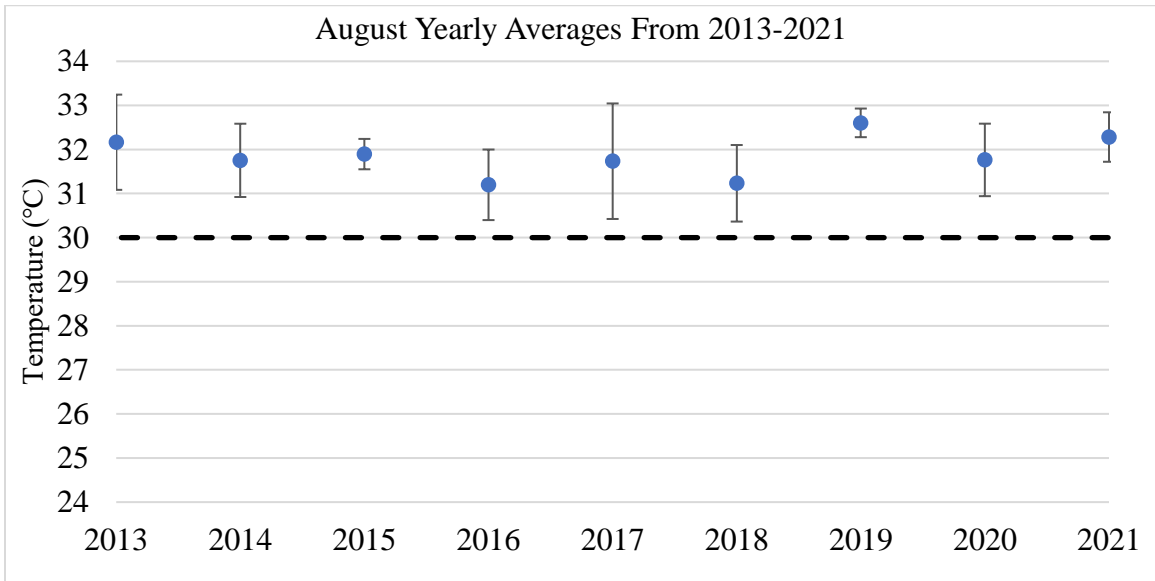


Figure 10. August Yearly Averages From 2013-2021.

Discussion

In contrast to other sea turtle species, the Kemp's ridley has a limited distribution and a single primary nesting beach. The majority of nesting for this species occurs on approximately 30 km of nesting beach located at Rancho Nuevo, Mexico. This species also exhibits arribada nesting behavior, which involves the synchronized mass nesting of large numbers of female turtles. The nesting season for the Kemp's ridley ranges from April through August, with a majority of nests being laid between late-May and mid-June. A typical nesting season includes several arribadas which results in episodic production of nests. Therefore, Kemp's ridley nests may experience a relatively limited thermal environment, in contrast to other sea turtle species with world-wide distributions of nesting beaches. Further, due to their critically endangered status, many of the natural nests at Rancho Nuevo are translocated to a protected area of beach (i.e., egg corrals) in an effort to enhance hatchling production. The thermal qualities of the Kemp's ridley nesting beach impact the proper embryonic development of incubating eggs and affect their temperature-dependent sex determination. Therefore, the environmental temperatures at the Rancho Nuevo nesting beach are essential to the ecology and conservation of the Kemp's ridley sea turtle. As such, it is imperative to evaluate and monitor nesting beach temperatures. The current beach temperatures at Rancho Nuevo also represent important baseline data for evaluating the impact of future changes in environmental temperature on the Kemp's ridley. The current study documented the thermal characteristics of the nesting beach and egg hatcheries at Rancho Nuevo. The

results provide insight in regards to the impact on temperature-dependent sex determination and hatchling production.

The results indicate that sand temperatures in the egg corrals during 2021 are increasing during May, reach approximately pivotal temperature around mid to late-May, and then remain relatively high throughout the remainder of the nesting season with the exception of weather events that cool the beach (LeBlanc et al., 2012b). The results also showed that the secondary egg corrals exhibited similar temperature trends and temperature ranges to Corral Central (Figure 2). These findings suggest that the initial nests of the season (e.g., April through early May) would be conducive to the production of male hatchlings, whereas the remainder of the nesting season is above the pivotal temperature, indicating the production of females. However, there may be episodic production of males due to weather event-related cooling the beach temperatures, as exemplified by the decrease in temperatures starting at the end of June.

The results also indicate that beach temperatures from 2021 showed similar trends to the egg corrals, however, the beach temperatures were significantly cooler than those in the egg corrals. The temperatures from the secondary nesting beaches showed similar temperature trends and varied from approximately 27°C early in the nesting season (May) to 33°C or greater in August.

During the 2021 nesting season, nesting began in April with the majority of nesting occurring in May through early-June. The nesting season exhibited two major arribadas, on May 5 and June 6, respectively. Both beach and egg corral temperatures (at nest depth) during May through August in 2021 were generally at or above pivotal

temperature. Therefore, the majority of nests, including those from the major arribadas in 2021, experienced temperatures that would be conducive to the production of female biases (LeBlanc et al., 2012b).

The beach temperatures during 2021 at the primary nesting beach at Rancho Nuevo were compared to the secondary nesting beaches located throughout Tamaulipas (Figure 5). All beaches were typically within approximately 1-2°C of one another and showed similar patterns in temperature dynamics. The majority of the secondary nesting beaches were warmer than Rancho Nuevo, and none of the beaches were continuously cooler than Rancho Nuevo.

Temperatures within the egg corrals at Rancho Nuevo as well as on the nesting beach during 2021 were relatively high for the majority of the nesting season. In fact, temperatures in the egg corrals periodically neared 34°C, and beach temperatures approached 33°C. These temperatures are nearing potential lethal temperatures for sea turtle development. Previous studies of various sea turtle species state temperatures in the 35°C range are lethal (Valverde et al., 2010; Santidrián Tomillo et al., 2012). Furthermore, a variety of studies showed that high but non-lethal incubation temperatures can decrease hatchling fitness (Matsuzawa et al., 2002; Glen et al., 2003; Segura and Cajade, 2010; Fisher, Godfrey, and Owens, 2014). During recent years, the egg corral temperatures have been monitored in real-time and have been partially shaded in an attempt to decrease embryonic mortality and enhance fitness. The effects of shading the corral are exemplified in Figure 1 in which there is an obvious dichotomy in temperatures in shaded vs un-shaded portions of the corral starting in approximately mid-July.

(Matsuzawa et al., 2002; Glen et al., 2003; Valverde et al., 2010; Segura and Cajade, 2010; Santidrián Tomillo et al., 2012; Fisher, Godfrey, and Owens, 2014).

Rancho Nuevo average daily beach temperatures from 2021 were compared to previous years (2013-2020) and results indicated consistent seasonal temperature trends. As exemplified by the average monthly temperatures (Figures 6-10), the coolest temperatures occur early in the season (April) and gradually increase (May) to rise above the pivotal temperature (June) and remain high near the end (July-August). The end of the season shows temperatures that are consistently above the pivotal temperature and potentially approach lethal temperatures (i.e., embryonic mortality).

Although the seasonal trends in beach temperatures were relatively similar at Rancho Nuevo from 2013-2021, significant variation was detected in the magnitude of the temperatures between the years. Further, average daily beach temperatures varied significantly by year and regression analysis indicated an upward (positive) trend in temperatures.

Collectively, the results of this study indicate that beach temperatures at Rancho Nuevo are currently at or above the pivotal temperature of sex determination for the majority of the nesting season, and periodically near lethal temperatures. Further, the data are consistent with the hypothesis that beach temperatures are increasing at Rancho Nuevo. This could potentially be problematic in an attempting to adapt to future environmental changes. The IPCC (Special Reports SP1.5, 2019) currently predicts that temperatures could rise approximately 1-4°C (depending on the model) over the next century. If Rancho Nuevo experiences a temperature increase of that magnitude, it could

result in extreme biases as well as high levels of embryonic mortality. It is currently not clear if or how the Kemp's ridley could naturally adapt to such a scenario. Some authors have suggested that increasing environmental temperatures could initially be advantageous (Matsuzawa et al., 2002; Glen et al., 2003; Valverde et al., 2010; Segura and Cajade, 2010; Santidrián Tomillo et al., 2012; Fisher, Godfrey, and Owens, 2014) by increasing the production of females. However, temperatures at Rancho Nuevo are already relatively high and conducive to female biases. It is plausible that this species could alter the seasonal timing of its nesting in an attempt to adapt. It is not clear to what extent they would be able to compensate for increasing environmental temperatures through a seasonal shift in nesting. Alternatively, the Kemp's ridley could attempt to utilize alternative nesting beaches with cooler beach temperatures. However, the current study was unable to identify any such nesting beaches to the north and south of Rancho Nuevo in Tamaulipas, Mexico. Additionally, the Kemp's ridley's sex determination system could evolve a higher pivotal temperature, but its pivotal temperature is already relatively high for a sea turtle. Further, the predicted change in environmental temperatures may be too rapid in comparison to the time required for the evolutionary changes in sex determination. Regardless, the results of this study indicate that the Kemp's ridley is extremely susceptible to global climate change. Its conservation and survival could be severely affected by increases in environmental temperatures. As such it is imperative to monitor and evaluate the conservation status of this species and the thermal characteristics of its nesting beach over the next several nesting seasons.

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ESTIMATING A HATCHLING SEX RATIO PRODUCED ON THE KEMP'S RIDLEY
SEA TURTLE'S (*LEPIDOCHELYS KEMPII*) PRIMARY NESTING BEACH AT
RANCHO NUEVO, MEXICO

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In preparation for Herpetological Conservation and Biology

Format adapted for thesis

Abstract

The current study monitored beach and egg hatchery temperatures on the primary nesting beach of the Kemp's ridley sea turtle in order to estimate hatchling sex ratios produced during the 2016 nesting season. Temperatures were monitored during the 2016 nesting season in the egg hatcheries (i.e., egg corrals) and on the nesting beach in locations where the majority of nesting occurs. This included a series of temperature transects over approximately 20 km of nesting beach at index locations. All data loggers were buried at mid nest depth. Nesting data were collected during the 2016 nesting season by the Binational Kemp's Ridley Recovery Program. This included the lay date and emergence date for each of 11,386 nests that were translocated to egg corrals during the 2016 data. The temperature data together with the nesting data were utilized to calculate the average incubation temperatures for each nest during the approximate thermosensitive period of sex determination. This information was then used to estimate the sex ratio produced in each nest based on previously published data on the thermal reaction norm of temperature-dependent sex determination for the Kemp's ridley. The results suggest that the egg corrals would have produced a significant female bias during the 2016 nesting season (estimate of 88.5% female). Additionally, a hypothetical sex ratio was predicted for the nesting beach which suggested a near 1:1 sex ratio (estimate of 50.4% female) would have been produced in those same nests if they were left on the natural nesting beach. It is plausible that the production of a female-biased hatching sex ratio could be enhancing the recovery of this critically endangered sea turtle.

Introduction

The Kemp's ridley sea turtle (*Lepidochelys kempii*) is historically the most endangered sea turtle in the world and came close to extinction in the mid-1980s (Pritchard and Marquez, 1973; Wibbels and Bevan, 2016). The Kemp's ridley is distributed in the Gulf of Mexico and along the Atlantic coast of the United States. In contrast to other sea turtle species, it has a single primary nesting beach located at Rancho Nuevo, Mexico. Approximately 80% of the nesting for the entire species occurs at this beach (Wibbels and Bevan 2016). In 1978, the U.S./Mexico Binational Kemp's Ridley Conservation Program was initiated to prevent the extinction and enhance the recovery of the species (Frey et al., 2014; Wibbels and Bevan, 2016). Due to the critically endangered status of this species, this program has included the translocation of the majority of nests to protected egg hatcheries (i.e., egg corrals) to enhance the protection of nests and production of hatchlings.

The use of a single primary nesting beach by the Kemp's ridley limits the thermal variability experienced by nests in this species. Further, the Kemp's ridley exhibits arribada nesting behavior in which synchronized episodic mass nesting events occurs. This concentrates nests temporally and further limits the thermal variability of nests in this species. The thermal environment of sea turtle nests impacts various aspects of embryonic development, including developmental rate, temperature-dependent sex determination, hatchling fitness, and hatching success (i.e., embryonic mortality).

The purpose of the current study is to monitor incubation temperatures on the primary nesting beach for the Kemp's ridley and estimate their impact on hatchling sex

ratios. Specifically, temperatures in the egg corrals are used to predict the overall hatchling sex ratio produced in the egg corrals during the 2016 nesting season. Further, the nesting beach temperatures are used to evaluate what sex ratio could have been produced in these nests had they not been translocated to the egg hatcheries. This hypothetical sex ratio provides insight on the impact of the egg corrals and also the natural sex ratio produced from the species' primary nesting beach. Monitoring the temperatures and estimating sex ratios at Rancho Nuevo are essential to evaluating the current and long-term impacts of global climate change on the Kemp's ridley.

Methods and Materials

Temperature Monitoring

HOBO data loggers were deployed on April 1 and retrieved on August 31 during the 2016 nesting season. Data loggers were programmed to record temperatures hourly for the entire nesting season. All data loggers were buried at 35 cm deep, the average mid-nest depth for a Kemp's ridley nest (Márquez, 1994; Eich, 2009).

Data loggers were placed into three egg hatcheries (i.e., egg corrals) at Rancho Nuevo. Three egg corrals are utilized at Rancho Nuevo. A relatively large number of nests each year are translocated into these egg corrals in order to enhance egg production by avoiding predation. The main egg corral (Corral Central) is located near the center of the Rancho Nuevo nesting beach and turtle camp. The other egg corrals are located 5 km north (Corral Norte) and 5 km south (Corral Sur) of the turtle camp. Nine data loggers were deployed in the largest egg corral (Corral Central) and 6 data loggers were deployed

in Corral Sur and Corral Norte. In the 2021 nesting season, the data loggers were deployed during the middle of May and recovered in mid-August.

In order to monitor beach temperatures, data loggers were used on a series of beach transects that spanned approximately 30 km of the nesting beach. Temperature transects were conducted at approximately 1.5 km intervals for a total of 17 transects. Two data loggers were utilized per transect in the area of beach where the majority of nesting occurs (i.e., at the base of the dune and approximately 10 meters seaward from the dune). At the end of the nesting season, all data temperature data was downloaded onto HOBOWare and were exported into Excel datasheets for archiving.

Monitoring and Translocation of Nests

As part of the Kemp's Ridley Recovery Program, a team of biologists utilize all-terrain vehicles to patrol approximately 20 km of beach multiple times daily for up to 5 months (April-August) each year at Rancho Nuevo, Mexico. This team attempts to record all nests and to move the majority of nests to egg corrals. All nests in the egg corrals are then monitored daily for their entire incubation period and upon emergence, hatchlings are collected and released into the Gulf of Mexico. Data from each nest (including lay date and hatch date) are recorded.

Model for Estimating Sex Ratios

A model was developed to predict sex ratios for individual nests using corral temperatures. Previous studies in turtles with TSD, indicate that sex determination is sensitive to temperature during the approximate middle third of development (Eich, 2009). In the current model, the middle third of incubation was calculated for each nest

based on lay date and hatch date. The hatch date was calculated as the emergence date minus 4 days, since hatchlings typically remain an average of approximately 4 days in the nest prior to emerging (Roberge, 2017). Average temperature during the middle third of incubation was calculated based on temperature data from the egg corrals or nesting beach, using the dates associated with the middle third for each nest.

The average temperature during the middle third was then utilized to predict the sex ratio in each nest based on the previously published reaction norm for the Kemp's ridley (LeBlanc et al., 2012). A conservative approach was adopted for predicting sex ratios using five categories. Based on the TSD reaction norm of the Kemp's ridley; 1) if the average temperature during the middle third was below 28°C, the nest was predicted 100% male, 2) if it was in the range of 28-30°C, the nest was predicted to be male-biased and assigned a value of 75% male, 3) if the average temperature was at 30°C (the pivotal temperature) a 50:50 ratio was predicted, 4) if it was between 30°C and 32°C, the nest was predicted to be female-biased and assigned a value of 75% female, 5) if it was above 32°C, it was predicted to be 100% female. Using that model, a hatchling sex ratio was predicted for nests that were translocated to egg corrals during the 2016 nesting season at Rancho Nuevo.

This sex ratio prediction model was incorporated into an Excel spreadsheet to automate the process in order to efficiently estimate the sex ratios from thousands of nests during the 2016 nesting season. This model automatically generated a variety of parameters that characterized the incubation and thermal environment of each nest. This included: the incubation duration (in days); length of the middle third of incubation (in

days); the start and end dates of the middle third; average temperature during the middle third; minimum and maximum temperatures during the middle third; and standard deviation of the average temperature during the middle third.

Results

Temperature Data

All data loggers were retrieved from the egg corrals and nesting beach at Rancho Nuevo during early September of 2016. The data loggers were downloaded using the HOBO Computer Software (HOBOWare), and all data was exported to Microsoft Excel for archiving and analysis. Daily averages were calculated for each data logger and those temperatures were utilized in the sex ratio prediction model.

Sex Ratio Prediction Model

During the 2016 nesting season, 11,386 nests were collected by the field team and translocated to egg corrals at Rancho Nuevo, Mexico. The 11,386 nests were run through the sex ratio prediction model. The results indicated that 5,409 nests were predicted to have a 100% female bias, 4,662 were predicted to be female-biased (assigned a value of 75% female), 1,310 were predicted to have a male-bias (assigned a value of 75% male-bias), and 5 nests were predicted to have a 100% male bias. The overall predicted sex ratio for these 11,386 nests that were translocated to egg corrals was estimated to be 88.45% female.

The sex ratio for the 11,386 nests was also predicted based on the beach temperature data in order to estimate a hypothetical sex ratio from the natural nesting

beach. The results from that analysis indicated that 0 nests were predicted to have a 100% female bias, 5,742 were predicted to be female-biased (assigned a value of 75% female), 4,351 were predicted to have a male bias (assigned a value of 75% male bias), and 1,293 nests were predicted to have a 100% male bias. The overall predicted sex ratio for these 11,386 nests that were translocated to egg corrals was estimated to be 50.43% female.

Discussion

The findings from this study provide a large-scale prediction of a yearly sex ratio produced from the primary nesting beach for the Kemp's ridley sea turtle. Overall, a sex ratio of 88.5% female was predicted for the 11,386 nests placed in the egg corrals. This represents the majority of nests produced by this species during the 2016 nesting season (Pat Burchfield pers. comm.). Previous studies, suggest that a female-biased hatchling sex ratio may be a long-term component of the Kemp's ridley recovery program at Rancho Nuevo (Wibbels 2007; Eich, 2009). This program has consistently translocated eggs to protected egg corrals during every year of the program. Further, the primary egg corral (Corral Central), where the majority of nests are translocated, has been located in the same location since the program started in 1978. The location of Corral Central is a relatively elevated portion of the beach that is generally protected from flooding during high tides. This location can result in higher nest incubation temperatures in comparison to the nesting beach (Eich, 2009; this thesis Chapter 2). The results of the current study also indicate that if those 11,386 nests were left on the nesting beach, it would have significantly decreased the female bias that was predicted for the egg corrals (Fisher

Exact Test; $p < 0.001$, see discussion below of hypothetical sex ratio prediction for the nesting beach). Thus, the Kemp's ridley program represents an example of a large-scale long-term manipulation of a hatchling sex ratio in a critically endangered species. It is not clear if a female-biased hatchling sex ratio is maintained through the various age classes of the population. However, there is some evidence suggesting a female bias in various age classes of the Kemp's ridley population (Wibbels, 2007). The impact of a female-biased sex ratio predicted in the current study is speculative, but a variety of models suggest the production of a female bias could be enhancing the recovery of this species through increased production of eggs in future years (Matsuzawa et al., 2002; Glen et al., 2003; Coyne and Landry, Jr., 2007; Valverde et al., 2010; Segura and Cajade, 2010; Santidrián Tomillo et al., 2012; Fisher, Godfrey, and Owens, 2014). However, it is plausible that if the female bias becomes too extreme, it could have a negative impact on the reproductive output of the population if males become a limiting factor.

The female-biased hatchling sex ratio predicted for the egg corrals could also reflect potential changes in environmental temperature, including the possibility of global climate change. There is some recent evidence that temperatures may be increasing at the primary nesting beach at Rancho Nuevo (this thesis Chapter 2). The results of the current study indicate that temperatures in the egg corrals at Rancho Nuevo are relatively high and are often at or above the upper range of the transitional range of temperatures (TRT) for the Kemp's ridley. Thus, the potential changes predicted by the IPCC (of 1-4°C) over the next century would not only result in extreme female biases, but would also result in higher embryonic mortality and decreased hatchling fitness.

The results of the current study also predict a theoretical sex ratio for the natural nesting beach of 50.4% female. Previous studies have suggested that population sex ratios in reptiles with TSD can produce a variety of population sex ratios and may not always conform to a 1:1 sex ratio (Shine, 1999) that is often predicted by evolutionary theory (Fisher, 1930). In the case of sea turtle populations, a variety of sex ratios including many female-biased sex ratios have been previously reported (Wibbels, 2007; Fuentes et al., 2011; Jensen et al., 2018;). Furthermore, recent studies suggest that sex ratios in some sea turtle populations may be becoming female-biased due to the impact global climate change. The hypothetical hatchling sex ratios predicted for the natural nesting in the current study suggest that the seasonal temperatures at the Rancho Nuevo nesting beach during the 2016 nesting season were not conducive to the production of strong female bias; rather, the natural temperature regime would have produced a near 1:1 sex ratio. This could potentially be a prime example of the production of a balanced sex ratio in a sea turtle population. Further, it is of interest that although a wide variety of potential nesting areas are available throughout the Gulf of Mexico, the Kemp's ridley utilizes a single primary nesting beach that is conducive to the production of a balanced sex ratio. It would be of interest to evaluate if the long-term temperature trends on the Rancho Nuevo nesting beach would consistently be predicted to produce a balanced sex ratio.

It is noteworthy that the current study utilized average temperature during the middle third of incubation as a predictor of sex determination. A variety of studies in reptiles with TSD suggest that the thermo-sensitive period of sex determination

approximates the middle third of incubation (Yntema, 1979; Bull & Vogt, 1981; Pieau & Dorizzi, 1981; Yntema & Mrosovsky, 1982; Ferguson & Joanen, 1983; Bull, 1987; Webb et al., 1987; Wibbels et al., 1991; Valenzuela, 2001; Godfrey & Mrosovsky, 2006; Eich, 2009; Fisher et al., 2014). Further, several studies have addressed the potential impact of variable temperatures on TSD (Valenzuela, 2001; Georges et al., 1994; 2004; 2005; Delmas et al., 2008; Bowden et al., 2014). Additionally, some studies have attempted to refine the middle third of development for sex determination studies relative to the constant impact of daily temperature fluctuations on embryonic development (Girondot, 1999; Georges et al., 2005; Girondot & Kaska; 2014), as well as develop optimal methodologies for reaction norms (Abreu-Grobois et al., 2020). It would be of interest in future studies to conduct an evaluation, based on the egg corral temperatures, that utilizes and compares the various results predicted from the multiple models. The egg corral temperatures observed in the current study were relatively high with 47.5% of the nests having average temperatures during the middle third of incubation that were above the transition range of temperature (i.e., 100% female range) and 40.9% that were between the pivotal and the upper range of the TRT. Considering these relatively warm temperatures, is possible that the data from the egg corrals at Rancho Nuevo, could generate similar results using the various methodologies.

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FINAL DISCUSSION

Many life history aspects of the Kemp's ridley sea turtle are impacted by environmental temperature. Of particular importance the pan-specific migration to the western Gulf of Mexico, including a single primary nesting beach, limits the thermal environments of the nests in this species. The incubation temperature of the nest affects embryonic development including sex determination, hatchling fitness, and embryonic mortality. Current and future temperatures at Rancho Nuevo, including those associated with global climate change, could impact all of these aspects of embryonic development. Therefore, it is essential to monitor the impact of environmental temperatures on Kemp's ridleys nesting beaches. The current thesis addresses this topic on multiple levels including the documentation of the thermal environment on the primary nesting beach as well as evaluating the impact of temperatures on sex determination.

Chapter 1 of this thesis, evaluated long-term hatchling sex ratio production in the Kemp's Ridley Recovery Program at Padre Island National Seashore, Texas (PAIS). This conservation program protects and incubates nests of the critically endangered Kemp's ridley sea turtle by utilizing egg hatcheries. The incubation temperatures utilized in this project affect the hatchling sex ratios. Histological analysis was utilized to evaluate the sex of hatchlings that were found dead in the nest over an 18-year period (2002-2019). The results revealed a female-biased sex ratio in each of the years analyzed. This female bias represents a large-scale manipulation of hatchling sex ratios in an endangered

species with TSD. Some ecological studies suggest that a female-biased sex ratio could be enhancing the recovery of the Kemp's ridley by increasing the reproductive output of this species.

Chapter 2 of this thesis documented the current thermal environment at the Kemp's ridleys primary nesting beach, during the 2021 nesting season. This included the evaluation of beach temperatures and egg hatchery (egg corral) temperatures. The results from the egg corrals revealed a seasonal trend in temperature dynamics which could potentially correspond to the production of males early in the nesting season that transitions to the production of females during mid to late nesting season. The results also suggested that major rain events during the season could potentially cool the nests and temporarily enhance the production of males. The data also indicated that beach temperatures exhibited the same trends as those in egg corrals, but they were significantly cooler. Beach temperatures were also compared to those from minor nesting beaches to the north and south of Rancho Nuevo. In general, all beaches showed similar seasonal trends, but there were differences in the magnitudes of temperatures between beaches.

In addition, chapter 2 included the comparison of monthly temperature data from 2021 to previous years (2013-2020). In general, there was a standard seasonal trend in which average temperatures were lowest in April and gradually increased throughout the remainder of the nesting season. Average temperatures were consistently below the pivotal temperature in April. During May, the temperatures increased, but were still below the pivotal temperature. By June, the average yearly temperatures rose above the pivotal temperature, and during July and August the average yearly temperatures

remained above the pivotal temperature. A slight positive relationship was detected between year and average daily temperature during the nesting season (2013-2021).

In chapter 3, a model was developed to predict sex ratios based on the average daily temperature during the middle third of the incubation period. This model was then used to gain insight on the potential sex ratio produced over an entire nesting season at Rancho Nuevo. Lay date and hatch date data from the 2016 nesting season was used in the model along with data from the previously published sex determination reaction norm for the Kemp's ridley. Hatchling sex ratios were predicted for 11,386 nests that were placed in the egg corrals during the 2016 nesting season. The results suggest a significant female bias was produced in the egg corral. The model was also used with temperature data from the nesting beach, to generate a hypothetical sex ratio for the natural nesting beach based on the 11,386 nests. The results predicted a near 1:1 sex ratio would be produced on the beach. The findings from this chapter, suggest that the translocation of nests from the beach to egg corrals is significantly enhancing the production of females. This suggests that the use of the egg corrals in the Kemp's ridley recovery program at Rancho Nuevo may represent a large-scale manipulation of hatchling sex ratios in this species.

Collectively, the results from this thesis document that the Kemp's ridley recovery program can produce a variety of sex ratios including some that are relatively extreme. Current data suggests the production of significant female biases in the Kemp's ridley program at Rancho Nuevo. The results indicate that temperatures are at or above pivotal for a majority of the nesting season. Further, the projected increases associated with global climate change could result in extreme sex ratio biases, decreased hatchling

fitness, as well as embryonic mortality at the Kemp's ridley's primary nesting beach.

These findings exemplify the need to monitor and evaluate environmental temperatures

and their impact on the Kemp's ridley nesting beaches.

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