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## paper: biology

### Evaluating Sex Ratios of Hawksbill Hatchlings on St. Croix, U.S. Virgin Islands

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#### **Abstract**

Like all sea turtles, the hawksbill (Eretmochelys imbricata) has temperature-dependent sex determination (TSD) in which the temperature during the middle third of egg incubation period determines the sex of the hatchlings. TSD can produce a wide range of sex ratios, and those sex ratios can impact the ecology and conservation of sea turtles. Buck Island (U.S. Virgin Islands) is a natural nesting beach for hawksbill sea turtles and thus represents an ecological model for examining natural sex ratios produced from TSD. Further, Buck Island is a major nesting site for the hawksbill, so the sex ratio is of conservational importance since it affects the recovery of this endangered species. The purpose of this study was 1) to evaluate nest temperatures in order to estimate hatchling sex ratios and 2) to verify sex ratios via the histology of hatchings found dead in the hawksbill nests on Buck Island during the 2007 nesting season. The average temperatures of the middle third of incubation were used to predict the sex ratios produced by each nest. Analysis of the nest temperature data suggest an overall strong female-bias with 25 female-biased nests, one male-biased nest and one nest with a pivotal temperature yielding a 1:1 sex ratio. Histological analysis also suggested a female-bias with approximately 92% of hatchlings identified as female and approximately 8% identified as male.

#### Introduction

Many reptiles and all sea turtles display temperaturedependent sex determination (TSD) (reviewed by Wibbels, 2003). Sea turtles have a MF (male/female) pattern meaning warmer incubation temperatures produce females while cooler incubation temperatures produce males. (Mrosovsky, 1994). Previous studies suggest that TSD can produce a wide variety of sex ratios and those sex ratios could significantly impact the reproductive ecology and conservation of endangered sea turtle populations. Therefore it is important to evaluate and monitor hatchling sex ratios produced from nesting beaches (Mrosovsky, 1994; Wibbels, 2003). The current study investigates hatchling sex ratios of the hawksbill sea turtle. Hawksbills typically inhabit coral reef environments in tropical regions of the Atlantic and Pacific oceans (Meylan and Donnely, 1999). Hawksbill turtles are an endangered species in large part due to their carapace, which is a popular world trade item because of its thick and ornate shell scutes (Carr, 1952; Meylan and Donnely, 1999). A previous study of TSD in hawksbills in Antigua revealed that temperatures of approximately 28.5 °C and below will produce 100% males and above approx. 30.3°C yields 100% females and the estimated pivotal temperature of the hawksbill is approx. 29.2°C (Mrosovsky et al., 1992).

The current study evaluates hatchling sex ratio being produced by hawksbill sea turtles on Buck Island, which is located several km off of St. Croix in the U.S. Virgin Islands. Buck Island is a major nesting beach for hawksbills in the Caribbean and consists of an undeveloped island with several natural nesting beaches. Thus, this study investigates naturally occurring sex ratios on an undeveloped and uninhabited island. Hawksbills are well known for nesting in various types of habitat. There are several distinct nesting beaches on Buck Island (Figure 1). The current study evaluates nest

temperatures and hatchling sex ratios from each of the four beaches and in three different beach zones (open beach, seaward vegetation, and beach forest) within each beach.

#### **Methods and Materials**

This study was conducted during the 2007 hawksbill nesting season on Buck Island, U.S. Virgin Islands. As indicated above, Buck Island is a major nesting beach for hawksbill sea turtle in the Caribbean, and is an uninhabited and undeveloped island. Incubation temperature in hawksbill nests were recorded using HOBO data loggers from Onset Computer Corporation (Poccassett, MA). The data loggers have a precision of approximately +/- 0.3° Celsius and are calibrated in a laboratory incubator to ensure their accuracy. Data loggers were programmed using Hoboware Pro software to record temperature at 1 hour intervals for the entire duration of incubation. The data loggers were placed in the center of the egg mass in each nest by National Park Service (NPS) while the female turtle was nesting. Some nests were relocated by NPS to other areas on the nesting beach for this study. These nests were in danger of being flooded because of their original location on the nesting beach. Nest temperatures were examined from nests on four different beaches on Buck Island: South Shore (SS), Turtle Bay (TB), West Beach (WB), and North Shore (NS). Nests were monitored by NPS and once the hatchlings emerged, the data loggers were collected and sent back to UAB for data analysis. For these data, incubation durations were estimated and the middle third of incubation was identified because this is when temperature sensitive period (TSP) of TSD occurs. The maximum, minimum and average middle third temperatures were calculated. The average temperature during the middle third was used to determine the sex ratio of the hatchlings. The temperatures were used to predict sex ratios based on a previous study done on incubation

temperatures of the hawksbill in the Caribbean which showed that temperatures below approximately 28.5°C would produce all male, above approx. 30.3°C produced all females and a pivotal temperature of approx. 29.2°C (Mrosovsky et al., 1992). Accordingly, nests with average middle third temperatures of 28.5 °C or higher were expected to yield 100% male hatchlings, 30.3°C or higher temperatures were expected to yield 100% female and nests with average middle third temperatures of 29.2°C were expected to yield a 1:1 sex ratio. Average middle third temperatures between pivotal temperature, 29.2°C, and 28.5°C were expected to generate male-biased nests, and average temperatures between pivotal and 30.3°C were expected to generate female-biased nests. Nests were located at different beaches on Buck Island and the type of beach zone was noted for each nest. Location was recorded to see if it had any effect on incubation temperatures.

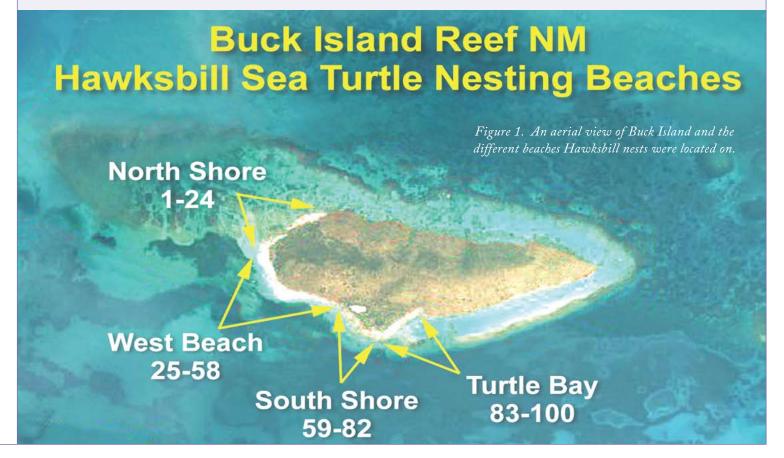
In addition to temperature analysis, sex ratio information was obtained through the histological evaluation of hatchings that were found dead in the nest after all live hatchlings had emerged. In the field, dead hatchlings were collected, partially dissected, and then preserved in buffered formalin to protect the tissue and prevent decay. In the laboratory, the kidney/gonad complex was dissected from each hatchling and then processed with standard paraffin histological techniques (Humason, 1972). The structure of the gonad was examined under a compound microscope to evaluate the development of the cortical and medullary regions in order to verify if the

tissue was an ovary or testis (Yntema and Mrosovsky, 1980; Wibbels, 2003).

#### Results

Temperatures were examined in a total of 27 nests. Average temperatures during the middle third of incubation, along with the predicted sex ratio for each nest are shown in Table 1 for all nests examined during the 2007 nesting season. Examples of nest temperature throughout the incubation period are shown for each of the four beaches, north shore, west beach, south shore, and turtle bay, in Figures 2-6 respectively.

A total of 399 hawksbill hatchlings were examined via histology in this study from the 2007 nesting season on Buck Island. Table 2 shows a summary of the results. Of the hatchlings examined approximately 49.6% were verified, but the tissues from approximately 50.4% of the hatchlings were too decomposed to determine their sex. Of the hatchlings whose sex was verified histologically, 92% were female and 8% were male (Table 2). The only beaches with nests that produced several male hatchlings were TT1 and TT4 (Table 1, 2 & Figure 2, 5). Table 1 indicates a 100% female bias for TT4 nests; however, histological analysis showed some males hatchlings were produced there despite the warmer incubation temperatures. The male hatchlings from nests on beach TT1/North Shore were also in beach forest zones indicating that these may be cooler locations than open beach and seaward vegetation zones (Table 1, Figure 2). Average incubation temperatures and the overall predicted sex ratio can be seen in Table 1.



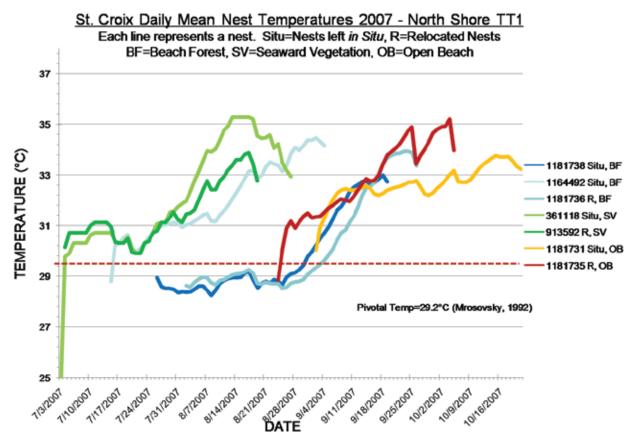
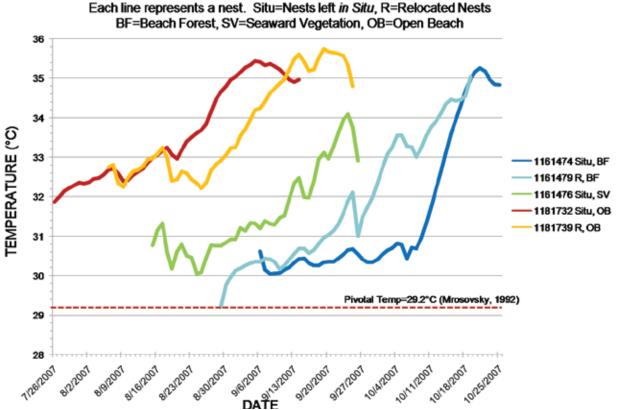


Figure 2. Nests located on the North Shore. This chart shows average incubation temperatures and predicted sex ratios.

The pivotal temperature is marked by the red, dashed line.

St. Croix Daily Mean Nest Temperatures 2007 - West Beach TT2



# St. Croix Daily Mean Nest Temperatures 2007 – West Beach TT3 Each line represents a nest. Situ=Nests left in Situ, R=Relocated Nests BF=Beach Forest, SV=Seaward Vegetation, OB=Open Beach

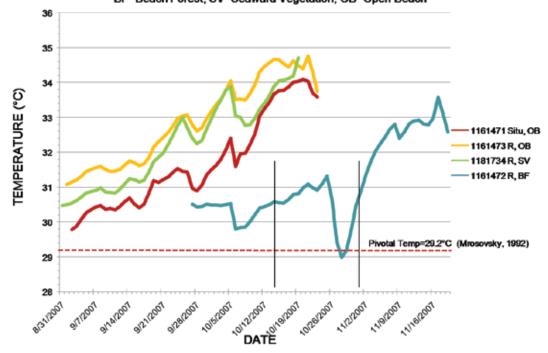
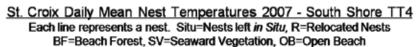


Figure 4. Nests located on West Beach. This chart shows average incubation temperatures and predicted sex ratios. The drop in temperatures between October 26–30 for 1161472 is most likely due to the early stages of hurricane Noel and significant rainfall.

This is an example of weather affecting middle third incubation temperatures.



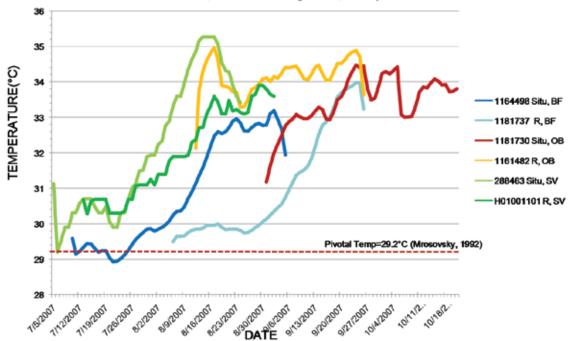


Figure 5. Nests located on South Shore. This chart shows average incubation temperatures and predicted sex ratios.

Nests located in open beach areas had higher overall incubation temperatures than other nest areas.

	Incubation Beach	Original Beach	Relocated		Incubation	TSP		Predicted
	Deach	Deach	Beach		incubation	Mid 1/3	Mean	Tredicted
TT#			Zone	SN#	Duration	Dates	Temp	Sex Ratios
								Male Bias
								(very close to
TT1	NS	NS	in situ, BF	1181738	56	8/14-9/1	29.04	piv temp)
TT1	NS	NS	in situ, BF	1164492	52	8/1-8/18	31.76	100% Female
TT1	NS	NS	in situ, BF	1164499	Est. 51	7/31-8/16	32.17	100% Female
	NO	\A/D	Dalas DE	1181736/	<b>5</b> 0	0/04 0/0	20.20	Unbiased (piv
TT1	NS	WB	Reloc., BF	1161736	56 50	8/21-9/8	29.20	temp)
TT1	NS	NS	in situ, SV	361118	56	7/22-8/9	31.94	100% Female
TT1	NS	NS	Reloc., SV	913592	47 50	7/20-8/4	30.87	100% Female
TT1	NS	NS	in situ, OB	1181731	50	9/19-10/5	32.58	100% Female
TT1	NS	NS	Reloc., OB	1181735	43	9/7-9/21	32.77	100% Female
TT2	WB	WB	in situ, BF	1161474	50 	9/23-10/9	30.60	100% Female
TT2	WB	NS	Reloc., BF	1161479	52	9/15-10/2	31.50	100% Female
TT2	WB	WB	in situ, SV	1161476	43	8/29-9/12	31.23	100% Female
TT2	WB	WB	in situ, OB	1181732	Est. 51	8/12-8/28	33.32	100% Female
TT2	WB	NS	Reloc., OB	1181739	51	8/23-9/8	33.29	100% Female
TT3	WB	WB	in situ, OB	1161471	52	9/19-10/6	31.45	100% Female
TT3	WB	NS	Reloc., OB	1161473	53	9/19-10/6	32.99	100% Female
TT3	WB	NS	Reloc., SV	1181734	50	9/17-10/3	32.41	100% Female
				44044=0		10/15-		4000/ = 4
TT3	WB	TB	Reloc., BF	1161472	54	11/1	30.51	100% Female
TT4	SS	SS	in situ, BF	1164498	58	7/29-8/17	30.64	100% Female
TT4	SS	ТВ	Polos PE	1161737/	52	8/23-9/9	30.35	100% Famala
	SS	SS	Reloc., BF	1181737		9/17-10/4		100% Female
TT4			in situ, OB	1181730	Est. 52		33.92	100% Female
TT4	SS	TB	Reloc., OB	1161482	46 54	8/27-9/11	34.15	100% Female
TT4	SS	SS	in situ, SV	288463	51	7/22-8/7	31.72	100% Female
TT4	SS	NS	Reloc., SV	H100101	Est. 52	7/30-8/16	31.97	100% Female
TT5	TB	TB	Reloc., BF	1164500	54	8/20-9/6	30.37	100% Female
TT5	TB	TB	in situ, SV	1181729	55	8/19-9/6	32.02	100% Female
TT5	TB	TB	Reloc., SV	889243	49	8/9-8/25	30.44	100% Female
TT5	TB	TB	Reloc., OB	1164496	Est. 53	8/3-8/20	33.74	100% Female
						Overall	24 74	
						Avg.	31.74	

Table 1. The highlighted data shows average mean temperatures during the middle third of incubation produced by the nests and the predicted sex ratio outcomes. The table indicates the beach zone and island location that the original nests (in situ) were located and where relocated nests were. TT1 represents North Shore, TT2 and TT3=West Beach, TT4=South Shore, and TT5=Turtle Bay.

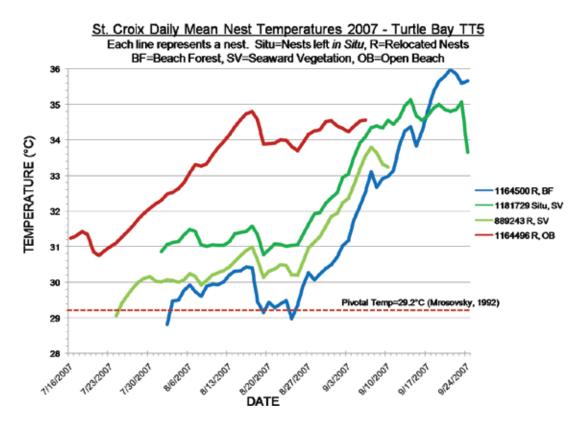


Figure 6. Nests located on Turtle Bay. This chart shows average incubation temperatures and predicted sex ratios. Nests located in open beach areas had higher overall incubation temperatures than the other nest areas.

TT#	Total Hatchlings	Female	Male	Undetermined
TT1	92	49	5	38
TT2	109	41	1	67
TT3	52	25	1	26
TT4	78	28	8	42
TT5	68	40	0	28
Total	399	183	15	201

Table 2. This table shows the total number of hatchlings for each beach and the number of female, male or undetermined hatchlings.

Undetermined indicates tissues that were too decomposed or degraded to identify. A strong female biased ratio can be seen.

#### **Discussion**

Most nests located on the North Shore/TT1 position were predicted to produce 100% female, although one nest was predicted to produce a male-biased and one nest was predicted to produce a 1:1 sex ratio. All of those nests were located in beach forest zone and three were in situ nests while the nest with a pivotal temperature had been relocated to BF from West Beach (Figure 2). All of the nests from the TT2, TT3, TT4 and TT5 locations were predicted to produce 100% females. Collectively, the data indicate that incubation temperatures are relatively warm and well above pivotal temperature in the majority of the nests examined. This clearly suggests an overall female bias (Table 1). Further, the location of the nests on the beach may affect incubation temperatures and thus sex ratios. For example, nests located in the more wooded, less open area on the North Shore may be cooler due to shading. The data indicate warmer overall temperatures for open beach areas, followed by slightly less warm seaward vegetation temperatures and beach forest areas having coolest overall temperatures for TT3, TT4, and TT5 beaches (Figures 4-6). No apparent trend could be seen for relocated nests. The results also provide examples of how weather events may play key roles in controlling nest incubation temperatures. For example, Hurricane Noel passed nearby Buck Island when it was a tropical depression in late October. Although the weather did not change the outcome of the sex ratio of hatchlings, incubation temperatures were cooler during that time period (Table 2, Figure 4). As seen in data logger 1161472 (Figure 4), a storm caused a decrease in nest temperature of approx. 3°C during the latter part of TSP, however this did not decrease below the estimated pivotal temperature of 29.2°C therefore that nest still had a female biased outcome.

In summary, these results reveal a female biased hatchling sex ratio on Buck Island for the 2007 nesting season. This is consistent with a previous study done on the island (Wibbels et al., 1999). The results also suggest that factors such as weather, location of a specific nesting beach, and location of a nest on a nesting beach may influence incubation temperatures (Table 1). The findings of this study are being forwarded to the U.S. National Park Service in St. Croix and will be used in the development of the management strategy for this endangered sea turtle.

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