

Loggerhead Sex Ratio Results 2002 and 2003
Summary of Northern and Southern Subpopulation Samples

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To date, we secured the necessary permits, collected hatchling loggerhead sea turtles from Northern and southern Subpopulations. After rearing them in the laboratory, sex was determined laparoscopically; sex identification criteria were verified with biopsy in 10% of the animals. Any mortalities were dissected and the gonads were sampled histologically. We verified the criteria used during laparoscopy and determined the sex of turtles from the 2002 and 2003 year classes. The sex ratios of those samples are provided separately here, then they are compared.

Additionally in 2002 and in 2003 we arranged for temperature loggers to be placed in nests. Not all loggers were retrieved when storm events caused loggers to be lost. In those cases, turtles from adjacent remaining nests on the same beach, that were deposited during the same time interval, supplied the sample of hatchlings. Abbreviations used throughout the report are:

Southern Subpopulation Sites

BR=FLBR: Boca Raton Florida
 HI =FLHI: Hutchinson Island, Florida
 JU = FLJU: Juno Beach, Florida
 MB = FLMB: Miami Beach, Florida
 ME = FLME: Melbourne Beach, Florida
 SA = FLSA: Sarasota Florida
 SN = FLSA: Sanibel/Captiva, Florida

Northern Subpopulation Sites

WI = GAWA: Wassaw Island, Georgia
 CI = SCCI: Cape Island, South Carolina
 KI = SCKI: Kiawah Island, South Carolina
 HH = SCHH: Hilton Head Island, South Carolina
 ON = NCON: Onslow Beach North Carolina
 CL = NCCL: Cape Lookout North Carolina

Sex Ratios: 2002

	average	SD	nests	# turtles
overall	0.71	0.3336	115	906
south	0.68	0.3573	70	554
north	0.75	0.2921	45	352
s. early	0.23	0.2451	18	101
s. mid	0.73	0.2743	29	239
s. late	0.97	0.0576	23	214
n. early	0.56	0.3240	10	59
n. mid	0.83	0.2397	20	148
n. late	0.77	0.2958	15	145
BR	0.76	0.2500	12	97
HI	0.69	0.4092	11	84
JU	0.76	0.3748	11	87
MB	0.65	0.3701	11	76
ME	0.63	0.3996	8	63
SA	0.71	0.3405	11	95
SN	0.45	0.4183	6	52
CI	0.89	0.1452	10	77
CL	0.54	0.4032	8	57
KI	0.79	0.2618	12	92
WI	0.75	0.2798	15	126

These means and standard deviations are based on nests that had at least 3 turtles per nest that were either dead, biopsied, scored by either discriminant analysis function, or were lapped after the cut-off date when accuracy was very high. These turtles will be referred to as verified.

Independent Samples t-test, assuming unequal variances ($F=5.25$, $p=0.024$) showed that there were not significant differences in the overall sex ratios between the north and south subpopulations. However, t-tests comparing the intra-season sampling periods between the two subpopulations did reveal differences. The south had significantly fewer females than the north for both the early ($p=0.047$) and mid intra-season samplings ($p=0.022$), but had significantly higher males than the north in the late season ($p=0.014$). These indicate that the differences balance out over beaches representing each subpopulation.

Univariate ANOVA found that there were significant differences between beaches ($p=0.007$) and between intra-season sampling (early mid late, $p<0.001$). Using a Tukey HSD post hoc test, the only significantly different beach comparison was Cape Island, SC (sex ratio=0.8921) to Sanibel, FL (sex ratio=0.4500, $p=.014$). The variance was so large at each beach, that other differences were not apparent.

Within season sampling. There were significantly fewer females in the early season samples (sex ratio=33.45% female) than in both the mid (sex ratio=78.94%, $p<0.001$) and late season sampling (sex ratio=88.59%, $p<0.001$).

Sex Ratios: 2003

	average	SD	# of nests	# of turtles	
Overall	0.6923	0.3368	49	477	exclude MB and BR12
North	0.4650	0.3463	22	214	
South	0.8776	0.1821	27	263	exclude MB and BR12
N. Early	0.5452	0.3435	9	87	
N. Mid	0.2352	0.2865	6	58	
N. Late	0.5587	0.3481	7	69	
S. Early	0.8476	0.2714	7	75	exclude MB and BR12
S. Mid	0.9341	0.1038	11	104	exclude MB and BR12
S. Late	0.8318	0.1775	10	84	exclude MB and BR12
ON	0.3167	0.3667	4	39	only E nests, 1 L
HH	0.3278	0.3675	8	77	
WI	0.6340	0.2677	10	98	
ME	0.8469	0.1929	9	87	
BR	0.9750	0.0500	10	86	exclude BR12
SN	0.8108	0.2265	9	90	
MB	0.0650	0.0929	5 (7)	26	

Nests with 2 or fewer turtles were excluded to avoid skewing averages. One Boca Raton nest and three Miami Beach nests were excluded (6 turtles: 1 Male and 5 Females).

Independent Samples t-test (SPSS v. 11) assuming equal variances ($F=0.55$, $p=0.46$) showed that there were significantly fewer females overall in the north subpopulation than in the south. However, t-tests comparing the intra-season sampling periods between the subpopulations showed the difference was not represented throughout the entire season. Only in the mid season sampling were there significantly fewer females from the north than the south ($p=0.001$). In the

early and late season samples there was no significant difference in the sex ratios between subpopulations. There was such a large difference in the mid season proportions of females (~ 0.7 difference) that it greatly affected the overall results. However, the difference may be due, in part, to the unequal sample sizes for the mid season (almost twice the number of nests sampled in the south than in the north). If the sample sizes were equal, it appears likely that there would be greater variance in the northern mid season sample.

Univariate ANOVA identified that there were significant differences among beaches ($p < 0.001$), but there were not significant differences among intra-season sampling ($p = 0.733$). Using a Tukey HSD post hoc test, there were many differences between beaches:

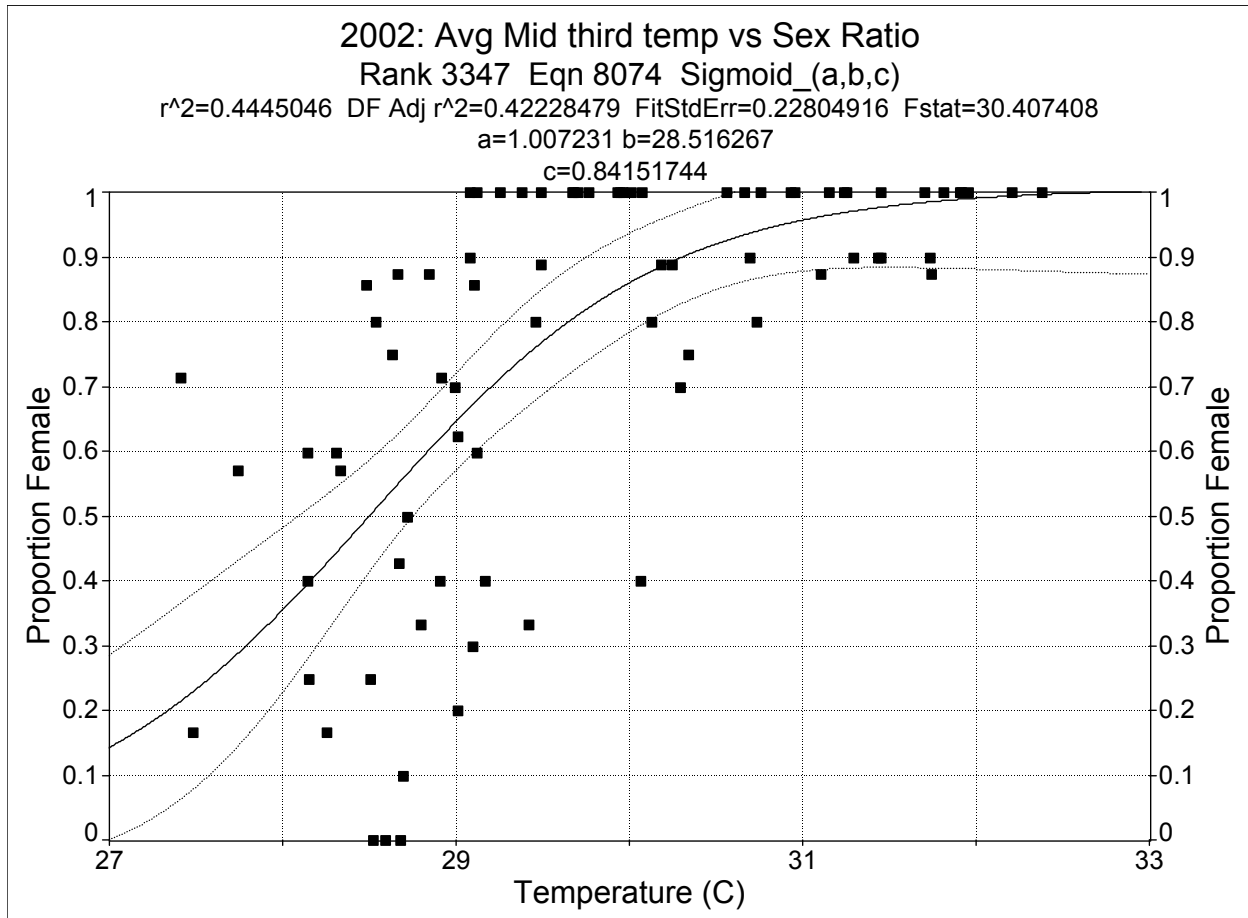
Beach 1	Beach 2	p	Beach 1 has:
Onslow Beach	Melbourne	0.004	fewer females
	Boca Raton	<0.001	fewer females
	Sanibel	0.009	fewer females
Hilton Head	Melbourne	<0.001	fewer females
	Boca Raton	<0.001	fewer females
	Sanibel	0.001	fewer females
Wassaw Island	Boca Raton	0.024	fewer females
	Miami Beach	0.001	more females
Melbourne	Miami Beach	<0.001	more females
Boca Raton	Miami Beach	<0.001	more females
Miami Beach	Sanibel	<0.001	fewer females

These results indicate that overall, in 2003 the north produced more males than the south, with the exception of Miami Beach. These results differ dramatically from those obtained in 2002.

Temperatures & Sex Ratios: 2002

Temperatures were retrieved from 103 nests. To determine how proportion females relate to changes 1°C changes in nest temperature, a sigmoidal regression was used to fit a curve of mean temperature of middle third vs. sex ratio of the 2002 data (Systat® TableCurve-2D v.5.01, 2002). The analysis was conducted on 79 of the 103 nests (all 79 nests had at least 5 verified turtles included in the sex ratio calculations to ensure sex ratios were accurate; 29 northern & 50 southern nests). The fit of the curve is not great ($r^2 = 0.445$) but is highly significant ($p < 0.001$). Based on the curve and 95% CI, the upper bound of the transitional range of temperatures (TRT) is ~32°C; lower bound of $\leq 27^\circ\text{C}$. The maximum effect temperature has on sex ratio is 30% change in percent female between 28°C and 29°C.

Fig.1. Scatter plot of the mean temperature during the middle third of incubation versus the sex ratio. All points represent at least 5 turtles. Sigmoidal regression curve is fit to the points with the 95% confidence intervals.



The points in figure 1 show a large amount of scatter. Just about 29°C, there is a range of sex ratios from 20% - 100% female. This is one of the first indications that temperature alone is not the controlling mechanism for determining sex.

Looking at Figure 1, it appears as though the range of temperatures that produce 100% females and those producing 100% males almost overlap. To better analyze this, the nests at each beach were divided into 3 categories: 100% female nests, 100% male nests, and both sexes present (mixed nests). Single-sex nests had at least 5 turtles in the nest that had been verified, while any nest that had at least 1 female and 1 male were categorized as a mixed nest (n=88 nests: 100% male nests n=3; mixed nests n=51; 100% female nests n=34). The daily average temperatures of the middle third were plotted and compared (Fig. 2). At the northern subpopulation beaches, both sexes were produced from nests with a middle-third daily average nest temperature ranging from 26.5°C – 33.0°C. Those beaches from the southern population had nests that produced both sexes with middle-third daily averages from 26.5°C - 33.5°C (Fig. 2). Overlap in the temperature ranges, from slight to complete, occurred between the single sex nests and those producing both sexes (Fig. 2). The clearest separation between the single-sex temperature range and the two-sex temperature range was at Boca Raton (FLBR), while total overlap occurred at Hutchinson Island (FLHI; Fig. 2), where the recorded range of nest temperatures producing 100% females was greater than the range producing mixed sex ratios.

Fig. 2. Box-plot of the range of daily average temperatures experienced in the nests of the study beaches during the middle third of incubation (n= # of nests). Beaches are arranged from north to south from Cape Lookout, NC (NCCL) to Miami Beach, FL (FLMB) and then north up the west coast of Florida from Sanibel (FLSN) and Sarasota (FLSA).

Range of Mid-third Daily Average Temperatures in 2002

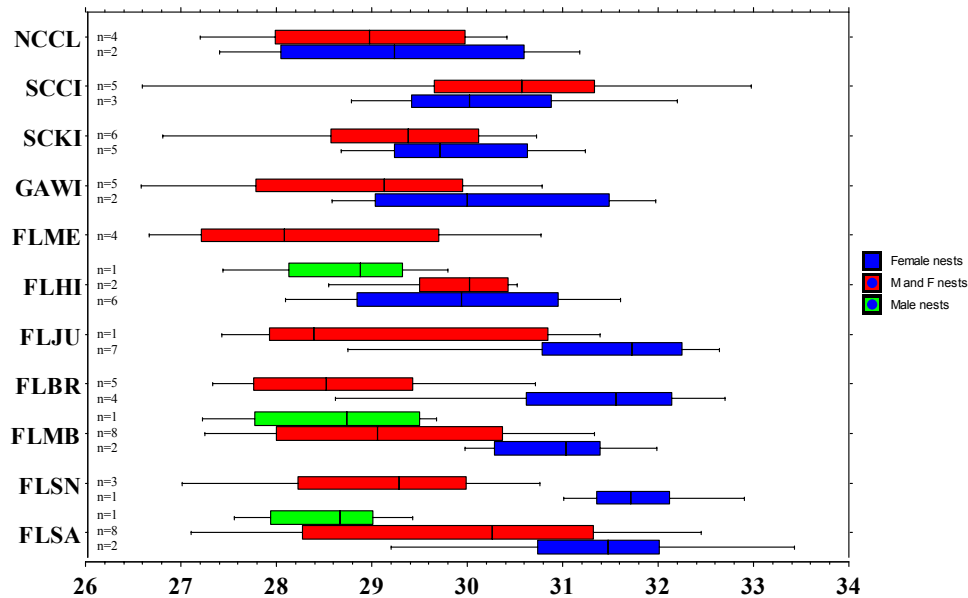
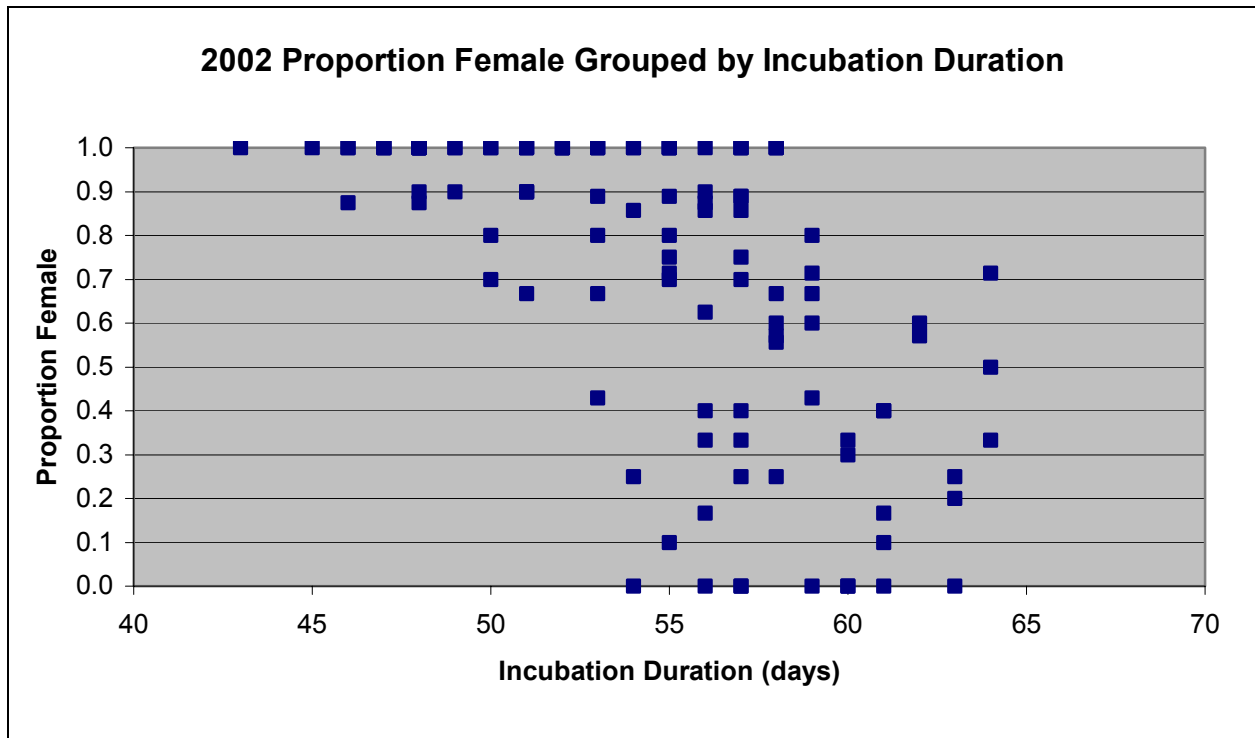


Figure 2, points to temperature alone as not the only controlling mechanism for determining sex.

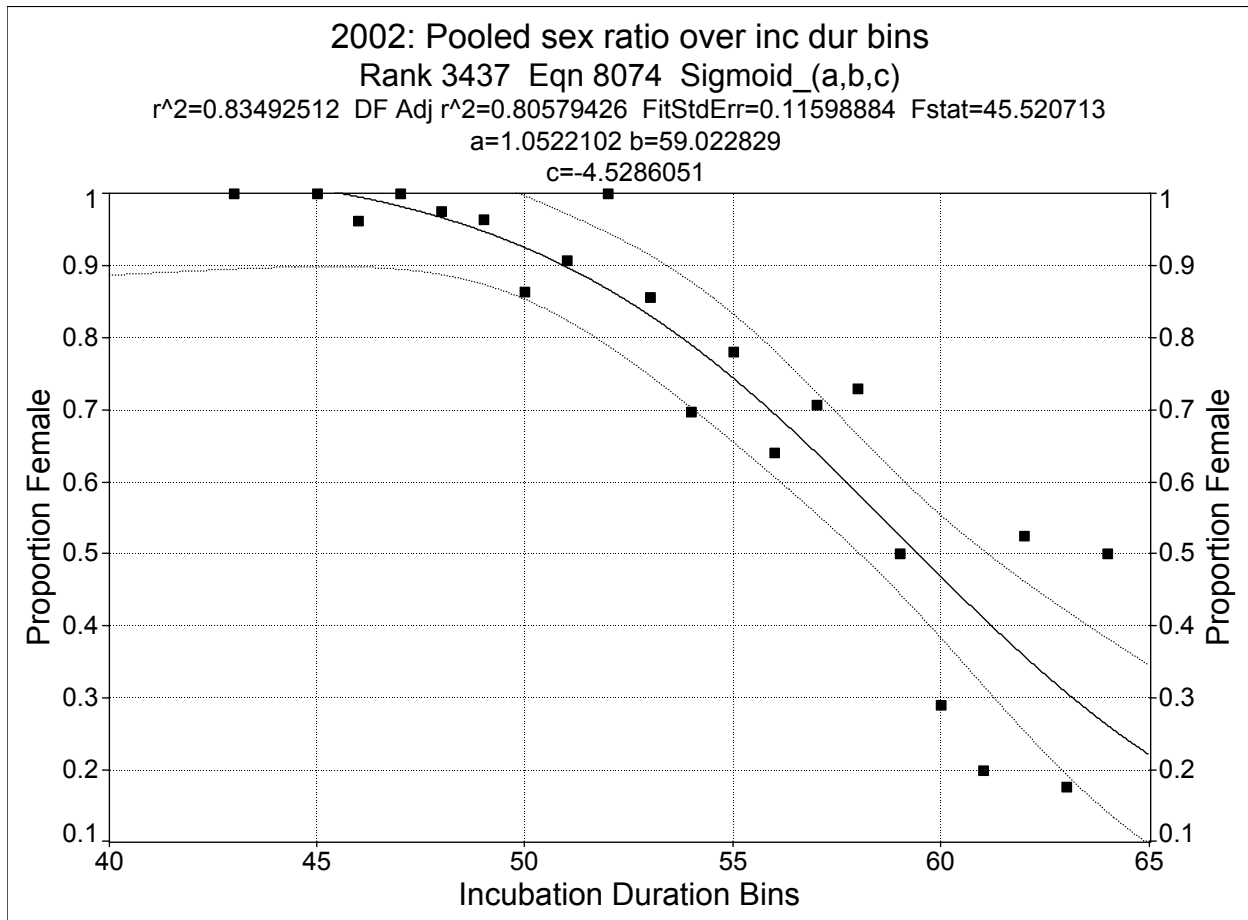
It is not just the temperature of the nests that display a lot of scatter; the incubation duration does as well. Figure 3 shows the incubation duration for nests plotted against the proportion female for each of those nests. Again, each nest had at least 5 turtles that had been verified to ensure the accuracy of the sex ratio. There is wide scatter over the different lengths of incubation that will produce a particular sex ratio, as well as a wide scatter over the sex ratios that are produced by a given incubation length. For example, nests producing 100% females ranged in incubation length of 43 – 58 days. There were 9 nests that incubated for 57 days, and produced sex ratios ranging from 0 – 100% female. Incubation duration is directly correlated to the temperature in the nest; at some locations incubation duration is an indirect way of determining the temperature. So again, but that cannot be directly related to hatchling sex. Figure 3 supports the hypothesis that temperature alone is not the controlling mechanism for determining sex.

Fig. 3. Scatterplot showing the spread of incubation lengths of nests in 2002, plotted against the proportion female produced by each nest.



However, if the incubation lengths are pooled into single day bins, and the pooled proportion female is plotted against the bins, a clearer picture results (Fig. 4). Fitting a sigmoidal curve to the graph, the fit is $r^2=0.835$ and is highly significant ($p<0.001$). Above 60 days of incubation, the scatter is large, with sex ratios ranging from <20% female, which is expected, to >50% female, which is not expected. This result was identified after pooling across nests so that we minimized the variation between nests to accurately predict the sex ratio based on the incubation length.

Fig. 4. Scatterplot of pooled sex ratios into single day incubation length bins. A sigmoidal curve and 95% C.I. are also shown.

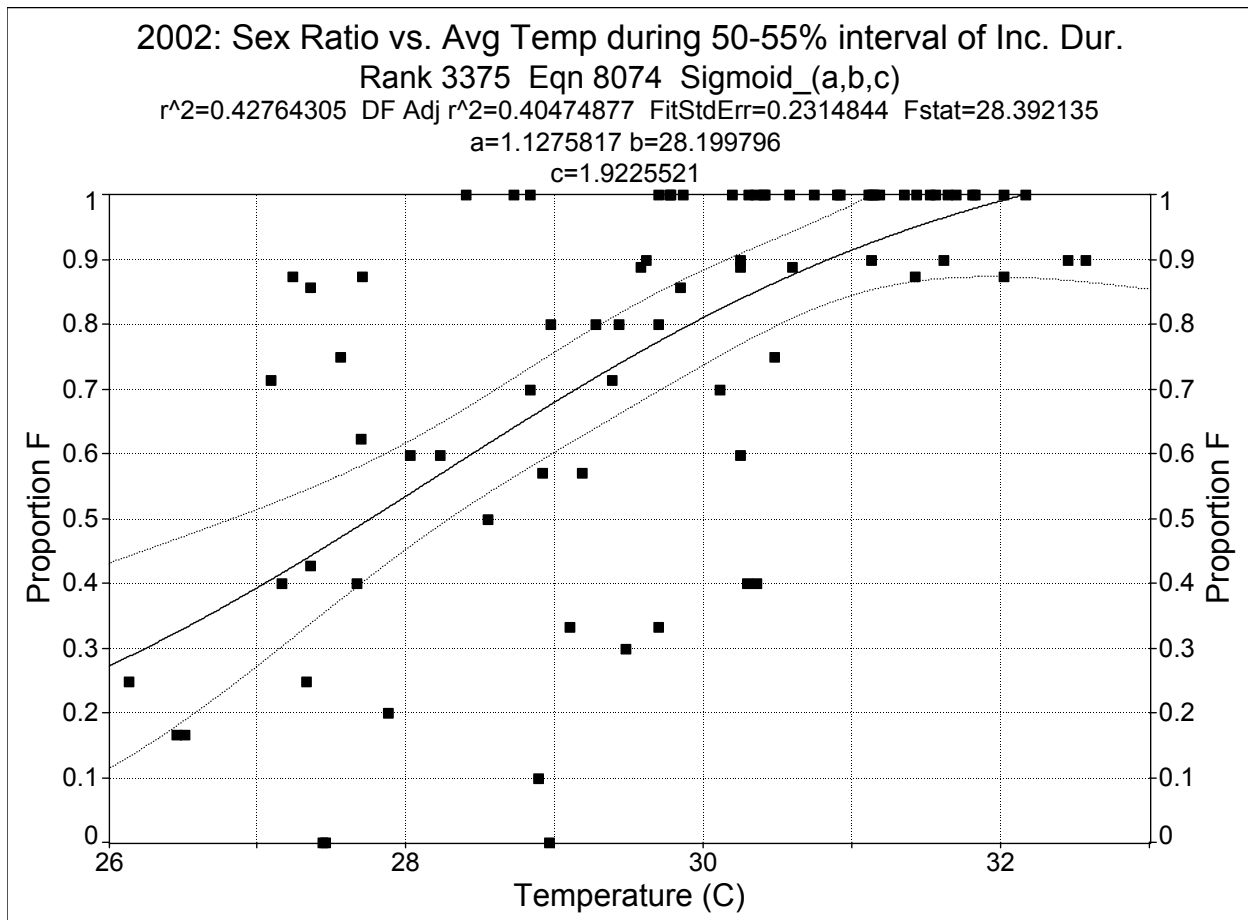


To determine if the average temperature over the entire middle third is the best predictor of the sex ratio, the incubation length was divided into 5% intervals, and the average temperature in each interval was plotted against the sex ratios produced (Fig. 5). Here we used the 79 nests with a least 5 verified turtles were used for this analysis. A sigmoidal curve was fit to each plot to determine the most accurate time frame for which temperature can best predict the sex ratio. Figure 5, shows the best-fit plot for the 2002 data, which was the average temperature during the 50-55% interval of the incubation length. The fit was not tight ($r^2 = 0.428$) but was highly significant ($p < 0.001$), and is not quite as good as the fit of the curve using the average temperature of the entire middle third ($r^2 = 0.445$). However, we not a few important observations in explanation.

1. The timing of the best-fit is late in the middle third. Previous studies had concluded that sex determination happened in the first half of the middle third. But those studies were based on constant temperature, lab-incubated nests. The current study involves natural, *in situ* nests, and indicates that maybe determination is later or that average temperature lags behind the realized temperature affecting the embryos. (This may be consistent with some of the results found by Georges et al. in which the extent of the fluctuations about a mean temperature produce very different apparent effects on the eggs than the mean alone would indicate).
2. The scatter is very large and wide, which indicates again that temperature alone is not the controlling mechanism for determining sex.

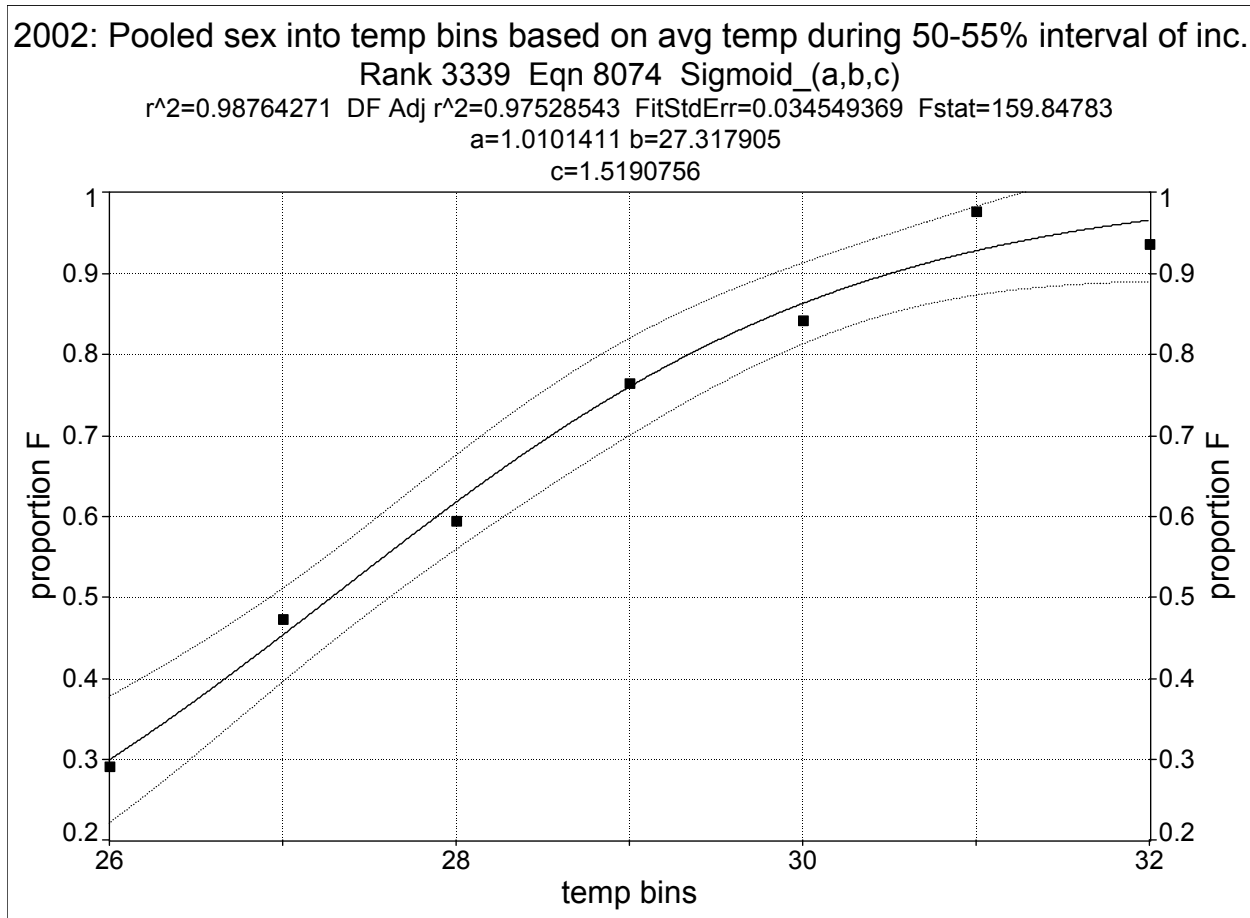
- Anchoring the curve at an origin of 24-26°, temperatures at which we did not get viable hatchlings, may force a better fit.

Fig. 5 Scatterplot of average temperature during the 50-55% interval of incubation length plotted against the proportion of females produced by the nest. Sigmoidal regression curve is fit to the points, along with the 95% C.I.



As with the incubation length, a much clear pattern can be seen when the sex ratios are pooled over single degree temperature bins. Working with the 50-55% interval, as it was the best predictor, the sex ratios were pooled across single degree temperature bins (Fig. 6). A sigmoidal regression curve was fit to the graph. The fit of the curve was excellent ($r^2=0.988$) and was highly significant ($p<0.001$). However, like the incubation durations, this fit was achieved only after pooling across nests (reducing sample variance). Thus the much variance among individuals and nests is too great to accurately predict the sex ratio based on the incubation temperature.

Fig. 6. Scatterplot of the average temperature during the 50-55% interval of incubation length with the proportion female pooled across nests into single degree temperature bins. Sigmoidal regression curve and 95% C.I. have been fit to the points.



Temperatures & Sex Ratios: 2003

Temperatures were retrieved from 29 nests. To determine how much change in proportion females can be attributed to 1°C interval changes in nest temperature, a sigmoidal regression was used to fit a curve of mean temperature taken from middle third of incubation vs. sex ratio of the 2003 data (Systat® TableCurve-2D v.5.01, 2002). The analysis was conducted on 28 of 29 nests (all 28 nests [16 northern & 12 southern] had at least 5 verified turtles included in the sex ratio calculations to ensure sex ratios were fairly representative). The fit of the curve is not ideal ($r^2=0.461$) but is highly significant ($p<0.001$). Based on the curve and 95% CI, the upper bound of the transitional range of temperatures (TRT) is ~32.5°C; lower bound of $\leq 28^\circ\text{C}$. The maximum effect temperature has on sex ratio is 25% change in percent female between 29.5°C and 30.5°C.

As in 2002, there was a large amount of scatter in the data. At about 30°C, the range of sex ratios spans from 0% - 100% female. This further strengthens the conclusion indicated by the 2002 results that temperature alone is not the controlling mechanism for determining sex.

It appears as though the range of temperatures that produce 100% females and those producing 100% males often overlap. To better analyze this, the nests at each beach were divided into 3

categories: 100% female nests, 100% male nests, and both sexes present (mixed nests). Single-sex nests had at least 5 turtles in the nest that had been verified, while any nest that had at least 1 female and 1 male were categorized as a mixed nest (n = 24 nests: 100% male nests n = 4; mixed nests n = 14; 100% female nests n = 6). The daily average temperatures of the middle third were plotted and compared (Fig. 7). At the northern subpopulation beaches, both sexes were produced from nests with middle-third daily average nest temperatures ranging from 25.0°C – 32.0°C. Those beaches from the southern population had nests that produced both sexes with middle-third daily averages from 26.0°C – 32.0°C (Fig. 7). Overlap in the temperature ranges, from slight to complete, occurred between the single sex nests and those producing both sexes (Fig. 7). There was no clear separation between the single-sex temperature range and the two-sex temperature range at any of the beaches in 2003. There was, however, total overlap at Onslow Beach (NCON; Fig. 7), where the recorded range of nest temperatures producing 100% males was greater than the range producing mixed sex ratios.

Fig. 7. Box-plot of the range of daily average temperatures experienced in the nests of the study beaches during the middle third of incubation (n = # of nests). Beaches are arranged from north to south from Onslow Beach, NC (NCON) to Boca Raton, FL (FLBR). Miami Beach was not included in this comparison as only late season nest were represented.

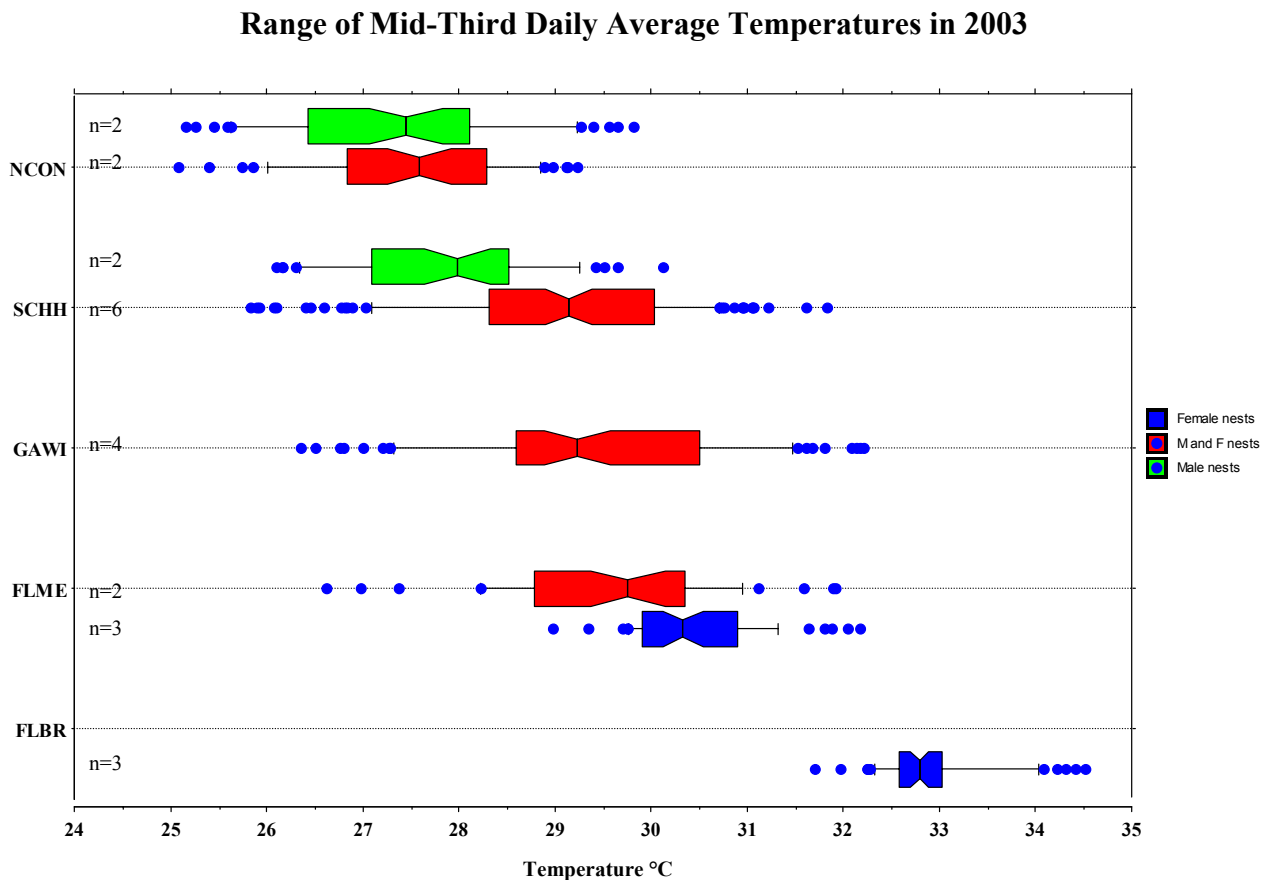
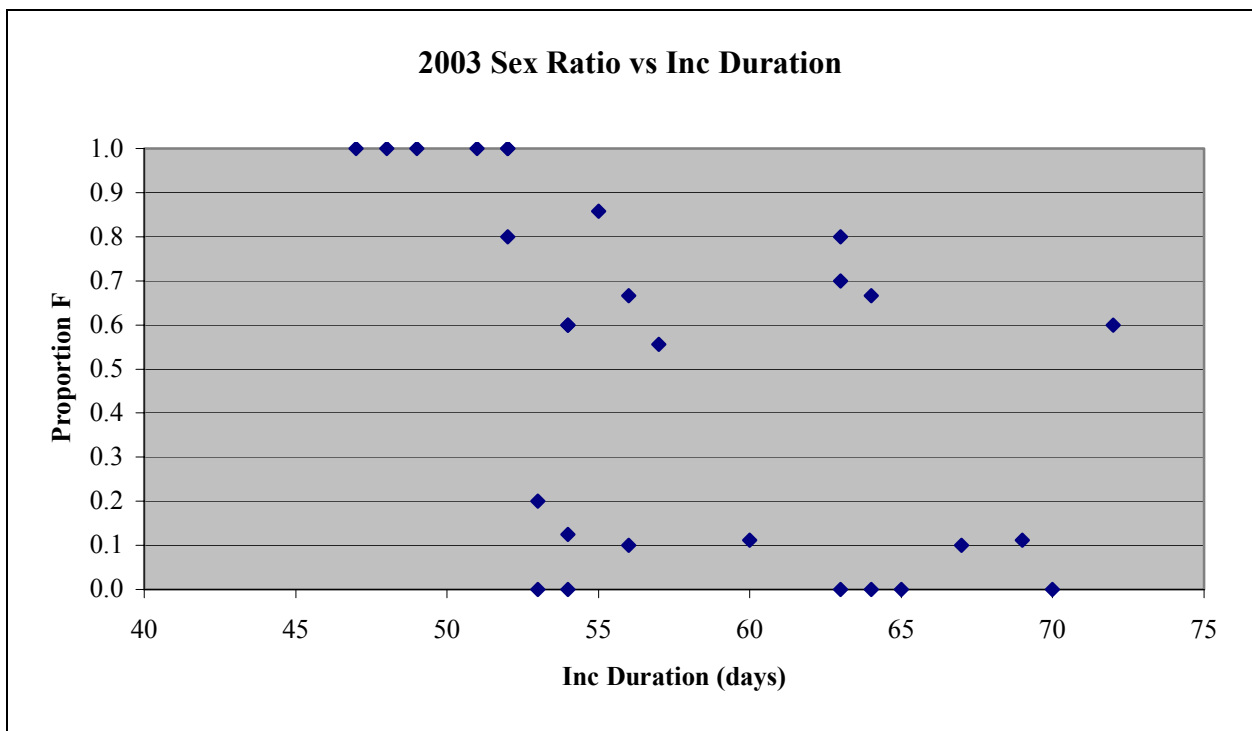


Figure 7 further strengthens the conclusion that temperature alone is not the controlling mechanism for determining sex.

As in 2002, it is not just the temperature of the nests that displayed a lot of scatter; the incubation durations do as well. Figure 8 shows the incubation durations for nests plotted against the

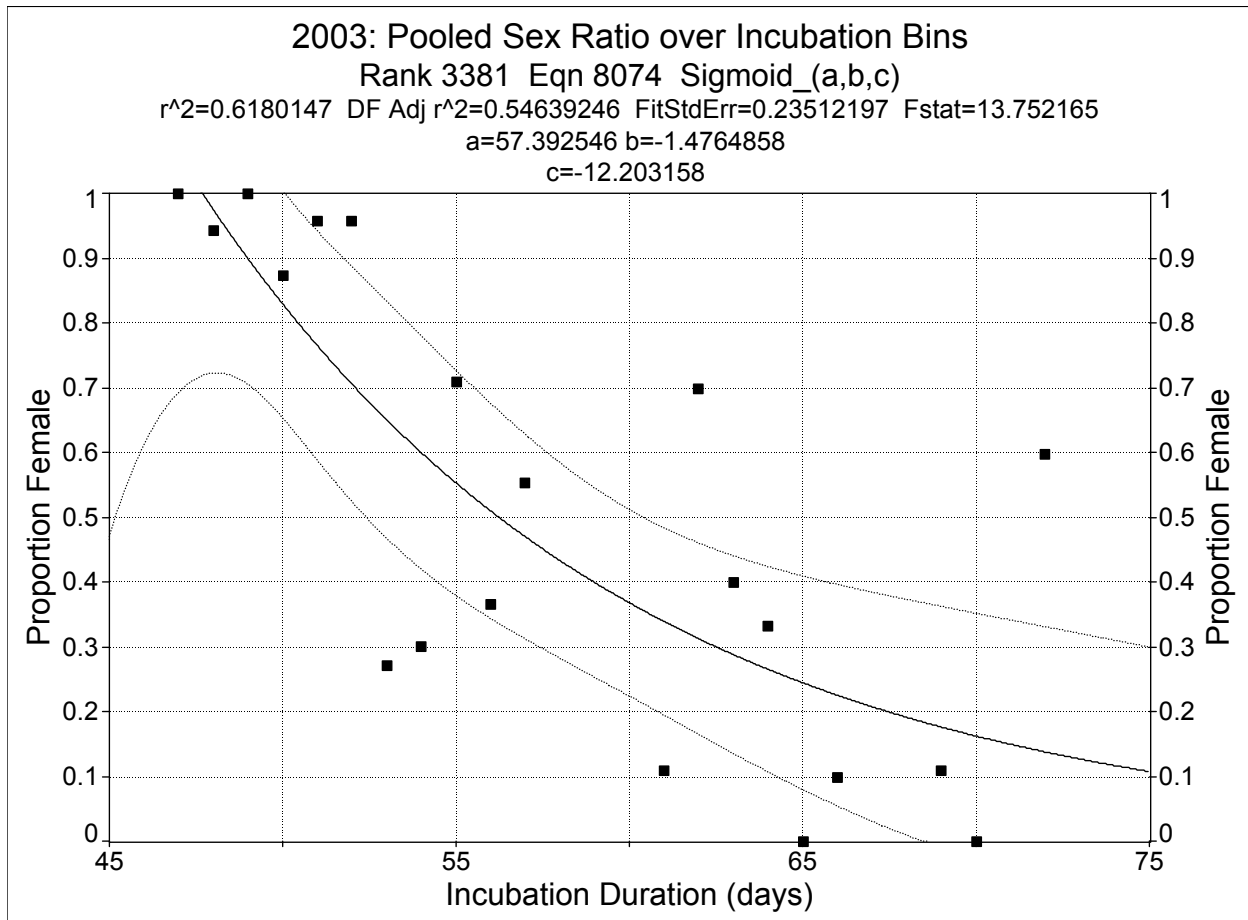
proportion female for each of those nests. Again, each nest had at least 5 turtles that had been verified to ensure good representation of the sex ratio. The scatter in 2003 is not as wide as it was in 2002, but this is most likely due to the lower number of nests (26 nests in 2003 vs. 110 nests in 2002). There still is scatter over the different lengths of incubation that produce a particular sex ratio, as well as a wide scatter across the sex ratios that are produced by a given incubation duration. For example, nests producing 0% females ranged in incubation duration of 53 – 70 days. There were 4 nests that incubated for 52 or 53 days, and produced sex ratios ranging from 0 – 100%. Just as in 2002, the 2003 incubation duration results contribute to the conclusion that temperature alone is not the controlling mechanism for determining sex.

Fig. 8. Scatterplot showing the spread of incubation lengths of nests in 2003, plotted against the proportion female produced by each nest.



Unlike in 2002, pooling the sex ratios into single day incubation bins, did little to reduce scatter. This is most likely because of the much lower sample size, compared to 2002. The fit of the sigmoidal regression curve is good ($r^2=0.618$) and is highly significant ($p<0.001$), but the pattern is not as well defined as it was in 2003 (Fig.9).

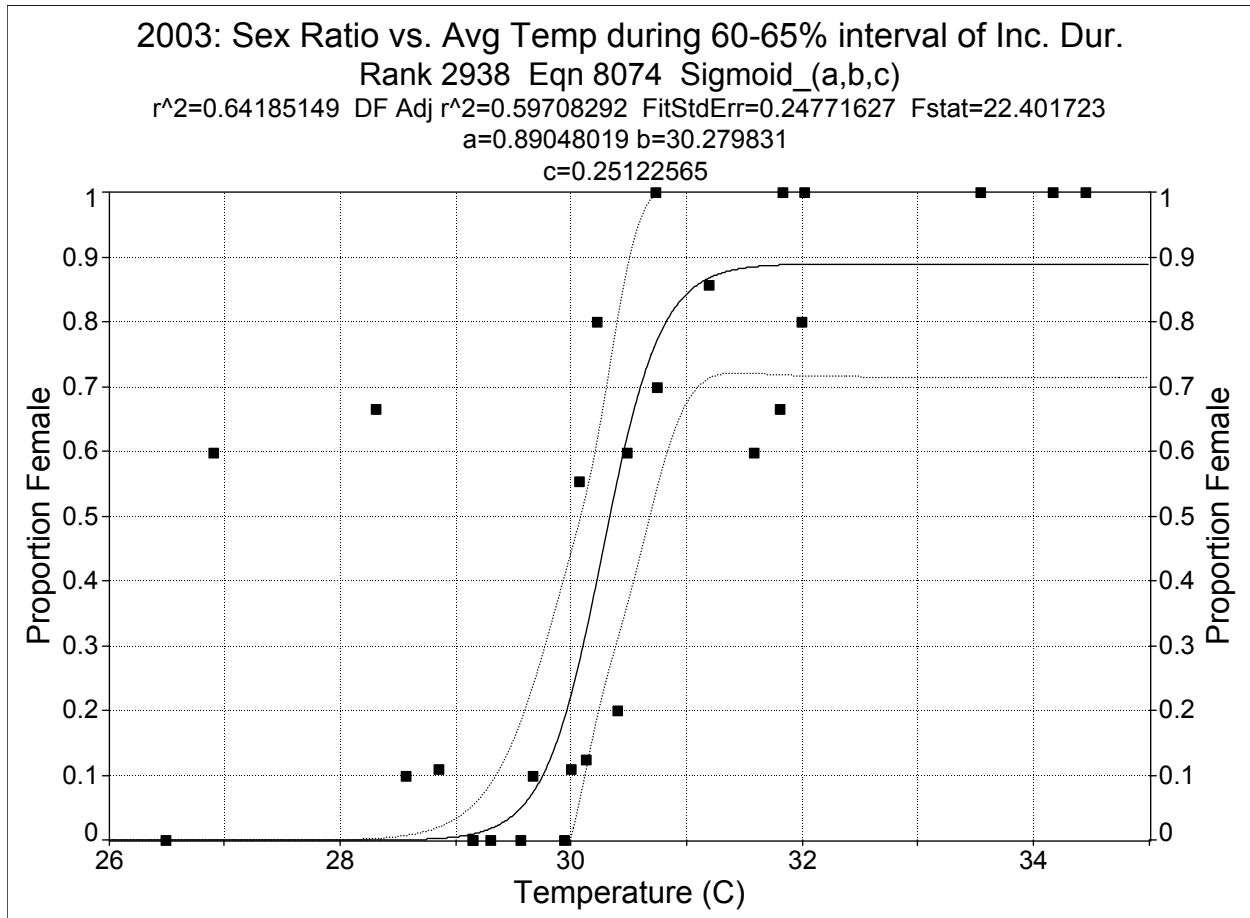
Fig. 9. Scatterplot of pooled sex ratios into single day incubation length bins. A sigmoidal curve and 95% C.I. are also shown.



To determine if the average temperature over the entire middle third is the best predictor of the sex ratio, the incubation length was divided into 5% intervals, and the average temperature in each interval was plotted against the sex ratios produced (Fig. 10). As in the previous analyses the 28 nests with a least 5 verified turtles were used for this analysis. A sigmoidal curve was fit to each plot to identify the time frame for which temperature can best predict the sex ratio. Figure 10, shows the best-fit plot, which was the average temperature during the 60-65% interval of the incubation length. In 2002 it was the 50-55% interval. The fit is good ($r^2 = 0.624$) and highly significant ($p < 0.001$), and is much better fit than the curve using the average temperature of the entire middle third ($r^2 = 0.461$). These results support the conclusions we found in the 2002 analyses.

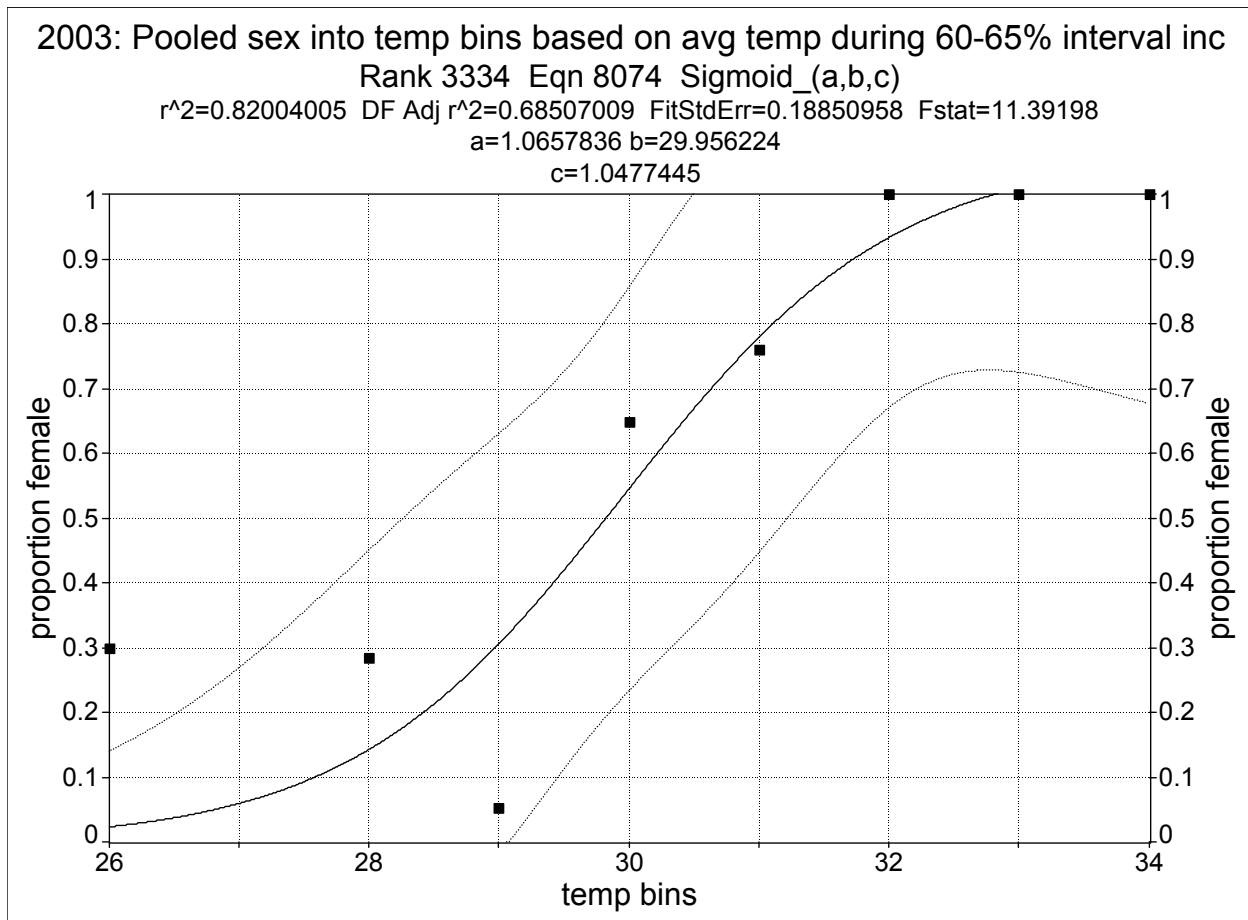
1. The timing of the best-fit in 2003 is even later in the middle third, possibly indicating that sex determination is later or that average temperature lags behind the apparent temperature in the nest, experienced earlier by the eggs.
2. The scatter is still large and wide, further strengthening the now unavoidable conclusion that temperature alone is not the controlling mechanism for determining sex.

Fig. 10 Scatterplot of average temperature during the 60-65% interval of incubation length plotted against the proportion of females produced by the nest. Sigmoidal regression curve is fit to the points, along with the 95% C.I.



When the data are pooled into single degree temperature bins, a clear pattern is seen. The fit of the curve is better ($r^2=0.820$), but is less significant ($p=0.014$; Fig.11). This is most likely due to the very wide confidence intervals and the variance that occurs at the cooler temperatures. The Wide confidence intervals also are a further indication that there is a lot of inter-nest variation. So, it is unlikely that one can accurately predict sex ratios based on temperature alone.

Fig. 11. Scatterplot of the average temperature during the 50-55% interval of incubation length with the proportion female pooled across nests into single degree temperature bins. Sigmoidal regression curve and 95% C.I. have been fit to the points.



Comparing both years

Five beaches were sampled in both 2002 and 2003. The following table summarizes some of the differences between the two years. The table lists the results of Independent samples t-tests, with the p-values are listed in the table

<u>Site</u>	<u>Sex Ratios</u>	<u>Inc. Dur.</u>	<u>Avg. Mid-Third Temps</u>
Overall	not different	not different	not different*
North	<0.001	<0.001	0.013
South	not different	0.002	not different*
Wassaw Island	not different	not different	not different
Melbourne	not different	not different	0.004
Boca Raton	0.015	0.024	<0.001
Miami Beach	not different	not different	not different
Sanibel	not different	not different	*

*missing some data; results may change slightly.

This table includes mixture of equal variance and unequal variance tests, used as appropriate for each comparison.

Independent samples t-tests were also used to compare the intra-season sampling periods within a subpopulation across years. In the north, one sampling period seems responsible for the difference seen. In the northern subpopulation, there significantly fewer females in the mid season sampling period in 2003 (sex ratio=23.52%) than in 2002 (sex ratio=88.68%, $p < 0.001$). There were no significant differences between the sex ratios of the other two intra-sampling periods. Again, the low number of females present in the mid-season of 2003 greatly affected the results. It was quite different for the southern subpopulation. All intra-season sampling periods were significantly different. For the early and mid season sampling, there were more females in 2003 than in 2002 ($p \leq 0.001$ for both). However, there were fewer females during the late season sampling in 2003 than in 2002 ($p = 0.002$). Together these balance so that there is no significant difference in the overall sex ratio of the south subpopulation between years.