

## Sex Ratios of Wild American Alligator Hatchlings in Southwest Louisiana

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**Abstract** - The sex of American Alligator (*Alligator mississippiensis*) hatchlings is determined by the egg temperature during the middle third of the 9–12 week incubation period. As a consequence, predictable sex ratios are possible for clutches incubated in constant temperatures in the laboratory, but naturally occurring sex ratios of American Alligator hatchlings from wild nests exposed to fluctuating temperatures are not well documented. Over a 5-year period (1995–1999), we determined the sex of American Alligator hatchlings from wild nests left in the field until after sex was irreversibly determined. A total of 6226 hatchlings from 232 naturally incubated wild nests showed a strong female bias (71.9% females, yearly range = 62.3–89.4% females). Most nests (64.2%) produced hatchlings of both sexes. Of the remaining clutches that produced exclusively one sex (83 nests), 78 nests produced all females, and 5 nests produced only male hatchlings. For the 2 years in which nest-cavity temperatures were known, higher temperatures led to production of significantly more male hatchlings ( $P < 0.001$  for both 1997 and 1999). Knowledge of natural sex ratios of hatchlings can aid in the management and harvest of this commercially valuable species, and in understanding sex-ratio bias in American Alligator populations.

### Introduction

Multiple crocodylian species, including *Alligator mississippiensis* Daudin (American Alligator; hereafter also Alligator) exhibit temperature-dependent sex determination (hereafter TSD; Ferguson and Joanen 1982, 1983; Lang and Andrews 1994). Refined laboratory studies with accurate temperature controls have examined this phenomenon in detail (see Western 1999 and references therein). The influence of temperature on the sex of developing Alligator hatchlings is complicated. Under constant temperature incubation in the laboratory, only a narrow range of intermediate temperatures produce males, with females being produced over a much broader range of both high and low temperatures (Lang and Andrews 1994). Specifically, laboratory incubation at a constant temperature of  $\leq 31$  °C produces only females, 32 °C results in mixed sex ratios, and 33 °C only produces males (Lang and Andrews 1994). Mixed sex ratios occur at 34 °C, and very high temperatures (35 °C) produce exclusively females; however, mortality is high at this temperature (only 11% embryonic survival at 35 °C; Lang and Andrews 1994) and thus the production of high-temperature females may rarely be seen in nests incubated in the wild. Therefore, the TSD pattern in American Alligators in a controlled laboratory setting is a bimodal distribution of exclusively females at low and at high temperatures, males

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at a narrow range of intermediate temperatures, and mixed sex ratios on either side of the male-producing temperatures. At constant temperatures, the incubation period (i.e., the time between oviposition and hatching) ranges from 63 days at 33 °C to 84 days at 29 °C (Lang and Andrews 1994). In American Alligators, the thermosensitive period when sex is irreversibly determined occurs during the middle third of incubation, or at approximately days 30–45 (Lang and Andrews 1994).

Less is known about sex ratios of Alligator hatchlings in the wild, where nests, eggs, and embryos are exposed to fluctuating temperatures (e.g., range of 23.3–32.8 °C; Joanen 1969). It is important for wildlife biologists to have knowledge of sex ratios of Alligators from all size/age classes for use in management of the population and establishment of harvest quotas (which may select for one sex rather than the other). In Louisiana, harvest programs exist for both eggs and sub-adult and adult Alligators.

Given the data from constant incubation thermal regimes in the laboratory, female-biased sex ratios might be predicted among hatchlings because only a narrow range of nest temperatures result in males. However, because natural nest temperatures vary throughout the day and season it is difficult to accurately predict whether naturally produced Alligator hatchling sex ratios will be biased toward males or females (Rhodes and Lang 1995, 1996). In fact, reviews of crocodylian sex ratios found that many populations of a variety of species examined are male-biased (Lance et al. 2000, Thorbjarnarson 1997). For example, in an extensive 6-year survey of over 3000 juvenile American Alligators at 11 sites in Louisiana, Lance et al. (2000) reported a significant male-biased (58%) sex ratio that varied by year and by site.

The inconsistency between lab-based predictions and field measurements suggest more work is required to determine sex ratios of Alligator clutches incubated naturally in wild nests. Information obtained from additional studies could be of great importance as projected global temperature changes affect wetlands and wetlands management (Withey and van Kooten 2011) and therefore Alligators. Regional climate changes could also affect many Louisiana Alligator “ranchers”, who are licensed to collect eggs from wild Alligator nests to provide stock for commercial Alligator farms (Elsey et al. 2001), as climatic factors affect the timing and degree of Alligator nesting, age of sexual maturity, and growth rates (Joanen and McNease 1987) as well as the sex ratio of hatchlings. Louisiana’s Alligator industry can be a \$60 million industry in peak market years (Louisiana Department of Wildlife and Fisheries 2009), and thus, data relative to Alligator ecology is of significant economic interest to managers and landowners. This study was initiated to evaluate naturally occurring sex ratios in nests of American Alligators in Louisiana. We also attempted to correlate nest-incubation temperatures during the thermosensitive period with resulting hatchling sex ratios.

## Methods

We conducted this study on portions of Rockefeller Wildlife Refuge, a 30,700-ha coastal marsh in Cameron and Vermilion parishes, in southwestern Louisiana. The

refuge boundaries and predominant vegetation were described by Joanen (1969); current marsh types and management units were described recently by Selman and Baccigalopi (2012). Nests were located by helicopter and marked with 3.05-m PVC pipes. We plotted nest sites on an aerial map for later use by ground crews. The great majority of nests were later accessed by airboat, with the exception of a few nests that were adjacent to roads and were approached by vehicle and on foot. During June 1995, we placed temperature recorders (Optic StowAway, Onset Computer Corp., Pocasset, MA) in 10 Alligator nests soon after oviposition. We also collected 16 other clutches late in incubation (after the thermosensitive period) to examine the hatchling sex ratio, for a total of 26 clutches examined in 1995. We expanded the study from 1996–1999, and placed temperature recorders in additional nests (2, 37, 42, and 40 nests with recorders in each successive year) and collected more nests in August of each year, after the thermosensitive period.

Nests were opened to reveal the egg cavity. If temperature recorders were placed in nests, 2 eggs were removed and sacrificed to determine the stage of incubation of the developing embryos by examining the size of the embryo (Ferguson 1985). This step was not done for nests that did not receive data loggers as embryos were not needed for aging, and instead the entire clutch was collected in August. We placed the temperature recorder near the middle of the clutch of eggs, with care being taken to minimize disturbance to nest integrity and not inadvertently rotate eggs. We re-covered the cavity with the original nest media, predominantly *Spartina patens*. We programmed the temperature recorders, which had a sensitivity of  $\pm 0.2$  °C, to take a reading every 15 minutes.

In August, after the thermosensitive period was completed, we revisited the nests containing temperature recorders (the only visit after the initial visit, to limit disturbance). We marked the eggs for upright orientation, packed them carefully in layers of nest media, and transported them to the lab at Rockefeller for artificial incubation (approximately 30.5–31.5 °C) until hatching (Joanen et al. 1987), which generally occurred one to three weeks later. We collected full clutches from numerous other nests not containing temperature recorders in the same manner during this time and transported them to the field laboratory for incubation under the same conditions as clutches that contained recorders.

During incubation and after hatching, each clutch was held in a separate container with nest media from its corresponding nest used for insulation and cushioning. Hatchlings were maintained by clutch until sex was verified. We determined sex for each hatchling by visual examination of the genitalia within the cloaca as described by Allsteadt and Lang (1995), using a lighted magnifying glass for clarity. J.W. Lang made all observations to minimize error, as determining the sex of hatchling crocodylians is challenging (Joanen and McNease 1978, Otano et al. 2010, Ziegler and Olbort 2007). In some cases, embryos that died late in incubation were saved for possible sex determination, if tissue degradation did not preclude it.

For nests that contained data loggers, we used a simple linear regression to determine if there was a relationship between mean daily temperatures in the nest cavity during the thermosensitive period for sex determination (days 30–45) and

the percentage of hatchlings within the clutch that were male. Unfortunately, a computer-system change led to loss of electronic temperature-data-logger files; however, nest-cavity-temperature data from 1997 and 1999 could be gleaned from quarterly reports provided by the second author as part of a research contract. We also evaluated ambient temperature data for the month of July (the thermosensitive period for sex determination at the study site occurs primarily in July) for each of the 5 years, to determine if there was a correlation between ambient minimum temperatures and overall hatchling sex ratio. Ambient temperature was recorded at the Rockefeller refuge headquarters weather observation station.

## Results

During the 5-year study, we evaluated a total of 6226 hatchlings and observed a strong overall female bias for hatchlings produced each year (Table 1). The percentage of females ranged from 62.3% in 1998 to 89.4% in 1995. Seventy-eight of 232 nests (33.6%) produced only female hatchlings, while there were only 5 of 232 nests (2.2%) that produced only males; all 5 of these nests were from the first 3 years of the study.

Data-logger temperatures from 1997 revealed that cool nest temperatures (29–31 °C) during the temperature-sensitive period produced 100% females, intermediate temperatures produced varying sex ratios (including 100% males produced in one clutch at 32.8 °C), 2 nests at higher temperatures (33–34 °C) produced 74 and 78% males, and the warmest nest (35 °C) produced predominately (79.2%) females (Fig. 1). There was a significant Pearson correlation coefficient ( $R$ ) of 0.772 such that increasing nest cavity temperatures led to development of more male hatchlings ( $P < 0.001$ ).

In 1999, one nest with a relatively cool initial temperature of 29 °C produced all female hatchlings. In contrast, a relatively warm nest with an initial temperature of 32.8 °C produced 66% males. The nest-cavity temperatures during the thermosensitive period again correlated with the percent males produced, with warm nests producing a majority of males ( $R = 0.566$ ,  $P < 0.001$ ), and the coolest nests producing only females. Despite a severe drought in spring/summer 1996 in southwestern Louisiana in which warm, dry conditions perhaps should have fa-

Table 1. Sex ratio of hatchling American Alligators, *Alligator mississippiensis* from nests on Rockefeller Refuge 1995–1999.

Study year	Number of males	Number of females	Total hatchlings or embryos	Total nests	Nests	
					producing 100% females	producing 100% males
1995	81 (10.6%)	683 (89.4%)	764	26	9	1
1996	254 (34.1%)	491 (65.9%)	745	32	13	3
1997	372 (25.7%)	1074 (74.3%)	1446	47	21	1
1998	510 (37.7%)	844 (62.3%)	1354	62	20	0
1999	531 (27.7%)	1386 (72.3%)	1917	65	15	0
Total	1748 (28.1%)	4478 (71.9%)	6226	232	78	5

vored the production of males (assuming high ambient temperatures cause higher temperatures in nest cavities), only 34.1% of the hatchlings were male from the nests studied ( $n = 32$ ). This was the second highest percentage of male hatchlings produced during the 5-year study.

The Pearson correlation coefficient for the relationship between the minimum ambient July temperature for the 5-year study period and the percentage of male hatchlings produced each year was 0.76,  $P = 0.14$ , indicating a non-significant but positive association between warmer minimum July temperatures and more male hatchlings.

### Discussion

In all 5 years of this study, a strong female-bias (71.9%) was noted among hatchlings produced from 232 clutches examined. This result is a marked difference to the male-biased juvenile sex ratio (58%) we have documented in tag-and-release efforts in southwestern Louisiana for some 3000 American Alligators from 1991–1995 (Lance et. al. 2000). Thus, there may be differential survival rates that tend to favor male juveniles; the faster growth rates of males (Elsey et. al. 1992, Joanen et. al. 1987) may give them a competitive advantage over juvenile females.

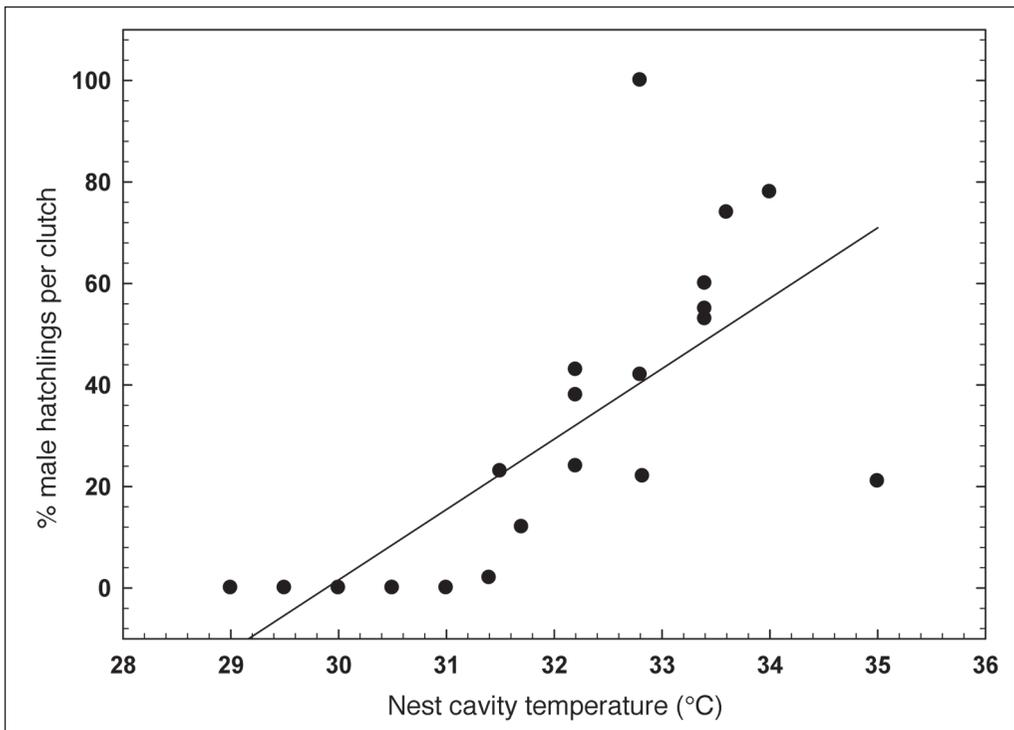


Figure 1. Nest temperatures ( $^{\circ}\text{C}$ ) from data loggers during the thermosensitive period (days 30–45) for sex determination for American Alligators (*Alligator mississippiensis*) from the nest cavity of wild alligator nests on Rockefeller Refuge in 1997, and the resulting sex ratio expressed as percent male hatchlings for each clutch. Increasing nest-cavity temperatures led to the development of more male hatchlings ( $R = 0.772$ ,  $P < 0.001$ ).

The few nests with higher nest-cavity temperatures led to the production of more male hatchlings. In the field, we rarely recorded the very high temperatures (35 °C) that lead to production of females in controlled laboratory experiments. Ambient air temperatures were also qualitatively associated with sex ratios of hatchlings produced, with higher temperatures leading to a higher proportion of males within a clutch. We anticipated ambient temperature would not be as rigorous a predictor of sex ratios as actual nest-cavity temperatures, but suspect that knowledge of these trends may assist in assessment of sex-ratio data in regions where nest-cavity-temperature data may not be available to researchers.

In a controlled laboratory setting at constant incubation temperatures, the pattern of percent males produced vs. incubation temperature is unimodal, with high proportions of male hatchlings at a single range of intermediate to high temperatures, and female production is bimodal, with high proportions of female hatchlings at high and low temperatures above and below the male-producing mid-range (Lang and Andrews 1994). In the present study, we observed the same phenomenon in wild Alligator nests in 1997, with the coolest nests producing all females, the intermediate temperatures producing more males, and the warmest nest (35 °C during the thermosensitive period) producing 79% females. These results are likely influenced by natural variability in field-nest temperatures leading to the production of some hatchlings of each sex in most years.

In a related study, results similar to ours were documented in coastal South Carolina (Rhodes and Lang 1995, 1996) where sex ratios of naturally incubated Alligator clutches (778 hatchlings from 25 nests) also exhibited a strong female bias (79.2%) in 1994. Likewise, in 1995 Rhodes and Lang (1995, 1996) monitored an additional 20 nests and the sex ratio of the resulting 648 hatchlings was also female biased (58.2%). The South Carolina study was continued for four more years, and in one year, 100% of hatchlings produced from all clutches evaluated were female (Lance et al. 2000). We are not aware of data on subsequent juvenile sex ratios in coastal South Carolina to evaluate sex-specific differential survival.

Growth rates in Alligators vary by sex; males tend to grow faster and attain larger maximum total lengths than females (Chabreck and Joanen 1979, Elsey et al. 1992, Wilkinson and Rhodes 1997, Woodward et al. 1995). Because survival in Alligators is size-dependent (Nichols et al. 1976, Rootes 1989), the sex of the hatchling (and resulting growth rate and size) will affect its survival rate. Thus, knowledge of the sex ratio of a population is essential to management of the species; particularly if harvest programs preferentially target one sex.

Detailed studies exist on the relationship between nest temperature, sex ratios, hatching success, and survivorship in other crocodylians, including *Paleosuchus trigonatus* Schneider (Dwarf Caiman; Magnusson et al. 1990) and *Caiman latirostris* Daudin (Broad-Nosed Caiman; Piña et al. 2003). In the closely related *Caiman crocodilus yacare* Daudin (Yacare Caiman), nests constructed of varying types of vegetation may have differing incubation temperatures (Campos 1993). Similarly, we also have preliminary data suggesting different types of vegetation can affect

nest-cavity temperatures, which would affect Alligator egg survival as well as hatchling sex ratio. This finding is interesting from an evolutionary standpoint, as perhaps female nest-site selection could influence hatchling sex ratio.

Many commercial Alligator egg ranchers in several southeastern states (Louisiana, Texas, and Florida) collect large numbers of wild Alligator eggs to incubate and then raise the hatchlings in captivity to produce juveniles for the international leather goods market. Being that the incubation period of Alligator eggs is brief, many of the eggs are often collected after the thermosensitive period for sex determination. Commercial Alligator ranchers/producers may prefer to obtain hatchlings of one sex or the other; males often grow faster, which could minimize production costs, whereas females may be preferred to ensure that mandatory releases to the wild will include females for future nesting on egg-collection sites (a juvenile head-start release program is a required part of the egg-collection permit in Louisiana [Elsey et al. 2001]). Thus, Alligator farmers may choose to coordinate egg collecting efforts in varying regions based on which eggs they prefer to incubate early (so they can influence the sex of the hatchlings by incubation temperature) and in which regions they might collect eggs after the sex has been influenced by ambient nest temperatures, such as areas of lower nest densities.

Differences in nesting habitats and seasonal weather patterns in various regions may influence hatchling sex ratios from nests incubated in the wild. For example, results from our study showed production of 89.4% females in 1995, which differs markedly from hatchling sex ratios in South Carolina the same year, in which more males (42% of 648 hatchlings) were produced (Rhodes and Lang 1995, 1996). Similar to the range in sex ratios we observed among years in Louisiana, a hot, dry summer likely contributed to the 42% males produced in South Carolina in 1995 as compared to the cooler, wetter summer in 1994 when only 20% males were produced at the same site (Lang 1995). Recent speculation about global climate change and possible effects on crocodylian reproduction and sex ratios is of interest (Rao et al. 2013). Indeed, some authors have speculated that global warming may affect reptiles exhibiting temperature-dependent sex determination, as altered incubation temperatures in nests could potentially result in skewed sex ratios or hatchlings of only one sex (Charruau 2012 and references therein), or that past climate-change influences on sex ratios may have contributed to selective extinction of some Archosaurs (Ferguson and Joanen 1982). However, normal expected cyclical weather patterns and sex-specific differential survival may ensure adequate production of both sexes of crocodylians, which have survived successfully for millions of years.

#### **Acknowledgments**

We thank all Louisiana Department of Wildlife and Fisheries employees who assisted with Alligator egg collection and incubation, including Phillip “Scooter” Trosclair, Jeb Linscombe, George Melancon, and Leisa Nunez. We thank Lisa Morris of the Department of Experimental Statistics at Louisiana State University for assistance with analyses.

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