Sex Ratios of Naturally-incubated Alligator Hatchlings: Field Techniques and Initial Results

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Abstract: In American alligators (*Alligator mississippiensis*), sex determination is dependent upon incubation temperature of the egg prior to hatching. Information about hatchling alligator sex ratios is equivocal, however. Moreover, numerous surveys of juveniles and adults show a male-biased ratio, although a female-biased ratio has been reported for hatchlings from Louisiana. We outline a technique for estimating sex ratios in naturally-incubated alligator nests, and present the initial results from impoundment habitats in South Carolina. In 1994, we located 33 nests and sexed and released 778 hatchlings from 25 nests. The observed sex ratio was 1M:3.8F. Hatchling sex ratios are likely to vary temporally and spatially; therefore, long-term studies in representative habitats are required.

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Data on sex ratio is a necessary component in any model designed to provide information about a given population structure. Likewise, this information is a prerequisite for the successful management of wildlife populations, including those of the American alligator. Sex determination in alligators, other crocodilians, and many turtles is dependent on the egg incubation temperature prior to hatching. In these species, the nesting environment has a profound effect on hatchling sex. Consequently, and unlike most bird and mammal species in which the neonatal sex ratio approximates one male to one female, the sex ratios of alligator hatchlings may not be one to one, and may vary with climate and/or location.

Previous studies of alligator sex ratios have relied on the live capture and sexing of juveniles and adults or on data from harvested adults. Although some of these studies apparently were biased by the methods employed (Giles and Childs 1949, Hines and Woodward 1980, Joanen and McNease 1987), nearly all surveys conducted over the past 3 decades throughout the range of alligators have resulted in male-biased sex ratios for juveniles and adults (Palmisano et al.

1973, Murphy 1977, Joanen et al. 1984, Brandt 1991, Kinler and Taylor 1992, Rootes and Chabreck 1992). In addition, a detailed analysis of recent surveys of juvenile alligators in coastal Louisiana indicates sex ratios vary markedly from year to year and in different locations (R. Elsey, pers. commun.). While these investigations suggest the neonatal sex ratios of alligators from wild nests may have been male-biased, it is difficult to rule out the possibility of age-specific mortality and/or sex-specific habitat preferences affecting the results of alligator surveys.

In marked contrast, Ferguson and Joanen (1983) concluded the sex ratios of hatchling alligators in coastal Louisiana were strongly female-biased; i.e., 83% female, based on 4 years of nesting (1977–1981) and over 8,000 juveniles subsequently sexed. In their study, eggs were removed from wild nests after 5 weeks, artificially incubated, and juveniles were sexed 2 years later. The results of Ferguson and Joanen (1983), due to time of collection and incubation temperatures, however, may not reflect sex ratios in nature (Mrosovsky and Provancha 1992, Rootes and Chabreck 1992, Lang and Andrews 1994). Earlier reports had indicated the pattern of temperature-dependent sex determination (TSD) in alligators occurs early in incubation and results in females at low incubation temperatures (<32 C) and males at high temperatures (>32 C) (reviewed in Deeming and Ferguson 1991). More recent studies, however, indicate TSD occurs during the middle third of incubation and females are produced at both high (>33 C) and low (<32 C) temperatures, while males are produced predominately at intermediate temperatures (32–33 C) (Lang and Andrews 1994).

The South Carolina alligator population has not been commercially exploited for approximately 30 years, and therefore may be classified as "stable." Alligator sex ratios may be different in "stable" versus "recovering" populations; i.e., South Carolina vs. Louisiana (Deeming and Ferguson 1989). Further, most alligator nesting in South Carolina occurs on remnant dikes and berms (Rhodes unpubl. data) in wetland impoundments (Wilkinson and Rhodes 1992). In Louisiana, nests on dikes were reported to produce a preponderance of male hatchlings (Ferguson and Joanen 1982, 1983). If this relationship holds for South Carolina, then many high-ground nests may contribute to a male-bias in hatchling populations.

At present, it is not clear whether alligator sex ratios at hatching are biased. If so, is recruitment skewed towards males or females? To what extent do hatchling sex ratios vary with annual climate and/or nesting habitat? Prompted by the new information about the pattern and timing TSD in alligators (Lang and Andrews 1994) and by a new technique for sexing live hatchlings (Allsteadt and Lang 1995), our objectives were to outline a practical methodology for determining alligator sex ratios in the field and to initiate a long-term study of alligator sex ratios in coastal South Carolina.

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Methods

The study was conducted on the Santee Coastal Reserve, a 10,118-ha area on the north-central coast of South Carolina. Mean annual precipitation is about 127 cm, and mean January and July temperatures are 8.9 C and 25.6 C, respectively. The annual number of frost-free days is 260–290 (S. Sidlow, S.C. Dep. Nat. Resour., pers. commun.). For 1994, seasonal and annual temperatures were average and annual rainfall was above normal (D. Smith, state climatologist, S.C. Dep. Nat. Resour., pers. commun.).

The study was restricted to the 1,421-ha managed impoundments portion of Santee Coastal Reserve known as the Cape. Vegetative cover within impoundments was typically a mixture of emergents (e.g., giant cordgrass *Spartina cynosuroides* and saltmarsh bulrush *Scirpus robustus*), submerged aquatics (e.g. *Ruppia maritima*), and open water. Trees and shrubs were sparse, except on dikes. Surrounding wetlands were tidal and subject to extreme salinity variation (00 to 35 ppt) due to periodic discharges into the Santee River from the Santee-Cooper lakes.

Aerial nest surveys were conducted in late July 1994, about a month after nesting. Impoundments were thoroughly searched between the upland borders and the perimeter dikes. Nests were located using 3 observers (including the pilot) from a Hughes 500 helicopter flying at low altitudes (15.3–30.6 m) and an average speed of 64 km/hour. Nests locations were plotted on an aerial photograph (scale 1 cm = 158.4 m).

In early August, these nests were located on the ground. Nesting habitats were defined as impoundment dike, impoundment berm (or shelf of dike), remnant dike or berm within impoundment, impoundment bed (floor of flooded impoundment), natural creek edge, or other. Data recorded at nests included time of day, presence or absence of eggs, nest temperature at center of clutch (nearest 0.1 C), nest material type, and features of the nesting habitat.

Because the thermosensitive period (when incubation temperature determines sex) occurs during the middle third of development, or from about Day 25 from date laid to Day 50 under normal conditions (Lang and Andrews 1994), eggs were collected from nests during the latter third of development (third week of August). At this time, nest temperature was again recorded and clutch size noted. Length and width of eggs were measured to the nearest 0.1 mm using digital calibers and weighed on a digital scale to the nearest 0.1 g. Eggs, by clutch, were arranged in ventilated 41.6 l plastic storage boxes with nesting material. If fire ants (*Solenopsis saevissima*) were present in nest material, we replaced natural nest material with cyress (*Cyperus spp.*) shavings. Storage boxes were stacked in open air and periodic temperature checks were taken to ensure non-lethal temperatures. Nest material was wetted as needed.

Once hatching was complete, hatchlings, by clutch, were removed to separate 0.6 × 0.6 m open-air raceways containing water and basking areas. All remaining eggs were opened, and late stage embryos and dead hatchlings were sexed by internal examination of gonads. Live hatchlings were sexed by genitalia inspection as described by Allsteadt and Lang (1995), and live hatchling total length and snout-vent length (nearest 1.0 mm) and weight (nearest 0.1 g) was recorded. Hatchlings were released at original nest site in early September, within 2–3 weeks of egg collection.

Nonparametric Mann-Whitney tests were used to test for differences in the distribution of hatchling sex ratios and of high and low alligator nest temperatures. A Chi-squared analysis was used to test if observed sex ratios differed from an expected 1M:1F sex ratio. Statistically significant differences were identified at the 95% level of confidence ($P \leq 0.05$).

Results

A total of 35 nests (1 nest/41 ha) were located during aerial surveys, including 33 active nests, 1 false nest, and 1 nest which could not be found by later ground search. Twenty nests were located on remnant dikes or berms within impoundments, 8 were located on the beds of impoundments, and 5 were located along natural creek margins within impoundments. Primary nest vegetation was giant cordgrass, but nests at low marsh elevations contained black needlerush (*Juncus roemerianus*) and saw grass (*Cladium* spp.).

Of the 33 active nests, 10 hatched before eggs were collected. A total of 1,007 eggs was collected from the remaining 23 nests. Average clutch size was 43.8 ± 1.4 (SE). Clutches from 2 nests, 1 containing 52 eggs and another with 22 eggs, failed to hatch. Hatching success, defined as the total eggs hatched divided by total eggs, was 71.8%. Twenty-one nests hatched a total of 711 young which were subsequently released (Table 1). In a number of instances, adults and/or juveniles were seen in the vicinity of the nests within hours following hatchling release.

Sex ratios, by nest, ranged from 100% females to 100% males, for the 25 clutches sampled. Fourteen of these were all female; only 4 nests had male-biased sex ratios (Table 1). There was no difference in the distribution of sex ratios from nests hatched before egg collection and collected nests ($Z = 0.940$, $P = 0.347$). Likewise, the distribution of sex ratios with respect to total hatchlings (live and dead) for each clutch did not differ from live hatchlings for each clutch ($Z = 0.106$, $P = 0.916$). Overall, the number sexed (and sex ratios) for total hatchlings (live and dead), live hatchlings, and dead hatchlings were 177M:648F (1M:3.67F), 161M:616F (1M:3.82F), and 16M:32F (1M:2F), respectively. In each category, sex ratios are significantly different from an expected 1M:1F sex ratio (total, $X^2 = 268$, 1 df, $P < 0.0001$; live, $X^2 = 254$, 1 df, $P < 0.0001$; dead, $X^2 = 5.33$, 1 df, $P = 0.021$).

Table 1. Clutch size, hatching data, sex ratio, and number of alligators sexed and released from naturally-incubated nests, Santee Coastal Reserve, South Carolina, 1994.

Nest No.[a]	Hatch date	Clutch size	Hatch success	*N*	Sex ratio (% males)	Nest height
6[b]	234			13	31	H
24[b]	234			21	43	H
14	235	37	89.2	31	0	H
1[b]	235			12	25	H
3	235	50	90.0	44	59	H
19	236	50	78.0	39	0	L
25[b]	236			21	0	H
32	236	47	85.1	40	0	H
16	236	39	82.1	32	35	H
31	236	40	90.0	36	89	H
21	236	45	91.1	41	95	H
22	237	44	50.0	21	0	H
30	237	45	80.0	34	29	L
2	239	51	90.2	46	0	H
5	239	52	63.5	32	0	H
18	239	41	82.9	33	0	L
20	239	49	63.3	31	0	H
35	239	43	74.4	32	31	H
29	241	40	80.0	30	0	L
17	244	37	89.2	33	0	L
27	245	41	92.7	38	0	L
28	246	49	87.8	43	0	L
13	248	49	81.6	40	25	H
11	249	42	26.2	11	100	H
4	254	42	61.9	24	0	H
26		22	0			
33		52	0			

[a] Missing nests were either false, never located, or hatched before egg collection.
[b] Collected hatchlings from early-hatched nests.

Nests were characterized by habitat into high versus low elevations within the impoundments. Mean core temperature of low nests (30.9 ± 0.5 C, $N = 7$) was lower than mean core temperature of high nests (31.7 ± 0.4 C, $N = 17$) ($Z = 2.51$, $P = 0.012$). More females were produced in low nests (4% M) relative to high nests (29% M) ($Z = 1.77$, $P = 0.0761$). In contrast, nests that produced greater than 50% M ($N = 4$) were associated with relatively warm temperatures (31–34 C) and high elevations (Table 1). These nests tended to hatch earlier and had high survivorship. In this study, sex ratio was not correlated with clutch size.

Discussion

The methodology we followed is simple, inexpensive, and effective. The key elements include: 1) location of a representative sample of nests after egg deposition, ideally in typical habitats, 2) collection of eggs from each clutch after sex is determined (>50 days after eggs laid), but before eggs hatch (60–75 days after eggs are laid), 3) determination of relevant nest parameters, including nest temperature, clutch size, and nest location, 4) incubation, 5) sex determination of all live hatchlings by cloacal inspection of the genitalia, and, if possible, sex determination by examining gonads of all dead young, and 6) release of hatchlings at the original nest site as soon as possible, usually within 2–3 weeks of egg collection.

In our study, we attempted to obtain unbiased sex ratios from naturally-incubated alligator nests. This approach has the advantage of providing direct information about neonatal sex ratios in the area sampled, and eliminates the potential biases associated with assessing sex ratios of older-aged cohorts. In addition, our methodology provides a premise for studying possible correlates of hatchling sex ratios with nest temperature, nest location, climatic factors, etc.

We were able to monitor the sex ratios from 25 naturally-incubated nests by determining the sexes of the resultant hatchlings, and then releasing the young. The overall sex ratio of live hatchlings was female-biased (1M:3.8F or 21% M), and differed from previously reported male-biased ratios of surveyed juveniles and adults (Murphy 1977, Fuller 1981, Wilkinson 1983, Brandt 1991, Kinler and Taylor 1992, Rootes and Chabreck 1992, R. Elsey, pers. commun.). These earlier studies were conducted in different years, locations, and habitats. When incubation conditions vary, there is no *a priori* reason to assume that hatchling sex ratios will be equivalent.

A large majority of alligator nests in South Carolina are located on impoundment dikes or on remnant interior dikes (Rhodes unpubl. data). Ferguson and Joanen (1982, 1983) noted that nests located on levees tended to be hotter and drier than low-marsh nests and produced mostly males. Likewise, we found nests classified as low to be cooler and produce mostly females; nests classified at higher areas in our study produced a higher percentage of males, but were still female-biased overall.

The summer temperature and rainfall values for 1994 near our study site were similar to normals for the previous 30 years. Mean monthly temperatures and precipitation were only slightly higher for the 3 summer months; but precipitation during the month of June, at the beginning of nesting, was more than twice normal monthly value. This may have contributed to higher than average water levels, lower than average nest temperatures, and a relatively high proportion of all-female nests.

In future studies, we plan to employ a 3-part strategy for assessing alligator sex ratios: 1) determine hatchling sex ratios annually from representative nests, 2) correlate sex ratios with nest temperatures and related climatic factors, and 3) monitor the sex ratios of previously sexed, known-aged cohorts of juvenile alligators in these habitats. Because the outcome depends on the immediate thermal environment of the nest, hatchling sex ratios are likely to vary in different years, locations, and habitats. In order to assess hatchling alligator sex ratios, long-term studies in representative habitats will be required.

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