EFFECTS OF INCUBATION TEMPERATURE ON SEX RATIOS IN PINE SNAKES: DIFFERENTIAL VULNERABILITY OF MALES AND FEMALES

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Sex ratios of vertebrates have important evolutionary consequences in terms of mating systems and parental care, affecting the relative fitness of individuals. Biased sex ratios can occur at fertilization and at different points in the life cycle. Sexual differences in mortality after birth have been documented in a number of species including humans (Wingard 1984), ground squirrels (*Spermophilus beldingi*; Sherman and Morton 1984), sheep, horses, cattle, birds, and fish (Comfort 1979), and insects (MacArthur and Baillie 1932; Deevey and Deevey 1945). Moreover, male mortality is generally greater than female mortality at all stages, resulting in unbalanced sex ratios favoring females (Caswell and Weeks 1986). In addition to sexual differences in mortality after birth, primary sex ratios and differential mortality before birth (or hatching) can affect later sex ratios. In this discussion, we use primary sex ratio as the ratio at fertilization, secondary sex ratio as that at birth (or hatching), and tertiary sex ratio as that at sexual maturity (after Lincoln et al. 1982; Murray 1984).

The mechanisms of sex determination clearly affect primary sex ratios. Sex determination and sex differentiation have been extensively studied in most vertebrate groups (Bacci 1965; Ohno 1967, 1979; Reinboth 1975; Standora and Spotila 1985). Heteromorphic sex chromosomes are common in mammals and birds, less common in reptiles, and uncommon in amphibians and fish (Bull 1980). Presumably the earliest origins of gonochorism involved environmental control, and genotypic sex determination evolved later (Mittwoch 1975).

We examine sex ratios at different stages in the life cycle of a reptile to determine whether an environmental variable such as temperature might affect secondary sex ratios. In reptiles, sex determination occurs by two mechanisms: genetic factors in snakes and in some lizards and turtles (Gorman 1973; Vorontsov 1973; Bull 1985b), and environmental factors such as humidity (Gutzke and Paukstis 1983) and incubation temperatures in some turtles (Yntema 1976, 1979;

Yntema and Mrosovsky 1980; Morreale et al. 1982; Bull 1985*a*; Caillouet and Duronslet 1985), lizards (Charnier 1966), and alligators and crocodiles (Ferguson and Joanen 1982, 1983; Webb and Smith 1984). Even within a group, the mechanism of sex determination varies; for example, some turtles do not exhibit temperature-dependent sex determination (Bull 1985*b*).

In reptiles with temperature-determined sex differentiation, some temperatures produce all males and others produce all females, with a pivotal temperature producing equal sex ratios under laboratory conditions of constant temperature (Yntema and Mrosovsky 1982; Standora and Spotila 1985). Field studies have now confirmed that similar, disparate sex ratios occur in nature with different and varying temperature regimes (daily cycles; Ferguson and Joanen 1982; Bull 1985*a*). Sex ratios in temperature-dependent species are controlled by sex differentiation during development rather than by differential mortality between the sexes (Bull and Vogt 1979). This observation is difficult to test if embryos are aborted early in development when they cannot be effectively sexed.

Most reports indicate that gender in snakes is determined genetically (Vorontsov 1973), with a resultant 50:50 primary and secondary sex ratio (Shine and Bull 1977). Known exceptions are four male-biased secondary sex ratios (*Agkistrodon contortrix*, Fitch 1960*a*; *Elaphe quadrivirgata*, Fukada 1960; *Notechis scutatus*, Shine and Bull 1977; *Pituophis melanoleucus*, Gutzke et al. 1985), and one female-biased secondary sex ratio (*E. climacophora*, Fukada 1956). Gutzke et al. (1985) found a higher proportion of males in both hatchlings and adults over a 2-yr period. Because some mortality resulted from the range of laboratory incubation temperatures, they excluded clutches with any mortality from their sex-ratio analysis. By excluding clutches suffering mortality, they may have missed differential pre-hatching mortality. Furthermore, the biases in secondary sex ratios reported above may be a result of pre-hatching mortality, rather than biased primary sex ratios. Biased adult sex ratios occurred in 19 colubrid and viperid species; males were favored in populations of 9 species; females in 9; and in one species, for which data on two populations are available, a different sex was favored in each population (Parker and Plummer 1987).

Given the differential effect of incubation temperature on sex determination in some reptiles, decreased hatching rates at temperature extremes, and differential environmental effects on subsequent mortality of the sexes, we hypothesized that developmental temperature might effect mortality of both sexes during development. In the present study, we attempt to determine whether there was an additional sexual difference in mortality as a function of incubation temperature. Sex ratios could be equal or biased at fertilization, followed by equal or differential mortality during development and the subsequent life of the animal. The primary sex ratio and subsequent mortality rates determine the sex ratio at any stage in the life cycle. Here we examine primary sex ratios (insofar as possible), differential mortality during development, and sex ratios in a population of pine snakes (*P. melanoleucus*).

The primary sex ratio could be equal and followed by equal mortality (fig. 1, *top*, line A) or by differential mortality (fig. 1, *top*, line B), or pine snakes could
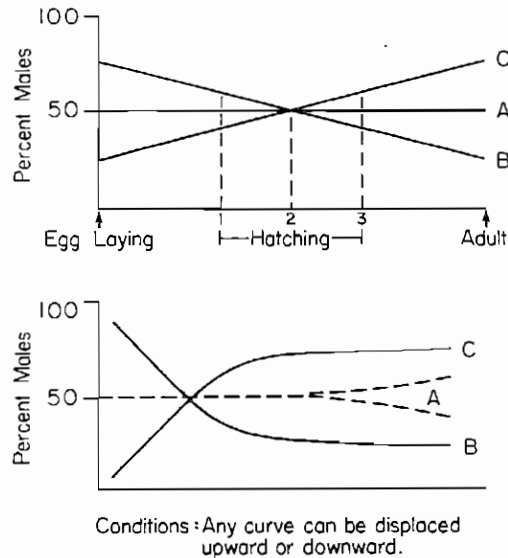
FIG. 1.—Schematic for sex ratios when primary sex ratio is at unity followed by equal mortality (*top*, line A) or unequal mortality (*top*, lines B or C). *Bottom*, Sex ratios when primary sex ratios are biased (lines B, C) followed by unequal mortality. The secondary sex ratio depends on the time of hatching.

have an unequal primary sex ratio followed by differential mortality (fig. 1, *bottom*, lines B and C). Depending on when hatching occurred, sex ratios at hatching could be biased toward one sex (fig. 1, *top*, lines 1 or 3) or equal (fig. 1, *top*, line 2). Unequal primary sex ratios followed by equal mortality would simply move line A up or down (fig. 1, *top*). If the relationships are not linear, the curves may resemble those at the bottom of figure I, but the resulting unequal sex ratios still remain.

Using a combination of experiments and observations of wild snakes, we examine the relationship of incubation temperature and sex ratios in pine snakes. We studied sex ratios of pine snakes in four groups: (1) adults in nature over an 11-yr period; (2) hatchlings in nature over a 4-yr period; (3) hatchlings from broods hatched in a laboratory at ambient temperatures (daily cycle of temperatures revolving around 24°–30°C); and (4) hatchlings from broods hatched under controlled constant temperatures of 21°, 23°, 26°, 28°, 30°, 32°, and 33°C.

METHODS

Pine snakes were studied in Ocean, Atlantic, and Burlington counties, New Jersey, from 1976 to 1986. Populations in the three counties were separated from each other by 20 to 80 km. Individual populations inhabited areas of about 80–90 ha. Suitable habitat was searched three to five times a week from early April until late November. Searching consisted of turning over logs and other debris, and searching known hibernacula and nesting sites. Snakes were sexed by hemipenes

eversion or tail size and length (see Schaefer 1934; Fitch 1960b; Gregory 1983). Snakes were counted in the sample only when they were first located.

From 1983 to 1986 the fates of 12 clutches in natural nests were followed, and the ambient temperatures were recorded either initially (1–3 days after egg laying), weekly, or continuously. For all three years, ambient temperatures were taken 1–3 days after egg laying. From 1978 to 1986, 37 clutches of pine snake eggs were collected immediately after oviposition and incubated at ambient temperatures (24°–30°C with normal daily cycles) in a laboratory. Following hatching, all snakes were sexed by eversion of hemipenes.

From 1984 to 1986, 68 clutches were incubated at constant, controlled temperatures. In 1984, 11 clutches were incubated at 21°, 26°, and 30°C; in 1985, 30 clutches were incubated at 22°, 28°, and 32°C; and in 1986, 27 clutches were incubated at 23°, 28°, and 33°C ($\pm$ 1°C for all treatments). All clutches were from known females, divided into three equal groups of eggs, and placed at each of the three temperatures each year. Eggs from a given clutch were incubated in individual plastic boxes (16 × 30 × 9 cm; Packard et al. 1981); eggs were placed on damp sand and covered with moist sphagnum moss. Eggs were checked every other day to ensure proper humidity and to assess mortality. To ensure constant hydric conditions (that is, wet sand, moist sphagnum, and adequate humidity), each box was provided with a mixture of equivalent amounts of dry sand and water, and equal amounts of water were periodically added to each cage. Although this procedure would produce some variation in hydric conditions, this variation should not be critical since Gutzke (pers. comm.) reported no evidence that hydric conditions over a wide range affect sex determination in this species. The hydric environment does not affect hatching success in the conspecific bull snake, but eggs in moist or wet conditions produce larger hatchlings than those in dry conditions (Gutzke and Packard 1987). Whenever an embryo died (egg moldy or discolored), it was dissected and sexed if possible. Hatchlings were placed in individual boxes following hatching and were sexed by hemipenes eversion. Infertile eggs (or embryos that died early in development), identified by candling or dissection, were removed from the sample.

We then examined sex ratios (1) in pine snakes that were wild-caught, by year and size; (2) in hatchlings from nests incubated in the wild, by year and incubation temperature; (3) in hatchlings from clutches incubated in the laboratory under controlled cycling temperatures, by year and temperature; and (4) in hatchlings from eggs incubated in the laboratory under controlled, constant temperatures, by year and temperature. Sex ratios were determined by dividing the number of males by the number of females. Comparisons among groups were made with Kruskal-Wallis χ^2 tests. (Unless otherwise noted, we give means $\pm$ one standard deviation.)

RESULTS

Pine Snakes in Nature

Sex ratios of wild-caught pine snakes varied by year (table 1). In most years, the sex ratios were skewed toward females or were at unity. For all years combined,

TABLE 1

Sex Ratios (males:females) for Pine Snakes Captured in the New Jersey Pine Barrens, 1976–1986

		No. of Females		Sex Ratio	
Year	Males	Non-Gravid	Gravid	Non-Gravid Snakes	All Female Snakes
1976	0	1	—	—	—
1977	3	5	0	0.60	0.60
1978	6	17	10	0.35	0.22
1979	8	8	1	1.00	0.88
1980	1	1	2	1.00	0.33
1981	6	10	25	0.60	0.17
1982	8	7	7	1.14	0.57
1983	3	5	1	0.60	0.50
1984	3	3	3	1.00	0.50
1985	2	3	7	0.67	0.20
1986	12	19	21	0.63	0.30
TOTAL	52	79	55	0.66	0.39

Note.—The snakes are active ones located from May through October. For all years combined, the same percentages of both sexes were captured in May, when they might be mating (about 30%), and July (about 25%), when females are nesting during the breeding and egg-laying periods (Burger and Zappalorti, unpubl. data).

TABLE 2

Sex Ratios (males:females) for Pine Snakes by Size Class in Nature in New Jersey

	All Snakes Encountered					Non-Gravid Snakes		
Snout-Vent Length (cm)	N	%	Sex Ratio	χ^2	P	Sex Ratio	χ^2	P
25–85	6	11	0.78		NS	0.78		NS
86–105	18	35	0.23	18.7	0.001	0.30	11.3	0.001
106–125	20	39	0.54	5.7	0.02	1.36		NS
Over 125	8	15	0.40	4.6	0.05	0.46	3.2	NS

sex ratios (excluding gravid females) were skewed toward females ($\chi^2 = 5.2$, df $= 1$, $P < 0.02$). When all gravid females (most females found in June and early July) were included, the sex ratios were more dramatically skewed in favor of females in every year and for all years combined ($\chi^2 = 23.9$, df $= 1$, $P < 0.001$).

Sex ratios also varied by size class, with more females in all size classes over 86 cm when all snakes are considered and more females in the 86–105-cm size class when gravid females are eliminated (table 2). A disparate sex ratio in the largest-size class suggests either that adult females are larger or that fewer males live as long to attain fully adult lengths.

Hatchlings Incubated in Nature

Nest temperatures varied in nature during the 3 yr of nest monitoring. For pine snakes hatched in nature, sex ratios were at unity for nests incubated at a mean

TABLE 3

SEX RATIOS (MALE:FEMALE) OF HATCHLING PINE SNAKES INCUBATED IN THEIR OWN NESTS AND IN THE LABORATORY WITH DAILY TEMPERATURES CYCLING AROUND THE MEAN ($\pm$ 3° C)

Incubation Temperature (°C)*	Year	No. of Clutches	No. of Eggs	Sex Ratio
In nature				
27.1 ± 2.6	1986	7	57	1.10
28 ± 2.2	1983–1984	3	21	1.10
32.1 ± 2.5	1985	2	19	2.80
In laboratory				
24	1986	2	18	0.50
25	1983	2	22	0.57
25	1986	9	90	1.20
26	1984	3	42	0.68
28	1978	8	82	1.00
28	1986	5	28	1.15
28	1985	3	25	1.78
30	1986	5	31	1.21

* For all cases, the temperature cycled around the average temperatures given here. In nature, the temperature for 1983–1984 was based on mean temperatures from one measurement in each of the 37 nests. For 1985, in nature, the temperature was based on mean temperatures from these two nests recorded 18 times per nest during incubation (the mean ± standard deviation is given). (The temperatures were taken by the method described for 1983–1984, 33.3° ± 2.4°C.) For 1986, it was based on mean (± standard deviation) temperatures from seven nests from continuously recording thermometers. (Temperatures were taken by the method described for 1983–1984, 27.6° ± 2.4°C.)

temperature of 27°–28°C and were slightly biased toward males for nests incubated at 32°C (table 3).

For most natural nests, all embryos that developed hatched. However, in 1986, late August and September were cooler than usual, and six fully developed embryos, five of which were male, failed to hatch (sign test, $P < 0.05$). When these eggs were dissected, they contained fully formed snakes with full color patterns. These eggs were in late clutches that did not hatch until late September and experienced unseasonably cold and rainy conditions in August and September.

Hatchlings Incubated in the Laboratory

Eggs were incubated in the laboratory under cycling and under constant temperatures (tables 3, 4). Under cycling incubation temperatures, hatchling sex ratios were related to incubation temperature, with male-biased sex ratios increasing with increasing mean temperatures (Kendall's $\tau = 0.55$, $N = 34$, $P < 0.001$; table 3, fig. 2).

At constant, controlled temperatures no eggs hatched when maintained at 21°C. However, when the temperature of eggs incubated at 21°C was raised to 23°C at 70 days, the sex ratio of hatchlings was biased toward females ($\chi^2 = 5.4$, df = 1, $P < 0.02$). At other temperatures, the sex ratios of all embryos (hatchlings and those that failed to hatch) were not significantly different from unity (table 4). The percentage of eggs hatching increased with increasing incubation temperature up
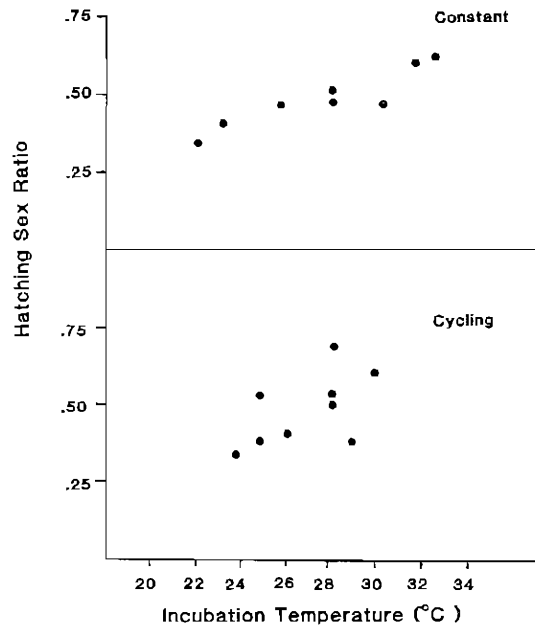
Fig. 2.—Relationship of sex ratio at different incubation temperatures in the laboratory under a cycling regimen and under constant-temperature regimens.

TABLE 4

Effect of Constant, Controlled Incubation Temperature on Hatching Rate, Incubation Period (mean ± standard deviation), and Sex Ratio of Pine Snakes

Temperature (°C)	Year	No. of Eggs	% Hatching	Days until Hatching*	Sex Ratio Hatchlings	Sex Ratio Embryos
21	1984					
Remained at 21		15†	0	—	—	—
Raised to 23‡		19	47	103±8.4	0.11	0.73
23	1985	73	78	96.9±7.2	0.54	0.66
23	1986	66	90	87.6±5.4	0.68	0.57
26	1984	43	87	73±3.4	0.86	0.87
28	1985	73	93	63.8±2.7	1.00	0.97
28	1986	97	99	62.3±3.6	0.96	0.96
30	1984	39	97	60±2.9	0.90	0.95
32	1985	62	77	58.3±6.2	1.40	1.14
33	1986	64	87	54.4±3.6	1.24	1.46

Note.—Infertile eggs were removed from the sample.
* Mean ± one standard deviation.
† Eggs dried before 70 days.
‡ Temperature raised at 70 days.

TABLE 5

DIFFERENTIAL MORTALITY OF PINE SNAKE EMBRYOS
INCUBATED AT CONSTANT TEMPERATURES

TEMPERATURE (°C)	No. THAT DIED		% OF DEAD EMBRYOS THAT WERE MALES
	Males	Females	
21	7	2	78
22	9	6	60
23	4	0	100
26–30	5	5	50
32	5	9	36
33	1	4	20

to 30°C and began decreasing thereafter (table 4, fig. 2). Incubation period decreased with increasing temperature.

For the embryos that died after sex differentiation, differential mortality by sex occurred (table 5). At low incubation temperatures, more males than females died; and at high incubation temperatures, more females died ($\chi^2 = 7.72$, df $= 2$, $P < 0.02$). In general, the embryos that died were fully developed and had weights and snout-vent lengths that did not differ significantly from those of the hatchlings (χ^2 tests).

DISCUSSION

Differential Mortality and Sex Ratios

In many animals, males suffer higher mortality than females, often resulting in biased sex ratios or differential survival (Comfort 1979). Depending on primary sex ratios, differential mortality of the sexes may result in biased sex ratios with fewer males than females for some part of the life cycle. In this study of pine snakes, sex ratios were at unity or biased toward females for 11 yr for some size classes. This result conforms to published reports for most vertebrates (Caswell and Weeks 1986, see below) and for most snakes (Parker and Plummer 1987). However, we also found that male and female embryos suffered differential mortality as a function of incubation temperature.

Males suffered higher embryonic mortality at low incubation temperatures, and females at high temperatures. Such differential mortality adversely affecting embryos of both sexes has not been reported previously, although differential male mortality has been reported for human fetuses (Ciocco 1940). Differential mortality between fertilization and birth (or hatching) is often difficult to determine anatomically because at fertilization primary or secondary sex characteristics are not evident. For species with clear chromosomal determination of sex, such determinations could be made but have not usually been made for nonhumans. In this study, most embryonic mortality occurred late in development, making it possible to determine sex. Similar determination of sex before hatching would be difficult in most birds (despite contrary reports from the poultry industry), because primary and secondary sexual characteristics are not well developed even at hatching.

The presence of higher embryonic mortality rates for both males and females (at different ends of an environmental gradient) suggests that secondary sex ratios could be skewed in either direction at hatching depending on developmental conditions. Furthermore, sex ratios may be skewed at the latitudinal extremes of the breeding range for a given species. Thus, in the Northern Hemisphere, at northern extremes more females might be produced, and at southern extremes more males.

The mechanism for sex determination in some reptiles and sexual differences in mortality may be related, in that for both there seems to be a pivotal temperature (or more than one temperature) at which one sex or the other is affected. In pine snakes, males suffered higher mortality at low temperatures, and females suffered higher mortality at high temperatures. However, this differential mortality has not been examined in other species. Three patterns are evident in environmental sex determination: (1) females develop at low temperatures, and males at high temperatures; (2) the converse of pattern 1; and (3) females develop at low and high temperatures, and males develop at intermediate ones (review in Head et al. 1987). Similar differences may occur for sexual differences in mortality, but at present our data support the first condition: females suffer lower mortality at low temperatures, and males suffer lower mortality at high temperatures. If the evolutionary reason for environmental sex determination relates to producing less fit males or females at the extremes (see discussion in Webb and Smith 1984), then the results of our study may illustrate some of the characteristics of the less fit sex at different temperatures.

Ferguson and Joanen (1983) demonstrated that in alligators (*Alligator mississippiensis*), females develop at a lower incubation temperature and are heavier than males (that develop at a higher temperature). This weight difference allows females to grow faster in the first year and mature faster sexually, an advantage for this population. Thus, Ferguson and Joanen provided a selective advantage for environmentally determined sex in terms of the Charnov and Bull (1977) model.

For fish, amphibians, and reptiles for which incubation conditions vary depending on conditions of oviposition sites, environmental variables may affect secondary sex ratios. This may be particularly true for fish and amphibians that deposit their eggs in water of varying temperatures. In such species, either sex may suffer higher mortality at the extremes of the temperature gradient. Conceivably, even in homeotherms with a relatively constant fetal environment or incubation regime (in birds and monotremes), slight differences in environmental gradients such as temperature could result in differential mortality of the sexes. This possibility could be tested in birds by varying incubation temperatures and then determining sex ratios at birth using sex chromosomes. Similar effects might result from variable hydric (moisture) conditions (see Gutzke and Packard 1987). Our results provide the first clear example of higher embryonic mortality rates of both males and females (but at different ends of the temperature continuum), suggesting a possible mechanism for different secondary sex ratios in vertebrates.

Sex Ratios in Nature

In this study, sex ratios of wild-caught snakes were either biased toward females or were at unity in every year. In no year did we find more males than

females. Even when gravid females are removed, sex ratios were biased toward females or at unity. These results suggest either that there are indeed more female pine snakes in our Pine Barrens study site or that the method of capture results in a biased sex ratio. Recently. Head et al. (1987) suggested that sustained or global climatic change would severely affect species with environmental sex determination and could effectively extinguish one sex or the other. Although less dramatic, differential mortality of the sexes, as reported in this paper, could likewise have severe results if climates changed.

Several reasons for a bias in capture methods exist: (1) males are less easy to see because they are more cryptic, better hidden, or move less than females; (2) males remain in the denser or wetter areas of the Pine Barrens, outside of our study site; and (3) females are easier to find because they are searching for nesting sites and engaged in nesting. The biased sex ratios are not due to female nesting behavior because the bias remains after gravid females have been removed from the sample. This result is even more dramatic because most males were also located in May and June, when females are searching for sites and laying eggs (Burger and Zappalorti 1988). Thus, to remove females but not males from these months should bias the results toward males (and it does not).

Since males and females are indistinguishable by color pattern, they are equally cryptic. However, males often remain in densely vegetated parts of the Pine Barrens, and they may remain within logs where their presence can be determined only when they are wearing radio transmitters (Burger and Zappalorti 1988). When we radio-tracked pine snakes, we found that particular females were located more often than particular males, suggesting that males move out of the study site and are less easy to locate even when they are in the study site (underground or within logs, making radio transmission difficult). Moreover, when we excavated seven hibernacula, we found a sex ratio of 0.91 for 42 snakes in 1986 (Burger et al., in press) and of 0.74 for 40 snakes in the same hibernacula in 1987 (unpubl. data). However, even using sex ratios from hibernacula is problematic because we found that some had mostly one sex whereas others were evenly mixed.

Our results contrast with those of Gutzke et al. (1985), who reported 95 male and 45 female bull snakes over a 2-yr period (sex ratio of 2.11). They found that 33% of the females were gravid, compared to 41% in the present study. Thus, for non-gravid bull snakes in their study, the sex ratio was even higher (3.17). Since bull snakes and pine snakes are conspecific, although their ranges are discontinuous, we had expected to find similar sex ratios. We suggest that differences in sex ratios among years and localities may relate to differences in incubation temperatures affecting hatching sex ratios (see below).

Sex Ratios of Experimental Clutches

For hatchlings incubated under cycling or constant controlled temperatures, secondary sex ratios were related to incubation temperature. At low temperatures, secondary sex ratios were biased toward females; at high temperatures they were at unity or biased toward males. However, the primary sex ratio (embryos that hatched and those that failed to hatch) did not differ significantly for different temperatures. Thus, incubation temperature does not determine sex in pine

snakes. At low temperatures, some males did not hatch; and at high temperatures, some females did not. When embryos were dissected, they contained partially or fully formed snakes with complete color patterns. There are developmental explanations for the failure of fully formed snakes to cut through the shell, but not for sexual differences in mortality as a function of incubation. Many reptilian embryos die if cooled at the beginning of incubation, or they develop cephalic or other abnormalities (Hubert 1985). High temperatures cause developmental abnormalities in male chick and quail embryos (Stoll 1948; Lutz-Ostertog 1966), and at low temperatures in *Lacerta viridis* endocrine function of the testes weakens (Raynaud and Pieau 1972). Temperature extremes affect inversion of male and female phenotypes in a number of amphibians and reptiles (Raynaud and Pieau 1985). Mortality at the end of development may be attributed to oxygen deficiencies (Yaron 1985), but this does not account for differential mortality of the sexes. Similarly, chronic dehydration and other stresses reduce the ability of hatchlings to absorb yolk during the hatching period (Ewert 1985), but this does not suggest a sexual difference. Joanen and McNease (1977) suggested that increased death at hatching may result from weakened hatchlings (as a result of transporting eggs) or abnormal toughness of the shell and its membrane. Although these are possible explanations for mortality at hatching, they do not account for sexual differences in mortality because all eggs were handled similarly.

Nonetheless, our data suggest that differences in nest temperatures, particularly during the critical pre-hatching period, could result in sexual differences in hatching rates for pine snakes. Such differential hatching could lead to disparate sex ratios in nature among years and among localities. In New Jersey, nest temperatures in early July vary from year to year, providing a potential for differential mortality. Sexual differences in mortality with incubation temperature could result in more males in areas with warmer summer temperatures (perhaps as in Nebraska; Gutzke et al. 1985) and more females where summer incubation temperatures are cooler (perhaps as in New Jersey). Similarly, Tinkle (1961) reported a north-south gradient in the percentage of males in a turtle (*Sternotherus odoratus*) that is consistent with differential mortality of the sexes as a function of incubation temperature. Furthermore, since lower incubation temperatures resulted in lower hatching rates, constraints on incubation rather than winter mortality and winter temperatures may limit the northern range of pine snakes.

The results of this study indicate that (1) determining sex ratios from only captured animals is difficult because of differential movement and behavior; (2) hatching sex ratios, but not primary sex ratios, are affected by incubation temperatures in pine snakes; (3) differential mortality of males and females occurs as a function of incubation temperature in pine snakes; and (4) lower mortality of both sexes in response to different parts of an environmental gradient might be affecting secondary sex ratios in vertebrates exposed to different environmental conditions during development.

SUMMARY

Sex ratios in vertebrates are the result of primary sex ratios and subsequent mortality rates. Mortality rates are generally higher in males, leading to differen-

tial longevity and biased sex ratios. Sex determination by heteromorphic sex chromosomes is common in mammals and birds and less so in other vertebrates. In many reptiles, sex is determined by incubation temperature, although in snakes, sex is determined genetically. Snakes are usually believed to have a male:female primary sex ratio of unity, although a male-biased sex ratio has recently been reported for hatchlings and adults.

Here, sex ratios from 134 adult pine snakes observed over an 11-yr period in nature indicate a 0.39 sex ratio for all snakes and 0.66 for all non-gravid snakes. By size class (generally indicative of age), ratios varied (from smallest to largest snakes) from 0.78, 0.23, 0.54, to 0.40. For 37 clutches incubated at room temperature (cycling temperatures), the sex ratio varied yearly from 0.50 to 1.78. Under controlled laboratory conditions, sex ratios of hatchlings varied from 0.11 at an incubation temperature of 21°C to 1.40 at an incubation temperature of 32°C. However, primary sex ratios of all embryos (hatched and unhatched) did not differ significantly from unity at different incubation temperatures. These data indicate differential mortality of males and females during embryonic development as a function of incubation temperatures in pine snakes, potentially resulting in different hatching sex ratios in nature depending on environmental temperatures. These results suggest that biases in secondary or tertiary sex ratios could result from differential effects of an environmental gradient (temperature) on the sexes during development in poikilotherms.

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