SEASONAL AND ONTOGENETIC CHANGES IN THE SEX RATIO OF A POPULATION OF STINKPOTS (KINOSTERNIDAE: *STERNOTHERUS ODORATUS*)

THESIS

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,

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By

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ABSTRACT

SEASONAL AND ONTOGENETIC CHANGES IN THE SEX RATIO OF A POPULATION OF STINKPOTS (KINOSTERNIDAE: STERNOTHERUS ODORATUS)

by

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Current hypotheses suggest turtle populations with biased sex ratios are the result of sampling errors such as improper sex determination, trapping bias, and behavioral differences between the sexes. This study was undertaken to determine if the observed sex ratio of a population of stinkpots (Sternotherus odoratus) was an artifact of sampling biases. A total of 989 (637 males, 352 females) individual stinkpots were captured from 1996 through 2000 at Spring Lake, Hays County, Texas. The overall sex ratio based on all census techniques was significantly male biased (1.8:1; p < 0.001). The sex ratio changed across size classes from a male bias in the smaller classes to a balanced ratio in the largest size classes. Both hoop traps and dip nets used to sample the population produced male biased sex ratios (p < 0.001). Hoop traps were baited with a chicken neck or a mature female stinkpot bimonthly from May 1999 – May 2000 to determine if the biased sex ratio was due to mate searching behaviors by males. The sex ratio deviated from 1:1 during November (p < 0.05) when only 5 males were captured and January (p < 0.001) when an excess of males entered the trap baited with the female. The sex ratio for the entire year for each trap type did not deviate from 1:1. The experimental trapping reinforced the view that the sex ratio in this population is inherently biased and is not an artifact of sampling techniques.

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INTRODUCTION

Turtles are a good model for population studies because they are long-lived animals that can be permanently marked for future identification (Cagle, 1939; Dodd 1997). Several species of aquatic turtles have been studied for long periods of time within the turtle's natural distribution (Bury, 1979; Gibbons, 1990; Ernst *et al.*, 1994).

An overall or actual sex ratio has been defined as the relative proportion of males to females in a population; a functional sex ratio only includes reproductively mature adults. Both types of sex ratios are important parameters of natural populations because of the influence each sex can have on aspects of population dynamics such as intrasexual competition, annual egg productivity, and time spent searching for mates (Gibbons, 1990). Sex ratios were reported for a number of turtle populations in North America (Ernst *et al.*, 1994). Both even (1:1) and unbalanced (skewed towards either sex) sex ratios were recorded in natural turtle populations (Bury, 1979; Gibbons 1990).

Four biotic factors determine the actual sex ratio at any point in time in natural populations of turtles: 1) sex ratio of the hatchlings, 2) differential mortality of the sexes during all phases of the life cycle, 3) differential emigration and immigration, and 4) differential maturation rate (Wilson, 1975; Gibbons, 1990).

The incubation temperature of the egg determines the sex of most turtles (Ernst *et al.*, 1994). Two patterns of temperature-dependent sex determination (TSD) have been discovered in turtles (Bull and Vogt, 1979; Ewert and Nelson, 1991). Most emydine

turtles have a pivotal temperature above which females are produced and below which males are produced (Bull and Vogt, 1979). The second pattern of TSD in turtles has been found in several families including Chelydridae and Kinosteridae; males generally develop at intermediate temperatures and females develop at the extremes (Vogt *et al.*, 1982; Ewert and Nelson, 1991). If the only available nesting sites are situated at one of the temperature extremes an uneven sex ratio in the hatchlings may occur within a population.

Mature turtles exhibit dissimilar behaviors because of the different reproductive strategies associated with each sex (Andersson, 1994; Thomas *et al.*, 1999). Most male turtles actively seek females during the reproductive season. This behavior can increase the probability of males encountering a predator. Female turtles leave the water to lay their eggs during the nesting season, which will expose them to a suite of terrestrial predators, such as raccoons and Red-tailed hawks (Siegel, 1980; Ernst *et al.*, 1994). Either of the above or any other gender-specific behaviors can cause differential mortality between the sexes and alter the functional sex ratio.

Male turtles of some species are more likely to travel farther and move between populations than are female turtles (Gibbons, 1986). This movement should only affect populations on a local level and even sex ratios should be established among these populations over time (Gibbons, 1990). Gibbons (1990) attributed some of the seasonal and annual variations in the sex ratio of four populations of red-eared sliders (*Trachemys scripta*) to differential emigration and immigration between the sexes.

Gibbons (1990) suggested that the most important natural factor influencing a functional sex ratio in turtle populations was differential maturation rates between the

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sexes. The functional sex ratio will favor the earlier maturing sex (Gibbons, 1990). Several species of aquatic turtles exhibit extreme size dimorphisms at maturity (Ernst *et al.*, 1994). The male is generally the smaller, earlier maturing sex. Males of a particular hatchling cohort will mature sooner than the females in that cohort and add more males to the reproductive population, skewing the functional sex ratio of a population.

Although uneven sex ratios do exist in natural turtle populations, a biased sex ratio may also be the result of sampling error by the researcher (Gibbons, 1970). It is important to discriminate between the biological factors that create the actual sex ratio of a population and a sex ratio that simply appears biased because of sampling error. Several studies reported uneven sex ratios only to later find that sampling error caused the observed bias (Gibbons, 1970). Studies which report uneven sex ratios and do not account for sampling errors such as improper sex determination of young turtles, trapping techniques which have known sex biases associated with them, and seasonal differences in the behavior between the sexes (Ream and Ream, 1966; Gibbons, 1983; Dodd, 1997) do not increase the understanding of the population of interest.

Improper sex determination can cause a population to appear to have a biased sex ratio. Turtles generally do not exhibit secondary sex characteristics for several years after hatching, so the sex of relatively small turtles may be difficult to determine and individuals may be assigned to the wrong sex (Gibbons, 1970).

Different methods of turtle trapping have produced significantly different sex ratio estimates. Male painted turtles (*Chrysemys picta*), Eastern mud turtles (*Kinosternon subrubum*), and *T. scripta* were captured in higher proportions to females in aquatic hoop traps (Ream and Ream, 1966; Gibbons, 1983; Gibbons 1990). Female *C. picta* were

captured in basking traps in higher proportions than males (Ream and Ream, 1966). Terrestrial pitfall traps captured higher proportions of female *K. subrubum* (Gibbons, 1983). An even sex ratio was reported when adult *C. picta* were captured by hand or with dip nets (Ream and Ream, 1966).

The first turtle to enter a trap may determine the sex ratio of the entire catch (Cagle and Chaney, 1950). A mature female turtle captured in a hoop trap may be enough of a stimulus to lure males into the trap during the breeding season (Cagle and Chaney, 1950). Frazer *et al.* (1990), successfully lured *C. picta* into hoop traps baited with a mature female and both male and female spotted turtles (*Clemmys guttata*) were captured in traps baited with decoys resembling *C. guttata* (Mansfield *et al.*, 1998). These studies show that sex ratios of turtles taken in traps have the potential to be skewed if other individuals were already present in the trap.

The stinkpot (*Sternotherus odoratus*) (Testudines: Kinosternidae) is a small (carapace length to 137mm), bottom-dwelling, durophagous musk turtle found in slow moving waterways throughout the Eastern and Southern United States (Conant and Collins, 1991; Ernst *et al.*, 1994). Male stinkpots, unlike most turtles, mature at the same age (3 years) regardless of size; females generally mature at carapace lengths of 65 mm, which can take from 2 - 7 years (Tinkle, 1961). The breeding season of *S. odoratus* usually lasts from January through March although some year around breeding was recorded in some populations (Ernst *et al.*, 1994). The nesting season of *S. odoratus* begins in April and extends through July (Ernst *et al.*, 1994).

Sex ratios were established for a number of populations of *Sternotherus odoratus*. Tinkle (1961), Bury (1979), and Gibbons (1990) compiled the ratios and found both even and unbalanced sex ratios. The stinkpot is an excellent model for a sex ratio study because 1) it does not exhibit extreme size differences between the sexes, 2) stinkpots are relatively easy to catch, and 3) the sex of a stinkpot can be determined within a year after hatching (F. Rose, pers. com.) as males have a long, thick tail ending in a terminal hook, which extends past the carapacial rim and females have a short, thin, tail ending in a pointed tip, which does not extend past the carapace (Conant and Collins, 1998; Ernst, *et al.*, 1994). Since the sex of this species can be determined so early in its life span a sex ratio can be monitored throughout its ontogeny.

The objectives of this study were 1) to establish a sex ratio for the population of stinkpots at Aquarena Center, Hays County, Texas, intensively sampled from 1996 – 2000, and 2) to determine if the sex ratio estimate is an intrinsic property of the population or an artifact of sampling error.

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MATERIALS AND METHODS

Spring Lake is an 8.5 ha reservoir fed by approximately 200 artesian springs issuing from the Edwards Aquifer along the Balcones Escarpment in Hays County, Texas (Seaman, 1997). These springs form the headwaters of the San Marcos River. Water at the spring source has low turbidity and a relatively constant temperature $(21 - 22.5^{\circ} \text{ C})$ (Groeger *et al.*, 1997). The lake is approximately 630 m in length from its origin to two artificial spillways. The shoreline on the western side of the lake has steep banks that descend abruptly to water depths of 6.5 m (Aguirre, 1999). The eastern shoreline is curbed in stone and concrete to about 350 m downstream from the main springs, forming the grounds and the walkway for an educational center.

A lentic slough converges with the main lake about 450 m downstream from the springs. The slough receives water from ill-defined spring sources, backflow from the spillways, and during a high rainfall event, from the Sink Creek drainage (Aguirre, 1999). A deep muddy substrate is present throughout the slough. A channel (1.5 - 4.5 m deep) runs along the eastern shore. The slough is flanked by a golf course on both the western and eastern sides and by a softball field near its confluence with the main lake (see Aguirre, 1999 for a thorough description of Spring Lake and Aquarena Center).

In 1994 Southwest Texas State University acquired Aquarena Center and proceeded to implement policies compatible with education and research (Seaman, 1997) and turtle trapping began at Spring Lake in 1996. Spring Lake sustains several

populations of turtles including the stinkpot, red-eared slider, Texas river cooter (*Pseudemys texana*), and the common snapping turtle (*Chelydra serpentina*).

Stinkpots were captured along the periphery of the lake by dip nets, hoop traps (usually baited with raw chicken or fish), and they were captured with each method at least once a month throughout the 4-year study with more extensive trapping during the spring and summer. Some individuals were gathered by hand from a canoe in both the main lake and the slough. Each individual received unique carapacial notches. Straightline carapace lengths (CL), carapace widths (CW), plastron lengths (PL) were recorded along with the weight and sex for each individual.

The mean straight-line measurements for each sex were compared using t-tests to determine if one of the sexes was larger. The critical statistical values were from Draper and Smith (1998). The population sample was divided into eight 10 mm size classes from 30 mm – 120 mm based on the carapace length. The largest size class was labeled 100 - 120 mm but the largest turtle was 111 mm. Sex ratios were established for each size class and for the entire population sampled. Sex ratios were tested for deviations from a 1:1 ratio with Chi square ($\alpha = 0.05$). Significance levels throughout are denoted as: * = p < 0.05; ** = p < 0.01; *** p < 0.001).

An experiment was designed to test the hypothesis that males were more likely to be captured in hoop traps because they were searching for females and that this behavior could affect the sex ratio estimate of the population. A series of 10 hoop traps were set bimonthly between May 1999 and May 2000. Five of the traps were baited with a chicken neck and five were baited with a mature female stinkpot. These traps will be referred to as the "food trap" and the "stinkpot trap." Mature females with a CL greater

than 65 mm (Tinkle, 1961) were used to bait the stinkpot trap. The females were collected in areas of the lake or slough that were not used for the experiment. The female was kept in a plastic container inside each hoop trap. A piece of foam rubber was attached to the container with PC • 7 heavy duty, waterproof epoxy paste (Protective Coating Co., Allentown Pennsylvania) so the trap would float. Half-inch holes were drilled into the container below the foam to allow water to circulate.

Traps were set in pairs along the periphery of the lake with at least 10 m between individual traps and at least 30 m between pairs. Trapping sites were not duplicated during the one year study. The traps remained in the water for 72 hours and fresh chicken was placed into each food trap every morning. The traps were checked at least three times a day. When turtles were captured the standard measurements (CL, CW, PL, weight, and sex) were recorded. Unmarked turtles were marked and the turtles were released near the capture site within 24 hours. The number and sex of the stinkpots captured during each trapping period was totaled. Sex ratios were established for each trap type (food or stinkpot) per month and a total sex ratio was recorded for the entire year for each trap. Sex ratios were also established for each month for the total number of stinkpots captured, regardless of the trap type and a total sex ratio was recorded for the entire entire year. The sex ratios were tested for deviations from parity using Chi square ($\alpha = 0.05$).

Hoop traps were set periodically between February 1999 and May 2000. Sex ratios were established per trap per night and a sex ratio was established for the total number of stinkpots captured (the experimental data was not included). Also, these data were used to create a matrix showing the number of instances certain combinations of

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each sex were captured (e.g., the combination of 1 male and 2 females were captured in a trap 9 different times) to determine if males and females were frequently captured together.

Every sex ratio established through trapping was tested for deviations to the sex ratio estimate established for the population using Chi square ($\alpha = 0.05$). These tests were performed to determine if the stinkpots captured by a particular trapping method or trapping period exceeded the proportions observed in the population.

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RESULTS

Between 1996 and 2000, 989 individual stinkpots were captured. The overall sex ratio was 1.8:1 (637 males, 352 females). This ratio deviates significantly different from a 1:1 ($\chi^2 = 82.72$, ***). The sex ratio was not constant across size classes (Figure 1). Between 50 – 89 mm the sex ratio is significantly male biased. Sex ratios of the two smallest size cohorts (30 – 49 mm) and two largest size cohorts (90 – 111 mm) size classes were not different from 1:1.

Males had CL measurements from 34.6 - 108.20 mm; female carapace length varied from 36.7 - 111.2 mm. Females were significantly larger than males in the straight-line measurements (Table 1). The weights were not compared between the sexes.

The sex ratio obtained from both trapping methods (dip net and hoop trap) yielded significantly male biased sex ratios (accurate trapping data were available on 963 individuals) (Table 2).

During the experimental trapping period (May 1999 – May 2000) 52 males and 37 females were captured in baited traps. The estimated sex ratio (1.4:1) did not differ from the theoretical 1:1 ratio ($\chi^2 = 2.22$, p > 0.10), nor the 1.8:1 ratio observed in the overall population ($\chi^2 = 1.41$, p > 0.10). The sex ratio did vary between months during the one-year survey (Figure 2). November and January were the only months in which the sex ratio differed from 1:1. The sex ratio also deviated from the sex ratio estimate



Figure 1. The relationship between stinkpot carapace length and sex ratio (secondary Y axis) of each size class. The line represents the proportion of males in each size class and the numbers associated with the line are the sex ratios for each size class. Sex ratios for each size class were tested for deviations from 1:1.

Measurement	Sex	n	Mean (mm) \pm s.d.	t-statistic	p value
CL	Male	637	86.1 ± 11.6	5.707	***
	Female	352	81.9 ± 10.6		
CW	Male	634	59.6 ± 5.4	16.607	***
	Female	351	53.6 ± 5.5		
PL	Male	633	54.3 ± 6.5	22.482	***
	Female	349	54.6 ± 7.4		
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Table 1. Mean straight line measurements in millimeters (mm) \pm standard deviation (s.d.) of carapace length (CL), carapace width (CW), and plastron length (PL) of *Sternotherus odoratus* captured at Aquarena Center since 1996.

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Table 2. Number of stinkpots captured per trap type. Sex ratios of each trap type were tested for deviations from 1:1. χ^2 = calculated chi square value.

Trap Type Males Captured		Females Captured	χ^2	p value	
Hoop Trap	363	187	56.3	***	
Dip Net	256	157	23.7	***	

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Figure 2. Total number of stinkpots captured during the experimental trapping period (May 1999 – May 2000), regardless of trap type. Sex ratios for each month were tested for deviations from 1:1.

(1.8:1) of the overall population during July and May 2000 when females outnumbered males and in January when the number of males exceeded what was expected from the population.

Fifty-eight turtles (34 males, 24 females) were captured in the food trap during the one-year period. The overall sex ratio of stinkpots for the food trap did not vary significantly from 1:1 ($\chi^2 = 1.72$, p > 0.10) or 1.8:1 ($\chi^2 = .845$, p > 0.10), but it did vary among months (Figure 3). In November no females were captured, therefore the sex ratio estimate of the trap varied from 1:1 ($\chi^2 = 5$, *).

The stinkpot trap yielded 31 (18 males, 13 females) stinkpots during the entire year, which was not significantly different from 1:1 ($\chi^2 = .806$, p > 0.10) or 1.8:1 ($\chi^2 = .543$, p > 0.10). The sex ratio estimate deviated from 1:1 only in January when males were captured in excess ($\chi^2 = 7.36$, **) (Figure 4).

Stinkpots were also captured in hoop traps baited with raw chicken or fish between experimental trapping periods. In 157 non-sequential trap nights between February 1999 and May 2000, 285 (189 males, 96 females) stinkpots were captured. This 1.9:1 sex ratio estimate is significantly different from 1:1 ($\chi^2 = 30.35$, ***) but not from 1.8:1 ($\chi^2 = .456$, p > 0.10). Males were captured without females in a trap 56% of the time, females were captured without males 21% of the time, and males and females were taken together 23% of the time (Figure 5).



Figure 3. Number of stinkpots captured in hoop traps baited with a food source. Sex ratios for each month were tested for deviations from 1:1.



Figure 4. Number of stinkpots taken in hoop traps baited with a mature female stinkpot. Sex ratios for each month were tested for deviations from 1:1.

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of males present in a trap

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Figure 5. Number of instances a specific combination of turtles was caught in a series of hoop traps set from February 1999 through May 2000 (e.g., 61 records of 1 male caught in a trap without a female).

DISCUSSION

The overall sex ratio estimate of the population of *S. odoratus* at Spring lake is male biased with approximately 1.8 males to 1 female. Both trapping methods (hoop trap and dip net) used to sample the population produced male biased sex ratios, which did not differ from the sex ratio estimate of the population. Hoop traps have traditionally captured higher proportions of males (Ream and Ream, 1966; Gibbons, 1983; Gibbons, 1990). Males also were captured in greater proportions in hoop traps at Aquarena Center. Males were also captured in greater proportions to females and more often in hoop traps without females during the supplemental trapping period from February 1999 – May 2000 (Figure 5). Dip nets were shown to capture an even sex ratio in adult painted turtles in Lake Mendota, Wisconsin (Ream and Ream, 1966); while at Aquarena Center, dip nets produced a male biased sex ratio of stinkpots.

The results from the experimental trapping reinforce the male biased sex ratio estimate of the population. Neither trap type (food or stinkpot) produced a sex ratio which deviated from 1:1 for the entire year. The sex ratio of each trap type did vary among months. The sex ratio of the food trap deviated from 1:1 in November when no females and only 5 males were captured (Figure 3). The sex ratio of the stinkpot trap deviated from 1:1 during the breeding season (January) when males were thought to be seeking females for mating purposes (Figure 4). If there is an equal probability of capturing each sex, except during the breeding season when a mature female caught in a

trap was enough of a stimulus for males to enter the trap, then the sex ratio estimate of the population would approximate the actual sex ratio in the population.

The sex ratio changes across size classes. There was an observable ontogenetic shift from a male bias in the smaller (assumed younger) size classes to a balanced ratio in the larger turtles (CL between 90 - 111 mm). Other than sampling error the sex ratio at any given instant in a natural population can only be the result of 1) the sex ratio of the hatchlings, 2) differential emigration and immigration of the sexes, 3) differential mortality of the sexes during all phases of the life cycle, and 4) differential maturation rates (Wilson, 1975; Gibbons, 1990).

The male biased sex ratio in this population might exist in the hatchlings. Male stinkpots develop at 25° C; females develop at both warmer (28° C) and cooler (23.5° C) temperatures (Vogt *et al.*, 1982). The average temperatures during the nesting season from 1980 - 1999 in San Marcos, Texas, are 19.8° C (April); 24.2° C (May); 27.6° C (June); and 29.3° C (July) (Anonymous, 1980 – 1999). Stinkpot nests were found at Aquarena Center relatively close to the water. Most nests were located within 10 - 11 m of the water, but always under dry conditions. Some eggs protruded through the soil surface and were partially covered with topsoil and leaf litter.

Currently, it is not known whether male stinkpots emigrate from Spring Lake. Spring Lake is the headwaters of the San Marcos River, so the decrease in numbers the larger males could be due to individuals leaving the lake and moving down the river to other populations.

Differential mortality could also cause the sex ratio to shift. Since males mature earlier, they will have to face the rigors of being adults sooner than females. Male

stinkpots seek females for reproduction. In this population with fewer females, males may have to travel further distances to find a mate. This behavior could increase the probability of encountering a predator. The probability of intrasexual competition between males for mates is higher in this population because there are fewer females and this could also increase mortality among the males of this population.

From hatching to sexual maturity a turtle can increase 3- to 20-fold in length; once maturity is reached growth slows down (Zug, 1993). Male stinkpots mature several years earlier than females (Tinkle, 1961). Immature females in the same age cohort will continue to grow at this juvenile rate while male growth will slow down, therefore females will increase in size quicker and more females will be in the larger size classes. Also, males in the larger size classes may be considerably older than the females in the same size class. Males may not live as long as females, so fewer males would reach the larger sizes.

The sex ratio estimate of this population was not a result of sampling biases. Several sets of trapping data were used to reinforce the male biased sex ratio estimate. The biased overall sex ratio and the shift across size is a result of the four biological factors acting in concert. Future studies in this population should include: 1) establishing a sex ratio estimate for the hatchlings and determining the sex determining temperatures for stinkpots at Aquarena Center, 2) examining the ages of both sexes of stinkpots in the largest size classes to determine if males are significantly older than females.

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