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Monitoring juveniles across years reveals non-Fisherian sex ratios in a reptile with environmental sex determination

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ABSTRACT

Question: Sampling biases influence the estimation of sex ratio in species with environmental sex determination (ESD). Are these biases the sole reason for the apparent sex ratio in such species?

Methods: Over a 6-year period, we studied maturing painted turtles (*Chrysemys picta*) through mark and recapture in a complex of ponds in northern Virginia. To avoid the biases associated with the measurement of primary and adult sex ratios, we monitored individuals over multiple years and measured sex-specific capture and survival rates.

Results: We observed a significant female bias that cannot be attributed to climatic variance or differential survival, capture, or age at maturity.

Conclusions: The observed female bias cannot be explained by existing evolutionary models and is consistent with a lag in evolutionary response to the loss of male-producing environments resulting from human influences on vegetation cover.

Keywords: environmental sex determination, mark–recapture, sex ratio, turtle.

INTRODUCTION

Fisher (1930) first predicted that population sex ratios should be unity at equilibrium when the cost of producing each sex is the same. The prediction stems from the notion that when individuals of one sex become more numerous in a population, selection will favour production of the minority sex, driving the sex ratio back to 1 : 1. While most animal populations are characterized by even investment in the sexes, biologists have identified conditions that should skew population sex ratios away from 1 : 1, and empirical observations have largely supported these theoretical predictions (West *et al.*, 2002). Because theory predicts that most sex ratios should be 1 : 1, the detection of biased primary population sex ratios is of great interest to researchers studying sex allocation.

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Bull and Charnov (1988, 1989) first characterized the trend towards female-biased primary sex ratios in reptiles and noted that 'the failure of sex-ratio theory to explain reptilian sex ratios stands in sharp contrast to its empirical success in other groups and thus warrants special attention'. The skewed sex ratios of turtles and crocodilians are associated with environmental sex determination (ESD), and extreme female biases, in excess of 90%, have been reported in some species. In reptiles with ESD, temperature is the primary environmental factor influencing nest sex ratios. Low temperatures produce males in most turtles ['pattern 1a' temperature-dependent sex determination (Ewert and Nelson, 1991)], intermediate temperatures produce males in some turtles and nearly all crocodilians ('pattern 2'), and high temperatures produce males in some crocodilians and some lizards ('pattern 1b').

Although sex ratios in animals with ESD have received much attention in empirical studies (Bull and Charnov, 1988; Korpelainen, 1990) and theoretical analyses (Bull and Charnov, 1989; Freedberg and Wade, 2001, 2004), the measurement of accurate primary sex ratios in the field is often confounded by several obstacles. In reptiles, nest sex ratios are generally extrapolated through nest temperature data or by sacrificing a subset of hatchlings emerging from nests. These estimates can be greatly affected by spatial sampling bias because, for instance, unshaded warmer nests may be easier to locate than cooler obscured nests (Mrosovsky and Provancha, 1992). In addition, yearly variation in ambient temperatures can create biased population sex ratios in individual years, even if the mean sex ratio of a population is unbiased. In map turtles, which produce females at warm incubation temperatures, reported female-biased hatchling sex ratios in two separate years (Vogt and Bull, 1984; Bull, 1985) may be attributed to atypically warm temperatures during July (the month during which sex is determined) in both years (NOAA weather records). Similarly, the 11 : 1 (female : male) sex ratio observed in an Illinois population of snapping turtles (*Chelydra serpentina*) in 1999 (Kolbe and Janzen, 2002) occurred in a year in which mean July temperatures were the warmest recorded in Illinois over the last 20 years. The strongly male-biased hatchling sex ratio (16 females : 41 males) collected over a 12-year period for *Chrysemys picta* in Long Island, New York (Zweifel, 1989) can be attributed to broods from 1967 when 21 of 22 hatchlings sexed were males and the mean July temperature recorded in New York City was 1.3°C cooler than average. Although studies of adult sex ratios avoid these issues associated with sampling nests, adult sex ratios are not likely to reflect primary sex ratios due to sex differences in capture ability, migration, age at maturity, and survival (Gibbons, 1990; Lovich and Gibbons, 1990; Steen and Gibbs, 2004). These challenges associated with the accurate measurement of primary sex ratios are applicable to a wide array of taxa characterized by ESD (Korpelainen, 1990).

We studied the sex ratio of juvenile painted turtles (*Chrysemys picta*) to determine whether females are investing equally in the production of male and female offspring as predicted by Fisherian theory. To circumvent the sampling biases associated with measuring nest or adult sex ratios in other studies of ESD animals, we monitored maturing juveniles through mark–recapture over several years. This methodology allows us to avoid sampling biases associated with temporal and spatial variation in nest temperatures. In addition, high recapture rates allowed us to detect any sex differences in survival and capture likelihood, enabling us to more accurately measure the population primary sex ratio.

METHODS

We studied the painted turtle, *Chrysemys picta*, from 1998 to 2003 in a multi-pond mark–recapture programme at Blandy Experimental Farm in northern Virginia (78°3′40″W, 39°5′40″N). *Chrysemys picta* is a small, aquatic turtle found throughout much of the United States and southern Canada. This species exhibits temperature-dependent sex determination with only males produced at incubation temperatures below 27°C and females produced at incubation temperatures above 30°C (Ewert and Nelson, 1991).

The study area consisted of several ponds within a single, easterly flowing drainage in an agricultural landscape (see Bowne, 2002, for detailed site description). The number of ponds varied annually with rainfall from a maximum of nine ponds in 1998 and 2003 to a minimum of two ponds in 2002. This pond complex has existed since at least 1937. We captured turtles with hoop traps (Nylon Net Company, Memphis, TN) from mid-April to mid-September of each year, except for 2002. In 1998, we set traps every week for two consecutive days. In 1999, 2000, 2001, and 2003, we set traps every two weeks for three consecutive days. In 2002, trapping was restricted to two three-day sessions in June. The capture effort over this 6-year study consisted of approximately 4500 trap nights (annual mean ± standard deviation = 750 ± 412).

Upon capture of each individual, we measured the length of the plastron, right third foreclaw, and precloacal tail with dial calipers and weighed each turtle with a Pesola hanging scale. We also recorded sex and the number of annuli. Every turtle was given a unique identification code by filing marginal scutes and was classified into one of five age-sex classes (adult male, adult female, subadult male, subadult female, and juvenile). A turtle was restricted to a single age-sex class per year but could change classes between years. Males were differentiated from females by a longer precloacal tail, longer foreclaws, and a less domed carapace. Individual males with third right foreclaw length of ≥10 mm were considered adult males (Frazer *et al.*, 1993). Females whose plastron length exceeded 118 mm were scored as adult females. We based this criterion on the size of the smallest gravid turtle detected by X-ray of 112 females at Blandy Experimental Farm (D.R. Bowne, unpublished data). Subadults of both sexes possessed at least three annuli. We considered turtles to be juveniles if they had two or fewer annuli.

Population parameters

Bowne (2002, 2003) established that movement between ponds by each age-sex class in this study system was frequent enough to treat all of the distinct ponds as one demographic population. We therefore pooled capture data across ponds and among trapping sessions within a single year. For each individual turtle, we recorded its age-sex class and whether it was captured or not captured in a given year. By recapturing a marked juvenile in subsequent years, we were able to follow its maturation and determine its sex.

We estimated probabilities of capture (p) and survival (ϕ) for populations of juveniles and adults of each sex using the Cormack-Jolly-Seber (CJS) model for open populations (Cormack, 1964; Jolly, 1965; Seber, 1965) in the program MARK 4.2 (White and Burnham, 1999). In the CJS model, survival and capture rates are estimated by following the fates of individual animals released previously (Nichols *et al.*, 2000). Two groups of juveniles exist, those whose gender was determined when they were recaptured as subadults or adults and those whose gender was not determined because they were not recaptured at a sexable age. We were most interested in detecting differences in survivorship and capture probability between the sexes, so only those individuals that were recaptured and sexed were included in the analysis. The analyses for juveniles and adults are designed to detect any changes in survivability.

Table 1. Constructed models for the juvenile-adult turtle data set ranked from best to worst

Model	AIC_c	ΔAIC_c	No. of parameters	Deviance	Bootstrap GOF *P*-value	ĉ (± 1 standard error)
1. $\phi_{tj} p_{tj} \phi_{ta} p_{ta}$	1708.32	0.00	20	166.37	0.12	1.14 (0.03)
2. $\phi_{cj} p_{tj} \phi_{ca} p_{ta}$	1709.13	0.81	12	179.52	0.06	1.11 (0.03)
3. $\phi_{t.} p_{t.} \phi_{ta} p_{ta}$	1719.28	10.96	30	158.56	0.15	1.09 (0.03)
4. $\phi_{t.} p_{t.}$ (Global)	1726.22	17.90	40	146.42	0.12	1.15 (0.04)
5. $\phi_{tj} p_{cj} \phi_{ta} p_{ca}$	1855.00	146.68	12	325.39	—	—
6. $\phi_{cj} p_{cj} \phi_{ca} p_{ca}$	1912.96	204.64	4	399.59	—	—

Note: Survival (ϕ) and recapture (p) probabilities are modelled as either time-dependent (t) or time held constant (c) for each of the four age-sex classes (.), juveniles (j), or adults (a).
AIC_c = a second-order version of Akaike's information criterion. ΔAIC_c = difference in AIC_c from lowest value (the best model) to the one of interest. GOF = goodness of fit. ĉ = overdispersion parameter.

We constructed and analysed six biologically meaningful models for the juvenile and adult data set (Table 1, Appendix 1). The fit of data to each CJS model was analysed using a bootstrap goodness-of-fit test with a run of 100 simulations in MARK (White and Burnham, 1999). To further test for overdispersion, we estimated the overdispersion parameter, c, for the global model by the median ĉ procedure in MARK 4.2 (White and Burnham, 1999). We selected the most parsimonious model using AIC_c, a second-order version of Akaike's information criterion (AIC) (Sugiura, 1978; Burnham and Anderson, 2002). The lowest AIC_c value that differed by at least 2 from the next lowest was considered the best model (Burnham and Anderson, 2002).

We determined age at maturation by monitoring first- and second-year juveniles until they reached sexual maturity. Using a *t*-test, we analysed sex differences in age at maturity for 17 females and 10 males that were monitored from juveniles to sexually mature adults during the course of study. We obtained maximum July temperature data for Blandy Experimental Farm for each year since 1984, the year such data were first recorded. Daily temperature in July has been shown to be a crucial variable in determining hatchling sex ratio (Vogt and Bull, 1984; Janzen, 1994). Formally incorporating yearly temperature into our analysis was not possible because of the composite nature of the juvenile age class.

RESULTS

The juvenile sex ratio was significantly female-biased. In every year (except the one when only six juveniles were sexed), female juveniles new to the population were more numerous than males, with an overall sex ratio (2.5 : 1) significantly different from 1 (d.f. = 1, $\chi^2 = 9.95$, $P = 0.0016$) (Table 2). Among new adults captured between 1998 and 2003, the sex ratio did not differ significantly from even (d.f. = 1, $\chi^2 = 0.95$, $P = 0.33$) (Table 1).

The global model (model 4) was one in which a survival and recapture probability parameter was estimated for male and female juveniles and adults at each time period. Three reduced models were far superior to the global model, and were a good fit (Table 1). Models 1 and 2 (Table 1) were approximately equivalent (i.e. changes in the AIC_c values

Table 2. Turtles captured in each year

Stage	Sex	1998	1999	2000	2001	2002	2003	Total
Juveniles[a]	Male	13	8	4	4	0	—	29
	Female	22	27	21	2	1	—	73
Adults[b]	Male	78	24	12	20	10	21	165
	Female	85	34	16	13	0	43	191

[a] Previously unmarked turtles that were originally captured as 1- and 2-year-old juveniles and subsequently recaptured as sexable subadults or adults.

[b] Includes previously unmarked adults as well as turtles originally captured as juveniles and subadults that had become sexually mature over the course of the study.

Table 3. Results for model 1 (see Table 1), in which the survival (ϕ) and recapture probabilities (p) for combined male and female juveniles and combined male and female adults are allowed to vary for each sampling period

Parameter	Age class	Sampling period	Estimate	Standard error	95% confidence interval	
					Lower	Upper
ϕ	juvenile	1998–1999	1.00	0.00	1.00	1.00
ϕ	juvenile	1999–2000	0.97	0.02	0.89	0.99
ϕ	juvenile	2000–2001	0.91	0.03	0.83	0.96
ϕ	juvenile	2001–2002	0.87	0.07	0.67	0.96
ϕ	juvenile	2002–2003	0.86	0.00	0.86	0.86
p	juvenile	1999	0.60	0.08	0.43	0.75
p	juvenile	2000	0.97	0.02	0.88	0.99
p	juvenile	2001	0.94	0.03	0.85	0.98
p	juvenile	2002	0.50	0.07	0.38	0.63
p	juvenile	2003	0.85	0.00	0.85	0.85
ϕ	adult	1998–1999	0.91	0.03	0.84	0.95
ϕ	adult	1999–2000	0.82	0.03	0.76	0.87
ϕ	adult	2000–2001	0.88	0.03	0.82	0.93
ϕ	adult	2001–2002	0.78	0.06	0.64	0.87
ϕ	adult	2002–2003	0.81	70.98	0.00	1.00
p	adult	1999	0.82	0.04	0.74	0.88
p	adult	2000	0.91	0.02	0.85	0.94
p	adult	2001	0.95	0.02	0.88	0.98
p	adult	2002	0.43	0.05	0.34	0.53
p	adult	2003	0.81	70.97	0.00	1.00

were less than 2), but model 1 had the lowest $\mathrm{AIC_c}$ and we thus examined this model in greater detail (Table 3). In model 1, the sexes of both age classes were combined when estimating survival and recapture probabilities, but allowed to vary across sampling periods. In model 3 (Table 4), male and female juveniles were modelled separately for survivorship and recapture probability, but the sexes for adults were combined. Model 1 was superior to models 3 and 4 (i.e. changes in the $\mathrm{AIC_c}$ values were greater than 2; Tables 1 and 4),

Table 4. Results for model 3 (see Table 1), in which the survival (ϕ) and recapture probabilities (p) for male and female juveniles and combined male and female adults are allowed to vary for each sampling period

Parameter	Age class	Sampling period	Estimate	Standard error	95% confidence interval	
					Lower	Upper
ϕ	juvenile female	1998–1999	1.00	0.00	1.00	1.00
ϕ	juvenile female	1999–2000	0.98	0.02	0.84	0.99
ϕ	juvenile female	2000–2001	0.87	0.04	0.76	0.93
ϕ	juvenile female	2001–2002	0.88	0.08	0.62	0.97
ϕ	juvenile female	2002–2003	0.84	45.11	0.00	1.00
ϕ	juvenile male	1998–1999	1.00	0.00	1.00	1.00
ϕ	juvenile male	1999–2000	0.95	0.05	0.72	0.99
ϕ	juvenile male	2000–2001	1.00	0.00	1.00	1.00
ϕ	juvenile male	2001–2002	0.85	0.12	0.46	0.97
ϕ	juvenile male	2002–2003	0.89	46.22	0.00	1.00
p	juvenile female	1999	0.59	0.10	0.38	0.77
p	juvenile female	2000	0.97	0.02	0.85	0.99
p	juvenile female	2001	0.95	0.03	0.84	0.99
p	juvenile female	2002	0.54	0.08	0.39	0.69
p	juvenile female	2003	0.84	45.00	0.00	1.00
p	juvenile male	1999	0.61	0.13	0.34	0.83
p	juvenile male	2000	0.95	0.05	0.71	0.99
p	juvenile male	2001	0.92	0.06	0.72	0.98
p	juvenile male	2002	0.42	0.11	0.22	0.64
p	juvenile male	2003	0.89	46.22	0.0	1.00
ϕ	adult	1998–1999	0.91	0.03	0.84	0.95
ϕ	adult	1999–2000	0.82	0.03	0.76	0.87
ϕ	adult	2000–2001	0.88	0.03	0.82	0.93
ϕ	adult	2001–2002	0.78	0.06	0.64	0.87
ϕ	adult	2002–2003	0.81	70.98	0.00	1.00
p	adult	1999	0.82	0.04	0.74	0.88
p	adult	2000	0.91	0.02	0.85	0.94
p	adult	2001	0.95	0.02	0.88	0.98
p	adult	2002	0.43	0.05	0.34	0.53
p	adult	2003	0.81	70.97	0.00	1.00

demonstrating that survivorship and capture probability did not differ significantly between the sexes for both juveniles and adults. Juveniles had lower recapture probabilities than adults, but slightly higher survivorship (Tables 3 and 4). This counter-intuitive finding of higher survivorship in juveniles than adults is most likely an artifact of our model only including those juveniles that survived to be sexed. The annual variability in recapture probability for both age classes probably resulted from the change in spatial dispersion of individuals as a response to pond drying and differing trapping effort.

There was a significant difference in age at maturity between the sexes, with females maturing a full year later (5.9 ± 0.9 years vs. 4.9 ± 1.2 years, $t_{25} = 2.57$, $P = 0.017$). The mean maximum daily July temperature over the years that produced the juveniles in this study was consistent with the mean maximum daily July temperature from 1984 to 2003 (Fig. 1).

DISCUSSION

Our results demonstrate a strong and significant female bias in a population of painted turtles. Furthermore, we were able to rule out factors that likely cause disagreement between the observed sex ratio and the actual primary sex ratio in other studies of animals with ESD. Specifically, the experimental design allowed us to eliminate sex differences in survival, capture rates, or age at maturity as an explanation for the observed sex ratio. Because we monitored juveniles, we avoided sampling biases associated with surveying nesting sites, and the length of the study and the observed July temperatures allowed us to rule out yearly thermal variation as a cause of the female bias. These results are in contrast to Fisherian expectations, indicating the presence of a factor biasing sex allocation towards females in this population.

The female bias was evident in the juvenile but not the adult sex ratio. This discrepancy can be attributed, in part, to the fact that males reached adulthood at a significantly younger age than females in our population, a factor known to affect adult sex ratios in turtles (Lovich and Gibbons, 1990). Furthermore, several of the adults are likely immigrants from nearby populations, and thus the adult sex ratio observed may not reflect precisely the primary sex ratio in our population. Also, it is important to note the adult sex ratios observed are a combination of many age cohorts at different times in the past, each with a potentially different primary sex ratio. Because *C. picta* can live 40 years (Wilbur, 1975), it is possible that the environmental conditions producing the juvenile cohorts in our study are different than the conditions producing the adults in our study. Environmental effects that influence hatchling sex ratio may be changing, thus shifting the magnitude of the female bias over the last four decades. We cannot rule out the possibility that there have been important changes over the previous decades that gave rise to the adult cohorts we see today.

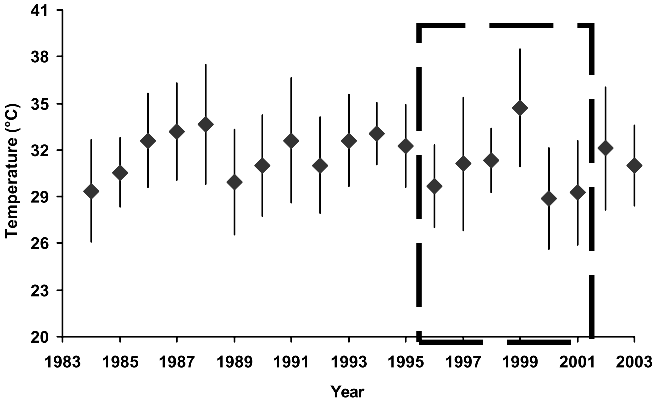

Fig. 1. Mean maximum daily temperature (°C) for July at Blandy Experimental Farm, Virginia. Boxed area indicates years in which the sex of juveniles from our study was determined. Error bars represent ± standard deviation.

There are several factors that may cause disagreement between the juvenile and primary sex ratios in studies of ESD species. Although we found no differences between the sexes in mortality of juvenile turtles, sex-specific mortality before the juvenile stage can affect the sex ratio, particularly when nest sex ratios are correlated with environmental conditions. For instance, sex-biased nest mortality may occur if the nests laid in the environment producing one sex experience a higher rate of predation or overwintering mortality. Neither factor is likely to meaningfully affect the findings in our study, as nest predation rates have been found not to differ between the sexes in *C. picta* (Valenzuela and Janzen, 2001) and overwintering mortality is likely infrequent at our study site due to relatively mild winters. Similarly, while differential embryonic mortality can potentially cause the primary sex ratio to differ from the juvenile sex ratio, a large-scale study of embryonic mortality in natural *C. picta* nests revealed a negligible difference in the mortality rates of nests producing each sex (Valenzuela and Janzen, 2001). There is also little evidence to assume sex-biased hatchling mortality in our study, as Zweifel (1989) reported that mortality of both sexes appears to be about the same in the early years in a long-term study of *C. picta*. Furthermore, Janzen and Morjan (2002) found no significant difference in first-year mortality between the sexes in *C. picta* ($\chi^2 = 1.73$, d.f. = 1, $P = 0.19$). While the existing data do suggest that there is strong agreement between the primary and juvenile sex ratios in our study, the potential for disagreement between primary and juvenile sex ratios does mean that researchers studying ESD species will need to account for phenomena such as sex-specific nest and hatching mortality that could confound meaningful interpretation of juvenile sex ratios.

Female-biased sex ratios under ESD

Primary sex ratios have been well studied in sea turtles, revealing a strong trend towards a female bias (Mrosovsky, 1994; Freedberg and Wade, 2001). The large number of multi-year studies spanning numerous species, geographic locations, and time periods suggests that sampling error is unlikely to explain this strong trend. Similarly, studies of crocodilians reveal a strong pattern of female-biased primary sex ratios (reviewed in Lance *et al.*, 2000; Freedberg and Wade, 2004). Although some researchers have estimated hatchling sex ratios in *C. picta* (Zweiffel, 1989; Janzen, 1994), most sex ratio studies in *C. picta* and other freshwater turtles have focused on adult sex ratios (reviewed in Ernst *et al.*, 1994). A strongly supported pattern of female-biased primary sex ratios is not as apparent in freshwater turtles as it is in sea turtles and crocodilians; however, several studies have reported female biases in freshwater turtles (Brooks and Nancekivell, 1984; Vogt and Bull, 1984; Bull, 1985; Schwarzkopf and Brooks, 1985; Kolbe and Janzen, 2002).

Several factors are predicted to cause female biases in reptiles with ESD. Homing to natal sites has been shown to favour a female bias in ESD species because female-producing nest sites are transmitted to subsequent generations at a higher rate than male-producing sites (Freedberg and Wade, 2001). Most evidence for this model comes from sea turtles, which migrate hundreds to thousands of kilometres to lay their eggs each nesting season. In all sea turtle species studied, significant genetic structure associated with nesting beaches indicates that females home to their natal beaches (reviewed in Bowen, 1995). While there is some evidence for natal homing in a non-migratory freshwater turtle, it is not known whether homing occurs at a scale that would favour female-biased sex ratios (Freedberg *et al.*, 2005). The genetic structuring with respect to nesting areas observed in *C. picta* can be attributed primarily to gametic correlations between offspring from successful nests rather than female homing (Scribner *et al.*, 1993). Similarly, although female *C. picta* show some nesting fidelity, they do so at a scale that is too great to produce repeatability in overstory vegetation (Janzen and Morjan, 2001; Valenzuela and Janzen, 2001). The lack of evidence for homing at a scale that can affect sex ratios in this species suggests it is unlikely that natal homing is responsible for the female bias we observed.

Male–male combat also can favour female-biased sex ratios in ESD species (Freedberg and Wade, 2004). Specifically, male combat and strong sexual selection favour the production of males in optimal developmental environments and females in suboptimal environments, a pattern that is predicted to lead to an overproduction of daughters (Frank and Swingland, 1988; Bull and Charnov, 1989). There is strong evidence in crocodilians and snapping turtles, taxa that exhibit male combat and dominance hierarchies, that male-producing incubation temperatures yield higher fitness. Conversely, this mechanism is unlikely to explain the female-biased sex ratio we observed in *C. picta*. In painted turtles and many other freshwater turtles, females grow considerably larger than males and there is no evidence for male combat or the production of females at sub-optimal temperatures (Berry and Shine, 1980; Freedberg *et al.*, 2001).

These models describe the optimal sex ratio in an evolutionary sense. However, environmental change could also alter the primary sex ratio and that sex ratio could be sub-optimal until the sex-determining system evolves to these altered conditions. Specifically, while Fisherian selection favours a threshold temperature and nesting behaviour that yield an even sex ratio, low effective heritability of these traits produces a lag in the evolutionary response to a skewed population sex ratio (Morjan, 2003). For instance, global climate change can theoretically lead to female-biased sex ratios (Janzen, 1994). However, mean July temperatures in Virginia increased less than 0.01°C between 1895 and 2004 (NOAA weather records, 2005), suggesting it is unlikely that global warming has contributed to the observed female bias.

Human activities can also alter vegetation cover shading nesting sites, limiting the opportunity for the production of offspring of one sex. In many reports of female-biased hatchling sex ratios in freshwater turtles, artificial clearing of vegetation has been noted as a potential factor contributing to warmer nesting environments (Brooks and Nancekivell, 1984; Vogt and Bull, 1984; Bull, 1985; Schwarzkopf and Brooks, 1985). Although the hatchling sex ratio for painted turtles nesting in an altered vegetation habitat in Illinois is unbiased (Janzen, 1994), the area includes both heavily shaded and unshaded locations, and thus it is not clear that the opportunity for production of offspring of either sex has been affected by human activity. The nesting environments in our study have been greatly affected by agricultural practices and are characterized by pastures and mowed fields. The available vegetation shading the nesting areas may thus offer substantially less shade than the flora historically associated with *C. picta* nesting areas, limiting the opportunity for male-producing nests.

This strong sex ratio bias stands in contrast to Fisherian theory, which predicts that a female excess should be accompanied by strong selection favouring the production of male offspring, driving the sex ratio back to unity. It is likely that the female bias we observed is not evolutionarily stable, but instead results from human manipulation of the nesting habitat and a subsequent loss of male-producing nesting locations. This observation adds to a growing body of studies of freshwater turtles where female biases can be attributed to either artificial clearing of vegetation or censusing during atypically warm summers. The trend noted for female biases in ESD reptiles may be more applicable to species with strong natal homing or male combat; a multitude of studies of largely undisturbed habitats spanning multiple years preclude these artificial influences as an explanation for the female excess. Future studies of primary sex ratios in animals with ESD that address these factors can provide valuable insight into the ecological and evolutionary factors shaping sex ratios under ESD.

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APPENDIX 1

We constructed models that were based on the life history of the turtles as well as variability of the study system. Adult and juvenile turtles are expected to differ in their survivorship and probability of capture, and all of the models emphasized this basic difference; juveniles and adults were treated separately. Within an age group, we were interested in differences between the sexes and so constructed models that treated the sexes either together (models 1, 2, 5, and 6) or separately (models 3 and 4). The availability of water, and to a lesser extent trapping effort, differed greatly among years. Consequently, we expected annual variability in survivorship and recapture probabilities. We tested for this variability by allowing each year to be treated individually for both survival and recapture (models 1, 3, and 4), held constant for each (model 6), held constant for survival (model 2), and held constant for recapture (model 5).