Temperature-dependent Sex Determination: Evolutionary Significance and the Adaptive Potential of Sea Turtles to Climate Change

by

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Environmental sex determination (ESD) in sea turtles occurs as temperature-dependent sex determination (TSD), whereby the sex of an individual is determined by external thermal cues. All sea turtles exhibit TSD type Ia, which means that higher temperatures produce females and lower temperatures produce males (Heredero Saura et al. 2022). Sex is determined during the thermosensitive period, when temperature cues induce the production of sex hormones that determine the sex of the embryo (Heredero Saura et al. 2022).

Pinpointing the evolutionary significance of TSD remains a challenge, despite the prevalence of TSD in sea turtles and other amniote vertebrates (Janzen & Phillips 2006). Sexing hatchlings requires sacrifice and histological examination of gonads (Lockley & Eizaguirre 2020); currently, however, six of the seven species of sea turtles are endangered, and three are critically endangered, which means that until alternative sexing methods are possible, our understanding of TSD in sea turtles—its evolutionary significance and the mechanisms for its maintenance—must remain restricted to laboratory incubation studies (Lockley & Eizaguirre 2006; Sea Turtle Conservation Bonaire, n.d.). In this paper, I will review the hypotheses that have been proposed so far for the evolutionary significance of TSD in sea turtles. I will then consider the plausibility of each of these hypotheses, particularly the Charnov-Bull model of differential fitness. I will also consider the risk that global warming poses for temperature-sensitive sea turtle populations around the globe and explore opportunities for future research.

My studies at the University of Chicago trace the effects of climate change around the globe, from the water-stressed regions of Pakistan to the rapidly eroding nesting beaches along the coast of Australia. The topic of this paper is particularly relevant to conversations about how climate change will affect the critical ecosystems on which all life depends. Without sea turtles,

marine ecosystems around the globe risk collapsing entirely; sea turtles are considered keystone species, which means that they play a critical role in the productivity and biodiversity of these ecosystems (The Leatherback Trust, n.d.). The opportunities for future research considered in this paper might inform the policy choices and conservation efforts that could save sea turtles—and thus the ecosystems that sustain millions of communities, human and nonhuman, around the globe—from extinction.

Evolutionary significance of TSD

The evolutionary significance of TSD in sea turtles remains elusive. Four hypotheses have been proposed to explain why sea turtles have maintained this process: (i) phylogenetic inertia, (ii) group adaptation, (iii) inbreeding avoidance, and (iv) differential fitness (Shine 1999). The phylogenetic inertia hypothesis proposes that there is no current evolutionary significance of TSD; it is merely an ancestral form of sex determination and is adaptively "neutral" (Shine 1999; Janzen and Phillips 2006). The group adaptation hypothesis states that TSD enables control over sex ratio and thus promotes group fitness. It has been argued, however, that this hypothesis is more of an explanation for sex-ratio bias than an explanation for TSD (Shine 1999). The inbreeding avoidance hypothesis, then, proposes that single-sex clutches—or, in other words, single-sex nests—reduce the risk of inbreeding among siblings.

Limited evidence exists for these three hypotheses. Instead, attempts to explain the evolutionary significance of TSD have mostly revolved around the final hypothesis, the Charnov-Bull model of differential fitness (Janzen and Phillips 2006).

Charnov-Bull Model

The Charnov-Bull model, or the differential fitness hypothesis, proposes that eggs should produce females when incubated at temperatures that promote high fitness for females, whereas eggs should produce males when incubated at temperatures that promote high fitness for males (Charnov & Bull 1977). In other words, according to this hypothesis, TSD produces males and females at their optimal temperatures, thereby maximizing offspring fitness. The hypothesis also assumes that hatchlings enter a "patchy" environment in which (i) there is local competition for mates, (ii) resources have differential value to males and females and are patchy in distribution, and (iii) predation or other sources of mortality are sex dependent (Charnov & Bull 1977).

Warner and Shine (2008) were the first to offer robust empirical support for the Charnov-Bull model. They found that the lifetime reproductive success, or fitness, of jacky dragons was highly correlated with incubation temperature—even after controlling for the confounding effects of sex and incubation temperature on offspring fitness by hormonally manipulating eggs. Warner and Shine incubated jacky dragon eggs at low (23-26°C), intermediate (27-30°C), and high (30-33°C) temperatures. Female jacky dragons are produced at low and high incubation temperatures, whereas both female and male jacky dragons are produced at intermediate incubation temperatures. Half the eggs in each of the three temperature regimes (23°C, 27°C, and 33°C) were treated with an aromatase inhibitor which produced males at all three temperatures. (This hormonal manipulation, the researchers note, had no discernible effect on the morphology or survival of the jacky dragons.) After the eggs hatched, the researchers observed the hatchlings in field enclosures for the next three-and-a-half years, the average lifespan of a jacky dragon. By the end of the study, the researchers found that males hatched from eggs incubated at intermediate, or naturally male-producing, temperatures sired

more offspring than did males hatched at low or high, or naturally *female*-producing, temperatures. They concluded that incubation temperature optimized the lifetime reproductive success of each sex, thus offering convincing evidence for the Charnov-Bull model.

However, the researchers also found that incubation temperature had negligible effect on offspring phenotypes and survival—although they did note that larger individuals, both males and females, produced more offspring than did smaller individuals. In sea turtles, incubation temperature seems to be highly correlated with offspring phenotypes and survivorship (Rivas et al. 2018); thus, the results of Warner and Shine's study should not be extrapolated to other TSD species, including sea turtles. It might be, if the Charnov-Bull model is true, that the functional outcomes initiated by a temperature signal vary among TSD species (Crews et al. 1994).

Indeed, Rivas et al. (2018) linked offspring phenotypes to incubation temperature in leatherback sea turtles. They relocated 48 leatherback clutches to a hatchery, where 24 of them were placed in the shade, and the other 24 were placed in the sun. All 48 clutches were placed at depths of 50, 75, and 90 cm. When the eggs hatched, the researchers determined the fitness of each hatchling by measuring the length and width of its carapace, its crawl speed, and its righting response time, or the time it took the hatchling to return to an upright position after being turned over on its carapace. The researchers found that, at all depths, hatchlings from cooler nests had faster crawl speed and righting response times than hatchlings from warmer nests; hatchlings from cooler nests also tended to be male. The researchers found that mean carapace length, mean carapace width, and mean weight of hatchlings were higher among the shaded clutches than among the unshaded ones. They suggest that morphology and locomotor performance, which seems to be determined by incubation temperature, might predict survivorship in leatherback sea turtles. To what extent the size and speed of a hatchling are robust indicators of its *lifetime*

fitness, however, remains unclear, as the researchers didn't follow the hatchlings throughout their lives. The link—if there is one—between phenotype, performance, and lifetime fitness is difficult to record, and this link has yet to be established in sea turtles (Janzen et al. 2006).

Maternal condition-dependent choice (MCDC) hypothesis

In 1996, Roosenburg proposed a revision of the Charnov-Bull model. The maternal condition-dependent choice (MCDC) hypothesis stipulates that (i) there is variation in maternal effect on offspring, (ii) variation in maternal investment, like nest site choice, differentially affects male and female offspring fitness, and (iii) the mother's reproductive success is determined by how her investment will influence the fitness of her offspring. This hypothesis suggests that TSD is a mechanism by which maternal investment, as opposed to incubation conditions *alone*, can influence offspring fitness.

Roosenburg (1996) tested the MCDC hypothesis in the diamondback terrapin turtle. Synthesizing demographic, observational, and experimental data collected between 1987 and 1992 in the Patuxent River, he found that female terrapins lay eggs that vary considerably between clutches, but not so much *within* clutches. Egg size is a key determinant of hatchling mass; it can determine how soon female terrapins reach sexual maturity, although it may not affect the timing of sexual maturity in male terrapins (Roosenburg 1996). Roosenburg found that females place larger eggs in warmer nests and smaller eggs in cooler nests. He also found that females from larger eggs may reach sexual maturity up to three years earlier than females from smaller eggs. The results of this study suggest that terrapins can discriminate among nest sites and, therefore, influence the sex of their offspring; in other words, egg size and nest site choice, or maternal investment, seemed to differentially affect male and female offspring fitness. The long-term relative benefit of large egg size and faster growth for females versus males, however,

is unclear (Morjan & Janzen 2003). Therefore, although Roosenburg's results offer convincing evidence for variation in maternal investment, the extent to which this variation determines offspring fitness remains vague. It is also unclear the extent to which egg size as a basis for nest site choice can be extrapolated to other TSD species, including sea turtles.

Indeed, Morjan and Janzen (2003) refute the hypothesis that a female manipulates offspring fitness through her investment. The researchers found no evidence that female painted turtles, like female diamondback terrapins, lay larger eggs in warmer nests, or nests with less vegetation cover. Observing a population of nesting painted turtles on the Thomson Causeway, an island in the Mississippi River, from 1995 to 1998, the researchers observed that, although mean egg size varied considerably, females laid eggs randomly in terms of vegetation cover. The researchers also looked for a potential correlation between egg size and nest temperature but found no such correlation. Thus, they concluded that egg size did not determine nest site choice for female painted turtles, although this correlation was observed in female diamondback terrapins (Roosenburg 1996). The researchers note, however, that in painted turtles, compared to diamondback terrapin turtles, larger egg size might not have a discernable advantage for one sex over the other. Indeed, the long-term benefit of large egg size for female painted turtles remains unclear; and regardless of which sex might be expected to benefit most from a larger egg size, females in this study did not match egg size to nest temperatures, which calls into question the generality of egg size as the basis for nest site choice.

It might also be argued that Morjan and Janzen measured the wrong variable, considering that maternal investment can be measured not only by egg size, but also by clutch size, yolk steroid hormones, and other variables (Morjan & Janzen 2003). Morjan and Janzen conclude that they cannot reject the MCDC hypothesis as long as there continues to be the possibility of

alternative mechanisms; thus, their study seems only to reject egg size as the basis of nest site choice for painted turtles, not the MCDC hypothesis itself. Studies suggest that nest site choice patterns in sea turtles, for example, may vary among species or location (Heredero Saura et al. 2022).

Heredero Saura et al. (2022) observed that green turtles tend to nest near previous nests. Monitoring nest sites on the Playa Cabuyal in Costa Rica over eight nesting seasons, they found statistically significant differences between the mean distances of nests placed by a single female and the mean distances of all nests placed by all females, which offers convincing evidence that green turtles do not choose their nest sites randomly. (20% of the females observed placed their nests relatively far from each other, but the researchers propose that spreading out nests could be advantageous for females when conditions in a particular nesting area become suboptimal.) The researchers found that most females preferred to nest under trees, which indirectly reduced the female-biased sex ratio. High temperatures also increase the risk of embryo mortality, so nesting under trees could, as well as reducing the female-biased sex ratio, increase hatchling output and produce more male offspring (Valenzuela et al. 2019). The researchers suggest that tree shading could benefit sea turtle populations in locations where climate change will cause average temperatures to rise (IPCC 2021).

Climate change: adaptive potential of sea turtles

The Intergovernmental Panel on Climate Change predicts that global warming will exceed 2°C during the twenty-first century unless there are "deep reductions" in greenhouse gas emissions (IPCC 2021, 14). If temperature cues determine sex ratios for TSD species, global warming poses a serious risk to sea turtle populations. Rising global temperatures could induce

an overproduction of female offspring, impairing population growth and thus exacerbating the risk of species extinction (Lockley & Eizaguirre 2020).

Jensen et al. (2018) found that green turtle populations are already becoming highly feminized. Using genetic markers and a mixed-stock analysis, they linked green turtles foraging in the Great Barrier Reef to the nesting beach on which they hatched. They found a moderate female sex bias in turtles deriving from the cooler, southern Great Barrier Reef nesting beaches (65-69% female); however, they found an extreme female bias in turtles deriving from the warmer, northern nesting beaches. 99.1% of the juvenile turtles, 99.8% of the subadult turtles, and 86.8% of the adult-sized turtles from these beaches were female. The researchers suggest that a single-sex population, or "complete feminization," is possible as global temperatures continue to rise (Jensen et al. 2019, 154).

Valenzuela et al. (2019) studied the effects of thermal fluctuations, predicted to increase with climate change, on painted turtles. They recorded hourly the thermal profile of a natural painted turtle nest in Iowa that produced only males; they then replicated the nest in a laboratory and, as expected, all the eggs produced males (NatMale-IA profile). To simulate the predicted feminizing effect of global warming on TSD species, they added 5˚C to each recorded temperature in the NatMale-IA profile, which produced all females (semiNatFem-IA profile). The researchers then modified both profiles (NatMale-IA and semiNatFem-IA) by increasing the temperature variance of each by ±2˚C, ±4˚C, and ±6˚C. They found that smaller fluctuations of ±2˚C and ±4˚C in both the male-producing (NatMale-IA) and female-producing (semiNatFem-IA profile) profiles had negligible effect on sex ratios. Wider fluctuations of ±6˚C, however, feminized all eggs in the male-producing profile, whereas eggs in the female-producing profile remained seemingly unaffected and produced, as expected, all females. Stretching both

profiles by $\pm6°C$, the researchers found, caused unidirectional sex reversal or, in other words, feminization and not masculinization. The researchers then tested whether adding $\pm6°C$ to natural nest profiles that produced all females would also lack sex reversal. They recorded the thermal profile of a natural painted turtle nest from the Nebraska Sandhills and another nest from the Algonquin Provincial Park in Ontario, both of which produced only females. The $\pm6°C$ modification was then applied to both thermal profiles in the laboratory. The researchers found considerable interpopulation differences in response to identical incubation conditions; the modified Nebraska profile (NatFem-NE $\pm6°C$) continued to produce all females, whereas the modified Ontario profile (NatFem-ON $\pm6°C$) induced 100% embryonic mortality.

The results of Valenzuela et al.'s study suggest, with convincing evidence, that increased thermal fluctuations with climate change could accelerate the rate at which TSD populations become entirely feminized. Interpopulation differences in response to identical natural-nest incubation conditions, however, could potentially mitigate this feminizing effect—but only if these differences among populations indicate genetic variation (Valenzuela et al. 2019). This variation, Valenzuela et al. propose, might enable the survival of some populations of TSD turtles who can produce males even at higher average global temperatures. However, to what extent the effects observed in this study, and therefore the possible adaptations to climate change observed, are taxonomically widespread remain unclear.

Heredero Saura et al. (2022) suggest that, among female green turtles, the preference for nesting in shaded areas could moderate the feminizing effect of global warming if the temperature of these shaded areas remains within the naturally male-producing range. Green turtles tend to nest under trees, where their nests are shaded, as opposed to other species like leatherback and olive ridley turtles who tend to nest in the sun (Heredero Saura et al. 2022). It

has been suggested that, among TSD species, nesting in cooler areas may be a behavioral adaptation to global warming (Morjan 2003), but it remains unclear whether sea turtle species other than the green turtle will undergo this behavioral adaptation. Future research is necessary to determine the adaptive potential of sea turtle populations to rapidly progressing climate change.

Conclusions

Climate change poses more risks to sea turtle populations than higher global temperatures. Sea level rise around the globe threatens sea turtle nesting habitat, and changes in precipitation frequency—that is, a decline in precipitation in some regions that is predicted to occur with climate change—also risks feminizing sea turtle populations (Valenzuela et al. 2019; Veelenturf et al. 2020). It is unclear whether adaptive responses to climate change, like nesting in cooler areas, will be widespread among sea turtles, considering the fast pace of climate change and the much slower pace of adaptive evolution (Valenzuela et al. 2019). Future research should continue to focus on how global warming is affecting sea turtle populations and whether they will be able to adapt to rising global temperatures and other consequences of climate change. This research has important policy implications and might elucidate where human intervention and conservation efforts could be crucial.

Works Cited

Charnov, EL and Bull, J. 1977. When is sex environmentally determined? Nature 266:828-830.

Crews, D; Bergeron, JM; Bull, JJ; Flores, D; Tousignant, A; Skipper JK; Wibbels, T. 1994.
Temperature-dependent sex determination in reptiles: proximate mechanisms, ultimate
outcomes, and practical applications. Developmental Genetics 15:297-312.

Davenport, J. 1997. Temperature and life-history strategies of sea turtles. Journal of Thermal
Biology 22:479-488.

Heredero Saura, L; Jáñez-Escalada, L; López Navas, J; Cordero, K.; Santidrián Tomillo, P. 2022.
Nest-site selection influences offspring sex ratio in green turtles, a species with
temperature-dependent sex determination. Climatic Change 170.
https://doi.org/10.1007/s10584-022-03325-y

Intergovernmental Panel on Climate Change. 2021. Climate Change 2021: The Physical Science
Basis.

Janzen, FJ and Phillips, PC. 2006. Exploring the evolution of sex determination, especially in
reptiles. Journal of Evolutionary Biology 19:1775-1784.

Janzen, FJ; Tucker, JK; Paukstis, GL. 2006. Experimental analysis of an early life-history stage:
direct or indirect selection on body size of hatchling turtles? Functional Ecology 21:162-
170.

Jensen, MP; Allen, CD; Eguchi, T; Bell, IP; LaCasella, EL; Hilton, WA; Hof, CAM; Dutton, PH.
2018. Environmental Warming and Feminization of One of the Largest Sea Turtle
Populations in the World. Current Biology 28:154-159.

Lockley, EC and Eizaguirre C. 2021. Effects of global warming on species with temperature-
dependent sex determination: Bridging the gap between empirical research and

management. Evolutionary Applications 14:2361-2377.

Morjan, CL. 2003. How rapidly can maternal behavior affecting primary sex ratio evolve in a reptile with environmental sex determination? American Naturalist 162:205-219.

Morjan, CL and Janzen, FJ. 2003. Nest Temperature is Not Related to Egg Size in a Turtle with Temperature-dependent Sex Determination. Copeia 2003: 366-372.

Rivas, ML; Esteban, N; Marco, A. 2019. Potential male leatherback hatchlings exhibit higher fitness which might balance sea turtle sex ratios in the face of climate change. Climatic Change 28:154-159.

Roosenburg, WM. 1996. Maternal Condition and Nest Site Choice: An Alternative for the Maintenance of Environmental Sex Determination? American Zoologist 36:157-168.

Sea Turtle Conservation Bonaire. c2016-2022. Why Are Sea Turtles Endangered?

https://www.bonaireturtles.org/wp/explore/why-are-sea-turtles-endangered/

Shine, R. 1999. Why is sex determined by nest temperature in many reptiles? Trends in Ecology & Evolution 14:186-189.

The Leatherback Trust. c2023. Ancient Animals.

https://www.leatherback.org/why-leatherbacks/ancient-animals

Valenzuela, N; Literman, R; Neuwald, JL; Mizoguchi, B; Iverson, JB; Riley, JL; Litzgus, JD. 2019. Scientific Reports 9. https://doi.org/10.1038/s41598-019-40597-4

Veelenturf, CA; Sinclair, EM; Paladino, FV; Honarvar, S. 2020. Predicting the impacts of sea level rise in sea turtle nesting habitat on Bioko Island, Equatorial Guinea. PLoS ONE 15:e0222251. https://doi.org/10.1371/journal.pone.0222251

Warner, DA and Shine, R. 2008. The adaptive significance of temperature-dependent sex determination in a reptile. Nature 451:566-568.