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The deleterious effects of global warming on the emergence success and unequal sex ratio of TSD sea turtles

## I.    ABSTRACT

Marine turtles are among a group of amniotes believed to have gender differentiation influenced by temperature. This phenomenon, also common amongst reptiles, is referred to as temperature-dependent sex determination (TSD). However, marine ecologists and turtle conservationists are just beginning to acknowledge the potentially devastating influence global warming may have on the survival of sea turtle populations – a class of vertebrates that is already dwindling due to anthropogenic stressors. This has led to a surge of research dedicated to the pursuit of the mechanisms and implications of temperature on the polyphenic sexual determination of turtles. With the increased interest into the effects of temperature on the sex commitment of embryos, coupled with the alarming increase in global temperatures, researchers are exploring questions at the center of this critical and intriguing topic: why is this feature conserved amongst oviparous (egg-laying) amniotes; how does temperature affect the sexual differentiation of embryonic sea turtles; what factors might have led to the development of genetic rearrangements responsible for this polyphenic trait; to what extent does rising global temperatures impact the emergence success of TSD marine turtles? This literature review seeks to arrive towards a better understanding of the threat rising global temperatures pose to the emergence and sex ratio of hatchlings, and thus adversely impacting the longevity of marine turtle populations.

## II. INTRODUCTION

Temperature-dependent sex determination (TSD) is a developmental phenomenon which has been observed among reptiles and turtles. As the name implies, the sex of the offspring is influenced by environmental temperature. As such, TSD is a polyphenic trait; polyphenism is a unique form of phenotypic plasticity, in which a single genotype has the potential to commit to the expression of distinct, mutually exclusive phenotypes, in response to environmental conditions, signals, or cues (Schlichting and Wund, 2014). TSD occurs through a complex process not fully understood by researchers. What has been accepted, however, is a period in the early development of turtle embryos, preceding sex determination, in which the temperature of incubation seems to have a profound effect on the sexual destiny of the organism. Appropriately, the thermosensitive period has been understood as the point in development when the embryo is committed to a sex, followed by the onset of gonadal differentiation. Most studies have ascertained this critical period to be within the 15th stage (middle-one third incubation) of embryonic development, also referred to as the postovulatory days (Wibbels *et al.*, 1991).

Researchers speculate that the thermosensitive period exists among certain oviparous (egg-laying) amniotes, such as reptiles and turtles, but is not a determining characteristic for mammals. In large part, this difference is due to the role of the external environment in incubating the embryos. Sea turtles are oviparous vertebrates that bury their clutches on shores, whereas mammals are viviparous organisms characterized by the development of the embryo occurring internally within the parent. A clutch refers to a group of eggs that an organism lays at one time. The clutch size varies between each species, but a single marine turtle clutch may range from 50 to more than 200 eggs. The breeding season and frequency of egg-laying varies significantly between each species as well, with some turtles such as *Natator depressus* (flatback

sea turtle) laying up to three clutches each of 50 eggs every year, and at the other extreme, *Dermochelys coriacea (*leatherback sea turtle) laying 10 clutches each of 100 eggs every three to five years (Hirth, 1980). The variability in clutch size and breeding frequency underlies the challenges each species face, with regards to the clutches persevering through predation and other environmental hazards.

Generally, the larger the size of the clutch the higher the likelihood of some of the embryos developing, emerging as juveniles, and surviving long enough to reach sexual maturity and reproduce. The regions of an egg are heavily demarcated, with each layer/structure fulfilling a specific function; the division of compartments ensures that the developing embryo is not only protected, but that wastes/toxins (carbon dioxide, urea, etc.) are sequestered from critical areas, and that the sea turtle is supplemented with the proper nutrients (fatty acids, oxygen, water, etc.) to undergo the concomitant biological processes required to mature throughout its 31 stages of development (Crastz, 1982). Compared to other oviparous amniotes, marine turtle eggs are softer and somewhat more malleable; the porous nature of the shells are integral in providing the necessary gas exchanges with the environment, and is key in the thermoregulation of developing embryos (Ackerman, 1980). As such, clutches face more environmental risks compared to mammalian embryos. These threats include the following: predation, dehydration, exhausting the finite nutrients in the yolk sac, and even being displaced from the nesting site as a result of a storm or other natural source.

A lesser known fact is that eggs are considerably more susceptible to changes induced by ambient temperatures. The primary reason why organisms establish nests is to shelter, and in most cases hide, the developing offspring from predators. However, some organisms mitigate the thermolabile dangers of conduction between the egg and ground, by ensconcing the eggs in nests

made from surrounding materials. Female sea turtles, in particular, are known to bury their clutches in sand or dirt, in order to establish a moist, low saline barrier around the porous shells and to foster an appropriate incubation temperature (Bustard and Greenham, 1968). This buffering not only serves as cushioning from the hard ground, but burying the eggs ameliorates fluctuations in temperatures more likely to occur when exposing the soft, fragile eggs to raw ambient conditions. For some oviparous animals, such as a growing number of marine turtle species identified by researchers, temperature also governs other aspects of development such as determining the sex of the embryo. Studies have generalized that the average temperature yielding predominantly sea turtle males ranges from 26 to 27 degrees Celsius, whereas the average temperature for females ranges from 29 to 32 degrees Celsius (Dimond and Mohanty-Hejmadi, 1983). A number of experiments take advantage of the thermosensitive period by manipulating the incubation temperature of clutches.

## III.    SUPPORTING LITERATURE

### iv.    Classifying trends associated with TSD

Organisms that undergo temperature-based sex differentiation display one of two polyphenic patterns: Type I (subdivided into A and B) or Type II. Organisms that follow the Type I pattern, such as marine turtles, exhibit a single transition zone as exhibited by the curve in Figure 1. The Type IA model consists of the majority of embryos developing males at lower temperatures, and females at higher temperatures, whereas the opposite trend is associated with the Type IB model. Various studies involving *Lepidochelys olivacea* (olive ridley sea turtle)*, Trachemys scripta* (pond slider), *and Chrysemys picta* (painted turtle) have suggested that most TSD turtles follow the Type IA model, as opposed to lizards and crocodiles which tend to exhibit Type IB (Shoemaker and Crews, 2009). The Type II pattern of TSD is characterized by the

majority of females hatching under extreme incubation temperatures, and has been observed across certain species of alligators and geckos. The evolution of such a pattern may be beneficial to populations that would otherwise be subjected under greater strains within environments that exceed normal incubation temperature(s). Under these extreme conditions, a greater percentage of females correlate with greater fecundity, which corresponds with an increase in the survival for the entire population. Hence, why many researchers in the field propose that the Type II pattern is the overarching form of TSD most common in nature, but since most experiments analyze these organisms by manipulating temperatures in only one extreme, the Type I pattern is more represented (Graves, 2008).
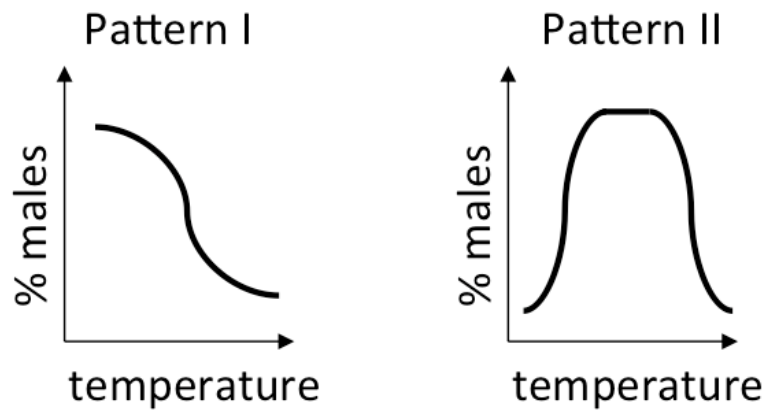
**Figure 1: TSD patterns**



**Figure 1: TSD oviparous vertebrates either follow the Type I or Type II model; most sea turtles follow the Type IA pattern, whereas most TSD reptiles follow Type II. [Shoemaker and Crews, 2009]**

### v.     TSD originating as a potential response to prehistoric climate change

Although the genotypic mechanism of TSD has yet to be elucidated, promising research has shed light onto the genetic trends associated with this polyphenism. A study by Shoemaker and Crews (2009) isolated two regulatory transcription factors as being involved in the development of urogenital ridges—primary gonadal constructs which later differentiate into male and female genitalia. The development of these gonad precursors are believed to experience significant changes in expression during the thermosensitive period. Among them, *Steroidogenic*

*factor 1* (*Sf1*) plays a domineering role in early gonadogenesis by mediating the transcription of key steroidogenic proteins and enzymes; the downstream targets of *Sf1* promote cellular proliferation, while simultaneously impeding cell apoptosis and concomitant bipotential gonad formation (Luo *et al*., 1994). *In situ* hybridization analysis of *T. scripta* gonads revealed that expressions of *Sf1* transcripts were noticeably greater at lower temperatures conducive towards producing males, but in *Chelydra serpentine*, gonadal expressions of *Sf1* rose at temperatures yielding a greater female ratio (Shoemaker and Crews, 2009). Although the elevated presence of *Sf1* in *T. scripta* and *C. serpentine* resulted in conflicting preference in sex ratios, it is apparent that atypically high expressions of *Sf1* transcripts are dependent on temperature and occur primarily within the thermosensitive period. Accordingly, it is likely that the downstream targets of *Sf1* transcripts undergo different cellular pathways throughout various TSD species. Regardless of its specific mechanism, the role of *Sf1* early in embryonic development remains a potent mediator of sexual differentiation for TSD organisms.

However, researchers are in a disagreement over whether the polyphenism exhibited by TSD sea turtles is a trait inherently encoded in the genome of certain marine oviparous, or if TSD originated as an evolutionary response triggered by dramatic shifts in the global environment. Turtles are a peculiar order of amniotes due to their highly varied number of chromosomes ranging from 28 to 66. Changes to regions of the genome can take place in many different forms: deletions, insertions, truncations, additions, or duplications; in certain cases, entire chromosomes can be deleted or duplicated. Over the passage of many millennia, these alterations compose the genetic make-up of an organism, and are inherited by the offspring if these mutations are favorable. Evolution occurs when the ubiquity of favorable genetic changes is so extensive, that a subset becomes a separate species capable of outcompeting its genetic

predecessors for resources, or in some cases, occupying a separate niche in the ecosystem. Although genetic mutations may occur at random, pervasive alterations in the genome of higher order vertebrates are usually in response to the environment. Typically, extreme rearrangements in the genome occur when the ecosystem(s), an organism has been accustomed to, undergoes a sudden or dramatic change severe enough to threaten the survival of that species. This form of adaptation increases the rate of survival for these species by changing the genome, subsequently manifesting into different phenotypic displays or behaviors better suited for that environment. Appropriately, a study proposed that the phylogenetic turtle chromosome rate of evolution exhibited a roughly twenty-fold increase, within the last 200 million years, coinciding with global temperatures reaching extremes (Valenzuela and Adams, 2011). Thus, it is likely that chromosomal rearrangements and temperature-dependent sex determination coevolved for marine turtles.

In this sense, TSD may have originally developed in response to the tumultuous changes in temperature. The genome of prehistoric oviparous amniotes may have acquired significant alterations/changes resulting in the preference of one sex over the other, which in a dynamic setting, may have increased the likelihood of survival for the entire species. For example, *D. coriacea*, and select species of alligators, were among the few vertebrates larger than 50 pounds that survived the Cretaceous–Tertiary (K–T) extinction event (Muench *et al*., 2000). It is possible that TSD could have been a crucial factor in the survival of *D. coriacea* clutches. In turn, this would have maintained the population and subsequent latency of leatherback sea turtles during this period of extreme global cooling. Researchers believe that around this time key transcription factors began to be expressed, and subsequently facilitated complex biological interactions culminating into this polyphenic trait. Among them is believed to be *Wilms' tumor 1* (*Wt1*),

which plays a role in the formation of sex gonads and is explicitly associated with the thermosensitive period. Genetic analyses have implicated *Wt1* in the upregulation of the sex-determining region of the Y chromosome, as well as the direct mediation of *Sf1* in certain species of amniotes, but studies have yet to be applied to species of marine turtles (Matsuzawa-Watanabe, 2003). It may be possible that the activity of the *Wt1* gene, in conjunction with *Sf1*, may be the missing link definitively attributing TSD to the phenotypic plasticity of sex chromosomes during the thermosensitive period, thereby inducing changes in chromosomal expression early in embryonic development.

### vi.     The temperature's role in determining sex is upstream biological factors

Other biological factors have been assumed to play a role in the sex determination of amniotic vertebrates before the thermosensitive period, but have since been refuted. Among them, some studies postulated that fluctuating aromatase levels coincided with the thermosensitive period, and thus had a greater impact on subsequent gonad differentiation than temperature; aromatase is a cytochrome P450 class enzyme involved in the conversion of testosterone to estradiol, which is the primary estrogen sex hormone for females (Carreau *et al.*, 2003). However, *in vitro* RT-PCR analyses, conducted by Murdock and Wibbels (2003), revealed that *T. scripta* aromatase mRNA levels were not significantly different at the 15th stage of embryonic development (thermosensitive period) between embryos that later emerged as male and female hatchlings (Murdock and Wibbels, 2003). Rather, the greatest discrepancy in the expression of aromatase occurred well after sexual differentiation, around stage 20 during the formation of the ovaries and testes (Figure 2). This finding was important considering that estrogen is a hormone found upstream in the majority of biological pathways leading to the specialization of primary and secondary female characteristics. Accordingly, if the effects of the

thermosensitive period precede the stage at which aromatase levels are significantly higher in females, then the effects of temperature may be the deterministic factor in the differentiation of sex amongst TSD organisms.

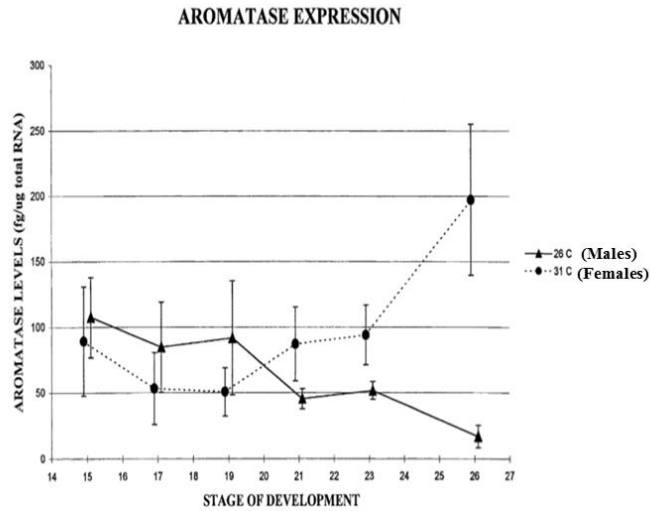**Figure 2: Embryonic aromatase expression during the thermosensitive period**



Figure 2: Graph showing the aromatase mRNA levels (in fg/μg of total RNA from AKG tissues) obtained as a result of the quantitative competitive RT-PCRs performed on total RNA isolated from AKGs taken at stages 15, 17, 19, 21, and 23, and at hatching (stage 26) at both male and female-producing temperatures. [Murdock and Wibbels, 2003]

**vii. Incubation temperature on the emergence of marine turtle hatchlings**

Recent studies have explored the intimate relationship between ecosystems where turtles lay their eggs, and the natural metabolic activity of sea turtle embryos. In particular, nesting sites have a demonstrable impact on the development of turtles, primarily due to the temperature of the sand affecting the incubation of the clutches. In addition to ambient temperatures modulating the temperature of the sand, metabolic heat generated from the developing embryos also contributes to the incubation temperature of clutches. Previous studies attenuated the role of elevated nest temperature as a result of heat stemming from embryonic metabolic activity, and its effect on TSD species. The arguments were made under the premise that metabolic activity occurred well after the thermosensitive period, around the emergence of juvenile sea turtles (Mrosovsky and Yntema, 1980). Such findings were apparent from the largest nesting site of

*Caretta caretta* (loggerhead sea turtle) in the Mediterranean, where researchers could only detect metabolic heat during the final third of the incubation period; the infinitesimal heat generated during the postovulatory days (middle-one third incubation period) was not significant enough to induce the feminization of the *C. caretta* clutches of interest (Zbinden *et al.*, 2006).

However, a two year comparative study by Maulany *et al.* (2012) revealed that elevated incubation temperatures not only corresponded with lower emergence rates of marine turtle hatchlings, but also led to an increased female ratio. The focus of the study was on *L. olivacea*; eggs were relocated to a secured turtle hatchery in Alas Purwo National Park, Indonesia where nests could be monitored without top-down stressors. The results showed that metabolic activity increased the maximum temperatures of the nests from two to six degrees Celsius, which presented a major concern considering that the projected lethal temperature for the clutches was 34 degrees Celsius. As depicted in Figure 3, the emergence success of the hatchlings was significantly lower in 2010 due to the maximum temperature (34 to 38 degrees Celsius), of the *L. olivacea* nests being well past the lethal range. In fact, the increased temperature was so detrimental to the development of the embryos that only 54.2 % of the eggs hatched—meaning that nearly half of the population did not survive (Maulany *et al*., 2012). The prolonged exposure to above lethal temperatures was hazardous to the embryonic development of the turtles, as reflected by the lower emergence success.

In the same study, a greater ratio of female hatchlings emerged in 2009 compared to 2010. This was influenced by the greater number of nests maintained in the upper temperature range conducive to female sex differentiation (29.5 to 31.0 degrees Celsius) during the middle-third period of incubation (thermosensitive period) (Figure 4). The turtles that emerged in 2009 had a female emergence approximately 14% greater than what was observed in 2010 due to the

2010 population being more skewed towards the cooler end of the temperature range (Maulany *et al*., 2012). As this study reflects, embryonic metabolic activity may not directly determine the sex of marine turtles, but such variations in temperature once perceived to be inconsequential, in fact has significant impact on the success of hatchling emergence.

**Figure 3: Emergence success of *L. olivacea* as a function of maximum temperatures over a three day span**
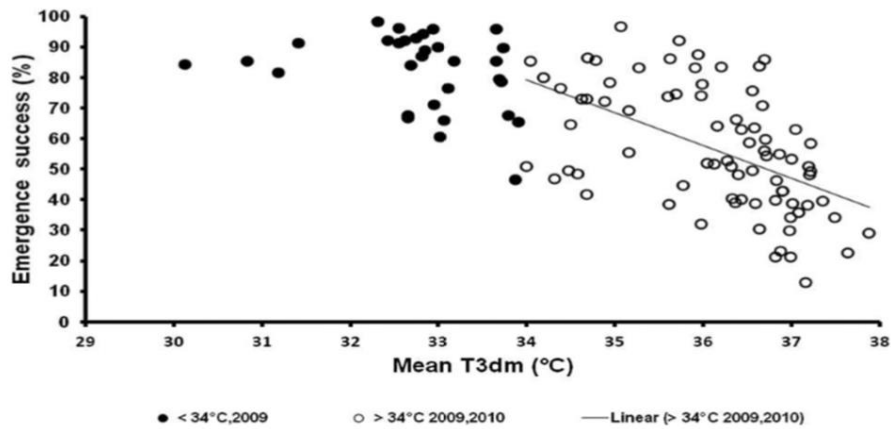


Figure 3: Mean emergence success of Olive Ridley turtles plotted against mean maximum temperatures over three consecutive days during incubation (T3dm). There was no relationship between T3dm and emergence success for T3dm < 34°C in 2009 ($R^2 = 0.089$, $P > 0.05$, $n = 31$ nests), but there was a relationship for T3dm > 34°C in 2009 and 2010 ($R^2 = 0.278$, $P < 0.001$, $n = 78$ nests). [**Maulany *et al*., 2012**]

**Figure 4: *L. olivacea* nest incubation during the thermosensitive period (days 17 -34)**
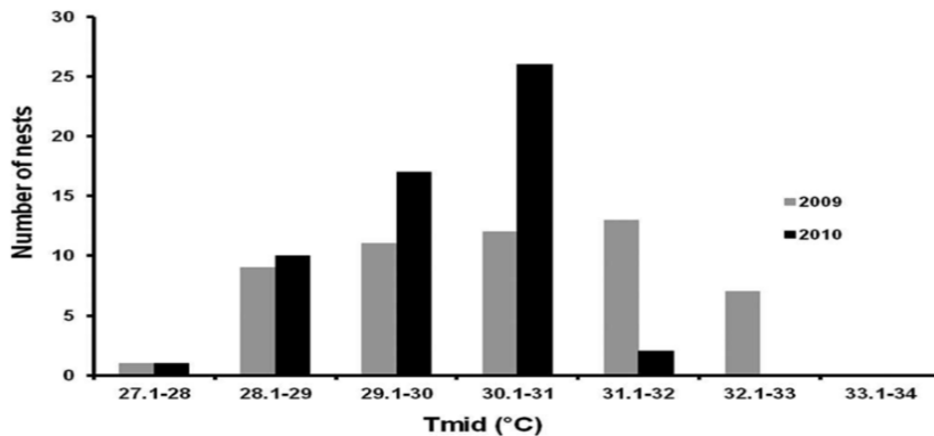


Figure 4: Frequency distribution of Olive Ridley turtle nests over each temperature interval during the middle third of incubation period in the Alas Purwo National Park hatchery (2009 and 2010 nesting seasons). [**Maulany *et al*., 2012**]

**viii.     TSD as a contingency for survival under sub-optimal conditions**

Another prominent theory related to the evolution of TSD, focuses heavily on the disproportionate male to female ratio, at certain temperatures, being a means of survival for populations under extreme environmental stress. A study by Spencer and Janzen (2014) revealed intriguing trends associated with a common sea turtle influenced by TSD, *C. picta*. The study was conducted under the assumption that *C. picta* followed the Charnov-Bull model; this theory proposed that the emergence of female sea turtles are more likely to dominate populations at warmer incubations where they are more adapted to survive, with the inverse being expected for populations with a higher ratio of males (Charnov and Bull, 1977). The emergence of *C. picta* hatchlings were analyzed during the cool and warm overwintering regimes—the period in the year when marine turtles wait out the winter due to a scarcity of resources and/or the presence of hazardous environmental conditions.

Accordingly, the two-fold results were in agreement with the Charnov-Bull model. At the warmer overwintering regime, embryos that would later differentiate as females consumed significantly less yolk ($p < 0.01$) compared to the male embryos, and the inverse occurred amongst males hatched in the cooler overwintering regime ($p < 0.01$); greater yolk consumption was linked to higher mortality brought on by greater metabolic rates (Figure 5). Indeed, attrition of biological functions commonly occurs when organisms are subjected under taxing conditions; embryos respond to the lower availability of viable energy by upregulating the metabolism of fatty acids stored within the yolk. The yolk reservoir sustains the developing sea turtle throughout the embryonic and fetal stages, and is even briefly retained after the juvenile has hatched, in order to meet the rigors of digging out nest and making it out to sea (Kraemer and Bennett, 1981). It was concluded that the embryos less fit for the overwinter regime prematurely

exhausted their nutrient supply, resulting in developmental arrest before the emergence of the

juvenile (Spencer and Janzen, 2014). The other moiety of the study revealed that *C. picta*,

hatched from single-sex clutches, developed significantly larger ($p<0.03$) carapaces, and thus

were physically larger when compared to mixed-sex clutches under both overwintering regimes

(Figure 6).

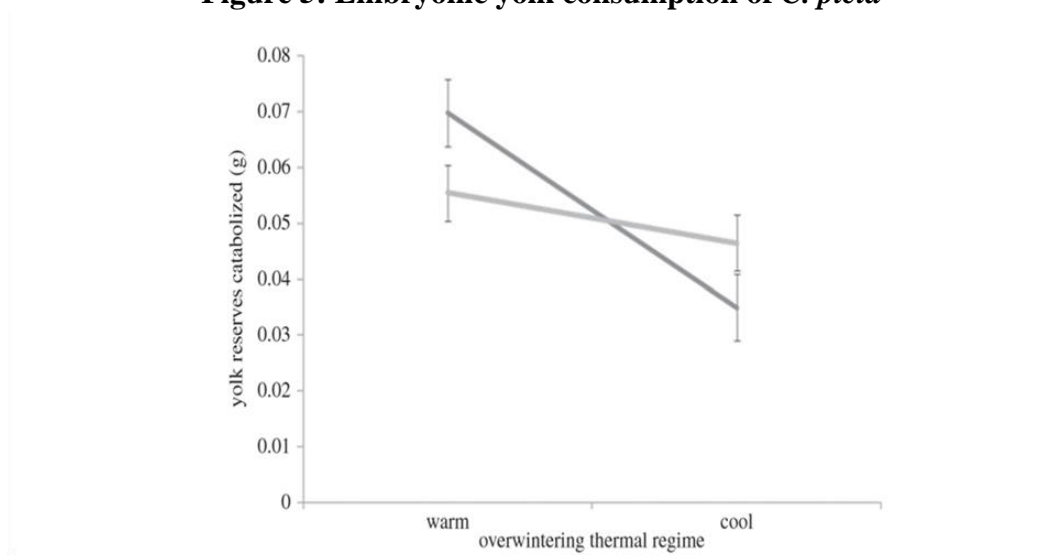**Figure 5: Embryonic yolk consumption of *C. picta***



Figure 5: Amount of yolk reserves consumed by male (black) and female (grey) hatchlings from predominantly single-sex nests of painted turtles in the different overwintering regimes ($\pm$ s.e). [Spencer and Janzen, 2014]

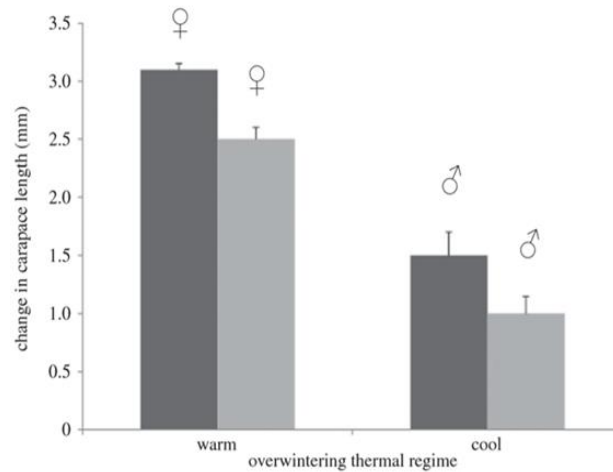**Figure 6: Comparison of *C. picta* hatchlings from single-sex and mixed-sex clutches**



Figure 6: Average change in carapace length between winter and spring in hatchlings from single-sex (dark grey) and mixed-sex (light grey) clutches in the cool (males) and warm (females) overwintering treatments ($\pm$ s.e). [Spencer and Janzen, 2014]

The findings presented by Sim *et al.* (2015) not only corroborated the conclusions of

Charnov and Bull (1977) and Spencer and Janzen (2014), but the data implicated above average

temperatures in the adverse motor function of marine turtles. The results revealed that *C. caretta*

hatchlings exposed to sub-lethal temperatures (above 34 degrees Celsius), over the final three

days of incubation, performed considerably worse in crawling ($p<0.02$) and swimming ($p=0.03$-

$0.04$) assays, when compared to hatchlings incubated below 34 degrees Celsius. It was also

determined that the hatchlings from sub-lethal incubations exhibited abnormal morphologies,

with regards to carapace width ($p<0.008$) and carapace size index ($p<0.01$), indicating that the

bulk of the skeletal-muscular development occurs within the final-third incubation period,

around the time of emergence (Sim *et al.*, 2015). Physical defects are as a major impairment in

the ability for a turtle to exercise proper locomotive functions; in turn, this hinders the sea

turtle's fitness capacity while swimming, mating, and escaping predation. When accounted as a

whole, TSD may be a process driven by optimal adaption to an environment that is dependent on

both the incubation temperature and metabolic activity of developing embryos. In addition, these

results suggest that the metabolic activity of embryos play a critical role in not only the sex ratio

and emergence success, but also the recruitment of marine turtle hatchlings.

## IX.    DISCUSSION

It is clear that the sex ratio of TSD sea turtles is highly susceptible to the incubation

temperature. Through a myriad of complex mechanisms affecting the development of embryos

during the critical thermosensitive period, environmental temperatures may sway the male to

female ratio of emerging populations to favor the emergence of hatchings of predominantly one

sex. Moreover, studies have isolated the thermosensitive period as being the earliest stage in

which the sex of a developing embryo is determined; popular theories involving the interplay of

hormonal factors, such as aromatase, have been revealed to have an effect after the development of the gonads. The thermosensitive period not only precedes the development of urogenital ridges, but has also been implicated in the process of gonadogenesis (Murdock and Wibbels, 2003; Luo *et al*., 1994). Other promising studies have suggested that TSD is a process in which temperature can trigger changes in the chromosomal genotype of sea turtles. This process— which geneticists believed to have evolved hundreds of millions of years ago in response to extreme fluctuations in global temperatures—contributed to the profound variation in chromosomal expression exhibited by modern, extant sea turtles (Matsuzawa-Watanabe, 2003; Valenzuela and Adams, 2011).

More contemporary studies have shifted away from proving the existence of TSD to quantitatively determining other temperature-related factors impacting the sex ratios of hatchlings. Indeed, ambient temperatures are merely one of the many temperature-related variables with the potential to affect TSD sea turtles. Heat conduction between the eggs and nesting sites, along with the incubation temperature of the sand or equivalent composition of a nest, also influences the determination of the sex. However, a variable which ecologists have only begun to consider as having a demonstrable impact on sex differentiation stems from the metabolic activity of embryonic marine turtles. The temperature of nesting sites are not only characterized by the temperature of the ambient and conductive environments, but also by the heat produced from the embryos following the thermosensitive period. Although embryos do not undergo significant activity in metabolism until well after the middle-third of the incubation period, the two to six degree Celsius increase in heat generated by metabolic activity may be significant enough to drive the overall temperature into lethal ranges and thus reduce emergence success (Maulany *et al*., 2012).

Similarly, a modest increase in temperature has also been known to induce the disproportionate sex ratio of hatchlings. Thus, the metabolic rates of the embryos seem to be a deterministic factor in the survival and maturation of embryos, which also coincides with temperature. In essence, the sex outcomes of TSD are not merely dependent on temperature, but also the metabolic efficiency of the developing embryos; for most species of sea turtles, males undergo more efficient metabolism under lower temperatures, whereas the inverse is apparent for females (Spencer and Janzen, 2014). In addition, hatchlings that emerged under sub-lethal temperatures tend to suffer from locomotive complications and morphological defects, as opposed to turtles hatched under optimal conditions (Sim *et al.*, 2015). Unfortunately, the likelihood of deformed, less fit hatchlings surviving to adulthood is highly implausible. All of these factors portray how fickle, susceptible, and ultimately vulnerable sea turtle hatchlings are in response to variations in temperatures.

Thus, the current dilemma of rising global ambient temperatures may prove to be more hazardous to the survival of sea turtles than previously believed. Scientists have estimated that mean global ambient and sea temperatures have already risen by 0.6 degrees Celsius within the last century alone, with some models projecting accelerated increases in the range of 0.3 to 7.5 degrees Celsius within the approaching century, depending on the extent of fossil fuel burning and carbon emissions (Levitus *et al.*, 2001; Zwiers, 2002). If left unchecked, sea turtle populations will be adversely impacted in two major ways: high emergence mortality/low emergence success of hatchlings and anisotropic sex ratios. With regards to the mortality rate of embryos, studies have already reflected that 2010 experienced temperatures, over three consecutive days, which not only exceeded the lethal incubation temperature of 34 degrees Celsius, but also resulted in almost 50% of emergence failure/mortality. Likewise, various

studies have reported that fluctuations in temperature as modest as 2 degrees Celsius were significant enough to yield sea turtle populations of a single sex (Dimond and Mohanty-Hejmadi, 1983). Popular regions around the world, such as Brazil and select areas of the Mediterranean, are estimated to undergo climate increases so severe, that total feminization of key species is likely to occur (Hawkes *et al*., 2007). Even worse, regions with already exceptionally high temperatures, such as southern Florida, are feared to exceed lethal incubation temperatures. Increases in temperature will only continue to exacerbate the strain placed on sea turtle populations.

## X.    CONCLUSION

Within the last century, global temperatures have experienced an increase significant enough to cause alarm for the natural environment and at-risk species, particularly marine turtles. Considering that turtles in general are facing a plethora of factors threatening reproduction, longevity, and recruitment, global warming may be added to this growing list of potential hazards to the well-being of marine turtle populations worldwide.

It is well-known that anthropogenic activities are often a detriment to sea turtles. Human encroachment into the natural habitats has invariably compromised mating and nesting sites where sea turtles are known to reproduce. Among most untenable ecosystems, human interference has a bottom-up disturbance on trophic ecological levels. For example, the destruction of sea grass beds, a staple food source for most marine turtles, is a common practice in areas where human territories overlap with natural habitats for turtles. In addition, industrial sites on the periphery of habitats inadvertently pollute the water, essentially corrupting the primary producers of these ecosystems. Over the last couple of centuries, more deliberate acts of exploitation, such as poaching, hunting, eating, and even the displacement of juveniles and

adults, has led to the extinction of several species of turtles, and has driven many more near the brink of extinction. Thankfully, modern endeavors to educate the public on the dangers of unabated human disturbances have ameliorated the wanton destruction of sea turtle species and natural habitats.

However, global warming should be at the forefront of conservationist concerns due to the pervasive influence of rising global temperature on sea turtle populations worldwide, even regions where human zones do not encroach on sites where marine turtles mate or establish nests. Fortunately, efforts are in place to mediate this seemingly insurmountable issue. Among them, some conservationists have manufactured artificial shade stations near coastal habitats, with the hope that female turtles select those cooler environments as nesting sites to lay their clutches. Still, such efforts may not be enough to reach the majority of TSD sea turtles in need. In order to obtain meaningful and widespread results in preserving these vulnerable species, legislation is necessary. For example, legal restraints can be established with the intent of reducing human encroachment and activities that affect nesting sites near coastal habitats.

In particular, beach nourishment programs pose an unprecedented threat to the emergence success and male to female ratio if not monitored properly. Beach nourishment programs aim to bolster coastal defenses against natural forces (storms, high tides, tsunamis, etc.) by replenishing the natural infrastructure (sand, sediment, etc.) lost due to natural erosion. Although rejuvenating the sand reservoir along receding shorelines yields several advantages, particularly serving as a buffer against storms and revitalizing beaches for human enjoyment, such a process becomes a hazard for marine turtles in two major ways. If the newly introduced material and native sand/sediment are not the same in terms of specific heat, size of the grain, texture, and overall composition, then sea turtle nesting sites become compromised. As such, the indigenous turtles

may lay their clutches in sand that retain more heat than normal, in essence, increasing the incubation temperature of the clutches. Another problematic area regards the tendency for the newly deposited sediment to harden. As a result, indigenous turtles may be incapable of digging through the superficial layer of the sand to bury their clutches; this is a severe detriment to the development of the embryos considering that burying clutches under sand aids in preventing dehydration and establishes proper incubation temperature. However, these issues can be averted if the highest degree of fidelity is maintained in monitoring sand replenishment procedures.

Above all, a deeper comprehension of TSD will allow for more specialized conservationist treatments for sea turtles. The most promising of which, involves the relocation of nests to hatcheries with controlled temperatures. Using this strategy, conservationists can raise clutches under incubation environments more conducive towards an equal sex ratio. Modern efforts are especially focused on isolating males, considering that under the projected model of increases in global temperatures, the availability of male sea turtles is expected to dwindle. Relocating juveniles to nurseries, even after hatching from natural settings, may be necessary since certain species of TSD turtles undergo energy intensive migrations from young ages; thus males are much more likely to perish based on studies revealing that males experience sub-optimal metabolism and growth under warmer incubations.

Future research can be aimed towards developing a model that ascertains the optimal incubation temperature for each endangered species of turtles, taking into account the variability in metabolic heat generation. Such a model would allow researchers to mimic ambient temperatures conducive towards high emergence, as well as an equal male to female ratio of hatchlings. Ultimately, by employing a conscientious approach to unraveling the intricacies of TSD, hope and optimism remains for the preservation of endangered marine turtles.

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