High beach temperatures increased female-biased primary sex ratios but reduced output of female hatchlings in the leatherback turtle

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ABSTRACT

Sex of offspring in most turtles is determined by temperature-dependent sex determination (TSD). In sea turtles, higher incubation temperatures produce female hatchlings and primary sex ratios are often highly female-biased. Because of the current rate of climate warming, highly female-biased sex ratios have raised concern among scientists and managers because populations might become too female biased for genetic viability.

We tested the effects of higher incubation temperatures on embryo and hatchling mortality and on sex ratios in a population of leatherback turtles (Dermochelys coriacea) in the eastern Pacific. The long-term study provided a large sample size in a location influenced by El Niño Southern Oscillation that resulted in highly variable climatic conditions between seasons. High temperatures reduced emergence success. Output of female hatchlings increased with incubation temperature as it reached the upper end of the transitional range (range of temperatures that produce both sexes) (30 °C) and decreased afterwards because high temperatures increased mortality of 'female clutches'. Effect of temperature on female hatchling output lessened female-biased sex ratios from 85% female primary sex ratios to 79% secondary sex ratios (sex ratios of total number of hatchlings emerged). If male turtles reproduce more often than females, operational sex ratios will be closer to 1:1. Female-biased primary sex ratios should not raise concerns by default, but climate change may still threaten populations by reducing hatchling output and increasing frequency of seasons with 100% female production. Clutch relocation to cooler conditions may alter sex ratios and should be used cautiously unless temperatures are so high that no hatchlings survive. In addition, it is unknown what differential survival of male versus female hatchlings may have on the eventual adult sex ratio after they enter the ocean and disperse.

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1. Introduction

Sex determination in reptiles occurs through two different mechanisms, genotypic sex determination (GSD) and temperature-dependent sex determination (TSD) (Janzen and Krenz, 2004). Primary sex ratios (sex ratios at hatching) in oviparous reptiles with TSD are determined by temperature during the incubation period (Valenzuela, 2004). High temperatures produce males

in some groups such as tuataras (Mitchell et al., 2010), and in other groups produce females, as in sea turtles (Standora and Spotila, 1985). In some species of lizards, turtles and crocodilians, males are produced at intermediate temperatures and females at extreme high and low temperatures (Valenzuela, 2004). The temperature that results in 1:1 sex ratios is known as the pivotal temperature and the time of incubation during which sex is determined is the thermosensitive period (TSP). There is also a range of temperatures that can produce mixed sex ratios and that is known as the transitional range (TR) (Mrosovsky and Pieau, 1991). Values over or under the TR result in 100% production of one sex. Pivotal temperature, TSP and TR can vary not only among species but also among populations.

Primary sex ratios in sea turtles are typically female-biased (Standora and Spotila, 1985; Godfrey et al., 1996; Broderick et al., 2001; Hawkes et al., 2007; Zbinden et al., 2007) and beaches that produce male-biased sex ratios due to low mean nest temperatures are rare (Steckenreuter, 2010; Katselidis et al., 2012). Moreover, a hatchling production of 90% or higher proportions of females are not uncommon at several nesting beaches (Mrosovsky and Provancha, 1992; Godfrey et al., 1999; Broderick et al., 2000; Godley et al., 2001; Sieg et al., 2011; Patino-Martínez et al., 2012). However, some of these studies were based on limited data-sets that may have failed to include multiple seasons or reflect climate and seasonal patterns throughout a number of nesting seasons.

One common problem frequently encountered in sea turtle studies of primary sex ratios is small sample sizes (e.g. ~15–20 nests), which rarely include more than one or two nesting seasons. Incubation temperature is highly correlated to air temperature (Hays et al., 2003) and air temperature fluctuates within and between seasons, more abruptly in some regions than in others. There is often seasonality in sex ratios, as temperature frequently increases throughout the nesting season, with males being produced early in the season and females towards the end (Standora and Spotila, 1985; Godfrey et al., 1996; Sieg et al., 2011). In some temperate areas, males are produced at the beginning and end of the nesting season while females are produced during the middle part (Katselidis et al., 2012). Therefore, larger sample sizes are needed to properly (1) characterize the variability in hatchling sex ratios intra and inter-seasonally and (2) estimate the overall sex ratios of hatchling output. Sex ratios within a single population can also differ among nearby nesting beaches where clutches experience different incubation temperatures (Broderick et al., 2001; Hays et al., 2003; Katselidis et al., 2012), as well as among nests on a single beach because of varied incubating environments (Godfrey et al., 1996; Broderick et al., 2000).

Previous studies suggested that temperature-induced differential mortality of sea turtle eggs and hatchlings could affect the estimation of sex ratios (Matsuzawa et al., 2002; Sieg et al., 2011). However, there are no studies that have combined analyses of egg and hatchling mortality and estimations of sex ratios. Moreover, sex ratios of juvenile and adult sea turtles have been recently found to be more male-biased than expected from the studies done on the nesting beaches. For instance, operational sex ratios (sex ratios of reproductive turtles in a season) were ~1:1 (female: male) in loggerhead turtles (*Caretta caretta*) (Hays et al., 2010), 1:1.4 and 1:1 in leatherback turtles (*Dermochelys coriacea*) (Stewart and Dutton, 2011 and Stewart and Dutton, 2013 respectively) and 1:1.4 in green turtles (*Chelonia mydas*) (Wright et al., 2012). Surprisingly, the latter study, which reported a greater proportion of male than female turtles reproducing in a season was done in Northern Cyprus, on a nesting beach where primary sex ratios were estimated as ~95% females. Likewise, a high proportion of juvenile and adult male loggerhead turtles occur at a foraging ground in the Mediterranean Sea (Rees et al., 2013).

Here we analyzed the effect of temperature on emergence success and as a result, on the sex ratios of hatchling leatherback turtles at Playa Grande, Costa Rica. This local population constitutes a good model because primary sex ratios are estimated as highly female-biased (Binckley et al., 1998; Sieg et al., 2011), and high temperatures reduce hatching success of eggs and emergence of hatchlings from the nest (Santidrián et al., 2009, 2012). Additionally, the long-term nature of the study (nine years) provided a large intra and inter-seasonal sample size in a location of highly variable environmental conditions caused by El Niño Southern Oscillation (ENSO) (Santidrián et al., 2012).

Because females are produced at high temperatures, we hypothesized that the negative effect of high temperature on hatching success and emergence rate would affect the overall female hatchling output (number of female hatchlings) from the beach. Therefore, secondary sex ratios, defined as the sex ratios of the total hatchling output produced on the beach over multiple seasons, will be less female-biased than previously thought, as a result of the effect of temperature at levels that produce a single sex on mortality of eggs and hatchlings.

2. Materials and methods

We collected data from leatherback turtle clutches at Playa Grande (10°20 N, 85°51 W), Parque Nacional Marino Las Baulas, North Pacific Costa Rica. This beach registers ~40–50% of all leatherback nesting events in the eastern Pacific Ocean and our laboratory has carried out population studies there since the late 1980s (Reina et al., 2002). The number of turtles included in this study only corresponded to females identified at Playa Grande during the nesting season. We excluded from the calculation turtles that were only seen at nearby Playa Langosta or nested outside the main nesting season.

We marked, monitored temperatures and excavated ~40% of all clutches laid at Playa Grande over nine nesting seasons, from 2004–2005 to 2012–2013 (Table 1). We placed type T thermocouples (24 gauge Cu–Cn) in the center of the clutch while the turtle was laying eggs to mark the nest and record temperatures. We took temperature readings in the afternoon (15:00–16:00 h) every other day during the incubation period using a BAT-12 thermocouple reader (±0.1 °C). Because of the low daily variation in temperature at leatherback nest depth (Binckley et al., 1998), we did not need to take temperatures at a greater frequency. We excavated nests two days after the first group of hatchlings was seen or hatchling tracks were found. Since most emergences occurred at night, excavations were conducted on the second afternoon after the emergence event was reported.

We estimated emergence success, mean temperature during the second third of incubation, primary sex ratios, hatchling output (number of hatchlings produced) and female hatchling output for each clutch included in the study. We used emergence success as an indicator of mortality (proportion of hatchlings emerged to the surface related to clutch size) because it combined hatching success (success during development) and emergence rate (success during emergence), and provided an ecologically relevant measure of hatchling production (Wallace et al., 2007).

To estimate emergence success (E), we used the formula $E = (S - (L + D))/(S + U)$, where S is number of hatched eggs (shells), U number of unhatched eggs, L number of live hatchlings found in the nest and D number of dead hatchlings found in the nest at excavation time (Santidrián et al., 2009). We consider that live hatchlings found at this time would not have completed emergence by themselves, since they are usually weak, dehydrated and struggle to walk and find the water when they are released.

Because TSP in leatherback turtles occurs in the second trimester of development (Mrosovsky and Pieau, 1991), we calculated mean temperatures between days 20 and 40 of incubation, which corresponded to the middle third of the average incubation period (60 days, Sieg et al., 2011). Pivotal temperature for this population is 29.4 °C, and the TR 29–30 °C (Binckley et al., 1998). To estimate primary sex ratios for each clutch, we gave each clutch a value between 0 (all male) and 1 (all female). Based on Binckley's curve, temperatures greater than 30 °C produced 100% females (sex ratio = 1) and temperatures lower than 29 °C produced 100% males (sex ratio = 0). For temperatures between 29 °C and 30 °C, we estimated sex ratios by 0.1 °C increments using Binckley's curve. We also calculated mean and standard deviation of emergence success and female hatchling output by 0.1 °C increments in mean