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Sex ratio estimates for species with temperature-dependent sex determination differ according to the proxy used

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ABSTRACT

Knowledge of the sex ratio of a population is crucial to understand their structure and dynamics. For species, such as marine turtles, with temperature-dependent sex determination, this knowledge provides a baseline in advance of climate change. Determining the primary sex ratio for marine turtle populations is challenging since offspring lack sexually dimorphic external characteristics. Therefore several proxies have been used to estimate the primary sex ratio of marine turtle populations. However, no study to date has compared estimations of sex ratio when using different proxies to determine the most accurate and to detect potential bias. To address this, we estimated the sex ratio of natural loggerhead, *Caretta caretta,* nests using 8 different proxies: two based on constant temperature equivalent (average of temperature or average temperature weighted by the growth of embryos during each time step) both for three developmental periods (the whole incubation, the middle third of incubation and the middle third of development) as well as two proxies based on incubation duration (duration of the whole incubation and of the middle third of development). Sex ratio estimates differed greatly depending on the proxy being used. Here we discuss the differences among proxies based on the biological relevance of underlying hypotheses and highlight the need for studies to accurately determine the thermosensitive period and to obtain appropriate estimates of embryo growth rate to estimate marine turtle sex ratio.

1. Introduction

Knowledge of the sex ratio of a population is crucial to understand their structure, dynamics and their evolutionary potential; this informs population viability analysis and effective population size calculations, are relevant to the management and conservation of species (Brook et al., 2000; Ewen et al., 2001). An understanding of primary sex ratio is also important in the context of a warming climate, where populations of species with temperature-dependent sex determination (TSD), may have their sex ratios altered with changes in temperatures (Stubbs et al., 2014; dei Marcovaldi et al., 2016).

Determining the primary sex ratio of populations can be challenging, especially for species that lack heteromorphic sex chromosomes and when offspring lack sexually dimorphic external characteristics. This is the case for marine turtles which exhibit TSD, where incubation temperature triggers the development of either testes or ovaries in embryos (Yntema and Mrosovsky 1980; Mrosovsky 1982; Janzen and Paukstis 1991), and they are not externally dimorphic until puberty (Miller 1997; Wyneken 2001; Ceriani and Wyneken 2008). Thus, reliable sexing of marine turtle hatchlings require the sacrifice of animals and histological examination of the gonad, which is difficult to justify for threatened species

Since empirical estimates of *in situ* primary sex ratios are difficult to obtain, several proxies (e.g., incubation temperature and duration) have been used to estimate the primary sex ratio of marine turtle populations (Girondot et al., 2010; Wyneken and Lolavar 2015). Wyneken and Lolavar (2015) recently categorized then based on how many steps are necessary to determine sex from the actual embryonic sex ratio, with an increase in proxy "distance" decreasing the reliability of the sex ratio estimate. First level proxies includes samples of hatchlings from nests, second level proxies includes the use of incubation temperature during the Thermosensitive Period (TSP) – developmental period for which temperature influences gonadal differentiation (Mrosovsky and Pieau 1991), and

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Table 1
Description of each proxy. Proxies 1–3 are averaged temperature CTE_A, proxies 4–6 are averaged temperature weighted by the growth of embryos CTE_W and proxies 7 and 8 are based on the duration of two developmental periods, respectively the whole incubation period and the middle third of development which corresponds to the thermosensitive period for sex determination (named TSP in this study).

Proxy ID	Proxy base	Temporal scale	Biological assumptions
1	Constant Temperature Equivalent averaged temperature (CTE_A) in °C	Whole incubation	Sex is determined only by temperature and during the whole incubation period.
2	Constant Temperature Equivalent averaged temperature (CTE_A) in °C	Middle third of incubation	Sex is determined only by temperature and only during the middle third of incubation period.
3	Constant Temperature Equivalent averaged temperature (CTE_A) in °C	Middle-third of development (TSP)	Sex is determined only by temperature and only during the thermosensitive period for sex determination (TSP).
4	Constant Temperature Equivalent averaged temperature weighted by the growth of embryos (CTE_W) in °C	Whole incubation	Sex is determined by the interaction between temperature and growth rate and during the whole incubation period.
5	Constant Temperature Equivalent averaged temperature weighted by the growth of embryos (CTE_W) in °C	Middle third of incubation	Sex is determined by the interaction between temperature and growth rate and only during the middle third of incubation period.
6	Constant Temperature Equivalent averaged temperature weighted by the growth of embryos (CTE_W) in °C	Middle-third of development (TSP)	Sex is determined by the interaction between temperature and growth rate and only during the thermosensitive period for sex determination (TSP).
7	Duration-based equivalent in days	Whole incubation (linearly proportional with the middle third of incubation)	Indirect measure of the effect of the thermal environment on both growth and gonadal differentiation throughout the whole incubation period.
8	Duration-based equivalent in days	Middle-third of development (TSP)	Indirect measure of the effect of the thermal environment on both growth and gonadal differentiation throughout the thermosensitive period for sex determination (TSP).

third level proxies includes, among others, incubation duration to infer TSP, the use of Constant Temperature Equivalent (CTE) to transform field incubation temperatures to a constant temperature that would result in the same developmental rate to determine sex ratio based on laboratory curves, and the use of climatic data to infer nest temperatures. It has been widely highlighted that these proxies need to be validated with actual sex ratio expressed in nests and that caution is necessary when interpreting sex ratio estimates from proxies (Girondot et al., 2010; Wyneken and Lolavar 2015). Unfortunately, for ethical, logistical and economic reasons validation is challenging (Gross et al., 1995; Wyneken et al., 2007), and consequently several studies have used and continue to use these proxies. Even though these proxies have been reviewed separately, no study to date has compared estimations of sex ratio when using different proxies to determine the most pertinent and detect potential bias. To address this, we estimated the sex ratio of natural loggerhead, *Caretta caretta,* nests using 8 different proxies and discuss the differences among methods based on the biological relevance of underlying hypotheses.

2. Materials and methods

2.1. Incubation temperatures and durations from field

The temperature of 43 nests of loggerhead turtles at Praia de Forte, Bahia, Brazil was recorded during the 2014–2015 nesting season (21st October 2014 to the 11th of March 2015). Five of them were removed from analysis because they showed abnormal temperature patterns that were likely due to predation and consequent exposure events. This region is an important loggerhead nesting area for the Southwest Atlantic loggerhead Regional Management Unit (Wallace et al., 2010) and an intensive study area for TAMAR (for information on TAMAR, see Marcovaldi and de Marcovaldi 1999). Of the 38 nests used for this study, 16 nests were monitored using Minilog data loggers (VEMCO Minilog-II-T, accuracy ± 0.1 °C, resolution = 0.01 °C) and 22 others with Thermochron iButtons (DS1922L-F5, accuracy ± 0.5 °C, resolution = 0.0625 °C). Dataloggers were placed at the middle of the clutches while females laid their eggs and removed just after hatchlings emerged. Incubation durations, are defined here as the time between when the eggs were laid and when the first hatchlings emerged. Air temperature at 2 m above the ground level and sea surface temperature in front of the beach were retrieved from the European Centre for Medium-range Weather Forecasts (ECMWF) which provide a global reanalysis of climate temperature for the last 37 years on earth at a 0.125° spatial resolution (ERA-Interim project), where models used historical records to provide smoothed temperature time hindcasts. We retrieved data every 6 h from the 1st January 1979 to the 31th December 2015 (latitude 12.375°S and longitude 38°W).

Temperature time series recorded within natural nests can be viewed as an autocorrelative process by which temperature recorded at time i depends on temperature at time i-1. Statistical properties of such time series are analyzed by autoregressive moving-average (ARMA) models which describe stationary stochastic process using two parameters, p and q (p is the lag for the coefficient of auto regression AR and q is the lag for the moving average MA). The ARMA model that best describe daily nest temperature was estimated for p = 0 or 1 and q = 0 or 1 (with 0 being time i and 1 being time i-1). We summed the Akaike Information Criterion (AIC) for each of the 38 temperature time series recorded in natural conditions for each of these p and q combinations. AICs are then compared to test which ARMA model best describes statistical properties of nest temperature time series.

2.2. Proxies for sex ratio estimations

The primary sex ratio, for each monitored nest, was predicted using 8 different proxies: 2 based on constant temperature equivalent average of temperature or average temperature weighted by the growth of embryos (in term of embryo size measured by straight carapace length during each time step) both for 3 developmental periods (the whole incubation, the middle third of incubation and the middle third of development) as well as 2 proxies based on incubation duration (duration of the whole incubation and of the middle third of development) (Table 1).