Metabolic heating in Mediterranean loggerhead sea turtle clutches

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Abstract

Offspring sex ratio is an important demographic parameter and, given its determination by incubation temperature in sea turtles, might be a key factor for their conservation under climate warming. An appealing approach to estimate hatchling sex ratios is to measure sand temperatures at nest depth and deduce hatchling sex ratios from a beforehand-established relationship of hatchling sex ratio and sand temperature. Such estimates will only be accurate though if metabolic heat produced by the embryos is considered. Judging whether metabolic heating has a potential effect on hatchling sex ratios without actually measuring temperature within clutches would greatly facilitate monitoring protocols. We tested for a relationship between the amount of metabolic heating and the number of developed embryos as well as clutch size in the largest known loggerhead sea turtle (*Caretta caretta*) population of the Mediterranean on Zakynthos (Greece). Temperatures were measured within 20 nests as well as at a reference site in the sand at nest depth. Metabolic heating was detected, but only during the last third of the incubation period did nests heat up considerably (1.6 °C on average) above the temperature of the surrounding sand. During the middle third of incubation, when sex is determined, the amount of metabolic heating was negligible. The amount of metabolic heating during the last third of the incubation duration was significantly correlated to the number of offspring developed to at least about 75% of incubation duration. This factor explained nearly 50% of variation in metabolic heating. Metabolic heating was also significantly correlated to clutch size. Given that clutch size within the Mediterranean is largest in Zakynthos loggerheads, we conclude that metabolic heating can be ignored in the estimate of hatchling sex ratios in Mediterranean loggerhead populations. These results thus provide the basis for a feasible monitoring of hatchling sex ratios in the loggerhead sea turtle in the Mediterranean.
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1. Introduction

Increase of temperature in reptilian clutches over that of the surrounding environment is a consequence of high metabolic activity during development and restricted thermal conductivity of the surrounding medium. Sea turtles bury sizeable clutches of large eggs in sandy beaches. The large amount of living tissue in these clutches combined with the relatively low thermal conductivity of the medium (air) between the eggs suggests that sea turtle clutches heat up during development above the temperature of the surrounding sand. Indeed metabolic heating has long been proposed to occur in sea turtle clutches (Carr and Hirth, 1961). Although the embryonic development of sea turtles has been studied in detail (Miller, 1985), only recently the availability of small data loggers has made it feasible to measure temperature within clutches during the entire

incubation duration with minimum disturbance to natural conditions. It seems that–as expected–detectable amounts of metabolic heating are a general characteristic of sea turtle clutches and that metabolic heating becomes evident around halfway through development and raises towards hatching (Godfrey et al., 1997; Booth and Astill, 2001; Broderick et al., 2001; Godley et al., 2001; Glen and Mrosovsky, 2004).

Metabolic heating in sea turtles is not merely an interesting topic in itself, but given the temperature-dependent sex determination of sea turtle embryos (Mrosovsky and Pieau, 1991), understanding what factors determine the amount of metabolic heating will improve monitoring protocols for these endangered species. Since sexing is not possible from external morphology in hatchling sea turtles, hatchling sex ratio estimates are traditionally inferred through indirect parameters (sand and within-nest temperatures or clutch incubation durations), based on their correlation with hatchling sex ratios established through histology of sacrificed hatchlings (Godfrey and Mrosovsky, 1997; Mrosovsky et al., 1999; Mrosovsky et al., 2002). Inferring to hatchling sex ratios through sand temperature measurements is a particularly appealing approach for several reasons, e.g., data can be collected with a minimum of working force. Meaningful hatchling sex ratio estimates can, however, only be obtained if a number of factors are considered, a major point of consideration being the average amount of metabolic heating. If metabolic heating is considerable, average clutch temperatures will systematically differ from sand temperatures at nest-depth. Indeed it has been suggested for several sea turtle populations that metabolic heating has a feminizing effect on hatchling sex ratios (Broderick et al., 2001; Glen and Mrosovsky, 2004). Therefore, to infer from sand temperature data to accurate hatchling sex ratio estimates, the amount of extra heat produced during the middle third of development must be known. Unfortunately, measuring metabolic heating is not all that simple, and monitoring hatchling sex ratios for a wide range of populations would be facilitated could metabolic heating be estimated from known population characteristics. It is likely, e.g., that the mass of developing tissue (i.e., the number and size of embryos) determines at least part of this variation. Two studies have indeed found correlations between the amount of metabolic heating and developed embryos/clutch sizes, both for the green sea turtle (*Chelonia mydas*). Broderick et al. (2001) found the amount of metabolic heating correlated positively with both clutch size and the number of hatchlings plus embryos that died during development in the Ascension Island population. However, the relationship they found may be driven by a few data points and should thus be interpreted with caution, as the authors state themselves. Booth and Astill (2001) also found that a surrogate of metabolic heating positively correlated with clutch size in Australia. However, their analysis is based on a very small sample (five clutches).

We investigated patterns of metabolic heating in Zakynthian loggerheads, the largest known population of the species in the Mediterranean (Margaritoulis et al., 2003) with a twofold goal: firstly, we simply aimed at estimating the amount of metabolic heating in Zakynthian loggerhead clutches as a prerequisite to estimate hatchling sex ratios from sand temperatures in this important nesting area. Secondly, we aimed to test for a reliable relationship between metabolic heating and developed embryos/clutch size, which could simplify estimates of metabolic heating in loggerhead populations within the Mediterranean and possibly on a larger scale.

2. Materials and methods

2.1. Study area

The Island of Zakynthos is situated in the Ionian Sea in the eastern Mediterranean. The climate is highly seasonal both in terms of temperature and rainfall, with high temperatures and almost inexistent precipitation during the summer months, while autumn and winter are characterized by lower temperatures, heavy rains and storms. The south-facing Bay of Laganas contains six discrete nesting beaches; the opening of the bay is ca. 12 km. Loggerhead sea turtles nest from the second half of May until the first half of August (Margaritoulis and Rees, 2003). Apart from a small nesting aggregation on the neighbouring Island of Kephalonia, the nesting beaches of Zakynthos constitute the northernmost loggerhead nesting area in the Mediterranean (Margaritoulis et al., 2003).

2.2. Data collection

The study was conducted on one of the beaches of the Bay of Laganas [Gerakas beach (37°42`N, 20°59`E)], during 2003 and 2004. Clutches we investigated were laid between 15 June and 24 July. To measure clutch temperature, we placed a temperature logger («Tiny-talk», accuracy ± 0.3 °C; Gemini Dataloggers, UK) into 20 nests (eight in 2003 and twelve in 2004). We programmed loggers to record temperature at 1 h intervals. Their precision was confirmed by comparing

temperature measurements taken with loggers placed next to each other. We inserted data loggers into the clutch in the early morning following the night a clutch was laid (i.e., within 2 to 6 h of the clutch being laid), temporarily removing part of the eggs from the egg chamber and placing the logger in the approximate centre of the clutch. This methodology was chosen based on a preliminary study where loggers were dropped while turtles were laying eggs, as we noticed that the device often rolled to the side of the clutch.

This was done at first light when the air temperature was still rather cold and humid and care was taken to replace sand in the original order and to work fast. Therefore, we have no reason to suspect that the positioning procedure or the data logger itself (the electronic device fits into a photographic film canister) influenced the incubation conditions of the clutches. Additionally, we measured sand temperature at average nest depth for Zakynthos (39 cm, ARCHELON internal reports, unpublished data) at one location within the same beach, at a distance of no more than 500 m from any study nest, for reference in both years of the study.

Nests were monitored daily by observation of tracks in order to detect hatching. The morning the first hatchling's track was recorded was considered as hatching date. The incubation duration (ID) was defined as the number of days from the date after the night a clutch was laid until the date of observation of the first hatchling tracks. We excavated nests 14 days after initial hatching, retrieved data loggers, categorized nest contents and determined hatching success (Miller, 1999). Additionally to the overall count of dead

embryos, the ones having reached at least about 75% of incubation duration were counted separately. All fieldwork was conducted during surveys by ARCHE-LON, The Sea Turtle Protection Society of Greece.

2.3. Data analysis

Nest temperature data of the time period between actual hatching and emergence, the latter being referred to as «observed hatching», estimated on 4 days (Godfrey and Mrosovsky, 1997), had to be excluded. Because sand temperature at nest depth can vary between nearby locations (i.e., the location of the study clutch and the location where sand temperature was measured), metabolic heating at any moment could not be simply calculated as the difference between clutch temperature and sand temperature.

Instead, as a first step, we determined the difference between clutch temperature and sand temperature per day for each day of the incubation duration. In a second step, the day-to-day change in this parameter was calculated. The baseline difference between nest temperature and sand temperature was considered as the average value of the difference between clutch temperature and sand temperature before its day-to-day change exceeded 0.3 °C (the precision of data loggers). We calculated metabolic heat per day as the difference between clutch temperature and sand temperature subtracted by the baseline difference as described above. To visualize the pattern of metabolic heating over the entire incubation period, we calculated the mean amount of metabolic heating during each 2%
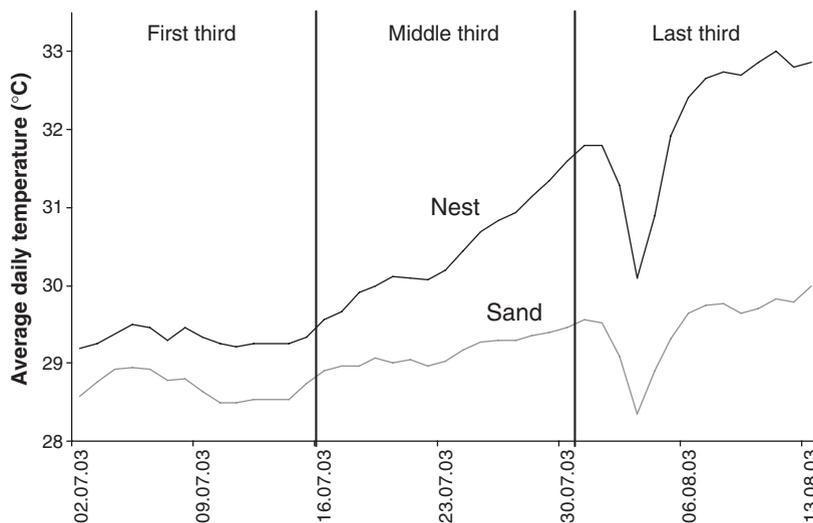

Fig. 1. Example of a typical clutch temperature profile and the related sand temperature profile from laying to actual hatching (4 days prior to observed hatching) to visualize temperature changes caused by metabolic heating. Note the effect of a storm at the start of August.
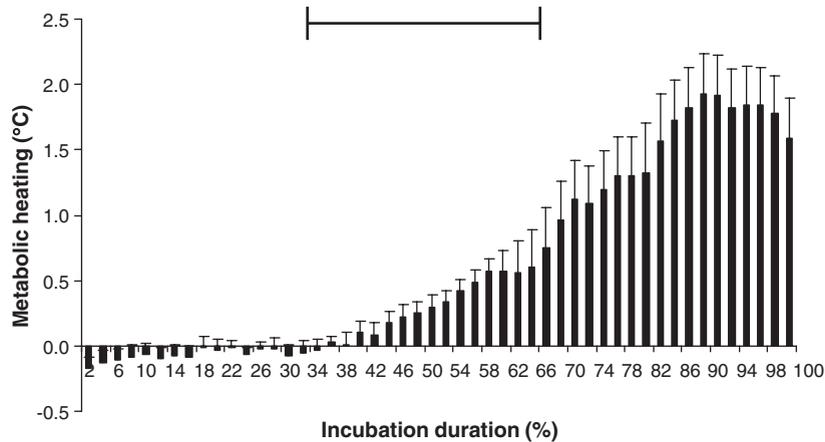
Fig. 2. Mean metabolic heating measured in eight clutches of 2003 according to relative advancement (%) of incubation. Error bars indicate SE. The horizontal bar depicts the second third of the incubation period.

period of the incubation duration. We tested for a relationship between the amount of metabolic heating and clutch size as well as the number of embryos that developed to at least about 75% of incubation duration (i.e., number of hatchlings plus number of late-stage dead embryos). To get an idea about the generalization of our results, we compared the data point of average metabolic heating vs. average clutch size of Cyprus loggerheads (Godley et al., 2001) to our data.

Apart from investigating metabolic heating, we verified the relationship between observed incubation duration and nest temperature—a basis to deduce hatchling sex ratio estimates from incubation durations.

Statistical analyses were performed with the program JMP IN© (version 4.04, SAS Institute Inc.).

3. Results

3.1. Metabolic heating

Clutch temperatures closely followed the course of sand temperatures during the first third of incubation as illustrated in the example (Fig. 1). The increasing discrepancy between nest and sand temperature afterwards is attributed to metabolic heating. The pattern of metabolic heating of the eight nests of 2003 (in 2004, sand temperature was recorded only from 12 July onwards due to loss of a data logger) over the entire incubation duration is shown in Fig. 2. Over the entire incubation period, these nests increased their temperature by, on average, $0.60 \pm 0.25\,°C$ ($\pm SD$) over that of the sand. During the first third of development, no metabolic heating could be detected ($-0.06 \pm 0.10\,°C$). During the middle third of development, the 20 clutches increased

their temperature by, on average, $0.20\,°C$ (± 0.20) above that of the surrounding sand. During the last third of incubation, metabolic heating accounted for on average $1.64\,°C$ (± 0.56) in all clutches studied. Because metabolic heating during the middle third of development was negligible, we only tested the effect of eggs developed to at least about 75% of incubation duration and clutch size on metabolic heating during the last third of development. Since the number of eggs developed to at least about 75% of incubation duration were highly correlated to clutch size ($F_{1,18} = 72.11$, $p < 0.001$, $R^2 = 0.80$), we tested each factor separately. The amount of metabolic heating was significantly correlated to both factors, although the number of eggs developed to at
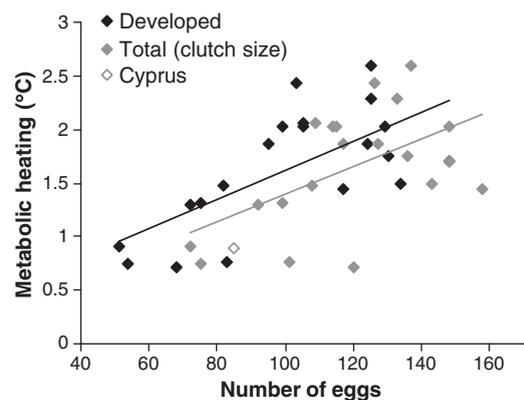

Fig. 3. Relationship between the amount of metabolic heating during the last third of development and the number of eggs developed to at least about 75% of incubation duration (metabolic heating = 0.013 × developed eggs + 0.27, black symbols) and clutch size (metabolic heating = 0.013 × clutch size + 0.12, grey symbols). The data point from Cyprus (grey outlined symbol) is an average of 15 clutches taken from Godley et al. (2001).
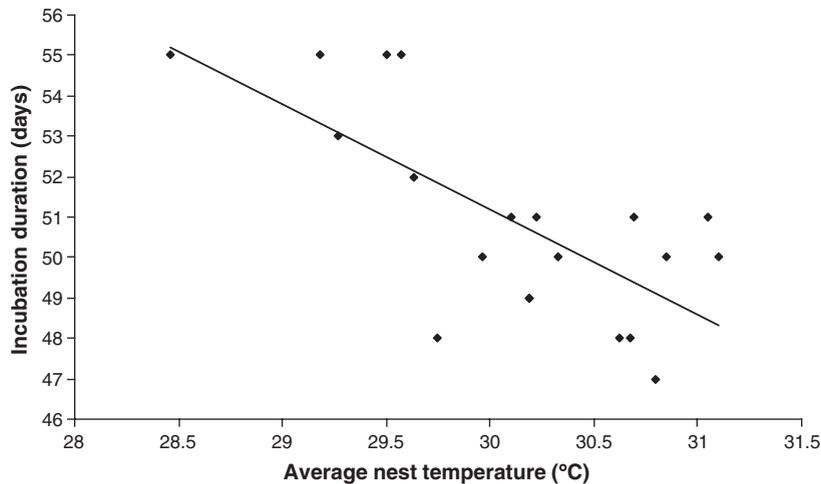
Fig. 4. Relationship between observed incubation duration and mean nest temperature for all study nests ($n=20$). Regression equation: Incubation duration $= -2.40 \times$ mean nest temperature $+ 122.46$.

least about 75% of incubation duration explained more of the variation ($F_{1,18}=15.22$, $p=0.001$, $R^2=0.46$, Fig. 3) than clutch size did ($F_{1,18}=7.41$, $p=0.014$, $R^2=0.29$, Fig. 3). The data point from Cyprus fits rather well into the relationship (Fig. 3). The amount of metabolic heating is significantly higher than in loggerhead sea turtle nests of northern Cyprus during the final third of development ($t_{33}=-4.09$, $p<0.001$), although not during the middle third ($t_{33}=0.076$, $p=0.94$).

3.2. Relationship between incubation duration and nest temperature

The negative correlation of incubation duration with average within-nest temperature was highly significant ($F_{1,19}=18.16$, $p<0.001$, $R^2=0.502$, Fig. 4), as was the correlation of incubation duration with average temperature during the middle third of development ($F_{1,19}=9.74$, $p=0.0059$, $R^2=0.35$).

4. Discussion

A better understanding of how sea turtle sex ratios are affecting population dynamics is needed to predict the effect of climate change on turtle populations. In the meantime it seems wise to monitor hatchling sex ratios. The fact that we found nearly half the variation in metabolic heating explained by the amount of developed eggs has implications for development of monitoring protocols for hatchling sex ratio. Tentatively, we suggest that whether or not metabolic heating is a significant factor shaping hatchling sex ratios does not have to be tested for each nesting area. Instead, the amount of metabolic heating in a given nesting area can be judged based on the results of a conspecific population and differences in the average number of developed embryos. It is obvious that this notion is based on rather little data and should certainly be verified in other populations before being widely applied. The fact that no such relationship was found in Cyprus loggerheads despite a comparable sample size is not necessarily challenging our result, but might simply be the outcome of low variation in factors. The most obvious difference between loggerhead clutches of Cyprus and Zakynthos seems the difference in clutch size. Whereas an average clutch in Zakynthos contains between 110 and 130 eggs, average clutches in northern Cyprus comprise only 75 to 83 eggs (Margaritoulis et al., 2003). The fact that we found a significant difference in metabolic heating between the data sets of Zakynthos and Cyprus with less metabolic heating detected in Cyprus is thus not surprising. The growth of embryo mass over time increasing with incubation time (Ackerman, 1997) closely matching the increase in metabolic heating underlines the close relationship between metabolic heating and embryo mass.

Although we could explain a large proportion of the variation in metabolic heating by number of developed embryos, what other factors are at play remains unresolved. It is likely that the conductivity of the surrounding sand plays a role in determining the amount of metabolic heating within the clutch (Speakman et al., 1998). Incubation of turtle eggs under controlled conditions could substantially increase our understanding of factors determining the amount of metabolic heating, but are restricted due to the conservation status of these species.

Experimental incubation of alligator eggs, a species whose sex is likewise determined by temperature, has revealed that eggs incubated in a clutch produced different sex ratios than those incubated individually; a result that was explained by differences in metabolic heating between the two treatments (Ewert and Nelson, 2003).

Our interpretation is based on results obtained from metabolic heating during the last third of development, whereas it is during the middle third that sex is determined (Mrosovsky and Pieau, 1991). The congruent pattern of metabolic heating over the incubation duration (this study; Broderick et al., 2001; Godley et al., 2001) suggests that average metabolic heating during the middle third of incubation can be reliably extrapolated from the amount of metabolic heating during the last third of the incubation duration. A further point of consideration is that we measured temperature in only one location of the clutch. It has been shown, however, that temperature in loggerhead clutches is not uniform for all eggs in a clutch (Godfrey et al., 1997; Hanson et al., 1998; Kaska et al., 1998; Booth and Astill, 2001; Houghton and Hays, 2001). Specifically, Godfrey et al. (1997) and Booth and Astill (2001) found the amount of metabolic heating higher in the centre than on the side of the clutch. Thus our results are likely to overestimate the amount of metabolic heating experienced by the average egg. This potential bias is not affecting the qualitative result that the average amount of metabolic heating during the middle third of development is negligible in our study.

Loggerhead clutches of Zakynthos being the largest amongst Mediterranean populations (Margaritoulis et al., 2003), we conclude that metabolic heating has no significant feminizing effect on Mediterranean loggerheads. Loggerhead clutches in other ocean basins are likely differently affected by metabolic heating, since Mediterranean loggerheads lay larger clutches with relatively small eggs compared to their body size than, e.g., Atlantic loggerheads (Tiwari and Bjorndal, 2000). It is thus likely that the specific relationship between developing embryos/clutch size and metabolic heating is different for other population groups.

Although sand temperatures and incubation durations remain indirect methods and as such potentially inaccurate, our results eliminate some of the uncertainty in the deduced hatchling sex ratio estimates.

Acknowledgements

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