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Metabolically-generated Heat of Developing Eggs and its Potential Effect on Sex Ratio of Sea Turtle Hatchlings

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Sexual differentiation of sea turtle hatchlings is influenced by the temperature at which the eggs are incubated (Raynaud and Pieau, 1985; Janzen and Paukstis, 1991). For all species of sea turtles, the general pattern of this thermal effect is the same: at a certain constant incubation temperature, called the pivotal temperature, an equal number of male and female hatchlings are produced. Incubation below or above the pivotal temperature will result in more males or females, respectively (Mrosovsky and Pieau, 1991). The range of incubation temperatures in which both sexes are produced (called the transitional range of temperature or TRT) is usually not more than 2–3 C wide, centered around the pivotal temperature (Mrosovsky, 1994). Incubation temperatures outside the TRT result in 100% male or female hatchlings.

For sea turtles, the adaptive value of this system of environmentally influenced sexual differentiation is poorly understood (Bull and Charnov, 1988; Mrosovsky, 1994). More data on sex ratios of hatchlings in natural conditions are needed to provide a solid database against which theories can be assessed. There are management and conservation implications of temperature-dependent sexual differentiation: almost any management procedure involving sea turtle eggs has the potential to alter the sex ratio of the hatchlings produced. For instance, ex situ incubation in Styrofoam© boxes, once widely used to protect the eggs and ensure high hatch rates, generally resulted in more male hatchlings because the temperatures in the boxes were usually cooler than those of the sand (Mrosovsky and Yntema, 1980; Mrosovsky, 1982; Morreale et al., 1982; Dutton et al., 1985). Of course, recognizing a change from the natural sex ratio implies that the natural sex ratio is already known. However, discovering natural sex ratios of sea turtles is an arduous task, because the nesting seasons on individual beaches can stretch for several months; weather and associated sand temperatures may change over this period, resulting in seasonal changes in sex ratios.

One method of estimating the overall sex ratio of hatchlings produced at an individual beach is to record mean daily sand temperatures. If the pivotal temperature is known, sand temperatures can be used to generate estimates of sex ratio. By combining information on sand temperatures and nesting density, one can estimate the sex of the hatchlings being produced at different times in the season. One difficulty with this procedure is that different zones of the beach may differ thermally; but this can be overcome by monitoring sand temperatures in all the different zones.

Another potential difficulty is that data on pivotal temperatures are usually generated in laboratory conditions. In contrast to the constant temperature incubation common to laboratory incubation procedures, there can be daily variation in sand temperature on turtle nesting beaches, especially at shallow depths; diel thermal variation can complicate simple estimates of sex ratio from daily mean temperature values (Bull, 1985; Georges et al., 1994). However, compared to nests of freshwater turtles, those of sea turtles are quite deep and hence subject to little daily variation in sand temperature (usually less than ±0.5 C; e.g., Morreale et al., 1982; Godfrey et al., 1996).

A third potential difficulty with using sand temperature as an index of sex ratio is that the eggs themselves produce metabolic heat during incubation (Carr and Hirth, 1961; Mrosovsky and Yntema, 1980; Standora et al., 1982; Maxwell et al., 1988; Maloney et al., 1990; Binckley, 1996). It is unclear whether metabolic

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warming overlaps with the thermosensitive period of sexual differentiation; if there is such overlap, metabolic warming could potentially influence the sex ratio. The latter problem could be avoided by discarding the method of using sand temperature to estimate sex ratio and instead relying on direct assessment of sex of the hatchlings. However, this method of sexing has the drawback of usually requiring the sacrifice of the hatchlings. If it is the case that metabolic warming overlaps with the thermosensitive period of sexual differentiation (roughly the middle third of incubation), then perhaps the temperature increase can be quantified. This value could be used as a correction factor to be added to sand temperature data, and would make predictions of sex ratios from sand temperature more accurate.

However, the onset of metabolic heat may not necessarily overlap with the thermosensitive period of sexual differentiation. To quantify the increase in temperature generated by incubating eggs and to relate this to the thermosensitive period of sexual differentiation, we monitored nest temperatures and nearby sand temperatures of leatherback sea turtles (*Dermochelys coriacea*). All work was done in the 1993 and 1994 nesting seasons, on Matapica Beach, Suriname. This dynamic beach is a major nesting area in Suriname for leatherback and green sea turtles (*Chelonia mydas*), and to a lesser extent, olive ridley (*Lepidochelys olivacea*) and hawksbill sea turtles (*Eretmochelys imbricata*) (for more details, see Schulz, 1975). Only natural nests of leatherback turtles were used in this study.

When leatherback nests were encountered within 12 h of deposition, we gently removed about 30%–50% of the eggs and placed temperature sensors in the middle and side of each nest, at similar depths to each other. All eggs (including small yolkless eggs, typical of leatherback nests) were returned to the nest, and the sand was replaced at the top and packed down. Approximately one meter away from each nest, parallel to the high tide line, a temperature sensor was buried in the sand at the same depth as the sensors in the nest. The sensors used were either Cu/Cn type K thermocouples, read with a Sensortek BAT 12 unit (Omega Inc., Stanford, Connecticut, USA), or digital thermistors (Godfrey and Mrosovsky, 1994). The sensors were usually read every 2–4 d, between the hours of 1000 and 1400, local time. The daily instantaneous sand temperature values were recorded for the probes within the nest, as well as for the reference probe in the sand nearby. Metabolic warming in each nest was calculated for each day by subtracting temperatures from the middle or side of each nest from the corresponding temperature of the reference site. The aim was to generate a profile of metabolic warming in the nests throughout development.

During incubation, individual nests were excavated once and one or two eggs next to the temperature probes were removed. The eggs were opened and (if viable) staged according to Renous et al. (1989). The aim was to compare the amount of metabolic heat in a nest with the stage of embryonic development of the eggs. The thermosensitive period for sexual differentiation in leatherback embryos is between stages 23 and 27 (Desvages et al., 1993). Once a nest was opened and sample eggs removed, no more data were collected from that nest, lest the opening of the nest altered the thermal environment or affected development (cf. Hendrickson, 1958).

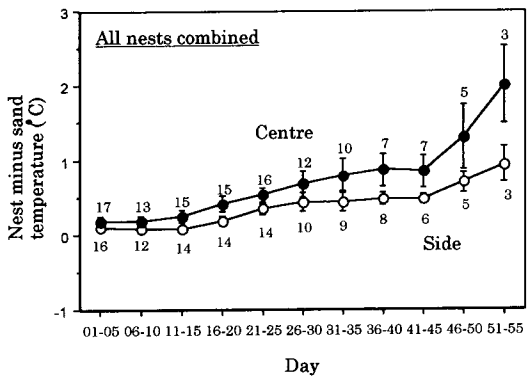

FIG. 1. Mean temperature difference between developing leatherback sea turtle eggs and surrounding sand. Temperature data are from thermosensitive probes placed in the approximate centers and sides of clutches; each clutch had a reference temperature probe placed in the sand, at similar depths to the nest probes, and one metre away from the clutch. For each clutch, temperature data for side and for center probes were collapsed into 5 d bins by averaging all temperatures recorded in those 5 d, shown is the mean temperature (±SEM) of all data from all clutches that contained viable eggs (see text). Values next to points refer to number of nests contributing to each point. Overall, temperatures were significantly warmer in the center of the clutches than in the sides of the clutches (paired t-test, 2 tailed, $P = 0.002$).

Seven nests in 1993 and 46 nests in 1994 were outfitted with temperature sensors. Of the 53 nests, 22 were lost, because either they were washed away by excessive high tides, or they were dug up by other nesting turtles, or they were poached. In addition, upon excavation and inspection of the eggs around the probes, some further nests had no viable eggs surrounding the side and/or center probes. Temperature data from probes that had no surrounding viable eggs were excluded from any analyses.

Metabolic warming in the leatherback nests was evident from very early stages and increased during development (Fig. 1). However, only after 25 d did the center of the nests on average become more than 0.5 C warmer than the sand. The eggs at the sides of the nests experienced less metabolic warming and became more than 0.5 C warmer than the sand only after day 45 (Fig. 1). Only four nests remained undisturbed until the very last stages of embryonic development, after which they were opened and the eggs surrounding the probes inspected. The temperature data from these late stage nests (of which one had no viable eggs around its center thermistor and another had no viable eggs around its side thermistor) are shown in Fig. 2.

From the eggs that were viable and staged, the majority of eggs experienced <1 C metabolic heat during the thermosensitive period for sexual differentiation (Fig. 3). During the thermosensitive period, there was no difference in mean amount of metabolic warming in eggs in the center of the clutches, +0.86 C, and

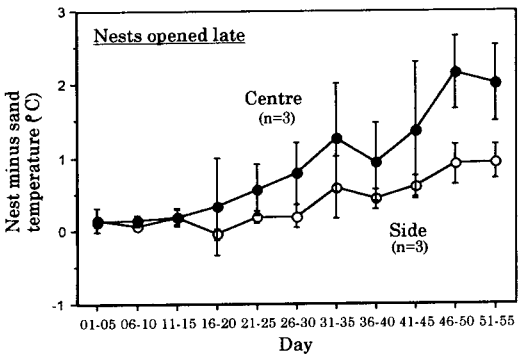

FIG. 2. Mean temperature difference (±SEM) between developing leatherback sea turtle eggs and surrounding sand, from nests that were not opened until just before hatching. Conventions are the same as in Fig. 1. Center temperatures were significantly warmer than side temperatures (paired t-test, 2 tailed, $P = 0.002$).

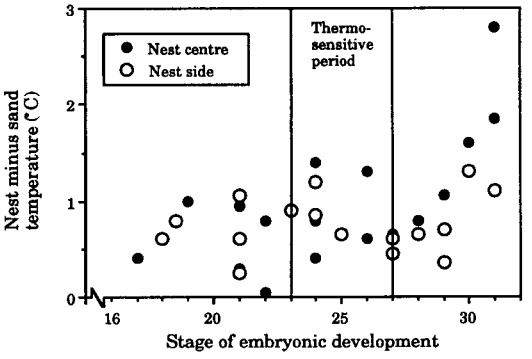

FIG. 3. The relationship between stage of development and amount of thermal difference between nest and surrounding sand. On a particular day, the temperatures in the nest (side and center) and the nearby sand were recorded, and then the nest opened. Eggs located next to the temperature probes were opened and staged according to Renous et al., (1989). Shown in the figure are the temperatures recorded on the day the eggs were opened.

those on the side, +0.78 C ($P = 0.192$, two tailed t-test, unpaired).

As incubation of leatherback eggs progresses, mean rate of oxygen consumption increases (Thompson, 1993), indicating an increase in overall metabolism during development. This increased metabolism coincides with an increase of self-generated heat in sea turtle nests (Fig. 1). The profile of the increase in metabolic heat is similar to that of the increase in oxygen consumption (Thompson, 1993), however, a strict comparison is probably not valid, given that the eggs monitored by Thompson were incubated at a constant temperature, while the eggs from this study came from nests laid at different times (and thus different thermal environments) during the season.

As expected, those eggs at the side of the nest experienced less metabolic heating on average than those at the center (Figs. 1 and 2). However, in general, eggs on the sides of the nests were still warmer than the nearby sand. From Figs. 1 and 2, it appears that the eggs in the center of the nests are >0.5 C warmer than the surrounding sand after day 25 of incubation. If the total incubation period is roughly 60 d, and the thermosensitive period for sexual differentiation is roughly during the middle third of incubation (Renous et al., 1989), then one might conclude that metabolic warming can have an effect on the sex of the hatchlings. However, this conclusion is simplistic, because nests from different thermal zones and times of year were grouped together. Some nests laid in cooler parts of the season would take longer to incubate, and so their thermosensitive period would occur later (in units of days) than that of nests laid in warmer parts of the season. Such grouping of nests is a common feature of previous studies of metabolic heat in sea turtle nests (e.g., Carr and Hirth, 1961; Maloney et al., 1990).

A more accurate estimate of the amount of metabolic warming that occurs during the thermosensitive period can be made from the data in Fig. 3, because eggs from different nests were opened and their embryonic stages were determined. Staging of the embryos allowed direct comparison of nests that experienced different thermal environments. The mean amount of metabolic warming experienced by incubating leatherback eggs during the thermosensitive period is 0.82 C (±0.09 SEM). Unfortunately, there are few data collected during the thermosensitive period in Fig. 3 (more than 50% of all potential data were lost during the field work). From the data available, there was no difference in the metabolic warming of eggs at the center and side of a nest, during stages 23 through 27, the thermosensitive period of sexual differentiation in leatherbacks (Desvages et al., 1993).

If there is differential heating of eggs in different parts of the nest (Figs. 1 and 2), then one might predict during incubation that some eggs will be subjected to more feminizing temperatures than others. However, it is unclear how often feminization from metabolic warming may occur. For leatherbacks, the TRT is between 29.0 C and 30.0 C; above and below these temperatures, all female and male hatchlings are produced, respectively (Rimblot-Baly et al., 1987). Therefore, for metabolic warming to have an influence on the sex ratio of a nest, that nest must be incubating in sand that is at or just below the pivotal temperature: if the sand is warmer than 30.0 C, then the hatchlings would differentiate into females regardless of metabolic warming. From Fig. 3, eggs were about 0.8 C warmer than the sand during the thermosensitive period. Therefore, metabolic warming has the potential to alter the sex ratio of leatherback hatchlings when the sand temperature is between 28.2 C and 30.0 C. However, at Matapica, Suriname, sand temperatures during large parts of the leatherback nesting season are often well above 30 C (Mrosovsky et al., 1984; Godfrey et al., 1996). Perhaps on other nesting beaches, the sand temperature at nest depth is closer to leatherback pivotal temperature for longer periods; in that case, metabolic warming might have larger effects on sex ratios.

Leatherback eggs have a small TRT relative to other

sea turtles (Mrosovsky, 1994). Metabolic warming in developing eggs of other species, should it occur during the thermosensitive period, may have a greater impact. Studies on other turtle species with large clutches of eggs should not ignore the potential impacts on sex ratios of metabolic warming.

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