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Temperature Dependence of Sexual Differentiation in Sea Turtles: Implications for Conservation Practices

ABSTRACT

Data on the effect of incubation temperature of the eggs on sexual differentiation in turtles are briefly reivewed. Even a change of 1 to 2° C can make a considerable difference to the sex ratio of the hatchlings. Current conservation methods include incubation of eggs in styrofoam boxes above ground, establishment of central hatcheries, incubation in reduced clutch sizes, and egg harvesting only during certain seasons. The thermal aspects of these practices are analyzed in turn. It is concluded that incubation of eggs in styrofoam boxes runs the risk of masculinizing turtle populations and that other practices may be affecting sex ratio in ways that cannot yet be specified. More work on this problem is urgently needed before unevaluated methods become accepted procedures.

Introduction

Sexual differentiation of a number of turtle species is affected by the incubation temperature of the eggs (Figure 1). At higher temperatures there are more females, at lower temperatures more males and, at least in the freshwater snapping turtle, *Chelydra serpentina,* at still lower temperatures more females again. The temperatures at which ratios between the sexes change rapidly we will refer to as pivotal temperatures. For the loggerhead sea turtle, *Caretta caretta,* the pivotal temperature is about 30° C (Figure 1). The method of sexing hatchlings of this species has been given by Yntema and Mrosovsky (1980) and speculations about the ultimate cause of having sexual differentiation dependent on temperature, and some theoretical ramifications of this phenomenon, are being advanced elsewhere (Mrosovsky, 1980). In this paper we are concerned with the practical implications for those involved in the conservation of sea turtles. Protecting eggs during incubation sometimes entails temperatures different from those prevailing in natural conditions. We will consider in turn the thermal aspects and possible effects on sex ratio of the following: 1) incubation
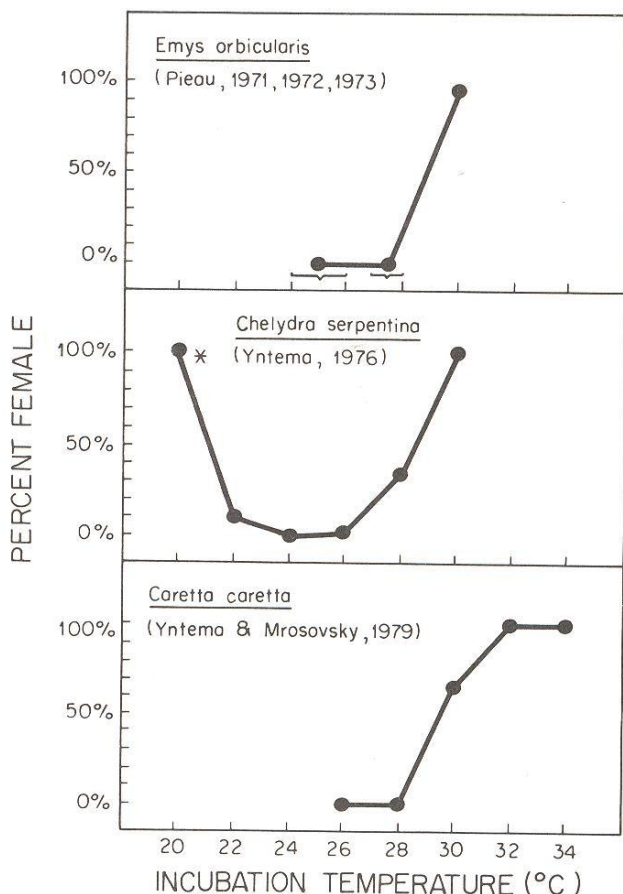
Figure 1. Sex ratio in 2 species of freshwater turtle (European pond terrapin, *Emys orbicularis,* and snapping turtle, *Chelydra serpentina*) and 1 species of sea turtle (loggerhead, *Caretta caretta*) from eggs incubated at different temperatures. Brackets show range of temperature prevailing. Star marks a group transferred to 26°C after 83 to 88 days at 20°C.

in styrofoam boxes above ground, 2) establishment of central protected hatcheries, 3) clutch size, and, 4) problems that might arise if eggs are harvested during close seasons.

Styrofoam Boxes and Incubation Duration

Styrofoam boxes, or similar forms of incubating eggs above ground in containers, have been widely used, for example, in Surinam, Cyprus, Mexico, the Caribbean and the United States. Incubation in styrofoam boxes generally takes longer than normal, presumably on account of lower temperatures (Schulz 1975, Marquez 1978). The two examples of this just cited will be considered quantitatively, but first it is necessary to be able to calculate temperature differences from the lengthened incubation periods. For this reference is made to Figure 2, showing the duration of incubation as a function of temperature for 2 species of marine

turtle (green turtle, *Chelonia mydas,* and loggerhead). The sample sizes are quite small at some points but the data are consistent enough to show that, as a general working rule over the ranges depicted, a 1° C lowering of temperature will be reflected in a 5-day increase in incubation time. This rule can of course be refined in the light of further data and can be made more complex to take into account the curvilinear relationship between temperature and incubation period that is obvious with wider temperature ranges (Yntema 1978). However, a simple rule may have value in field applications and is sufficient for the present argument.

With this rule in mind we can interpret the lengthening of incubation in styrofoam boxes. Table 1 shows data from Surinam. It seems likely that the differences between styrofoam boxes and the various procedures involving leaving the eggs in the sand, or replanting them, have been underestimated because, with buried eggs, incubation time includes time to emergence while, with animals in boxes only covered with a thin layer of sand (see Schulz, 1975), the time of hatching becomes apparent sooner. It therefore is likely, at least for leatherback (*Dermochelys coriacea*) and green turtles, that incubation temperatures in the styrofoam boxes were often 2° C lower than they would have been in the sand.

How would a 2° C drop influence the sex ratio? In Surinam the temperature of the sand at 80 cm depth, about the depth below ground level of the bottom of green turtle nests there (Schulz 1975), was 29 ± .5° C in a year that was not untypical as regards weather (Mrosovsky 1968). If the curve relating temperature and sex ratio for leatherbacks and green turtles is similar to the one shown for loggerheads in Figure 1, a 2° C drop could result in almost 100 percent of the hatchlings being male. However, to be concerned about a 2° C difference, it is not necessary even to assume that the curves for leatherbacks and green turtles in Surinam will be the same as those for the loggerhead clutch from Little Cumberland Island in the United States. All one has to assume is that the pivotal temperature for the Surinam turtles is close to temperatures commonly prevailing in natural nests, and that the shape of the curves relating incubation temperature to sex ratio are steep. They are steep in all the turtle species studied so far (Figure 1). A small temperature difference makes a considerable difference to sex ratio. The masculinizing effect of a 2° C drop could therefore be considerable.

The second example with styrofoam boxes concerns Atlantic ridley turtles, *Lepidochelys kempi,* a species perilously close to extinction. Attempts to boost the population of Atlantic ridleys in Mexico have included incubation of eggs in styrofoam boxes. Marquez (1978) reports that incubation took about 5 days longer in the boxes than in the sand (details on whether incubation

includes time for emergence not given). Using our simple rule for converting time to temperature, this would mean the boxes were 1° C cooler on average than the sand. However, Marquez suggests they were 2 to 3° C cooler. The discrepancy can possibly be resolved: the average temperature difference might have been nearer 1° C but at times differences of 2 to 3° C, or even more, occurred. More details about incubation duration and prevailing temperatures are needed to assess this matter properly. But if the curves relating sex ratio and incubation temperature are steep for the Atlantic ridley, as for other species studied (Figure 1), then even 1° C could result in an appreciable increase in the percentage of males.

In both these examples we have not asserted that there must have been changes in sex ratio, only that it is a possibility that should be very seriously investigated. There are two reasons at least for this caution. First, when eggs are exposed above ground in styrofoam boxes, the temperatures will fluctuate much more than when eggs are in the sand. At 80 cm depth, the sand can be an almost perfect constant-temperature incubator (Mrosovsky 1968). We do not know, therefore, whether a brief spell of warm temperature in a styrofoam box might protect the eggs against the masculinizing effects of generally lower temperatures. This question is not easy to answer with field studies involving uncontrollable fluctuations in ambient temperature levels. Laboratory work is needed. Second, if temperature in styrofoam boxes is considerably colder than in sand, it is possible that females will be produced. For the freshwater snapping turtle, *Chelydra serpentina*, the curve relating sex ratio to temperature is U-shaped (see Figure 1). The possibility that there are 2 pivotal temperatures in sea turtles needs to be investigated.

Because of these unknowns, there is a need for actual data on sex ratios from eggs incubated artificially. To date, the following information is available. At the Cayman Turtle Farm, Grand Cayman Island, where many
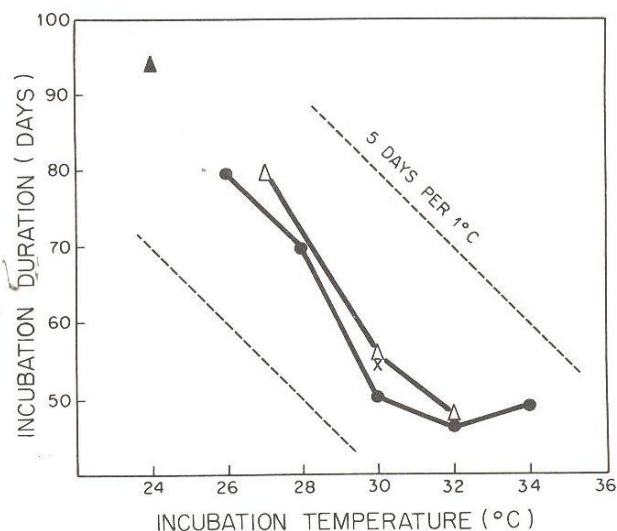

Figure 2. Incubation duration of sea turtle eggs as a function of incubation temperature. Dotted lines show slopes for a 5-day change in incubation duration per 1°C change in incubation temperature.

Open Triangle — *Chelonia mydas* (Bustard and Greenham 1968)
Closed Triangle — *Chelonia mydas* (Ackerman and Prange 1972)
Cross — *Caretta caretta* (Dimond 1965)
Circles — *Caretta caretta* (Yntema and Mrosovsky 1979)

In the last case, a variable 1 to 3 day correction has been added to the incubation times in the laboratory to cover what would probably have happened if the animals had also been at the various different temperatures during the 2 days spent in transit.

eggs have been incubated in a variety of ways, the sex ratio of green turtles varies greatly. Owens and Hendrickson (1978) give the following figures for the percentage females in different batches: 59, 83, 33, 17, 74, 97 and circa 100 percent. They suspect that incubation temperature varied in different seasons, hatcheries and containers.

Table 1. Mean incubation duration (days) of large samples of nests incubated in different ways in Surinam[1] and estimates of corresponding temperature differences

Species	In sand	In styrofoam boxes	Lengthening of incubation in styrofoam boxes	Estimated average degrees C cooler, styrofoam boxes
Leatherback (*Dermochelys coriacea*)	61–66	73	7–12	1.4–2.4°C
Green turtle (*Chelonia mydas*)	54–61	64–65	3–11	0.6–2.2°C
Olive ridley (*Lepidochelys olivacea*)	49–53	54	1–5	0.2–1.0°C

[1] From Schulz 1975, Tables 21 and 22.

Table 2. Onset of metabolic heating of eggs in green turtles (temperature within clutch exceeding sand temperature by >1°C)

Incubation duration in days	Days when metabolic heating begins	Percentage incubation period elapsed at start of metabolic heating	Reference
61	42	62	Carr and Hirth (1961)
68	34	50	Bustard (1972)

Location of Hatcheries and Nest-Site Selection

On Heron Island, Australia, incubation time for green turtle nests in shady areas exposed to the wind is about twice as long as that for nests incubating simultaneously in sunny protected areas (Bustard 1972). The period varies from 42 to 77 days, averaging 56 days; in seasons with much rain, nests have taken as long as 91 days to hatch. No details on sample sizes and methods were given. In his work on hawksbill turtles, *Eretmochelys imbricata*, Garnett (1978) failed to find any relationship between incubation duration and position of the nest in relation to shade.

Until nest-site selection is understood better and information has been obtained about sex ratios of hatchlings in undisturbed turtle populations, it may be difficult to design the ideal hatchery. On beaches where turtles nest in a variety of places, affecting incubation duration differentially, this factor should be considered in choosing a protected location for a central hatchery that may have relatively uniform thermal characteristics. A further reason for taking this matter seriously is that studies of freshwater turtle eggs incubated outdoors in natural conditions have shown that whether the eggs are in the sun or the shade and ambient temperatures do indeed affect sex ratio (Yntema, unpublished; Pieau 1975).

Clutch Size, Metabolic Heat and Critical Periods

In Malaysia, eggs of leatherback turtles are often buried in the sand in batches of 50 instead of in their natural clutches which average 84 eggs (Balasingam 1965, 1966). Apparently, hatching rates are better with 50 eggs. Because eggs produce metabolic heat, one should ask whether small clutches are cooler and so affect sex ratio.

Schulz (1975) reported that incubation times for green turtle eggs in styrofoam boxes with either 76 to 180 eggs per box or with 56 eggs per box were 65 days and 64 days, respectively. However, with natural nests, clutch size can affect incubation duration. Garnett (1978) found for hawksbill turtles in the Seychelles, that incubation (plus emergence) time was negatively correlated with clutch size (n = 44, r = .0.4, p < .01). However, in quantitative terms the effect was small, with 10 extra eggs in the clutch shortening incubation by less than half a day on average. Perhaps effects of clutch size are seen only in conditions where heat loss from the egg mass is relatively small. In cases where clutch size influences incubation duration (presumably through attending metabolic heating, although the relationship is not necessarily causal) would clutch size also affect the sex ratio?

Metabolic heating occurs mainly later on in incubation. Although heating up of the egg mass is gradual, it is convenient to be able to specify some day in incubation when this heating becomes important. We arbitrarily define the onset of metabolic heat as occurring when the egg mass becomes more than 1°C warmer than a control site in the sand at similar depth. Using this definition, it can be calculated that metabolic heating is not important until more than halfway through incubation (Table 2).

The critical period for sexual differentiation of the gonads in sea turtles has not yet been worked out, but this has been studied in snapping turtles (Yntema 1979). The exact critical period depends on the incubation temperature and details of the thermal schedules used. However, with all temperature regimes employed so far, the critical period does not start before stage 14 or continue after stage 20 of embryonic development is reached (for a description of embryonic stages see Yntema 1968). How many days it takes to reach stage 20 depends, of course, on the incubation temperature. Nevertheless, even at different temperatures, this stage is reached before halfway through the total incubation period (Table 3). If sea turtles are like snapping turtles, then the critical period for sexual differentiation occurs before metabolic heating becomes important. Clutch size should not, therefore, make much difference to sex ratio.

Before accepting this, it is necessary to study critical periods in sea turtles, especially in a unique species like the leatherback. Knowledge of the critical periods also is obviously of value in managing artificial hatcheries. It should also be pointed out that if critical periods for sea turtles turn out to be relatively early in incubation, before metabolic heating becomes important, it means that temperatures of sand adjacent to nests, rather than those within the actual egg mass itself, can be used to discover what temperature eggs in a given place or season are undergoing at the time

Table 3. Critical periods for sexual differentiation in snapping turtles (stages 14–20 of embryonic development)

Incubation temperature °C	Days to reach stage 14 (Yntema 1979)	Days to reach stage 20 (Yntema 1979)	Incubation period (Yntema 1978)	Percentage incubation period elapsed at end of critical period
26°	16	31	70	49%
30°	11	24	62	39%

of gonadal differentiation. This would simplify the application to field situations of information obtained in laboratory studies, with separated eggs incubated at a constant temperature.

Egg Harvesting and Close Seasons

Because sea turtles sometimes dig up the nests of turtles that have laid before, there are attractions in harvesting eggs that are laid early in a season. For instance, Pritchard (1978) has suggested that the logical method of exploiting olive ridleys, *Lepidochelys olivacea,* in Mexico would be to permit egg collection from the first *arribada* [synchronized mass nesting] or up to a certain date, because these eggs would be most liable to destruction by turtles laying later on. While this suggestion has obvious merit, especially when large arribadas are involved, it should nevertheless be evaluated in light of information about sand temperatures at different times in the nesting season and knowledge of the pivotal temperatures for these turtle populations.

Especially interesting problems arise with places where turtles nest all year round. For instance, in the Sarawak turtle islands (Talang Talang islands and Satang Besar) incubation plus emergence times of green turtles average 54 days during much of the summer: in February, during the monsoons, they average as high as 71 days (Hendrickson, 1958). This 17-day difference suggests a 3.4° C temperature difference if our simple rule is used. A more conservative estimate of 2° C can be made by referring to Bustard and Greenham's (1968) curve for green turtles in Figure 2 over the particular range of incubation periods involved. But even 2° C is enough to make a large difference to the sex ratio (Figure 1).

Green turtles also nest all year round in the Mozambique Channel on the island of Europa (Servan 1976). Incubation times range from 58 days in February, during the austral summer, to 85 to 99 days in June during the austral winter. Although sample sizes were small, it is clear that incubation can take at least 30 days longer at certain times of the year. Presumably temperature is largely responsible because not only is the sun weaker during the winter but the beaches are in shade during the morning (Servan 1976). Even if allowance is made for imperfection in our simple rule (which for 30 days

would mean a 6° C difference) when applied over large ranges, it does not seem at all unreasonable to suppose that there is a 4° C difference in incubation temperatures (Figure 2).

How do turtle populations evolve in such situations? Three possibilities will be mentioned. First, there might be a second pivotal temperature, as occurs in the snapping turtle (Figure 1). If this was fairly close to the upper one, it could permit some female differentiation, even at cooler times of year. Or, as suggested by Servan (1976) for Europa, different populations of turtles might use the island at different times of year. These populations might then have different pivotal temperatures. Perhaps this could even be used as a distinguishing characteristic. It is also possible that, although fewer turtles nest on Europa and the Sarawak turtle islands during the cooler seasons (Servan 1976, Hendrickson 1958) a high percentage of hatchlings produced then might be males. Should this be the case, there would be implications for egg harvesting schemes. For instance, eggs have been collected from Europa for turtle ranching (Fretey 1978). If this practice is to continue, it might be wise to consider spreading out egg collection over different seasons.

Furthermore, if clutch size turns out to be a significant factor in determining sex ratio, contrary to what we suspect at the moment, on the basis of the meagre information available ("Clutch Size. . .," above), then any seasonal changes in clutch size would also have to be taken into account.

Summary and Conclusions

A number of current conservation practices such as use of styrofoam boxes, close seasons, and establishment of central hatcheries, are likely to affect the sex ratio of sea turtles. The temperature changes involved, though slight in absolute terms, are probably large enough to affect sexual differentiation. But before this can be asserted with confidence, further work is needed. In particular it is important to:
• Learn about the effects of fluctuating temperatures such as are experienced by eggs above ground in styrofoam boxes.
• Discover what the pivotal temperature or temperatures are, and how much they differ in different species and populations.

• Locate the critical periods for sexual differentiation. There is a need to obtain at least some further information on the questions listed above now—before unevaluated methods become accepted procedures.

The possibility should also be considered that incubation in sand on a natural beach may provide the eggs with important benefits in addition to the correct temperature regime. Absorption of minerals and stimuli for imprinting are two possibilities that have already been raised (Simkiss 1962, Mrosovsky 1978). But there may be other facets of natural incubation that have not even been thought of yet. Certainly more research on the thermal aspects of incubation, as listed above, is urgently needed. But, in case we are not sufficiently in tune with the natural processes involved in incubation, we also advocate allowing at least some of the eggs in any conservation program to develop in the ground where the turtle laid them. In this context it is worth recalling the sentiments of Henry David Thoreau (1967) on the incubation of turtle eggs:

I am affected by the thought that the earth nurses these eggs. They are planted in the earth, and the earth takes care of them; she is genial to them and does not kill them. It suggests a certain vitality and intelligence in the earth, which I had not realized. This mother is not merely inanimate and inorganic. Though the immediate mother turtle abandons her offspring, the earth and sun are kind to them. The old turtle on which the earth rests takes care of them while the other waddles off. Earth was not made poisonous and deadly to them. The earth has some virtue in it; when seeds are put into it, they germinate; when turtles' eggs, they hatch in due time.

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