

Estimating the Time Between Hatching of Sea Turtles and Their Emergence From the Nest

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Sea turtles nest on tropical or temperate beaches. The eggs incubate in the sand, eventually producing hatchlings that emerge from the nest and scramble to the ocean. Several important physiological and behavioral events occur during incubation. These include temperature-dependent sexual differentiation during the middle third of incubation (Yntema and Mrosovsky, 1982; Raynaud and Pieau, 1985), and the typically nocturnal emergence of hatchlings from the nest, which appears to be gated by changes in sand temperature (Mrosovsky, 1968; Gyuris, 1993).

After hatching but before emerging, the hatchling turtles remain in the sand for a few days. This is an important stage for the hatchlings, allowing them time for improving behavioral synchrony in emergence from the nest, as well as for closing and straightening of the plastron and for absorbing the remnants of the yolk sac. Indeed, the greatest metabolism of residual yolk occurs while the hatchlings are still in the nest (Kraemer and Bennett, 1981). The interval between pipping from the egg and emerging from the nest has not been extensively studied and is not firmly established.

Investigation of the time between hatching and emergence has conservation and management implications. For instance, in some types of sand, sea turtles may have more difficulty digging to the surface after pipping. In the case of beach nourishment, a common technique used to stem beach erosion, sometimes the introduced sand is different from the naturally occurring sand and may initially be more compacted (Crain et al., 1995). This could lead to an increase in the emergence time, not only because the hatchlings must work harder to reach the surface, but also because greater exertion produces greater amounts of lactate in the body. High levels of lactate would be likely to require a longer resting period for the hatchlings just beneath the surface to allow for degradation of the lactate (Dial, 1987). The longer the period between hatching and emergence, the more residual yolk is likely to be used and the less yolk is left for the post-emergence period. This in turn might curtail the post-hatching frenzy, which is thought to be important in assisting the newly hatched turtles in moving away from a predator-filled shoreline towards a safer pelagic environment (Wyneken and Salmon, 1992).

All previously published studies that estimated the hatching-emergence interval relied on some sort of manipulation of the nest, e.g., digging into the nests prior to emergence to see if the eggs had hatched, or placing a glass pane on one side of the nests (see references in Table 1). We have developed a different and indirect technique that does not introduce any recording devices into the nest. This method uses hatching and sex ratio data from both naturally and laboratory incubated eggs. Specifically, we derived the estimated interval between hatching and emergence by calculating the difference in time between hatching of eggs in the laboratory and emergence of hatchlings in the field. To standardize the rate of development, we compared eggs and nests that produced similar sex ratios, since sex ratio and rate of development are correlated to incubation temperature (Mrosovsky and Yntema, 1980; Mrosovsky, 1988). Our study opportunistically analyzed sex ratio data from hatchlings that were utilized for other pivotal temperature and sex determination studies.

Materials and Methods. — Data on sex ratios and incubation durations for natural nests come from a number of studies on loggerhead (*Caretta caretta*) sea turtles (Mrosovsky et al., 1984b; Mrosovsky and Provancha, 1989, 1992; J. Provancha, S. Hopkins, and J. Richardson, unpubl. data). Data are from natural loggerhead nests laid in North Carolina, South Carolina, Georgia, and Florida, USA.

For our purposes, we defined incubation duration in natural nests as the number of days between the date of laying (day 0) and the date of the emergence of hatchlings from the nest. In all cases, freshly laid nests encountered in the morning were scored as being laid on the previous night. The location of each nest was marked, and several days before expected emergence, wire traps were placed over the nest at the surface of the sand, and checked daily in the early morning for hatchlings. If hatchlings in a trap were encountered, the nest was scored as having emerged the night before (the end of the incubation period). The sex ratio for each natural nest was calculated by determining the sex of 10 hatchlings selected randomly (although on occasion, fewer hatchlings were sexed from a particular nest; for details, see Mrosovsky and Provancha, 1989, 1992).

The data on sea turtle eggs incubated in the laboratory also came from clutches laid in the southeast USA (Mrosovsky, 1988). We defined incubation period for the eggs incubated in the laboratory as the number of days between the date of laying (day 0) and date of hatching. A turtle was considered hatched if its head and at least one flipper were outside of the eggshell. In a few cases, for example, if turtles appeared wedged against the side of the incubation container with their heads and most of their flipper exposed, they were scored as hatched, even though the tip of their flipper was still inside the egg.

Sex of hatchlings was determined by histological analysis of formalin-fixed gonads (see above references for details). One gonad from each hatchling was cut in half transversely and embedded in paraffin wax. Serial sections (10 μ m thick) from the cut end of the gonad were mounted on slides and stained with periodic acid – Schiff's reagent and Harris' haematoxylin, and examined under a light microscope. Sex of each hatchling was assigned by following the criteria of Yntema and Mrosovsky (1980).

For the incubation durations in the field, we used 76 loggerhead clutches. For the incubation durations in the laboratory, we used 389 eggs from 6 different loggerhead nests. Data were grouped into 1-day bins that spanned the range of incubation durations. For either laboratory incubated eggs or natural nests, we fitted a sigmoidal curve to the plots of incubation duration vs. sex ratio (% female), using Inplot 2.2 software (GraphPad, Inc.). For every sex ratio (2% female, 3% female, etc., to 98% female), we calculated the difference between the curves. The average of these values ($n = 97$) is the estimated mean hatching to emergence interval for these loggerheads.

Results. — Incubation durations in natural nests were longer than those of eggs incubated in the laboratory (Figs. 1 and 2). The mean difference between the two curves for all sex ratios was 4.1 days \pm 1.3 SD. At pivotal incubation duration (that duration which results in 50% female sex ratio, Mrosovsky et al., 1984a), this difference was also 4.1 days. This is considered to be the estimated mean hatching to emergence interval for these loggerhead turtles in the USA.

Table 1. Estimates of time between hatching and nest emergence (HE interval) of sea turtles. Mean interval for previous studies of *Caretta caretta* is 5.4 days. ^aRelocated or artificial nests. ^bNot stated.

Species	HE interval (days)	Sample size (nests)	Method	Reference
<i>Caretta caretta</i>	6	1	Excavation	Caldwell, 1959
	4-6	5 ^a	Plastic pole in nest, excavation	Kraemer and Richardson, 1979
	5	23	Temperature probe in nest	Webster and Gouveia, 1988
	5.7-5.9	18	Temperature probe in nest, excavation	Neville et al., 1989
	4-7	7 ^a	Glass-sided nest	Christens, 1990
<i>Chelonia mydas</i>	4.1	82	Sex ratios	Present study
	> 4	? ^{a,b}	Excavation	Hendrickson, 1958
	7	1 ^a	Glass-sided nest	Carr and Ogren, 1960
<i>Eretmochelys imbricata</i>	4-6	2 ^a	Glass-sided nest	Diamond, 1976
	6	1 ^a	Microphone, artificial nest	Raj, 1976

Discussion. — Based on our incubation sex ratio technique for loggerhead turtle hatchlings, we estimate that about 4.1 days on average are spent in the nest after hatching and before emerging on the beach surface. Estimates of this interval in natural nests range from 4 to 7 days (Table 1), and average 5.4 days, 31.7% longer than our estimate. Our definition of hatching obviously affects our estimates of the hatching to emergence interval. Gutzke et al. (1984) have suggested that in reptiles *pipping* (when the eggshell is first slit) is better than *hatching* as an index of the end of the incubation period, because it shows less variability. Although we did not systematically monitor pipping in all the laboratory incubated eggs, time to pipping is shorter than time to hatching: in two clutches of loggerhead eggs incubating at constant temperatures in a different study, hatching on average occurred 0.8 days after pipping ($n = 185$, range 0 to 2.5 days). This may be an overestimate, because eggs were inspected only twice a day. Pending further studies in loggerhead sea turtles, if the mean interval between pipping and emergence is required, then we suggest an approximate value of 5 days rather than 4 days.

Our estimate of the hatching to emergence interval depends on various assumptions, including that there is little daily fluctuation in temperatures of natural nests. Georges et al. (1994) found that an increase in the amplitude of the diel temperature cycle during laboratory incubation caused loggerhead eggs to become more female biased without an accompanying reduction in incubation duration. However, it is unlikely that this would have affected our estimates, for the following reasons. First, natural sea turtle nests are generally placed deep beneath the surface of the sand, and thus are subject to little daily variation in temperature, often less than 1°C overall (Morreale et al., 1982; Godfrey et al., 1996). In addition, we maintained relatively constant temperatures in all incubators during artificial incubation, usually with less than 0.5°C variation. Even if the temperature of the incubating eggs cycled around the mean as much as $\pm 1.0^\circ\text{C}$ per day, from the experiments of Georges et al. (1994), it can be determined that such variation during incubation would correspond to only 0.1°C increase in the “constant temperature equivalent.” This small increase in temperature would have only a slight effect on sex ratio.

Another assumption of the present method of estimating the hatching to emergence interval is that the eggs incubating in the laboratory were healthy and developing at similar rates, for a given temperature, as eggs in the sand on natural beaches. Etchberger et al. (1991) were able to lengthen by about 4 days the incubation period of freshwater turtle (*Trachemys scripta*) eggs independent of changes to the sex ratio by chronically decreasing the amount of oxygen that was circulated through the incubators. However, these effects were dependent upon extremely low levels of O_2 in the incubators (8%), which resulted in extremely low hatch rates. In contrast, our method of incubating eggs in the laboratory was designed to allow circulation of air throughout all egg containers, with the tops of the eggs exposed above the substrate (see Mrosovsky, 1988, for diagram). Our

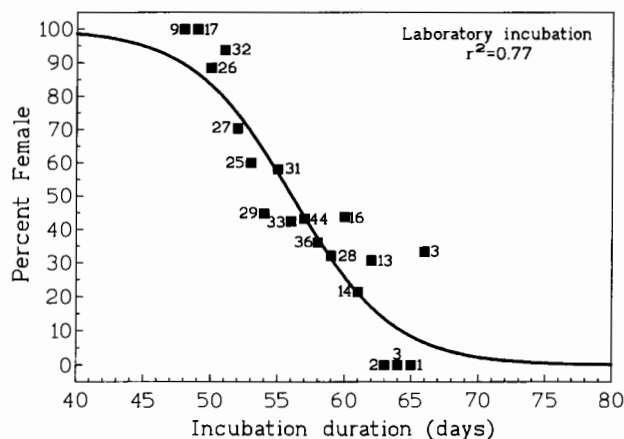


Figure 1. Sigmoidal curve fitted to data from loggerhead eggs incubated in the laboratory. Individual eggs (from 6 separate clutches) were incubated at different constant temperatures. The sex ratio of groups of eggs were sorted in one day intervals, according to incubation duration, which was defined as the number of days between laying and when the head and at least one flipper of the hatchling was outside of the eggshell. Values next to each point refer to numbers of eggs that contributed to each point. The curve was fitted with Inplot 2.2 software (GraphPad, Inc., San Diego, CA), with 0% and 100% female specified as lower and upper asymptotes. The laboratory pivotal incubation duration (i.e., that duration which results in 50% females) is 56.1 days, 4.1 days shorter than for natural nests (Fig. 2).

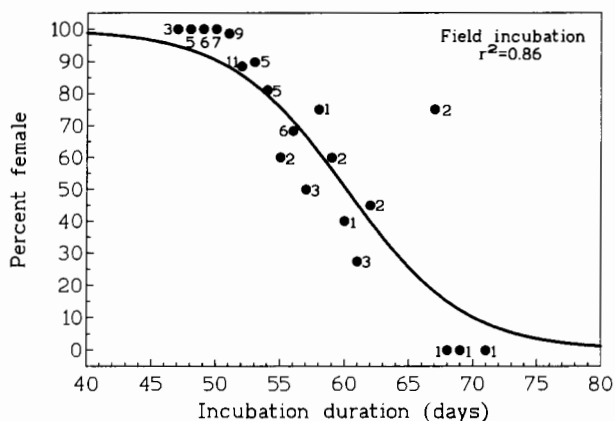


Figure 2. Sigmoidal curve fitted to the data from natural loggerhead nests in the USA. Individual nests were sampled for sex ratio. The sex ratio of groups of nests were sorted in one day intervals, according to incubation duration, which is defined as the time between laying and the time when hatchlings first emerged from the nest at the surface of the sand. Values next to each point refer to the number of sampled clutches that contributed to each point. The conventions of the curve fitting are the same as in Fig. 1. The field pivotal incubation duration (50% females) is 60.2 days, 4.1 days longer than for laboratory incubated nests (Fig. 1).

method is capable of giving high hatch rates (e.g., Marcovaldi et al., 1997), which would not have been expected if there had been oxygen deprivation (Ackerman, 1980; Etchberger et al., 1991). As well, in a different study using the same incubation methods (Marcovaldi et al., 1997), oxygen levels in the incubators remained between 20.2 and 20.7% (measured with a Servomex Oxygen Analyzer #572), which suggests that eggs previously held in these incubators were not oxygen deprived.

In terms of eggs incubating in the field, it is unlikely that the oxygen levels are so different from the laboratory incubation conditions as to contribute to a lengthening of the field incubation period. There are two reasons for this view. First, from the study by Etchberger et al. (1991), the hypoxic conditions needed to lengthen the incubation period produced a low survival rate (11%). Also, Ackerman (1980) noted a positive relationship between relative oxygen levels and hatching success for sea turtle eggs incubated in artificial nests. In contrast, for our study, the nests from which samples were collected in the field generally had high hatching success rate, which suggests that conditions were not hypoxic. Second, measured levels of O₂ in natural sea turtle nests remain between 14 and 19% for most of the incubation period, and drop to roughly 5% in the last few days before hatching (Ackerman, 1977). Presumably, hypoxia lasting only a few days would not result in lengthening of incubation, since hypoxia lasting the whole 60-day incubation period resulted in a lengthening of incubation by only 4 days for *Trachemys scripta* eggs (Etchberger et al., 1991). Of course, caution should be used when comparing results from different species, but these considerations suggest that the conditions between laboratory and field incubation are not greatly dissimilar.

A further assumption is that sex ratio is correlated with incubation duration. This assumption is supported by the high coefficients of determination of the fitted curves ($r^2 = 0.77$ and 0.86). Although the thermosensitive period for sexual differentiation is restricted to roughly the middle third of development, the prevailing sand temperatures of the first and last third of development are highly correlated with that of the middle third, because seasonal changes in sand temperature at sea turtle nest depth tend to be gradual rather than abrupt (e.g., Mrosovsky and Provancha, 1992). If the correlation between sex ratio and incubation duration is not high, and greatly influenced by a few points in a small data set, then the relationship between sex ratio and incubation duration would become less reliable.

Perhaps such factors account for the value of about 55 days pivotal incubation duration for naturally incubated green turtle clutches from Tortuguero, Costa Rica (Standora and Spotila, 1985, $n = 9$ nests, $r^2 = 0.48$). In the absence of information on laboratory incubation of Costa Rican green turtle eggs, it is not possible to estimate the hatching to emergence interval for this population of turtles. Webster and Gouveia (1988) used the relationship between nest temperature and sex ratio of naturally incubated and laboratory reared loggerhead turtle eggs to predict a 5–6 day hatching to emergence interval. Overall, the data from that study are difficult to evaluate, as they are available in abstract form only. One possible source of difference in their estimates is their use of laboratory incubation data from Mrosovsky and Yntema (1980), which not only was based on small numbers of eggs, but also had variable correction factors added to compensate for different transit times.

Returning to our own data, it is clear that not all the points fall perfectly on the fitted lines (see Figs. 1 and 2; see also Figs. 12 and 13 in Mrosovsky et al., 1984a), but some scatter is to be expected, because the sex ratios from natural nests were estimated not from full clutches but from samples of 10 per

clutch. Sampling error in the sex ratio would contribute to some of the variation in the data. In addition, we would expect there to be some differences in the pivotal temperatures in individual clutches, and such variations should translate into differences in pivotal durations (Mrosovsky, 1988). This is due to the strong relationship between sex ratio and incubation duration, which is an indicator of developmental rate. Consider two clutches that have different pivotal temperatures. If turtle embryos from the first clutch differentiate into females at lower temperatures than those from the second clutch, then the pivotal duration from the first clutch (with a lower pivotal temperature) should be longer than that of the second (with a higher pivotal temperature).

In contrast to previous work, the present method may be suitable for estimating the mean hatching to emergence interval of large groups of nests, rather than of individual clutches. It is interesting to note that there is a general correspondence between our hatching-emergence interval estimate and those obtained by methods involving intervention and/or translocation of individual clutches (Table 1). This suggests that such interventions do not have major effects on the hatching to emergence interval.

Knowledge of the average estimated hatching to emergence interval is potentially valuable for management purposes. For instance, the hatching to emergence interval can be used to generate predictions of hatchling sex ratios from nests with known incubation durations. Specifically, to the curve relating sex ratio and laboratory incubation duration, the hatching to emergence interval can be added to derive a field pivotal incubation duration and a corresponding curve relating sex ratio and field incubation duration (see Marcovaldi et al., 1997).

In conclusion, our estimates of the hatching to emergence interval are based on large sample sizes as well as on certain assumptions. However, until a method is developed of directly measuring the behavior of the hatchlings in individual nests without disturbance, the present procedure provides an indirect estimate of the time it takes marine turtle hatchlings to emerge from the nest after hatching.

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