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Validation of incubation duration as an index of the sex ratio of hatchling sea turtles

N. Mrosovsky, Cecília Baptistotte, and Matthew H. Godfrey

Abstract: One method of estimating the sex ratio of hatchling sea turtles is to use the incubation duration. Long and short durations imply low and high temperatures, respectively. In turtle species whose sex is determined by temperature, males are produced at low temperatures and females at high temperatures. This study assesses the validity of using incubation duration to estimate the sex ratio. Samples of hatchling loggerhead turtles (*Caretta caretta*) were collected from nests with known incubation durations, and sex was ascertained by means of histology. The sex ratio of groups of nests determined by histology was compared with that predicted from previous relationships between incubation duration and sex ratio. For conditions causing relatively long or relatively short incubation durations, the sex ratio could be predicted with considerable accuracy. For conditions causing durations nearer to the pivotal duration (that which gives 50% of each sex), predictions could be off by 10%, depending on the distribution of incubation durations, but it was still possible to determine whether ratios were highly skewed or approximately balanced. Estimating sex ratios of hatchling sea turtles from incubation durations is simple, cheap, and can be used retrospectively.

Résumé : L'une des méthodes d'estimation du rapport mâles : femelles chez des tortues marines fraîchement écloses est d'utiliser les données sur la durée de leur incubation. Des durées d'incubation longues supposent des températures fraîches, des durées courtes supposent des températures plus chaudes. Chez les tortues dont le sexe est déterminé par la température, les températures fraîches produisent des mâles et les températures chaudes, des femelles. Nous avons éprouvé la validité des estimations du rapport mâles : femelles par mesure de la durée de l'incubation. Des Caouannes (*Caretta caretta*) fraîchement écloses ont été récoltées dans des nids où la durée de l'incubation avait été mesurée et leur sexe a été déterminé par examen histologique. Le rapport mâles : femelles dans des groupes de nids où le sexe des individus a été déterminé histologiquement a été comparé au rapport prédit d'après les relations établies en fonction de la durée de l'incubation. Dans des conditions où les durées d'incubation étaient relativement longues ou relativement courtes, le rapport pouvait être prédit avec assez d'exactitude. Dans des conditions où les durées se rapprochaient de la durée critique (celle qui donne 50% de mâles et 50% de femelles), les prédictions pouvaient comporter des erreurs atteignant parfois 10% selon la répartition des durées d'incubation, mais il était encore possible de déterminer si les rapports étaient voisins de 1 où très différents de 1. L'estimation du rapport mâles : femelles chez les Caouannes à partir de la durée de l'incubation est une méthode simple et peu coûteuse qui peut être utilisée rétrospectivement.

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Introduction

Temperature-dependent sexual differentiation in marine turtles and other reptiles has generated much interest in the sex ratio of the offspring produced by these species. According to the principles outlined by Fisher (1930), frequency-dependent pressures should maintain primary sex ratios close to 1:1, provided that the parental costs of producing a female and a male are equal. Other assumptions implicit in Fisher's formulation are elaborated by Hamilton (1967) and Bull and Charnov (1988). Despite a bias against publishing data on primary sex ratios of 1:1 because of a perceived lack of interest in such results (Festa-Bianchet 1996), it appears that in most vertebrates, primary sex ratios are close to 1:1 (Maynard Smith 1980).

However, it is not always easy to determine offspring sex ratios. One of the challenges is to find a simple way of sexing animals at an age when they may lack sexually dimorphic characteristics. This is certainly true of hatchling sea turtles, whose sex has been ascertained only by invasive and (or) labour-intensive methods (Yntema and Mrosovsky

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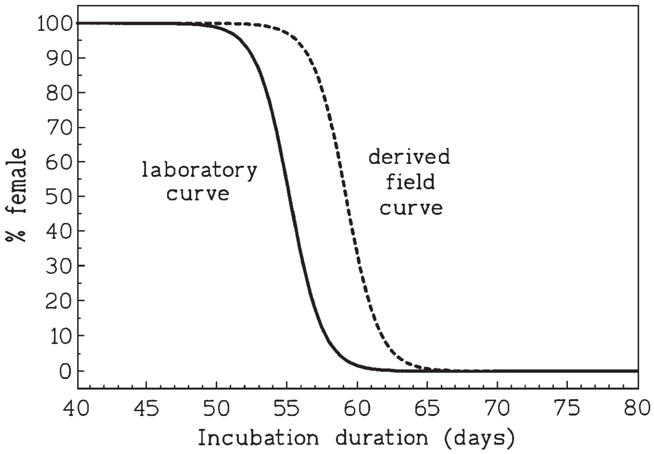
Fig. 1. Diagram of the method of estimating the sex ratio from the incubation duration in the field. First, a curve relating incubation duration and sex ratio is obtained for eggs incubated at constant temperature in the laboratory (Marcovaldi et al. 1997). This curve is then shifted to the right by a number of days corresponding to the interval between hatching and emergence at the surface, in this case 4.1 days. For ways of determining this interval see Godfrey and Mrosovsky (1997).



1980; van der Heiden et al. 1985; Rimblot-Baly et al. 1987; Gross et al. 1995). One possibility is to use hormone titres in the allantoic fluid left in the egg after hatching (Gross et al. 1995), but this has not produced consistent results in other hands (Hanson and Wibbels 1998). In field conditions, collecting allantoic fluid involves disturbing the eggs to separate them or obtaining samples before the fluid from different eggs mixes.

The possibility of using the rate of development as an index of egg temperature and thus of sex ratio in reptiles goes back some years (Mrosovsky and Yntema 1980; Mrosovsky et al. 1984; Standora and Spotila 1985). A measure of the rate of development is the duration of incubation, which in the field is the time elapsed between laying and emergence from the nest. Incubation duration could, in the future, provide a truly non-invasive way of estimating the sex ratio. Furthermore, the incubation duration can be obtained relatively easily, and indeed large sets of such data already exist for some turtle beaches.

A specific procedure for estimating the sex ratio from the incubation duration has been proposed by Marcovaldi et al. (1997), and this starts with laboratory studies to provide a curve relating the incubation duration of eggs to the sex ratio. This curve is then shifted to the right by a few days to reflect the fact that, in the laboratory, incubation durations do not include the interval between hatching from the egg and emergence at the surface (Fig. 1).

Using this method, Marcovaldi et al. (1997) estimated that sex ratios of hatchling loggerhead turtles (*Caretta caretta*) produced in Brazil were highly skewed toward females, especially in the state of Bahia. An extreme skew is consistent with the finding that for most of the nesting season the beach temperatures (Naro et al. 1999) were above the pivotal temperature, i.e., the constant temperature that gives 50% of each sex (Mrosovsky and Pieau 1991).

Nevertheless, direct validation of the use of incubation duration as an index of sex ratio is desirable because various assumptions are made in the method of Marcovaldi et al. (1997). We therefore collected samples of loggerhead turtle eggs in Brazil from clutches with known incubation durations. We estimated the sex ratio of these samples from these durations, using the methods in Marcovaldi et al. (1997), and compared the values with actual sex ratios determined by histology of the gonads.

Methods

Sampling

Ten hatchlings per nest were collected from 51 nests of loggerhead turtles on beaches in Espirito Santo (Comboios) and Bahia (Praia do Forte) in the 1996–1997 and 1997–1998 seasons. On beaches patrolled by Projeto Tartaruga Marinha (TAMAR), the national sea turtle conservation program in Brazil, nests are located the day after being laid and their position is marked with a numbered stake (Marcovaldi and Laurent 1996). A few days before hatchlings were expected to emerge from nests selected for the present study, plastic mesh was placed around the neck of the nest and anchored in the sand. These cages were checked daily in the early morning, and often several times in the night also. Hatchlings found emerged in the morning were scored as having emerged the previous evening. The same applied to newly laid nests found in the morning. Incubation duration was calculated as the time in days between laying and emergence. The aim was to obtain samples with a range of incubation durations. When emerged hatchlings were encountered, they were gently stirred by hand and 10 were picked at random; the rest were released.

Histology

Gonads were fixed in 8–10% formalin and transported to Canada with the appropriate CITES permits. The histological methods are given in Yntema and Mrosovsky (1980). Briefly, the gonad was cut in half transversely and one half was embedded cut side down in paraffin wax. Sections (10 μm thick) were mounted on slides and stained with periodic acid – Schiff's reagent (PAS) and Harris' haematoxylin. Ovaries had a thickened uneven cortex with a PAS-positive tunica albuginea between the cortex and the medulla. Testes had a very thin smooth cortex and immature seminiferous tubules in the medulla.

Analysis of data

Nests were grouped by incubation duration, and a mean of the sex ratios obtained from the histological examination for nests with the same incubation duration was calculated; this procedure provided points for the actual sex ratios. For the same incubation durations a sex ratio was derived from the curve of Marcovaldi et al. 1997 (see Fig. 1); this gave the predicted sex ratios. For both actual and predicted sex ratios, sigmoidal curves were fitted using Inplot 4.03 software (GraphPad, San Diego, California). Briefly, these curves are nonlinear regressions based on Marquardt's method, with a four-parameter logistic equation. A *t* test (Zar 1984) was used to assess whether the slope at the 50% female level of the actual curve was significantly different from that of the predicted curve.

Results

The percentages of females obtained for various incubation durations are given in Fig. 2. The pivotal incubation duration (the duration giving 50% of each sex; Mrosovsky et al. 1984; Godfrey et al. 1996) was 59.9 days, which is close to the predicted value of 59.3 days. When the percentages of

Fig. 2. Sex ratio of samples (10 hatchlings per clutch) at different incubation durations. The numbers beside the points show the number of clutches at that incubation duration. A sigmoidal curve is fitted to the points, with 0 and 100% specified as the lower and upper asymptotes.

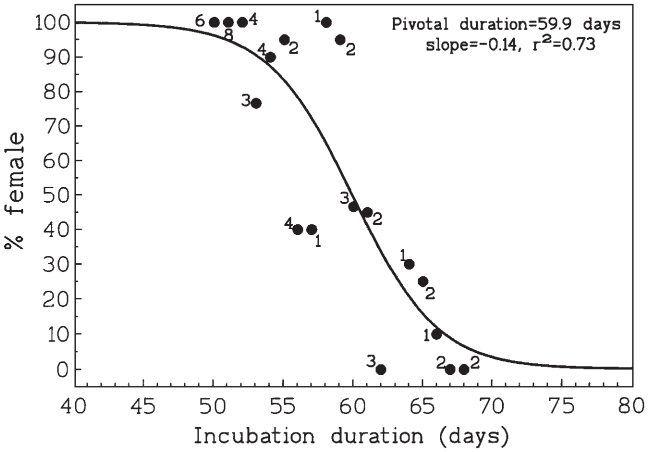
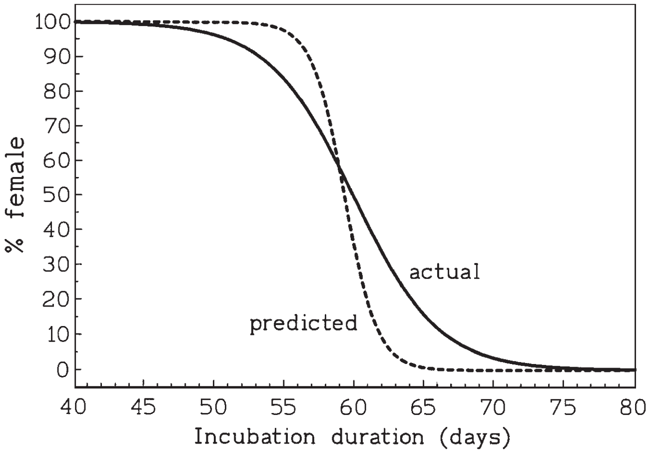


Fig. 3. Comparison of the curve from Fig. 2, based on actual sex ratios (solid line), with the curve for predicted sex ratios (broken line). The predicted curve was derived, as shown in Fig. 1, by adding the hatch-emergence interval to the curve fitting data obtained for Brazilian loggerhead turtle eggs incubated at constant temperature in the laboratory. The 50% female the curves points on give the pivotal incubation durations.



females were arcsine-transformed (Zar 1984), there was no significant difference between actual and predicted sex ratios (paired *t* test, $t = 0.43$, $p = 0.67$, df = 50). However, at the 50% female level, the slope of the actual curve (–0.142) was significantly different from that of the predicted curve (–0.365; $t = 5.4$, $p < 0.001$).

Discussion

The present method of estimating the sex ratio from the incubation duration is designed for groups of nests or populations, not for individual clutches. This is because a number of variables other than average temperature could influence the relationship between sex ratio and incubation duration. Short-lived but large temperature variations within the thermosensitive period, differences in clutch size and metabolic warming, and degree of compaction of the sand and difficulty in reaching the surface are examples of such variables. Daily variation in temperature is not included in this list because the depth of sea turtle nests provides a good thermal buffer. For example, at 60 cm depth, daily variation is generally <1°C (Godfrey et al. 1996; Naro et al. 1999); this makes virtually no difference to the constant temperature equivalent (Georges et al. 1994).

For groups of nests, laid over an extended season, aberrant results from some individual nests will, to some extent, cancel each other out. Despite individual variation, the correspondence between the actual and predicted curves in Fig. 3 shows that incubation duration can be used to predict the sex ratio for groups of nests. The accuracy of these estimates will, however, vary with the circumstances.

When incubation durations are mostly either long or short, giving sex ratios near asymptotic values of 0 or 100% female, the actual and predicted values are close, and incubation duration may be used with considerable confidence to estimate the sex ratio.

At intermediate incubation durations, even though the pivotal durations are close, there is some difference in the slopes of the curves (Fig. 3). This difference could affect estimates of sex ratio for groups of nests, depending on the distributions of their incubation durations. If these are split fairly evenly between those below and above the pivotal duration, then overestimates made from the predicted curve of the number of females at short durations will be cancelled out by underestimates of the number of females at longer durations. Estimates in such a case should be accurate because the pivotal values for the two curves are so close.

However, if incubation durations are mostly shorter or mostly longer than the pivotal duration, then the number of females could be overestimated or underestimated; the size of such misestimates would depend on how close the incubation durations were to those giving asymptotic sex ratios. For example, in the case of loggerhead turtles at Bahia, for which durations in the range of 51–54 days are common, the 92.6% females given by Marcovaldi et al. (1997) could be an overestimate. However, in the case of durations in the 51- to 54-day range, the divergence between the actual and predicted curves in Fig. 3 would only lead to overestimates in the order of 10%; the sex ratio of this population of hatchlings would still remain extremely biased toward females.

Moreover, the actual curve presented here is an approximation based on limited data. Some of the points in Fig. 2 are based on only one clutch, though most are based on two or more, and 18 incubation durations are represented. Perhaps if sample sizes were increased, the shape of the actual curve would become more similar to that of the predicted curve. It should also be recalled that only 10 hatchlings from a clutch were sampled, and this could have introduced sampling error (Ackerman 1997; Godfrey and Mrosovsky 1997). Larger sample sizes should reduce scatter.

The predicted curve is also an approximation. It assumes that the hatch-emergence interval is known and is reasonably constant throughout the season or from year to year. This may not be the case. Compaction of sand after rain might delay emergence (Hendrickson 1958). However, the sensitivity of sex-ratio estimates to changes in hatch-emergence interval can readily be assessed. For instance, Godfrey et al. (1999) calculated that even if the hatch-emergence interval were as brief as 1 day, the sex ratio of hawksbill turtles emerging in Bahia would still be highly skewed toward females (90.8%). It should also be recalled that the predicted curve is based on data from two clutches incubated in constant conditions. Although the results for these two clutches were similar, pivotal values do sometimes vary among loggerhead clutches (Limpus et al. 1985; Mrosovsky 1988).

Given such considerations, and limitations on sample sizes, the present correspondence between the actual and predicted curves is encouraging. However, it should not be assumed that this method will apply equally well to different beaches. If temperatures fluctuate in an unpredictable manner over short periods, perhaps because of rainstorms lasting a few days, then the sex ratio of embryos at their thermosensitive period may be influenced without much effect on incubation duration. In such cases, it is desirable to base estimates on data from a number of years. Other information, such as that on the consistency of the climate from year to year and whether temperature rises and falls in a fairly gradual way over the season, should be considered in determining how much weight to assign to estimates of incubation duration derived from a particular data set. In some cases, spot checks by histological methods may be feasible. But with reasonable caution, there is no reason why incubation duration, as an indirect measure of temperature, cannot be used on a variety of beaches with different climates to obtain first approximations of sex ratios.

Of course, it is hoped that a reliable, simple, cheap, noninvasive way of determining the sex of an individual turtle can be found. Until that time, incubation duration can provide some index of sex ratio for populations of turtles. This will assist in specifying sex ratios in population models more accurately than common assumptions of a 1:1 ratio. It could also be used retrospectively for beaches for which durations have been documented (cf. Standora and Spotila 1985). Incubation duration is therefore proposed more as an interim method, and also perhaps, as an adjunct to other methods, for taking macro-ecological snapshots rather than fine-resolution pictures.

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