Estimating sex ratios of loggerhead turtles in Brazil from pivotal incubation durations

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Abstract: A method of estimating natural sex ratios of hatchlings of species with temperature-dependent sexual differentiation from data on incubation durations is described. The method was applied to loggerhead turtles (*Caretta caretta*) nesting in Brazil. Data on incubation durations were collected from 11 nesting beaches monitored for up to six seasons. It was estimated that 82.5% of the loggerhead hatchlings produced were female. The strongly female-biased sex ratio in Brazil is similar to that found previously for loggerheads using beaches in the eastern U.S.A. This suggests that a female-biased hatchling sex ratio may be a feature of loggerhead populations.

Résumé : On trouvera ici la description d'une méthode d'estimation des rapports mâles : femelles à partir de données sur la durée de l'incubation chez des tortues néonates appartenant à des espèces à différentiation sexuelle déterminée par la température. La méthode a été utilisée chez des Caouanes (*Caretta caretta*) du Brésil. La durée de l'incubation a été mesurée dans les nids de tortues sur 11 plages de reproduction où les données ont été relevées pendant plusieurs saisons, parfois jusqu'à six. Nos estimations ont révélé que 82,5% des tortues à l'éclosion étaient des femelles. Ce rapport mâles : femelles très faible mesuré au Brésil est semblable à celui qui a été enregistré sur des plages de reproduction de cette tortue dans l'Est des États-Unis. Ce nombre démesuré de femelles chez les tortues fraîchement écloses est donc probablement une caractéristique des populations de caouanes.
[Traduit par la Rédaction]

Introduction

In many reptiles, sexual differentiation depends on the temperature of the eggs during a thermosensitive period in the course of incubation. This system of determining the phenotypic sex should make it relatively easy to vary the sex ratio. It has been argued that in order to understand the adaptive value of temperature-dependent sex determination, it might be instructive to know what the animals themselves are doing with this system, i.e., to know their sex ratios in nature (Mrosovsky 1994). However, it appears that there are considerable differences in the sex ratios of reptiles, ranging from close to 1:1 to more than 8:1 in favour of females (Mrosovsky and Provancha 1989, 1992; Janzen 1994; Chan and Liew 1995; Godfrey et al. 1996). Such species or population differences might in themselves be instructive if they could be correlated with some feature of life history, or with gender-related effects of incubation temperature (Bull and Charnov 1988).

The most extreme skew found so far is the approximately 90% bias towards female loggerhead turtle (*Caretta caretta*) hatchlings in Florida (Mrosovsky and Provancha 1989, 1992). Because 90% of loggerhead nesting along the Atlantic coast of the U.S.A. occurs in Florida (Murphy and Hopkins 1984; Shoop et al. 1985), even if all clutches laid north of

Florida produced 100% males, there would still be a heavy bias (>80%) in favour of females on the east coast of the United States.

Highly skewed sex ratios appear to challenge Fisherian sex-ratio theory, which predicts, given equal costs of producing a female or a male, that the sex ratio at the time parental investment ends should stabilize at 1:1 (Fisher 1930). Parental investment ends when the mother returns to the sea after laying eggs. Since the direction of sexual differentiation is determined after this time, during the thermosensitive period of incubation, the costs of producing male and female eggs must be close, if not identical. Therefore, according to classical theory, at hatching, the time nearest to the end of parental investment at which it is practical to sample and determine the phenotypic sex in such species (Mrosovsky et al. 1984a), the sex ratio should be balanced.

However, it is possible that in the case of turtles there are violations of some of the assumptions underlying this theory, such as random mating without population substructures that promote inbreeding (see Bull and Charnov 1988). Also, sampling error cannot be ruled out (Mrosovsky 1994), although this is unlikely in the case of the studies of loggerheads that extended over 5 years.

Another possibility is that loggerhead females from the eastern U.S.A. mate with males produced somewhere else, such as on the extensive beaches of Brazil. Females from one area could be balanced by males from another. The mitochondrial (mt) DNA of Brazilian and U.S. loggerheads is different (Bowen et al. 1994). This indicates that females born in Florida tend to return to nest in Florida and females born in Brazil tend to return to nest in Brazil. Although this evidence suggests that populations are separate, it is not critical, because mtDNA is instructive about the movements of females only. If large numbers of males originating in Brazil

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mated with females hatched in the U.S.A., this could produce a single breeding population with a less female-biased hatchling sex ratio than that in the U.S.A. alone. Separate male- and female-biased rookeries on this scale would be remarkable and would focus attention on the movements and mating of the males.

If, on the other hand, loggerheads nesting in Brazil are producing mostly female offspring, as happens in the U.S.A., that would suggest that this is a common feature of loggerhead populations. If similar huge female skews were found in both U.S. and Brazilian loggerheads, they would be harder to explain by invoking temporary disequilibria of sex ratios or sampling error. Such extreme sex-ratio biases in both the northern and southern hemisphere would constitute a body of data demanding an explanation based on sex-ratio theory.

Therefore, whether the data point to a geographic balancing of sex ratios or constitute an additional demonstration of female skews, it is of interest to estimate the sex ratios of loggerheads hatching on the beaches in Brazil.

Another reason for wanting to know more about the sex ratios of this population of loggerheads is that it has been suggested that an appropriate conservation measure is to skew sex ratios heavily in favour of females by deliberately warming the eggs (Vogt 1994) or by administering estrogens (Crews et al. 1994). If loggerheads in Brazil are already producing many more females than males, this would reinforce previous cautions (Wibbels et al. 1991; Mrosovsky and Godfrey 1995; Lovich 1996) about feminizing embryos. Indeed, if global warming occurs, a scarcity of males rather than of females might become a factor limiting population growth.

Thus, information on natural sex ratios is needed both to guide conservation programs and hatchery practices and to facilitate understanding of the population structure of loggerhead turtles and their apparently anomalous sex-ratio skews in the northwest Atlantic.

Unfortunately, determining sex ratios in hatchling sea turtles is not easy. The two main challenges are sexing and sampling. Like those of many reptiles, the neonates are not obviously sexually dimorphic. In the case of sea turtles, even the gross morphology of the gonad is not a reliable guide to the sex (Yntema and Mrosovsky 1980; Whitmore et al. 1985). Therefore, previous studies have had to fall back on time-consuming histology. Sex can now also be determined by assaying hormones in the allantoic fluid (Gross et al. 1995). Although this method is said to be noninvasive, it is necessary to invade the eggs before the turtles hatch, or remove the eggs from natural nests before hatching and separate them from each other. These procedures are often impractical, undesirable, or not cost effective on any scale in field conditions.

The lack of a simple reliable way of sexing is compounded by the large number of samples that are needed. Nesting seasons of sea turtles often extend over several months to half a year; during this time, temperatures, and therefore sex ratios, may vary. Worse still, thermal conditions may change from year to year. There are several documented cases of sex ratios in reptiles varying between years (Mrosovsky et al. 1984a; Rimblot-Baly et al. 1987; Bull and Charnov 1988; Janzen 1994; Godfrey et al. 1996). Therefore, to reduce the

chances of being misled by thermally atypical years, one needs to obtain information for a number of years. A further point is that most individual sea turtles do not reproduce in consecutive years. To obtain representative samples of nests and hatchlings from the adult population, it is preferable that studies span more than a year.

There is, however, a possible means of obviating some of these difficulties: the use of incubation durations as an index of sex. Conservation and management programs quite often collect data on incubation duration, in some cases over a number of years. This has been the case with the Brazilian Sea Turtle Conservation Program (TAMAR – IBAMA).

A large body of data shows that the incubation duration of turtle eggs is highly dependent on temperature (Bustard and Greenham 1968; Ackerman and Prange 1972; Mrosovsky 1988; Mrosovsky et al. 1992; Lewis-Winokur and Winokur 1995; Godfrey and Mrosovsky[2]). Sex ratios can be inferred either by converting durations to temperatures and then estimating the sex ratio from relationships between temperature and sex ratio (Mrosovsky and Yntema 1980; Standora and Spotila 1985), or directly from functions relating sex ratio to incubation duration (Mrosovsky et al. 1984a; Standora and Spotila 1985; Eendebak 1995). In the present case, because there are no data on sex ratios of Brazilian loggerheads, or any function relating their incubation durations to beach temperatures, we adopted a somewhat different approach.

First, from laboratory experiments with eggs incubated at different constant temperatures, we obtained values for the pivotal temperature and pivotal incubation duration for loggerhead clutches from Brazil. The pivotal temperature is that temperature giving a 1:1 sex ratio when eggs are incubated at constant temperature (for definitions see Mrosovsky and Pieau 1991). The pivotal incubation duration is the corresponding incubation duration. In the laboratory this is the time from egg laying to hatching. In contrast, in the field, the incubation duration determined as part of conservation and research programs refers to the time between egg laying and emergence of hatchlings from the surface of the sand. Therefore, to convert laboratory incubation durations to field incubation durations, one must add on the number of days taken by a turtle to reach the surface after it hatches from the egg.

For loggerheads the interval from hatching to emergence averages 4.1 days (Godfrey and Mrosovsky[2]). This value is for the U.S. population of loggerheads; it is assumed here that value is the same for loggerheads nesting elsewhere. (As this assumption may not be exactly correct, we also used some other values in our calculations to test the sensitivity of the estimates to this variable.)

Adding the hatch – emergence interval to the laboratory pivotal incubation duration provided a value for the field pivotal duration. This was then used as a standard against which to compare actual incubation durations obtained in the field, and so infer the sex ratio for these nests.

Incubation durations vary with the stage of the season because turtle nesting seasons generally last several months, during which the weather changes. Therefore, the actual durations were compared with the pivotal duration for differ-

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[2] M.H. Godfrey and N. Mrosovsky. Estimating the time between hatching of sea turtles and their emergence from the nest. Submitted for publication.

Fig. 1. Locations of the 11 beaches studied. BA, Bahia; ES, Espírito Santo; SE, Sergipe.
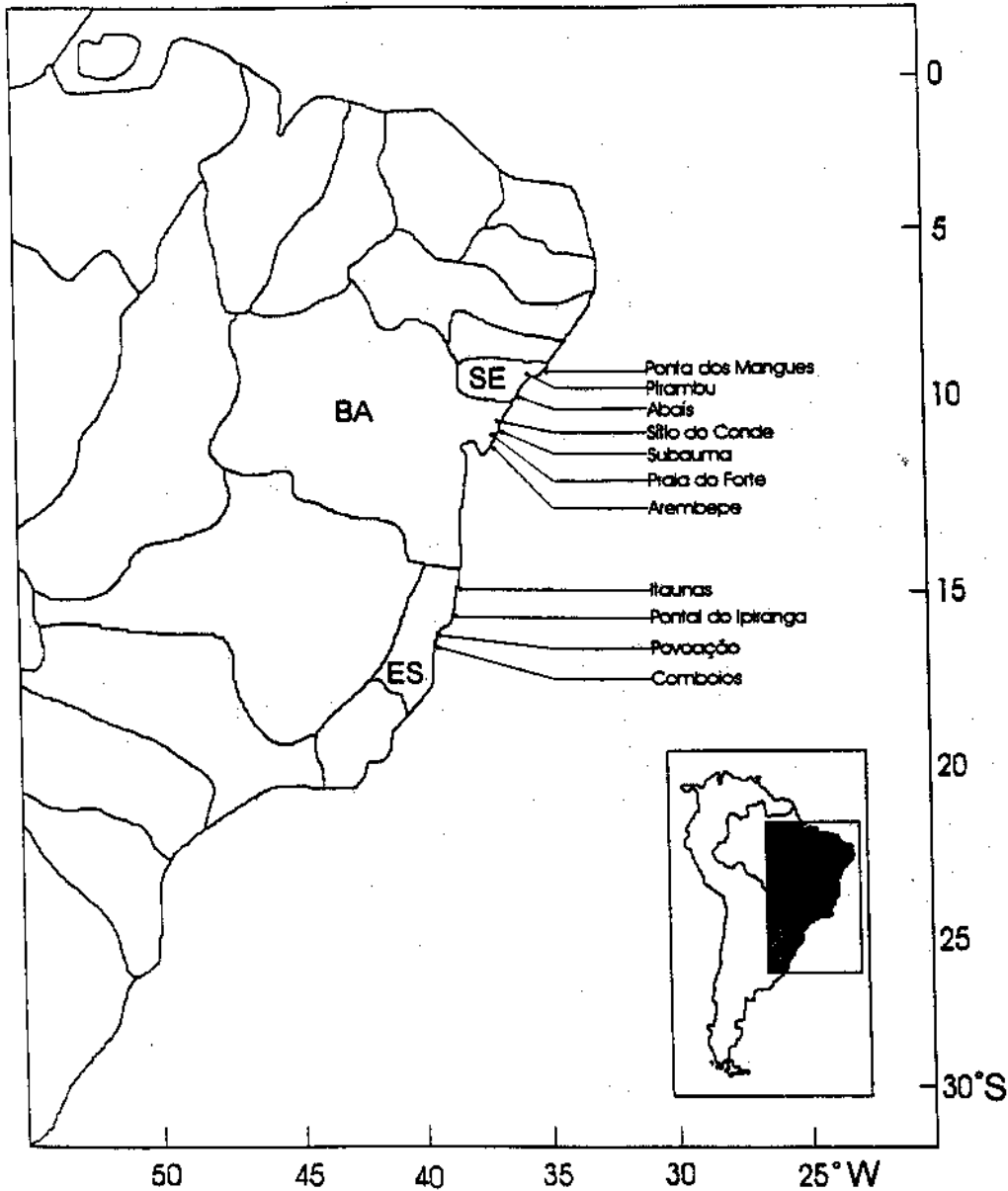
ent parts of the nesting season. This gave estimates of sex ratios of turtles produced during each part of the season. The numbers of turtles nesting in different parts of the season also vary. Data on seasonal nesting distributions for various beaches in Brazil have been collected by TAMAR. To obtain an overall sex ratio, the sex ratios produced at different times within the season were each multiplied by the number of nests laid at those times.

In summary, our procedure consisted of four steps: (1) Conducting laboratory experiments to obtain a value for the pivotal incubation duration. (2) Augmenting the laboratory pivotal incubation duration by 4.1 days to convert it to a field pivotal incubation duration. (3) Comparing this calculated field pivotal incubation duration with the actual incubation durations of nests. This was done for different periods within the nesting season to provide estimates of sex ratios produced at these different times. (4) Calculating the overall

sex ratio by taking into account the number of turtles nesting during different parts of the season.

Methods

Habitat and incubation durations in the field

Data on incubation durations were collected from 11 areas that are the main loggerhead turtle nesting areas on the coast of Brazil (Fig. 1). These 11 nesting areas were distributed over three states: Sergipe (Ponta dos Mangues, Pirambú, Abaís), Bahia (Sítio do Conde, Subauma, Praia do Forte, Arembepe), and Espírito Santo (Itaunas, Pontal do Ipiranga, Povoação, Comboios). For the beaches in Bahia and Espírito Santo, data were available from six consecutive seasons, starting with 1988−1989. For the beaches in Sergipe, data were available from four seasons, 1989−1990, 1990−1991, 1991−1992, and 1993−1994.

Nesting densities are, in general, greatest on the beaches in the state of Bahia. These beaches are often backed by plantations of

coconut palms (*Cocos nucifera*). The trees are usually relatively sparse or located far back from the nesting areas preferred by loggerheads; no major thermal effects from their shadows were detected in initial studies (Naro et al.[3]). Farther north, towards Sergipe, there are fewer plantations and the sand tends to become whiter and finer. To the south, in Espírito Santo, vegetation other than beach creeper is sparse near the nesting zone; sand grains tend to be larger and beaches more dynamic, with more wood on the beaches near river mouths. For more details see Bellini and de Almeida (1990), Santos (1993), and Marcovaldi and Laurent (1996).

Freshly laid nests found in the morning were scored as being laid on the previous night. From about 45 days after laying, nests were inspected daily in the early morning. Any emergence of hatchlings was scored as having taken place on the previous night. Incubation duration was calculated as the number of days between the night of laying and the night of emergence of the majority of the hatchlings. Only data from clutches left in situ on the beach are included here.

Nesting frequency

Data on nesting frequency (non-nesting crawls excluded) were available from all the 11 beaches mentioned for three seasons (1991–1992, 1992–1993, 1993–1994). Daily patrols were carried out from the start of October to the end of March. However, systematic patrols were not carried out in all 11 areas at the very start of the season, September. It was therefore necessary to base the percentage of a season's nests laid in that month on data for particular beaches. On one beach in Bahia, Praia do Forte, systematic patrols were made in the second half of September during 5 years (1990–1991 to 1994–1995; the minimum number of nests per season was 109). The mean percentage of the seasons' totals laid in the second half of September (16–30) in these years was 3.1%. From other work it is estimated that September as a whole accounts for close to 5% of the season's total (D'Amato and Marczwski 1993; Marcovaldi and Laurent 1996). We therefore assumed that 3.1% of a season's nests are laid in the second half of September and 1.9% in the first half, making a total of 5% for September as a whole. We assumed that these percentages were also appropriate for other beaches in Bahia.

The same percentages of nests were used for September in Sergipe. At Pirambú, 6.75% of all loggerhead nests were laid in the second half of September, based on data from 2 years, 1990–1991 and 1992–1993, during which regular patrols started in September. However, as the total numbers of loggerheads nesting in Pirambú in these years were so small, 22 and 11, respectively, it seemed preferable to use the figures from Bahia.

For Comboios in Espírito Santo, monitoring was carried out systematically in the second half of September for 4 years (1990–1991 to 1993–1994). Nesting from 16 to 30 September averaged 0.93% (the minimum number of nests for the whole season was 104). Based on the experience of biologists at Comboios, nesting in the first half of September is most uncommon (C. Baptistotte, personal communication). We therefore assigned 0.07% of the nests to this half-month period, to put nesting in the whole of September at 1% of the season's total. These values for Comboios were used for other beaches in Espírito Santo.

Even if the values for nesting in September are not quite correct, because so few nests are laid in this month, calculations of sex ratio in a season should only be in error by a few percent at most on this account.

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[3] E.F.S. Naro, N. Mrosovsky, and M.A. Marcovaldi. Thermal profiles of turtle hatcheries and nesting areas at Praia do Forte, Brazil. Submitted for publication.

Pivotal temperatures in the laboratory

A total of 200 eggs (100 from each of two clutches, mothers' tag Nos. BR3002/5038 and BR11915/11916) were collected at Praia do Forte, Bahia, on the night of 5–6 November 1995. They were kept in an air-conditioned room until about 4:00 p.m. the next day, and then taken to Toronto. The total time between laying of these two clutches and their being placed in temperature-controlled incubators in Toronto was approximately 32–35 h. On arrival, the majority of the eggs showed no signs of the white spot that is an early indicator of normal development (Blanck and Sawyer 1981).

Eggs were incubated in Precision Science incubators. Each egg was placed in a moist vermiculite and sponge substrate in a separate container. The methods of incubation have been described in detail for experiments with loggerhead eggs from the U.S.A. (Mrosovsky 1988). Exactly the same methods were employed here in order to make the data comparable. The only difference was that from day 36 onwards, small tubes were inserted into one of the incubators to permit measurement of oxygen levels with a Servomex No. 572 oxygen analyser. Between days 37 and 53, readings of oxygen levels within the incubator were taken in the morning almost daily, about 12 h after it was last opened. The analyser was calibrated weekly with pure nitrogen.

Temperatures in one incubator became erratic during the first few days. Therefore, on day 4, eggs were transferred to other incubators. The initial temperatures, which were within 0.8 and 0.4°C of the temperatures after day 4, were discounted because the eggs spent only 3 days after travel at the initial temperatures and this was well before the thermosensitive period (Yntema and Mrosovsky 1982).

A mercury thermometer with 0.1°C graduations lay on each shelf. Temperature groups were constituted retrospectively. For example, if the temperature on the top shelf of one incubator was very close to that on the lower shelf of another incubator, the eggs from these two shelves were combined.

All thermometers were calibrated against a Sybron/Taylor mercury thermometer with certified calibration against platinum resistance thermometers that had been calibrated by the U.S. National Bureau of Standards.

On day 32, a Yellow Springs Instruments No. 402 thermistor in a needle was inserted into one egg to assess evaporative cooling of the eggs (for methods see Mrosovsky 1988). The egg was 0.5°C (mean of five readings) cooler than a standard mercury thermometer placed nearby in the incubator. Therefore, a −0.5°C correction factor for evaporative cooling was applied to all temperatures taken in the incubators. This correction factor is the same as that used in previous work on loggerhead eggs from the U.S.A.

Sex was determined by microscopic examination of sections of the gonads. Histological methods are given in Yntema and Mrosovsky (1980) and Mrosovsky et al. (1984a).

Results

Pivotal temperature

The temperature that gave 50% of each sex was 29.2°C. There was little difference between the two clutches (Table 1). The result was unaffected if the eggs that were moved on day 4 were discarded. The overall hatch rate was 93.4%, eliminating differential mortality between the sexes as a factor in the estimation of the pivotal temperature.

Oxygen level

No indication of oxygen shortage in the incubators was detected. The mean level was 20.4% (range 20.2–20.7%; $n = 12$ readings).

Fig. 2. Curve used to obtain the pivotal incubation duration in the laboratory (i.e., duration giving 50% of each sex). The sigmoid curve was fitted by GraphPad Inplot4, assuming 100 and 0% asymptotes ($r = 0.988$); the number beside each data point shows the number of eggs at that duration.
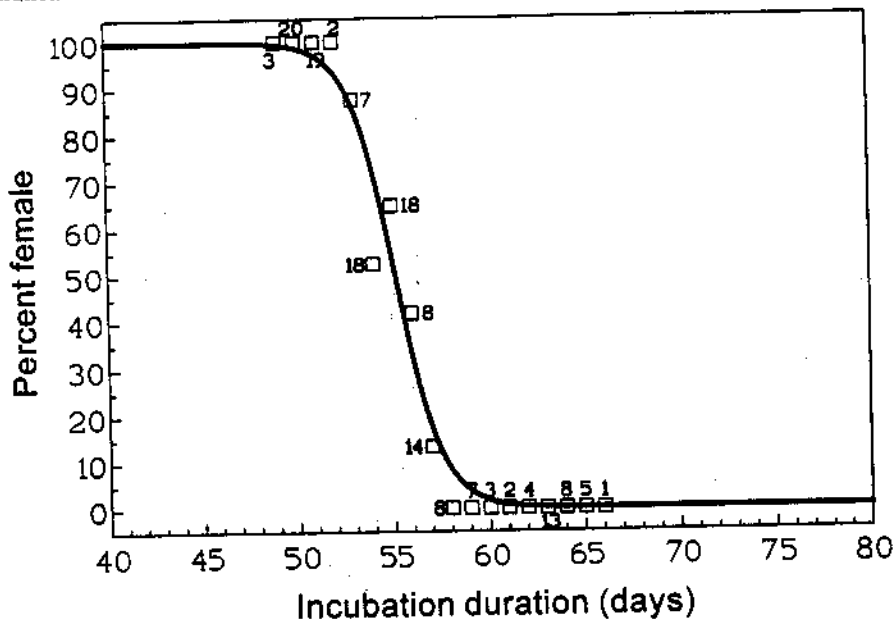

Incubation duration (days)

Table 1. Percentages of female Brazilian loggerhead turtles and incubation durations of eggs incubated at different temperatures.

	27.4 ± 0.3°C	28.0 ± 0.2°C	28.7 ± 0.3°C	29.0 ± 0.2°C	29.4 ± 0.2°C	30.6 ± 0.3°C
No. of eggs set						
Clutch P	8	15[a,b]	24[c]	15	15	22
Clutch Q	8	16[c]	22[c,d]	15	15	23
Females (%)						
Clutch P	0	0	20.8	23.1	73.3	100
	(8)	(12)	(23[e])	(14)	(15)	(22)
Clutch Q	0	0	25.0	42.9	60.0	100
	(8)	(15)	(19[e])	(13)	(15)	(21)
Both clutches	0	0	22.7	33.3	66.7	100
Incubation duration (days)	63.9	62.8	57.0	55.8	54.2	50.4

Note: Numbers in parentheses are numbers of turtles.

[a]Plus one additional egg that accidentally became desiccated early in the incubation period.
[b]Includes seven eggs that were moved from one incubator to another on the fourth day of incubation.
[c]Includes eight eggs that were moved from one incubator to another on the fourth day of incubation.
[d]Plus one additional egg used for assessment of evaporative cooling.
[e]Plus one sexed embryo taken from an egg that was opened because it appeared not to be viable.

Pivotal incubation duration

The pivotal incubation duration was 55.2 days in the laboratory (Fig. 2). Therefore, allowing 4.1 days for the hatch–emergence interval (Godfrey and Mrosovsky, see footnote 2), the pivotal incubation duration in the field would be 59.3 days (Fig. 3).

## Incubation durations and sex ratio in the field

The pivotal incubation duration of 59.3 days was added to graphs of actual incubation durations in the three states (Figs. 4–6). For each half-month, the percentage of each sex was calculated using the derived curve in Fig. 3 to convert each incubation duration into a sex ratio.

Nesting distributions

The nesting seasons and distributions of nests within seasons were similar among years, with differences of only a few percent at any time of year (Fig. 7). The nesting distributions

Fig. 3. Sex ratio as a function of incubation duration in the field (broken curve) derived from the laboratory curve (solid line from Fig. 3) shifted to the right by 4.1 days to allow for the hatch–emergence interval.
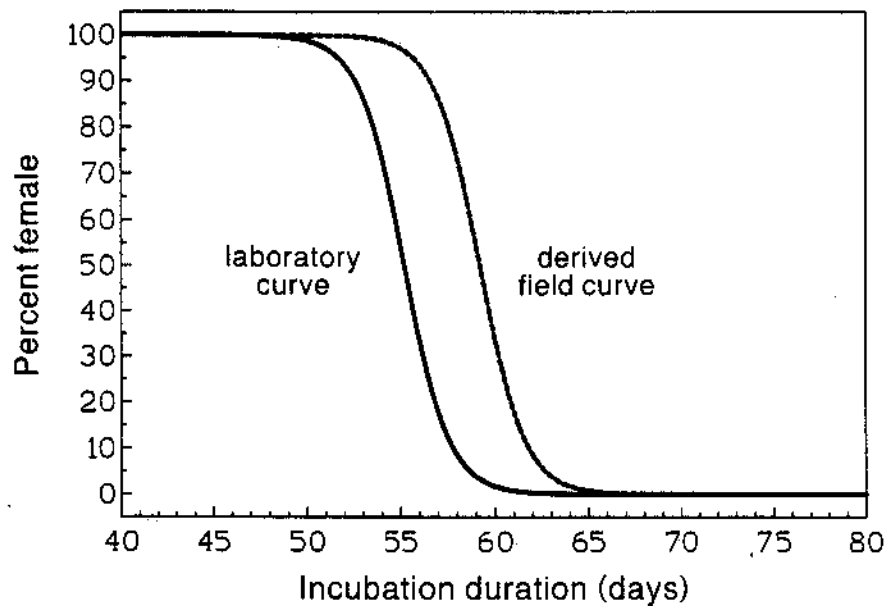
were also similar among the three states, except that at the start of the season in September, nesting in Espírito Santo was less (Fig. 8).

Discussion

Bahia: female skews in overall sex ratio

There are reasons for thinking that the sex ratio of logger-head hatchlings produced on the beaches of Bahia is highly biased towards females. First, for most of the season, incubation durations are below, often well below, the pivotal incubation duration. Second, data (Naro et al., see footnote 3) from Praia do Forte show that for much of the season, sand temperatures at turtle nest depth are higher than the pivotal temperature of 29.2°C obtained in our experiment described here. For most of the season, temperatures were above 30°C, readings of 31°C being common. Although measurements made on other beaches in Bahia and in more years would be desirable, the existing data on sand temperatures point to female-biased ratios. Third, the situation in Bahia is similar to that in Florida. The pivotal temperatures for loggerheads from Bahia and Florida are both close to 29°C; the sand temperatures in the two areas are also fairly similar (often above 30°C). Therefore, the sex ratios of hatchlings should be similar in the two areas. For Florida, there is direct evidence, from histology of the gonads, that about 90% of the hatchlings are females (Mrosovsky and Provancha 1989; 1992). On this basis it is highly probable that the loggerheads hatching in Bahia are also mostly females.

If there were large diel changes in temperature over the course of day, then it might be misleading to compare average temperatures in the field with pivotal levels from the laboratory. In Bahia, the temperatures varied over 24 h by only about 0.5°C at 60 cm depth and 2°C at 30 cm depth (Naro et al., see footnote 3). Such small temperature variations increase the "constant temperature equivalent" (Georges et al. 1994) by 0.3°C at most. Moreover, because diel varia-

tions raise the constant temperature equivalent, they would if anything increase the number of females.

We now estimate the extent of this bias quantitatively, taking nesting distributions into account. For each nest with an incubation duration, a sex ratio was assigned using the function relating incubation duration to sex ratio in Fig. 3. The average of all these sex ratios for each half-month bin was then calculated. The relative numbers of turtles in the different half-month bins (i.e., as a percentage of the total number of nests in the three seasons with nesting distributions) were then multiplied by the sex ratio. Summing the values from each bin gave an overall sex ratio of 92.6% female for Bahia.

Limitations and possible sources of error

1. The present method of estimating sex ratios is not appropriate for individual clutches. This is because the incubation durations of two clutches might be the same, but their sex ratios might differ because a temporary deviation from the average temperature might occur for one clutch during the thermosensitive period and for another clutch outside of it. In the present paper we estimate sex ratios for groups of clutches over several years. With sea turtles, whose nesting is spread over several months, any unusual perturbation of the generally gradual seasonal changes in temperature will occur during the thermosensitive period for only a subset of nests, and of these, a short perturbation should affect the sex ratios only of a further subset of nests that were close to the pivotal temperature. A longer unusual perturbation should also affect incubation duration, thus reducing the error in the estimate of sex ratio.

2. Systematic patrolling of many of the beaches during the years for which we present data started October 1 and ended March 31. If appreciable numbers of turtles nested before or after these dates, the number of male hatchlings might be underestimated, since September and April tend to be cooler than the main loggerhead nesting season. However, the seasonal nesting distributions (Figs. 7 and 8) and other infor-

Fig. 4. Incubation durations (mean ± 2 SD) for Bahia. Data are from all seasons combined. The numbers below each bar is the sample size for that half-month bin. The thin horizontal line is the pivotal incubation duration obtained in the laboratory. The thick horizontal line is the derived pivotal incubation duration for the field. Solid and open bars show estimates, derived from Fig. 3, of the percentages of females and males, respectively, for each half-month bin.
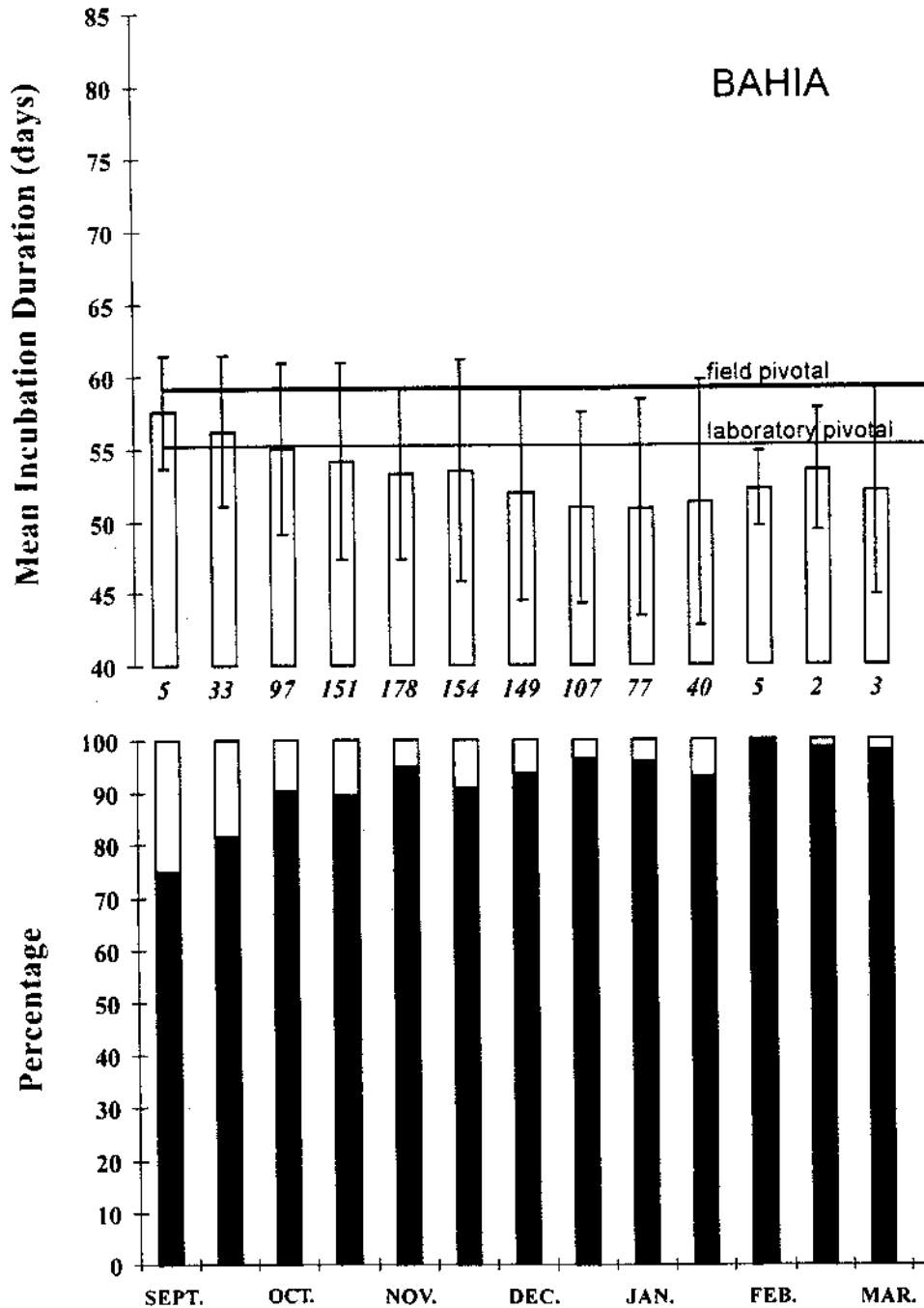
mation (see Methods) make it improbable that major nesting at either end of the season had been missed.

3. Pivotal temperatures were based on eggs from only two clutches. This is far from ideal for approximating a mean for the population. Yet this probably is not a major source of error. The pivotal temperature of 29.2°C for Bahia is close to 29.0°C, the pivotal temperature for loggerheads nesting on the east coast of the U.S.A. (n = 6 clutches; Mrosovsky 1988). The laboratory pivotal incubation duration obtained

here, 55.2 days, is close to the 56.1 days for loggerheads from the east coast of the U.S.A. (Godfrey and Mrosovsky, see footnote 2; n = 6 clutches). The pivotal temperature for sea turtles appears to be a conservative characteristic. So far, despite dissimilarities in experimental methods, all pivotal temperatures obtained for sea turtle species have been within a degree of 29°C (see the review table in Mrosovsky 1994; Georges et al. 1994). Finally, even if the population pivotal temperature were somewhat higher than the 29.2°C found

**Fig. 5.** Incubation durations (means ± 2 SD) for Espírito Santo. For details see Fig. 4.
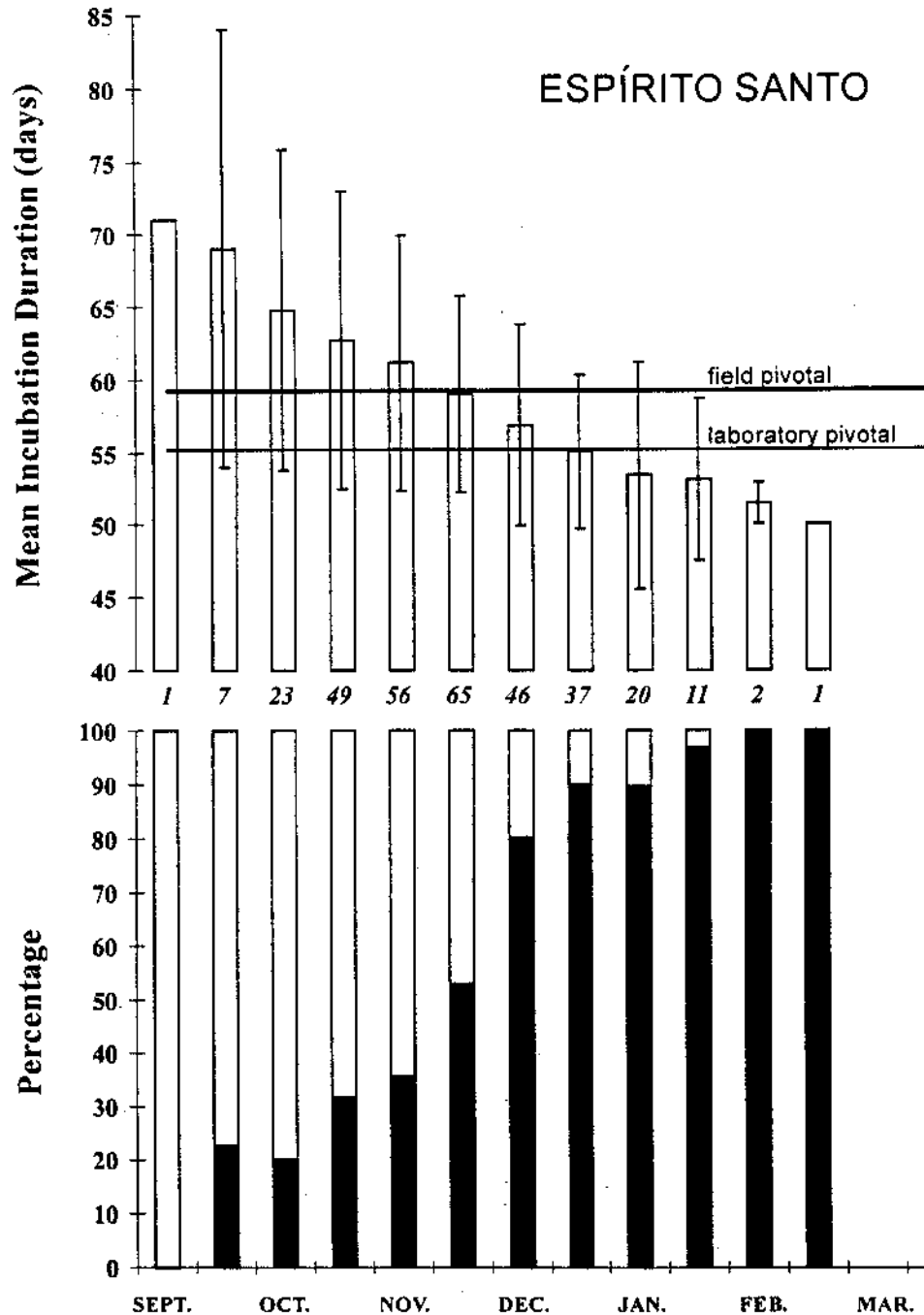
here, and the population pivotal duration somewhat shorter than the 59.3 days found here, the large skew towards females would be merely reduced, not abolished, because for most of the season the actual incubation durations were well below the pivotal incubation duration (Fig. 4).

4. If the hatch−emergence interval is overestimated (perhaps loggerhead hatchlings reach the surface sooner in Bahia than in Florida), the pivotal incubation duration of 59.3 days in the field will be too high and the extent of the female bias correspondingly overestimated. Suppose the hatch−emergence interval is 3.1 days rather than the 4.1 days that we have used, then the overall sex-ratio estimate for Bahia for all

years is reduced from 92.6 to 89.2% female. Again, because the actual incubation durations are well below pivotal levels for much of the season, the effect is not huge. Moreover, a 3.1-day hatch−emergence interval is shorter than any published values (reviewed in Godfrey and Mrosovsky, see footnote 2). It seems unlikely that the average could be shorter or even equal to 3 days.

5. In certain areas where there is a high risk of poaching or damage from tourists, all nests, wherever they are laid, are moved to safe places by TAMAR personnel. Removal of eggs in such cases should not bias the sample towards long or short incubation durations. In other places, however,

Fig. 6. Incubation durations (means ± 2 SD) for Sergipe. For details see Fig. 4.
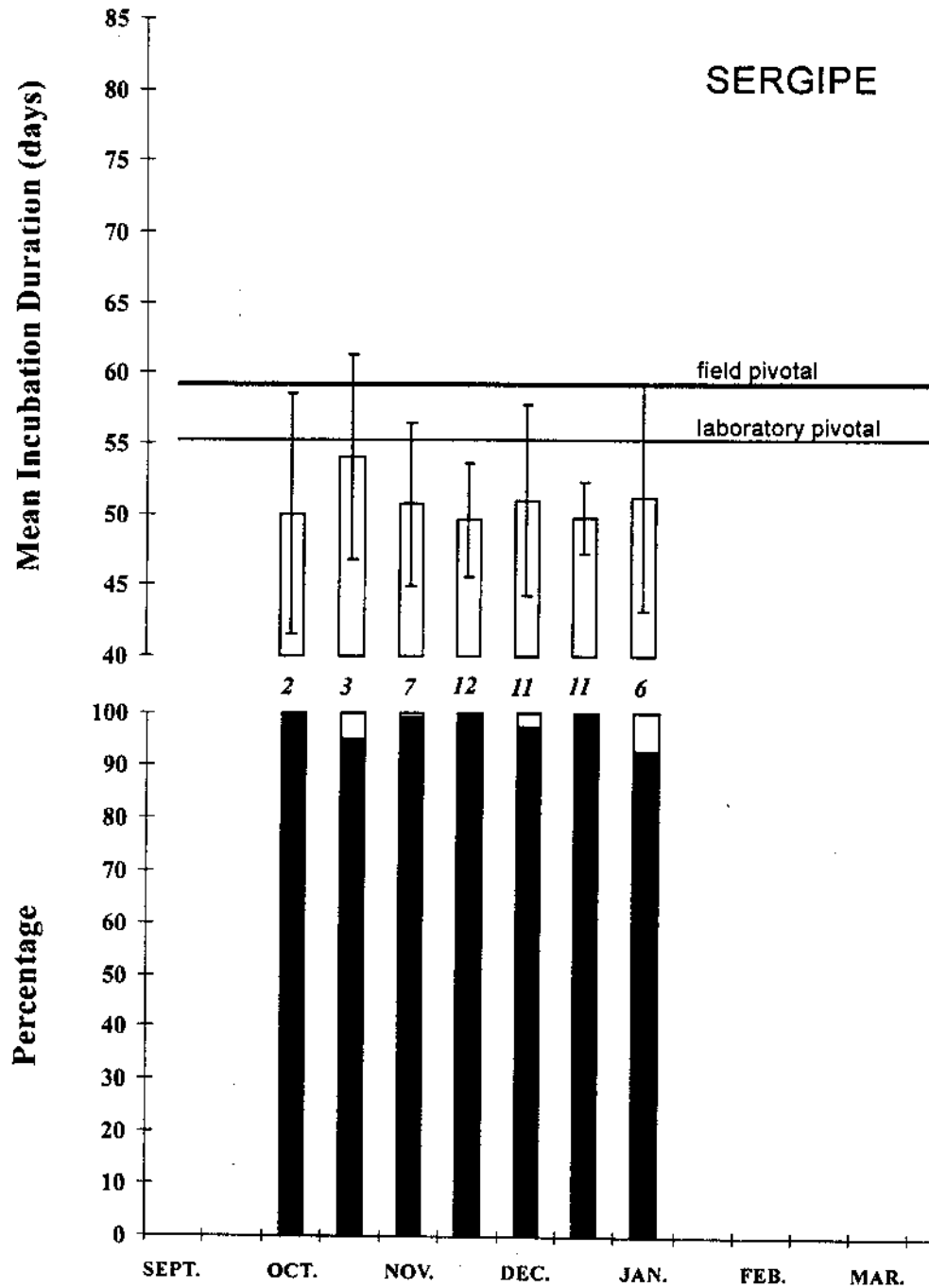

sometimes only clutches laid low down on the beach are relocated, while those higher up are left. The clutches moved are those laid so low on the beach that they are doomed by the high tide; they are known as "ninhos suicidas" in Brazil. Since this study concerns only nests left in situ, any relocated nests are excluded. However, it is not always possible to predict how high the water will come. Suppose that some of the relocated nests would, in fact, have survived if left in situ. Turtle eggs can withstand some washing over by seawater and still survive, though hatch rates are often reduced (Whitmore and Dutton 1985). Nests that are washed over are cooled (e.g., Mrosovsky et al. 1984a). Therefore if nests that

would have survived are moved, thus excluding their incubation durations from the sample, then the production of males could be underestimated. We do not have data to assess the extent of this source of error quantitatively. We believe that in most cases designating nests as doomed is correct because these judgements are usually made by people familiar with local conditions. Moreover, on many of these beaches, only a few nests are considered to be doomed by the sea.

Espírito Santo and Sergipe: south – north differences
In Espírito Santo, incubation durations were considerably longer and frequently above the field pivotal level (Fig. 5).

Fig. 7. Seasonal nesting distributions in Sergipe (a), Bahia (b), and Espírito Santo (c), expressed as a percentage of the season's total; the numbers in parentheses are the total number of nests in each season.
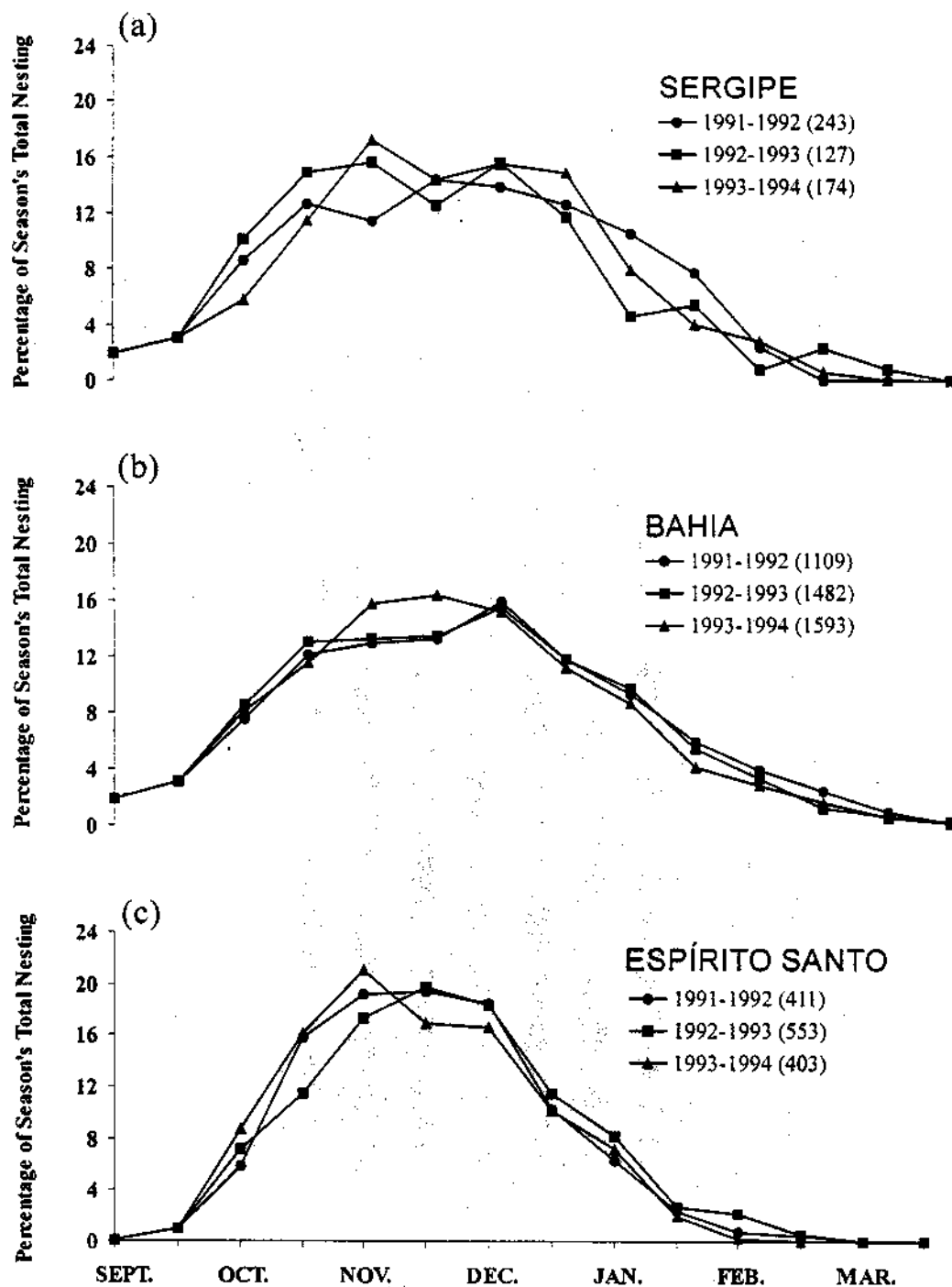

The overall sex ratio, calculated as above from the averages of all years' incubation durations and nesting distributions, was 57.3% female (50.0% when a 3.1-day hatch—emergence interval was used).

These calculations assume that pivotal incubation temperatures and durations are similar for loggerheads nesting in Espírito Santo to those reported here for Bahia. Obviously, data on this point would be desirable, but it is not an unreasonable assumption when one recalls that pivotal levels for sea turtles appear to be relatively conservative, and that no geographic differences in pivotal levels for loggerheads nesting from Florida to North Carolina have been detected (Mrosovsky 1988). If such differences exist they are probably small.

It would appear that the sex-ratio situation for loggerheads in Brazil and the U.S.A. has parallels. In both cases there is a major skew towards females in the nesting areas nearest to the equator. As one goes towards the poles, incubation durations increase (Figs. 4—6; Mrosovsky 1988; Schwartz 1989), and therefore, one might infer, the percentages of males also

Fig. 8. Comparison of seasonal nesting distributions in different states. Numbers for each half-month bin are expressed as a percentage of the total for 1991–1994.
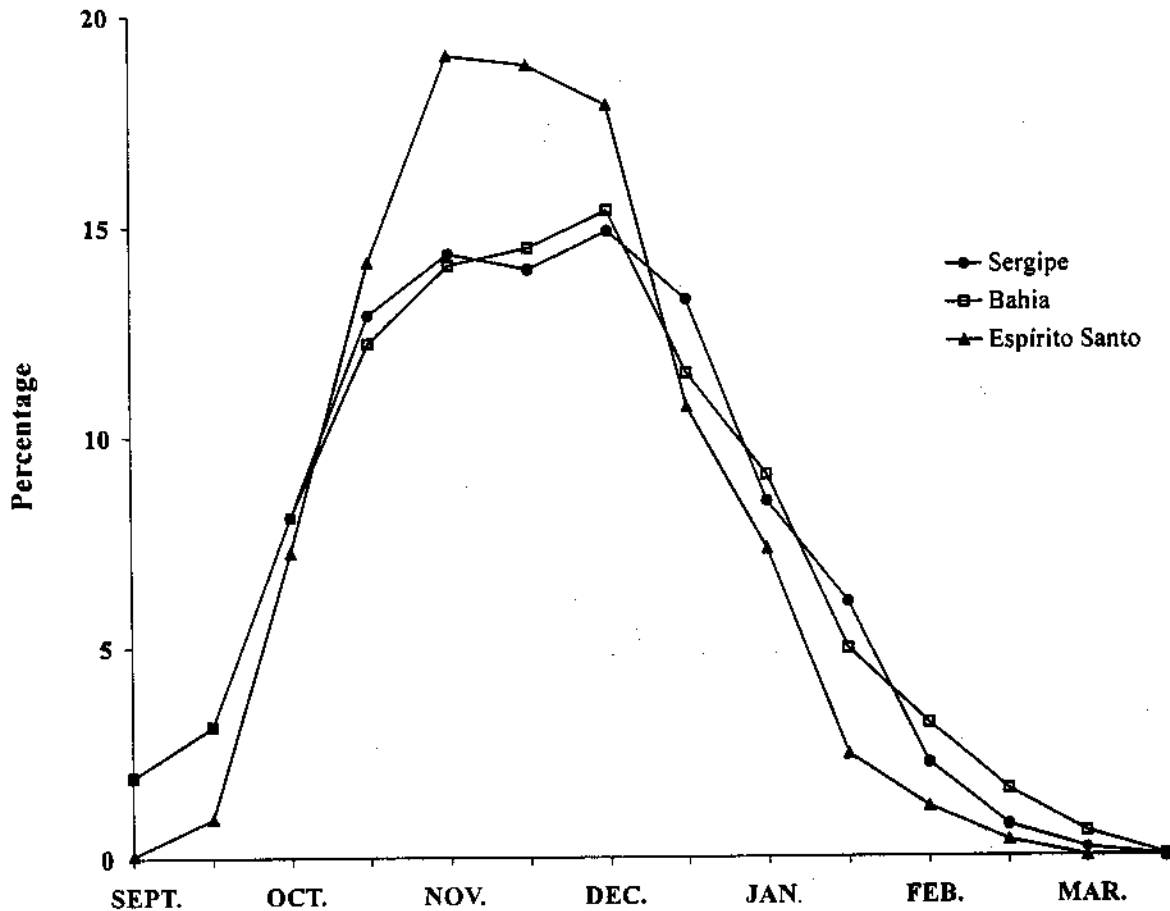
increase. Sexing of small samples of hatchlings from Georgia and South Carolina indicates that ratios are relatively balanced in these states (Mrosovsky et al. 1984b).

Sergipe

The data from Sergipe, at the northern end of the loggerhead nesting range in Brazil, are consistent with this. Incubation durations were generally short and well below pivotal levels, indicating a bias towards producing females.

The data for Sergipe cover fewer years, and sample sizes for incubation durations were smaller than those for the other states. No incubation durations were available for the beginning and end of the season (Fig. 6). Fortunately, few turtles nest at the end of the season, so that assumptions about sex ratios do not greatly influence the overall estimate. For missing values in the Sergipe data, the sex ratios calculated for Bahia were assumed to apply. The overall sex ratio in Sergipe was estimated at 96.9% female (94.5% when a 3.1-day hatch−emergence interval was used).

Sex-ratio estimates for different seasons

To learn if sex ratios varied greatly from year to year, we made separate estimates for each of the 3 years (1991−1992, 1992−1993, 1993−1994) for which we had nesting distribution data. This could not be done for Sergipe because of insufficient data on incubation durations for individual years.

In Bahia, the sex-ratio estimates for the 3 years were 89.3, 95.2, and 98.3% female, respectively. The higher the percentage of females, the more the production of the few males was restricted to the early parts of the season (Fig. 9). The average of these three sex ratios for the 3 years was 94.3% female. This is close to the sex ratio of 92.6% based on average incubation duration and nesting distribution data for all the years (see above).

In Espírito Santo there was more variation between the three seasons: the sex-ratio estimates for the 3 years were 53.5, 54.9, and 70.5% female, respectively (Fig. 10). This is another example of differences in hatchling sex ratios of turtles in different years. It is encouraging, however, that for Espírito Santo also, the mean of the three sex ratios for the three separate seasons, 59.6% female, is close to the sex ratio of 57.3% based on the average data from all the years.

Overall sex ratios for loggerheads in Brazil

To estimate the sex ratio of loggerheads produced in Brazil as a whole, as well as knowing the sex ratios for different areas, it is necessary to know the relative numbers of nests laid in different areas. This information is available for Sergipe, Bahia, and Espírito Santo for 4 years (Table 2). Combining the means for these years with overall sex-ratio estimates for these states gave 82.5% female (78.1% when a 3.1-day hatch−emergence interval was used).

Fig. 9. Seasonal sex production profiles for Bahia. In each season the total number of hatchlings produced is taken as 100%. The graph shows the percentages of this total produced in each half-month bin. Incubation duration data were not available for a few bins at either end of some seasons; in such cases the average duration for all years (Fig. 4) was used. These cases represented only 2.3–4.9% of nesting in any season. (a) Nesting distributions. (b–d) Estimated sex ratios for the 1991–1992, 1992–1993, and 1993–1994 season, respectively.

BAHIA
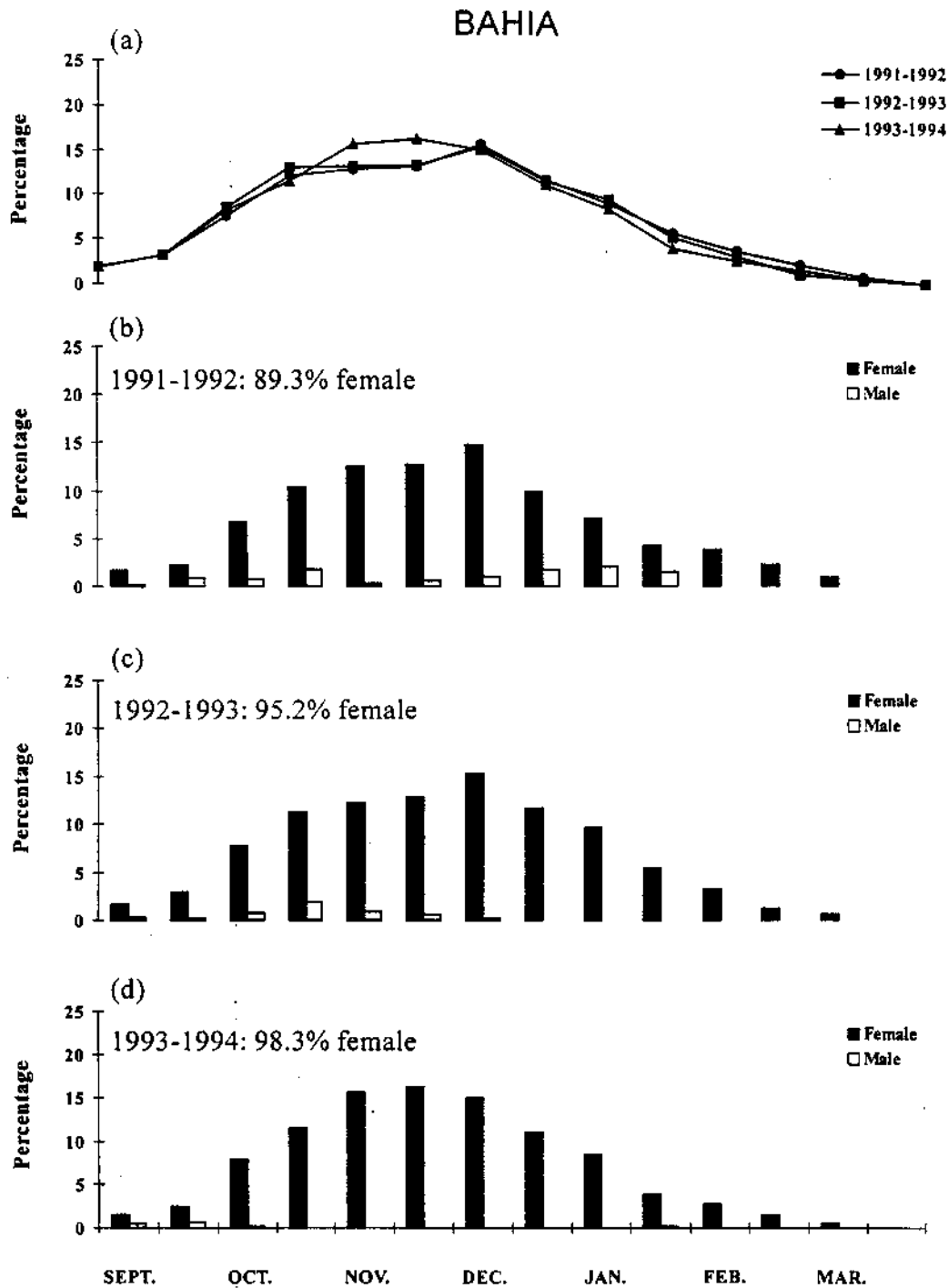
This calculation of the overall sex ratio for Brazilian loggerhead hatchlings should not be taken as a complete estimate but more as a starting point to be modified as more information is obtained. The Brazilian coastline is more than 8000 km long. TAMAR covers 1100 km, including the most important nesting areas, but not all of the beaches where fewer turtles nest. Also it is possible that new rookeries will still be discovered. For instance, in the state of Rio de Janiero, an area has recently been found where a few hundred nests per year are thought to be laid. Since this is south of Espírito Santo, it is likely to augment the production of males more than that of females.

Fig. 10. Seasonal sex production profiles for Espírito Santo. For details see Fig. 9. For a few bins at either end of some seasons, incubation duration data were not available; in such cases the average duration for all years (Fig. 5) was used. These cases represented only 0.3–2.7% of nesting in any season. (a) Nesting distributions. (b–d) Estimated sex ratios for the 1991–1992, 1992–1993, and 1993–1994 season, respectively.

ESPÍRITO SANTO
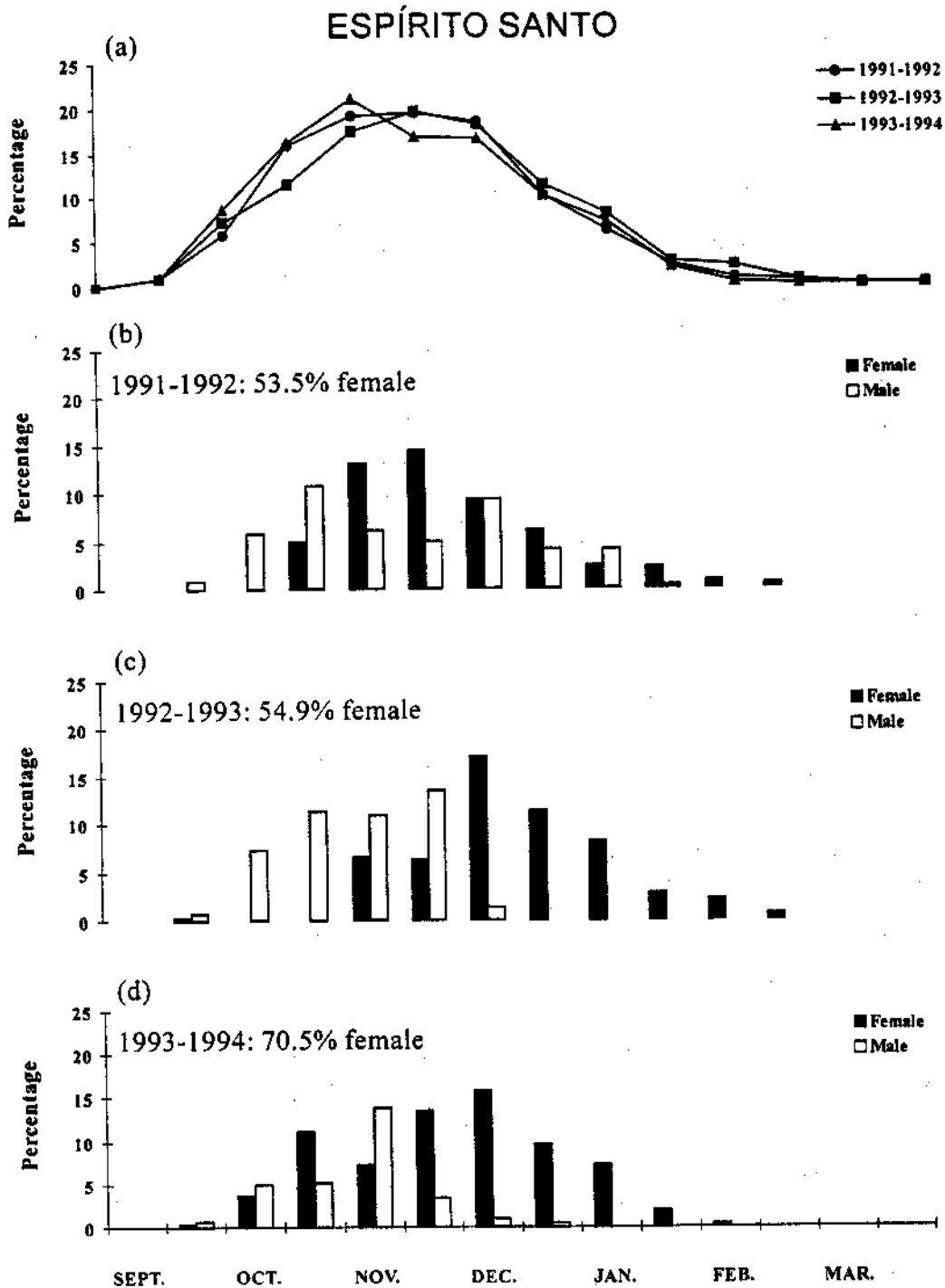
Despite these cautions, it is evident that many more loggerheads nest in the warmer, more northerly, female-producing areas, and that the overall sex ratio is strongly skewed towards females. To understand the demographic implications of this skew, it will be necessary to learn whether loggerheads in Brazil constitute one interbreeding population or subpopulations within their own rookeries.

Whichever the case, loggerheads nesting in Brazil do not produce enough males to balance the predominantly female output in the U.S.A.

Implications of skewed sex ratios

The present data on incubation durations in Brazil span 6 years and 11 beaches. The data indicating a skewed sex ratio

Table 2. Loggerhead nests laid in three states in Brazil as a percentage of the total (n) in these states.

Season	n	Sergipe	Bahia	Espírito Santo
1990–1991	1956	10.8	55.9	33.3
1991–1992	1861	12.8	59.3	27.9
1992–1993	2325	5.9	61.9	32.2
1993–1994	2342	7.2	67.7	25.1
Total (1990–1994)	8484	8.9	61.5	29.6

in Florida loggerheads were obtained over 5 years (Mrosovsky and Provancha 1992). Taken together, this evidence diminishes the likelihood that heavily female-biased ratios arise from sampling artifacts or peculiarities of particular nesting areas.

One may still invoke the possibility of a male-producing area elsewhere. The incubation durations of Yucatán loggerheads, 54–55 days (Zurita et al. 1993), do not suggest that many males are being produced in Mexico (cf. Figs. 2, 4, 5). But perhaps there are male-producing rookeries in Africa. The more areas in the western Atlantic that are shown to have female-biased outputs, the larger and (or) more male biased would any male-producing rookeries in Africa have to be to achieve balanced ratios. It seems more likely, with the data from the U.S.A. and now Brazil, that a female bias is a feature of loggerhead turtle demography. What life-history features might give rise to such a skew, and whether they exist in other turtle species, remain challenging questions.

The female-biased sex ratio of loggerhead hatchlings in Brazil, if it carries over to the adult stage, may have some bearing on hybridization between loggerhead and hawksbill turtles (Conceição et al. 1990). It has been pointed out, on the basis of mtDNA evidence, that so far, hybrids between these species have all been found to be crosses between female loggerheads and male hawksbills (Karl et al. 1995). Should this be substantiated with larger samples, it might be suspected that a greater availability of female loggerheads, combined with relatively indiscriminate mate choice by male hawksbills, was responsible. Far fewer hawksbills than loggerheads nest along the coast of mainland Brazil (D'Amato and Marczwski 1993).

It is suspected, on the basis of DNA studies, that some hybrids are capable of producing viable offspring (Bowen et al. 1996). If they are, and male hawksbills are contributing genes to the loggerhead population, then on Fisherian principles the sex ratio of pure loggerheads, when parental investment ends, would not be expected to be quite 1:1 but somewhat biased in favour of females. However, since hybrids are in any case rare, this is not likely to be a major effect. Activities of male hawksbills could hardly be responsible for the large bias towards production of female loggerheads in Brazil.

Implications for conservation programs

In Brazil, state and federal laws covering such things as building permits, access to cars, lighting, and designation of reserves already exist to protect turtle beaches, but enforcement is a continuing challenge. Where rookeries are small,

or when few turtles are nesting at the beginning of the season, there may be a temptation to economize on patrols or other conservation measures. If this is done, it should be with the realization that a higher percentage of male-producing nests may sometimes be left unprotected or unmonitored.

For instance, if there is little gene flow between turtles nesting in Bahia and those nesting elsewhere, then for the demographic health of the population, the few males produced in Bahia at the start of some seasons could be important. If, on the other hand, there is considerable gene flow between loggerheads nesting in the various states in Brazil, it would be prudent to make sure that both female- and male-producing regions are protected, even though fewer turtles nest in the latter.

In some areas, especially where there are many people, enforcement is not always practical at present, and TAMAR moves eggs to hatcheries. Concerns about sex-ratio distortion by the use of hatcheries (Mrosovsky and Yntema 1980) should not be exaggerated and lead to a general condemnation of this practice. Establishment of hatcheries and relocation of eggs on the beach are important ways of saving nests that would otherwise be destroyed. The details of each case should be examined. If incubation durations are generally shorter than pivotal incubation durations by a wide margin, as in Bahia (Fig. 4), then the use of hatcheries should have little effect on sex ratio. In the case of the hatcheries at Praia do Forte, Bahia, the likelihood of sex-ratio distortion is further reduced: both incubation durations (Marcovaldi and Laurent 1996) and sand temperatures (Naro et al., see footnote 3) are close to those on the nesting beach.

Applications of the present method

Given the wide availability of data on incubation durations, or the relative ease of collecting such data in places where turtle beaches are already being monitored, the present method of estimating sex ratios could be widely applied. It will give at least a first-order estimate, and can be made more accurate as more information becomes available on hatch–emergence intervals. It does require a knowledge of pivotal incubation durations. More research on this is needed, especially on the most appropriate ways of determining pivotal incubation durations and on their variation within and between populations. However, if pivotal levels for different populations turn out to be very close, it may eventually be possible to dispense with this step without much loss of accuracy. Even without refinement, the present method can already be used in cases where incubation durations differ considerably from pivotal levels. The incubation durations in Bahia, the main loggerhead nesting ground in Brazil, are generally so much below pivotal levels that it seems highly likely that there must be a large skew towards production of females in this region.

Acknowledgements

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