Pivotal temperature and predicted sex ratios for hatchling hawksbill turtles from Brazil

Matthew H. Godfrey, Adriana F. D'Amato, Maria Â. Marcovaldi, and N, Mrosovsky

Abstract: Like all other species of sea turtle, the hawksbill turtle (*Eretmochelysimbricata*)exhibits temperature-dependent sexual differentiation, with high incubation temperatures producing females and low temperatures producing males. Relatively little is known about the sex ratios of hatchlings produced by nesting populations of hawksbill turtles. Here we estimate the overall seasonal sex ratios of hatchling hawksbill turtles produced in Bahia, Brazil, during 6 nesting seasons, based on incubation durations, pivotal temperature, and pivotal incubation duration. The overall sex ratio of hatchlings produced in Bahia from 1991-1992 through 1996-1997 was estimated to be >90% female, which is more female-biased than estimated sex ratios of hatchling loggerhead turtles from Bahia and Florida, U.S.A. The biological and conservation implications of skewed sex ratios are discussed.

Resume : Comme toutes les autres especes de tortues marines, *Eretmochelys imbricata* subit sa differentiation sexuelle en fonction de la temperature, les temperatures d'incubation élevées donnant des femelles, les temperatures basses, des males. Il existe relativement peu d'informations au sujet des rapports males : femelles des tortues a l'eclosion au sein des populations reproductrices de cette espece. On trouvera ici une estimation saisonnière globale des rapports males : femelles des rejetons de cette espece a l'eclosion a Bahia, Brésil, au cours de 6 saisons de nidification, en fonction de la duree de l'incubation, de la temperature de pivotement et de la duree de la période de pivotement au cours de l'incubation. Le rapport global des rejetons produits a Bahia de 1991-1992 a 1996-1997 compte plus de 90% de femelles, ce qui constitue un pourcentage de femelles plus élevé que celui enregistré chez des rejetons de la Caouane a Bahia et en Floride, É.-U. L'impact de rapports males : femelles déséquilibrés sur la biologie des tortues et sur les programmes de conservation fait l'objet d'une discussion.

[Traduit par la Redaction]

Introduction

Sexual differentiation of sea turtles is influenced by the temperature of the sand in which the eggs develop: higher temperatures produce primarily female hatchlings and lower temperatures produce primarily male hatchlings (for review see Raynaud and Pieau 1985; Ewert and Nelson 1991). The range of temperatures resulting in mixed sex ratios is called the transitional range of temperature, and the incubation duration or constant incubation temperature that results in 50% of each sex is termed the pivotal duration or pivotal temperature, respectively (Mrosovsky et al. 1984; Mrosovsky and Pieau 1991). This system of temperature-dependent sexual differentiation (TSD) has been found in all species of sea

M.H. Godfrey.[1] Department of Zoology, University of Toronto, Toronto, ON M5S 3G5, Canada, and Projeto TAMAR and Fundação Pro-TAMAR, Caixa Postal 2219, Rio Vermelho, Salvador, Bahia, 40.210-970, Brazil.
A.F. D'Amato[2] **and M.Â. Marcovaldi.** Projeto TAMAR and Fundação Pro-TAMAR, Caixa Postal 2219, Rio Vermelho, Salvador, Bahia, 40.210-970, Brazil.
N. Mrosovsky. Department of Zoology, University of Toronto, Toronto, ON M5S 3G5, Canada.

[1]Author to whom all correspondence should be addressed (e-mail: protamar@e-net.com.br).
[2]Present address: Sociedade de Preservação, Pesquisa e Reprodução de Animais Silvestres, Caixa Postal 19, Lauro de Freitas, Bahia, 42700-00 Brazil.

turtles (Mrosovsky 1994), including the flatback turtle (*Natator depressus*) (S. Hewavisenthi, personal communication).

The existence of TSD in hawksbill turtles (*Eretmochelys imbricata*) was initially inferred from a report of a cold hawksbill turtle nest that produced only male embryos (Dalrymple et al. 1985), and from the sex ratios of dead hatchlings salvaged from hawksbill turtle nests in Barbados (Horrocks and Scott 1991). The pivotal temperature for 4 clutches of hawksbill turtles in Antigua was 29.2°C (Mrosovsky et al. 1992). More recently, Loop et al. (1995) reported that of 4 relocated hawksbill turtle nests that were monitored in Australia, 2 that were in the shade (i.e., cooler) produced more males than 2 that were in full sun, and Wibbels and Hillis (1995) found far more female than male dead hatchlings remaining in hawksbill turtle nests on Buck Island in the Caribbean. In none of these studies was a specific quantitative estimate of the overall hatchling sex ratio assigned.

In contrast, there have been extensive studies of the sex ratios of the offspring of loggerhead turtles (*Caretta caretta*). For the separate nesting populations of loggerhead turtles in the southeastern U.S.A. and Brazil, estimates based on data from 5 or more consecutive nesting seasons suggest that the sex ratios of hatchlings in both areas were strongly female-biased (Mrosovsky and Provancha 1992; Marcovaldi et al. 1997). Estimates of sex ratios spanning several years are preferable because the influence of anomalous weather patterns in a single year is reduced.

In the north of the state of Bahia, Brazil, hawksbill turtles share nesting beaches with loggerhead, olive ridley

(*Lepidochelysolivacea*), and green turtles (*Chelonia mydas*), although the latter 2 species tend to nest in relatively small numbers (D'Amato and Marczwski 1993; Marcovaldi and Laurent 1996). Estimates of the overall seasonal sex ratios of hatchling hawksbill turtles in Brazil would be interesting for several reasons. If hatchling hawksbill turtles there have a strong female bias in sex ratios, as hatchling loggerhead turtles do, this could have a variety of possible causes, including random fluctuation, global warming, etc. If, however, hawksbill turtle offspring have a more balanced sex ratio, this would stimulate further investigation of the reason for the difference in offspring sex ratio between species. In addition, there are ramifications in terms of conservation and management activities, including artificial incubation of eggs (Mrosovsky 1982; Morreale et al. 1982; Dutton et al. 1985) and induced feminization of turtle embryos (Crews et al. 1994; Vogt 1994; Mrosovsky and Godfrey 1995; Lovich 1996; Girondot et al. 1998).

For these reasons we investigated TSD in hawksbill turtles from Bahia. The overall aim of this study was to estimate seasonal sex ratios of hatchlings over a number of years. To do this we first established the pivotal temperature and pivotal incubation duration for this population. Then from records of nests laid in Bahia, we estimated the sex ratios of nests with known incubation durations by converting each duration to a sex ratio, after the method of Marcovaldi et al. (1997). These data were then combined with relative numbers of nests laid during each nesting season to produce an overall estimate of the sex ratio of offspring for different nesting seasons.

Materials and methods

Study area

Although hawksbill turtle nests can be found scattered along most of the coast of Bahia, the highest concentration occurs on the beach of Praia do Forte and, abutting it to the south, those of Arembepe–Interlagos. These beaches are relatively flat and backed mainly by coco palms (*Cocos nucifera*) and low-lying vegetation (for a description of these beaches see Marcovaldi and Laurent 1996; Silveira and Patiri 1995). Most of these beaches are termed intensive study areas, which TAMAR staff patrol daily during the nesting season, which runs from September to March (Marcovaldi and Laurent 1996). All nests are marked and, towards the expected end of the incubation period, checked daily for signs of emergence. The morning following emergence, all nests are excavated. In this way, the incubation duration and species are determined for each nest.

Collection of eggs and determination of the pivotal temperature and incubation duration

To calculate the pivotal temperature and pivotal incubation duration, eggs were collected from 2 clutches laid by hawksbill turtles on 28 January 1997. All 140 eggs were collected from clutch R, which was laid on Interlagos beach at 22:40 by a female hawksbill with tag numbers BR12301/BR12302. Clutch S was laid on Guarajuba beach at 10:30 by a female with tag numbers BR13424/BR13425; only 60 eggs were collected from this clutch. All the eggs were stored in Styrofoam boxes in air-conditioned rooms until approximately 16:00 on 29 January, when they were transferred to a travel box and brought to Toronto via airplane. The eggs underwent minimal disturbance and were not put through X-ray machines. They arrived in Toronto on 30 January and were placed in incubators by

11:30. The total time between laying and placement in incubators was 40 h for clutch R and 52 h for clutch S.

Each egg was placed individually on top of an indented damp foam sponge and surrounded by moistened vermiculite in a plastic container with air holes punched in the top of the container (for full description of the egg container see Mrosovsky 1988). The plastic containers were then covered with lids and randomly arranged on shelves in 5 incubators set at different constant temperatures. Each incubator had a large bowl of water below the bottom shelf to help maintain a high level of humidity. In addition, on days 15-16 and 36-37 of incubation, around 65 mL of deionized water was added to each individual plastic cup that held an egg.

On each shelf in each incubator we placed a mercury thermometer with $0.1\,^{\circ}C$ gradations encased in a tube of glycerol (for details of the calibration procedures for these thermometers see Marcovaldi et al. 1997). The thermometers were checked daily during the incubation period and from these daily recordings we determined the average temperature of each shelf over the whole incubation period. In addition, to ensure that the incubators were functioning normally we monitored the range of temperature over 24 h in each incubator with maximum-minimum thermometers.

Because of the relatively high humidity in the incubators, we calculated the cooling effect of evaporation on egg temperature. On days 33-37 of incubation a needle thermistor probe (YSI No. 402) was inserted into one egg. The temperature of the egg was then compared with that of a small beaker of glycerol placed next to the container of the egg being measured. This was repeated 8 times over 4 days, after which we opened the egg to confirm normal development. The average difference between the egg and the glycerol was $-0.25\,^{\circ}C$ to the nearest $0.25\,^{\circ}C$, therefore we subtracted $0.25\,^{\circ}C$ from the mean shelf temperatures to adjust for evaporative cooling.

After day 45 of incubation we checked each incubator twice daily for signs of pipping. An egg was considered to be pipped when the shell was slit and considered to be hatched when the hatchling had its head and at least one flipper outside of the shell. Immediately after hatching, the turtles were killed and the kidney-gonad complexes removed and placed in 8% formalin solution.

Histology and determination of pivotal temperature and pivotal incubation duration

Following fixation for at least 10 days, the kidney–gonad complexes were cut transversely at the midpoint of the gonad, run through a series of dehydrating baths, and embedded in paraffin wax. Groups of serial sections ($10\ \mu m$ thick) were taken from the cut end of the gonad and mounted on slides. These sections were then stained with periodic acid - Schiff's reagent (PAS) and Harris' haematoxylin. The sex of each hatchling was determined by examining it under a light microscope, using the criteria of Yntema and Mrosovsky (1980). Briefly, female gonads were characterized by the presence of a PAS-positive tunica albuginea in between a thickened convoluted cortex and a relatively homogenous medulla; male gonads were characterized by the lack of a tunica albuginea, a very thin cortex, and the presence of immature seminiferous tubules in the medulla. The gonads of 2 hatchlings had features of both sexes and were classified as showing signs of intersexuality. For the analysis of pivotal temperature and pivotal incubation duration, these 2 hatchlings were treated as "not females," based on the finding that in *Emys orbicularis*, another turtle species with TSD, hatchlings which show signs of intersexuality later function as mature males (Girondot et al. 1998).

To determine the pivotal temperature for hawksbill turtles, the mean incubation temperature for each shelf in each incubator was determined. In a few cases, the temperatures for individual shelves were similar, so data from these shelves were grouped together. The mean temperature of each shelf (or group) was plotted against

Table 1. Percentages of female hatchling hawksbill turtles and incubation durations of eggs incubated at different constant temperatures.

	Temperature (°C)[a]						
	27.9 ± 0.08	28.4 ± 0.15	28.9 ± 0.15	29.3 ± 0.09	29.5 ± 0.05	29.8 ± 0.07	30.4 ± 0.07
	(27.8-28.1)	(28.1-28.6)	(28.6-29.1)	(29.0-29.6)	(29.4-29.8)	(29.4-29.9)	(30.0-30.6)
No. of eggs							
Clutch R	12[b]	31	33	22[c]	22	10	10[b]
Clutch S	4	14	14	8	8*	6	6
Percent female							
Clutch R	0	0	16.1	10	36.4	80	100
	(n = 12)	(n = 29)	(n = 31)	(n = 20)	(n = 22)	(n = 10)[d]	(n = 10)
Clutch S	0	0	10	0	0	60	100
	(n = 3)	(n = 10)	(n = 10)	(n = 5)	(n = 7)[d]	(n = 5)	(n = 3)
Both clutches	0	0	14.6	8.0	27.6	73,,3	100
Incubation duration (days)	66.9	66.3	62.5	61.7	60.2	57,,5	56.7

Note: n is the number of sexed turtles that contribute to the estimate of sex ratio.

[a] Given as the mean ± SD, with the range in parentheses.

[b] Includes one sexed embryo from an egg opened early to check on development.

[c] Includes one egg that was used for assessing evaporative cooling.

[d] Includes one hatchling whose gonads exhibited signs of intersexuality.

sex ratio to determine the pivotal temperature. We used 2 different methods to derive the pivotal temperature. The first is the traditional method of interpolating between the 2 sex ratios that are closest to the 50% level (Mrosovsky and Pieau 1991). The second is based on maximum-likelihood analysis of all the sex ratios from the laboratory (Girondot 1999). An advantage of using the traditional method is that it provides consistency among the different studies on pivotal temperature; an advantage of Girondot's (1999) method is that it allows statistical comparison of pivotal temperatures among the different published studies (unpaired t test, two-tailed).

We calculated the incubation duration for each hatchling by counting the days between when the egg was laid and when it hatched. To determine the pivotal incubation duration we grouped the hatchlings by incubation duration and calculated the sex ratio for each incubation duration. We then plotted incubation duration against sex ratio for all eggs. Finally, we fitted a regression line (based on Marquardt's method, with a four-parameter logistic equation; Inplot 4.03 software, GraphPad, San Diego, Calif.) through all the points; the incubation duration corresponding to the point where the fitted line crossed the 50% female axis was taken as the pivotal incubation duration,

Estimating sex ratios for individual nests from previously collected data

Beginning in 1980, on all beaches that it monitors, Projeto TAMAR has maintained records of nesting events, including data on incubation durations. Incubation duration in the field is defined as the time between when a clutch is laid and when the hatchlings emerge from the nest and scurry to the ocean; this has also been termed the emergence period. Because the incubation duration is inversely related to the overall temperature, it is also inversely related to the proportion of females that are produced in the nest. Using the data from incubating hawksbill turtle eggs in the laboratory, we plotted sex ratio against incubation duration and used this relationship to predict the sex ratio for individual in situ hawksbill turtle nests laid during the nesting seasons between 1991-1992 and 1996–1997.

However, because hatchlings from natural nests require time to move through the sand after hatching before emerging from the nest, unlike those incubated in the laboratory, it was necessary to add a correction factor to the curve relating sex ratio to incubation

duration produced from the laboratory data (cf. Marcovaldi et al. 1997). On average, at least 4 days are needed by hatchling sea turtles to emerge from the nest after hatching (Godfrey and Mrosovsky 1997), so we used 4 days as a correction factor. In case the average hatching-to-emergence interval for hawksbill turtles in Brazil is shorter than 4 days, we also used 1, 2, and 3 days as correction factors and predicted sex ratios of nests from incubation durations using these values.

For all nesting seasons there were no data on incubation durations for 1-16 October and 17–31 March, although, on average, 0.5 and 0.6%, of the seasonal total of nests are laid during these periods; therefore, to calculate the average sex ratio for all seasons we substituted the sex ratios for 17-31 October and 1-17 March, respectively.

We calculated the confidence interval surrounding the overall mean sex ratio for 1991-1997. Using the bootstrapping technique we generated 1000 data sets by randomly sampling the original data set of incubation periods. The 1000 data sets were used to derive 1000 overall sex ratio values, from which we computed a 95% confidence interval.

Results

Pivotal temperature and pivotal incubation duration

Of the 200 eggs that were maintained at different constant temperatures for the complete incubation period, 171 hatched (85.5% emergence rate). Six of the embryos that did not hatch were very close to term, so we were able to successfully identify their sex (Table 1). Using the traditional method of calculating the pivotal temperature (cf. Mrosovsky and Pieau 1991), we found little difference between the 2 clutches: 29.6°C for clutch R and 29.7°C for clutch S ($p = 0.26$, two-tailed t test; Zar 1996); for all hatchlings the incubation temperature that produced 50% of each sex was 29.65°C, which was essentially identical with the value derived by means of maximum-likelihood analysis (29.66 ± 0.06°C (mean ± SD); cf. Girondot 1999). This pivotal temperature is significantly higher ($p < 0.0001$) than that found for loggerhead turtles clutches from the same beaches, 29.15 ± 0.06°C (Marcovaldi et al. 1997), and sig-

Fig. 1. Relationship between sex ratio and incubation duration for hawksbill turtle eggs in Bahia, Brazil. The solid line is based on laboratory incubation at constant temperature. The broken line was derived by adding a correction factor of 4 days to account for the time elapsed between hatching and emergence (see the text). The nonlinear curve was fitted using Inplot 4.03 software, with the upper and lower asymptotes set at 100 and 0% female, respectively.
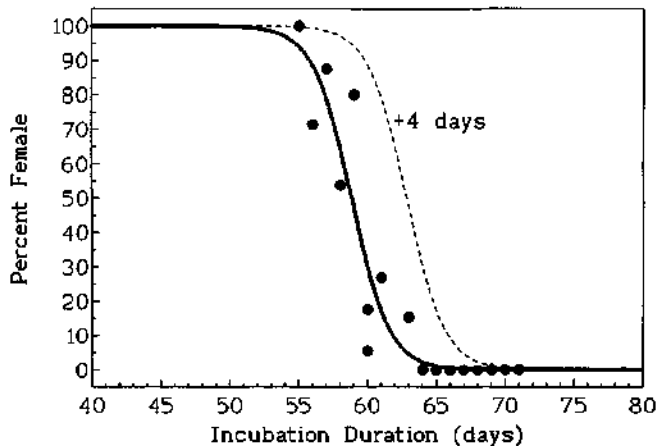
nificantly higher ($p < 0.0001$) than that found for hawksbill turtles from Antigua, 29.27 ± 0.05°C (Mrosovsky et al. 1992).

The incubation period in the laboratory that resulted in 50% of each sex was 58.8 days (Fig. 1). Adding 4 days to allow for the time it takes the hatchlings to dig through the sand and emerge on the beach from natural nests (Godfrey and Mrosovsky 1997), the pivotal incubation duration in the field was estimated at 62.8 days. This derived field curve was used to predict the sex ratios of hatchlings from individual natural nests with known incubation durations. On average, hawksbill turtle eggs took longer to develop than loggerhead turtle eggs incubated at similar temperatures (Fig. 2).

Relative nesting frequency and estimated overall sex ratios

The relative numbers of hawksbill turtle nests laid during the different nesting seasons studied were similar across years (Fig. 3). To calculate the overall sex ratio for a particular season we multiplied the estimated sex ratio for each half-month period by the relative numbers of nests laid in the same half month and then summed all the half-month periods in the season. For each of the seasons the predicted sex ratios were strongly female-biased (>90% female; Table 2). In case the 4-day correction factor we used is inappropriate for hawksbill turtles, we also calculated the overall seasonal sex ratios for all seasons using 1-, 2-, and 3-day correction factors. This had little effect on the outcome: assuming a 1-day correction factor in deriving the field incubation duration curve, the seasonal sex ratios were still heavily female-biased, >90% female production being the mean for all seasons (Table 2, Fig. 4).

Discussion

Natural hawksbill turtle nests in Bahia have probably produced almost exclusively female hatchlings since at least

1991–1992 according to present estimates. This conclusion is supported by other data. First, the pivotal temperature found here was 29.6°C and sand temperatures recorded in Praia do Forte are often greater than 31°C (Maciel et al. 1999). Thus, hawksbill turtle nests in this area would be expected to produce mostly female hatchlings. Second, loggerhead turtle nests laid on the same beaches in Bahia produce a majority of female hatchlings (Marcovaldi et al. 1997); the pivotal temperatures of the 2 species are roughly similar and both species nest during the summer months. Because the peak of nesting for hawksbill turtles, January-February, occurs later in the season than that for loggerhead turtles, November-December (Marcovaldi and Laurent 1996), hawksbill turtle eggs are exposed to somewhat higher sand temperatures as warming continues over the nesting season in Bahia (Maciel et al. 1999). It is tempting to speculate that the slightly higher pivotal temperature for hawksbill turtles represents an adaptive adjustment to keep their offspring sex ratios from becoming too skewed. A similar speculation was made about the slightly higher pivotal temperatures for leatherback turtles (*Dermochelys coriacea*) (29.25°C) and green turtles (28.75°C) sharing nesting beaches in Suriname (Mrosovsky et al. 1984). However, more recent investigations suggest that the pivotal temperature for green turtles in Suriname is nearly as high as that for leatherback turtles (Godfrey 1997; Girondot 1999). Speculation about adaptive adjustments of pivotal temperature should be tempered by recalling that pivotal temperatures recorded for species from both Suriname and Bahia are based on only a few clutches. In addition, there were some methodological differences among the various studies, such as the use of different values to correct for the evaporative cooling of eggs in the incubators. Moreover, in the case of hawksbill and loggerhead turtle eggs in Bahia, the sand temperatures are so high that nearly all hatchlings produced are female, regardless of any differences in pivotal temperature.

Comparisons between loggerhead and hawksbill turtles in Bahia are complicated by the existence of hybrids between the 2 species (Conceição et al, 1990). Bass et al. (1996) noted that 10 out of 14 samples of DNA from hawksbill turtle hatchlings from Bahia contained genetic markers thought to be specific to loggerhead turtles; this suggests that hybridization is a relatively common here. What is not known is whether this hybridization has resulted in greater similarity in physiological and behavioural features of these species. For instance, there does not appear to be any striking difference in areas of the beach selected by loggerhead and hawksbill turtles for nesting in Bahia, although the beaches there are mainly backed by coconut plantations and do not provide the dense vegetation preferred for nesting by hawksbill turtles in other regions (Diamond 1976; Ryder et al. 1989). However, a detailed study of nest-site preferences between these species in Bahia has yet to be done.

On the other hand, there are some clear differences between hawksbill and loggerhead turtles in Brazil. For instance, there is a difference in peak nesting times within a season (Marcovaldi and Laurent 1996). Also, the incubation durations differ. Hawksbill turtle eggs buried in a hatchery take longer, on average, to emerge than loggerhead turtle eggs buried nearby in the same hatchery (D'Amato and Marczwski 1993). Also, at similar constant temperatures in

Fig. 2. Relationship between temperature and incubation duration for hawksbill turtle (O) and loggerhead turtle (•) eggs incubated in the laboratory. A linear regression line is shown for each species. Values for loggerhead eggs are from Marcovaldi et al. (1997).

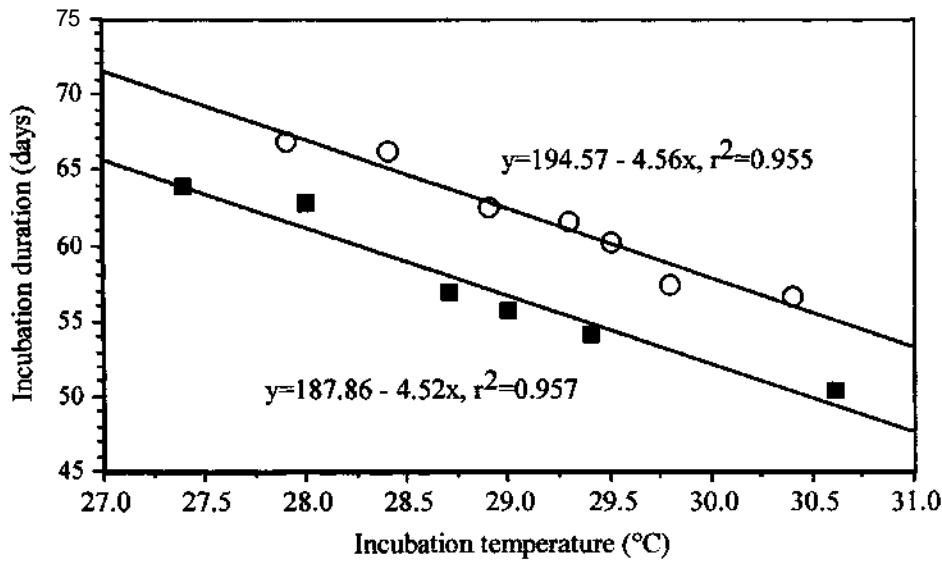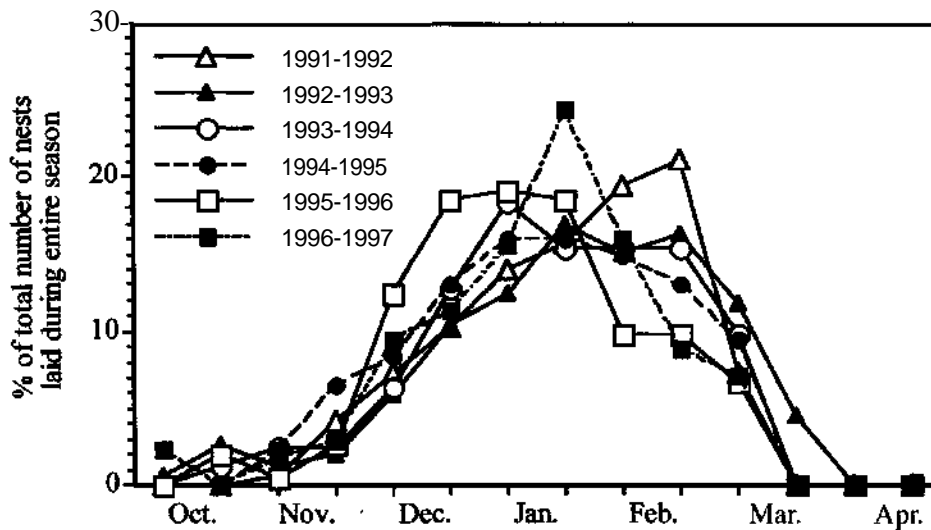$$y=194.57 - 4.56x, r^2=0.955$$

$$y=187.86 - 4.52x, r^2=0.957$$

Fig. 3. Relative number of hawksbill turtle nests per half month (expressed as a percentage of the season's total) during individual nesting seasons from 1991-1992 through 1996-1997, in Bahia, Brazil.

the laboratory (27.5–30.5°C), hawksbill turtle eggs take about 5 days longer to hatch (Fig. 2). Interestingly, this is opposite to what was reported by Ackerman (1997), who suggested that hawksbill eggs take 5 days less, on average, than loggerhead turtle eggs to develop to term when incubated in constant conditions.

One limitation of this study is that the sex ratios are based on only 2 clutches. It is possible that there is variation in pivotal temperatures (and hence pivotal incubation durations) for the population of hawksbill turtles in Bahia, which could alter the estimates of sex ratio based on incubation duration. To date, however, relatively little variation in pivotal temperatures of marine turtles has been found (Mrosovsky 1994). Also, sand temperatures in Bahia are generally well above 31°C during the nesting season for hawksbill turtles (Maciel et al. 1999; unpublished data), so this beach would produce almost all females regardless of among-clutch varia-

tion in pivotal temperatures. The method of converting incubation durations of clutches into sex ratios of hatchlings was recently tested by comparing it with the more traditional method of using histological analysis of the gonads of hatchlings to estimate sex ratios (Mrosovsky et al. 1999). For loggerhead turtle nests, there was concordance between sex ratios both predicted from the incubation duration and calculated by means of histology, i.e., there was no significant difference overall. Continued studies of sand temperatures on the nesting beaches and perhaps direct sexing of a sample of hatchlings would help to reinforce the present findings.

Biological implications of female-biased sex ratios

Eggs laid by at least 2 species of sea turtles nesting in Bahia have produced mainly females (Marcovaldi et al. 1997; this study). Evidently, the frequency-dependent pressures of skewed sex ratios as described by Fisher (1930)

Fig. 4. Average relative number of hawksbill turtle nests (expressed as a percentage of the season's total) laid per half month from 1991-1992 through 1996-1997 (bars and left-hand vertical axis). Also shown are the mean sex ratios (percent female) per half month (curves and right-hand vertical axis) for all nesting seasons, based on 1- to 4-day hatch-to-emergence intervals (see the text). Overall seasonal sex ratios for individual years with the different correction factors are listed in Table 2.
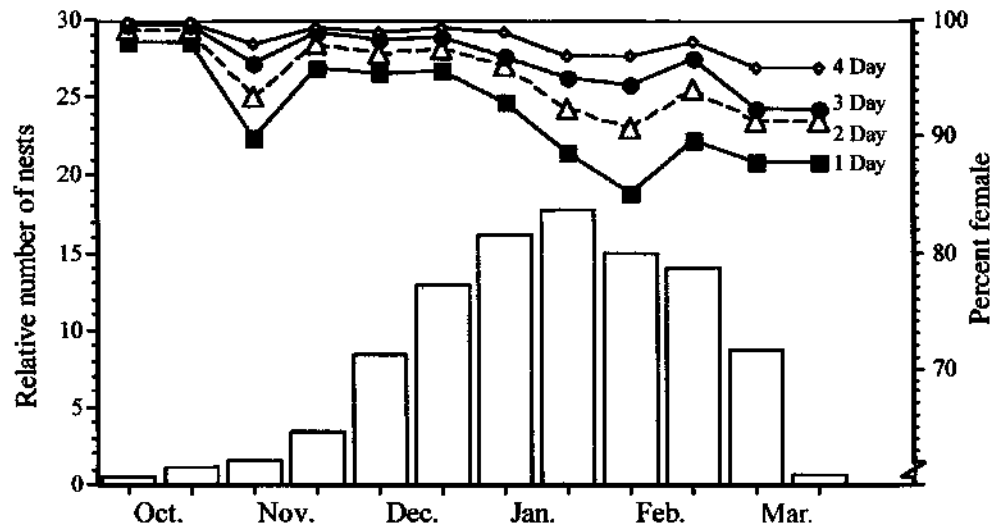

Table 2. Estimates of overall sex ratios (percent female) of hatchling hawksbill turtles produced in Bahia, Brazil, based on 1-, 2-, 3-, and 4-day correction factors (CF; see the text for details).

Nesting season	n	1-day CF	2-day CF	3-day CF	4-day CF
1991-1992	165	84.2	90.5	94.6	97.1
1992-1993	153	96.4	97.9	98.8	99.6
1993-1994	234	91.4	95.1	95.5	98.6
1994-1995	168	97.1	98.5	99.2	98.8
1995-1996	194	95.8	97.6	98.8	99.4
1996-1997	168	77.4	83.1	87.5	90.7
Mean for all seasons		90.8	94.2	96.2	97.9

Note: n is the number of all nests laid that season. For 1992-1993 nesting season, no incubation durations were available for 16–30 November or 1-14 February, so we substituted the mean incubation duration for all nests laid during these half-month periods in the other seasons. The mean overall sex ratios for all seasons were calculated by averaging within each half-month period the sex ratios for all seasons and multiplying these values by the mean percent nesting for that half month. The 95% confidence interval for the overall sex ratio estimate with the 4-day CF, based on the bootstrap technique (see Methods), is 97.8-98.0.

have not been strong enough to force these sex ratios closer to equality. Bull and Charnov (1988) described a number of conditions that might contribute to the production of biased sex ratios of offspring, such as local competition for mates between related males. However, since many features of the natural history and demography of sea turtles are unknown, it is not clear whether sea turtles meet any of these conditions. Nevertheless, given the current data we can begin to formulate simple questions about the implications of biased sex ratios.

Systematically collected data on sea turtle nests from Bahia are available only from 1991-1992 onwards, although incubation durations have been recorded since 1983. For each season between 1983-1984 and 1990-1991, the mean incubation duration for hawksbill turtle nests was <55 days, which suggests that a female-biased hatchling sex ratio has been common for almost 20 years. It is possible that the large number of female hatchlings translates into a female-biased adult sex ratio. If so, one question that can be asked

is where are the males coming from to mate with these females? In the case of loggerhead turtles, whose nesting extends along almost the whole coast of Brazil, relatively more males are produced on beaches in Espírito Santo and farther south than in the northern states of Bahia and Sergipe (Marcovaldi et al. 1997). Some of these males produced in the south might migrate north to mate with the females produced there. This possibility can be tested by investigating whether or not there is regular interchange of nuclear DNA between the northern and southern populations of loggerheads.

In the case of hawksbill turtles this scenario is more problematic, since nesting of hawksbill turtles in Brazil is sparse outside of Bahia (Marcovaldi and Laurent 1996) and elsewhere on the Atlantic coast of South America; e.g., very few hawksbill turtle nests are laid each year in the Guianas (Schulz 1975). However, the Caribbean supports a number of nesting populations (Groombridge and Luxmoore 1989). It is possible that males produced in the Caribbean migrate

Table 3. Emergence rates from natural nests compared with estimated sex ratios of different populations of hawksbill and loggerhead turtles.

	Location	Sex ratio (% female)	Emergence rate (%)	Reference
Loggerhead turtles	Bahia, Brazil	82.5	72-73	D'Amato and Marczwski 1993; Marcovaldi and Laurent 1996
Hawksbill turtles	Bahia, Brazil	>90	38-61	D'Amato and Marczwski 1993; Marcovaldi and Laurent 1996
	Antigua	Not highly female-biased	82	Hoyle and Richardson 1993; Mrosovsky et al. 1992

to Brazil to mate with females there and vice versa. Based on comparisons of the pivotal temperature and nesting-beach temperatures in Antigua, it has been argued that hawksbill turtle nests laid in Antigua do not produce hatchlings with a highly female-biased sex ratio (Mrosovsky et al. 1992; Mrosovsky 1994). In contrast, nests laid in the U.S. Virgin Islands are thought to produce an abundance of females (Wibbels and Hillis 1995). Nevertheless, a study using mixed stock analysis to determine contributors to foraging grounds in Caribbean waters showed no evidence of migrants from Brazil (Bowen et al. 1996). A further possibility is that there is interaction between female hawksbill turtles from Brazil and males from the African continent; there has been a single recorded incident of a tagged female from Brazil being recovered in Senegal (Marcovaldi and Filippini 1991).

Implications of skewed sex ratios for conservation and management

Is the highly female-biased sex ratio of hatchling hawksbill turtles in Bahia beneficial to the population? Clearly, there is little need to increase the number of female offspring in Bahia, as suggested by Vogt (1994). However, because hatcheries are used in Bahia to protect nests that would not otherwise survive, the question arises as to whether the thermal conditions in these hatcheries should be actively managed. Currently, TAMAR follows a policy of matching the thermal conditions of its hatcheries with those of natural nesting areas on the beaches. The similarity in incubation durations and sand temperatures between beach and hatcheries indicate that the nests incubated in the hatchery produce primarily females (D'Amato and Marczwski 1993; Maciel et al. 1999).

However, there are other possible strategies for managing turtle hatcheries. It could be argued that a relative scarcity of males (due to a highly female-biased sex ratio of the hatchlings) might result in a decreased incidence of multiple paternity in turtle clutches and (or) a reduction in the total time spent mating, which could lead to a reduction in hatching success (see Wood and Wood 1980). There is some indication that hawksbill turtle clutches in Bahia have a lower hatching success rate than loggerhead turtle clutches laid on the same beach and hawksbill turtle clutches laid in Antigua (Table 3). It would be interesting look into the possible relationship between hatching success and the incidence of multiple paternity in sea turtles in general.

Another possible consequence of the extreme bias towards female hatchlings is an effect on the frequency of hybridization. In experiments on hybridizing anurans, the number of

hybrid matings increased when the relative availability of males from the non-conspecific population also increased (Bergen et al. 1997). Perhaps there is a correlation between the relative availability of male loggerhead and hawksbill turtles and the level of mating between female hawksbill and male loggerhead turtles.

It was suggested previously that pairing of male hawksbill and female loggerhead turtles might result from the relative abundance of female loggerhead turtles (Marcovaldi et al. 1997). We suggest that pairing of male loggerhead and female hawksbill turtles is equally likely. A factor in both types of pairing could be that there are fewer nesting hawksbill turtles relative to nesting loggerhead turtles: roughly 7 times more loggerhead than hawksbill turtle nests are found in this region (Marcovaldi and Laurent 1996). Whether or not hybridization is desirable is difficult to evaluate. More information is needed on the hatching success and reproductive output of hybrids.

These speculations should not be taken as calling for an increase the production of male hatchlings by managing the thermal environment of the hatcheries (cf. Mortimer et al. 1993; Chan and Liew 1995), but as counselling that the issue of sex ratios in conservation should not be considered in isolation from questions of hatch rates, hybridization, multiple paternity, and movement of turtles between beaches. Nevertheless, estimating natural sex ratios is important for assessing management practices, and according to the present estimates, the sex ratio of hawksbill turtle hatchlings produced in Bahia is extremely female-biased.

References

Ackerman, R.A. 1997. The nest environment and the embryonic development of sea turtles. *In* The biology of sea turtles. *Edited by* PL. Lutz and J.A. Musick. CRC Press, Boca Raton, Fla. pp. 83-106.

Bass, A.L., Good, D.A., Bjorndal, K.A., Richardson, J.I., Hillis, Z.-M., Horrocks, J.A., and Bowen, B.W. 1996. Testing models of female reproductive migratory behaviour and population structure in the Caribbean hawksbill turtle, *Eretmochelys imbricata*, with mtDNA sequences. Mol. Ecol. 5: 321-328.

Bowen, B.W., Bass, A.L., Garcia-Rodriguez, A., Diez, C.E., van Dam, R., Bolten, A., Bjorndal, K.A., Miyamoto, M.M., and Ferl, R.J. 1996. Origin of hawksbill turtles in a Caribbean feeding area as indicated by genetic markers. Ecol. Appl. 6: 566-572.

Bergen, K., Semlitsch, R.D., and Reyer, H.-U. 1997. Hybrid female matings are directly related to the availability of *Rana lessonae* and *Rana esculenta* males in experimental populations. Copeia, 1997: 275-283.

Bull, J.J., and Charnov, E.L. 1988. How fundamental are Fisherian sex ratios? Oxf. Surv. Evol. Biol. 5: 96-135.

Chan, E.H., and Liew, H.C. 1995. Incubation temperatures and sex-ratios in the Malaysian leatherback turtle *Dermochelys coriacea*. Biol. Conserv. 74: 169-174.

Conceição, M.B., Levy, J.A., Marins, L.F., and Marcovaldi, M.A. 1990. Eletrophoretic characterization of a hybrid between *Eretmochelys imbricata* and *Caretta caretta* (Cheloniidae). Comp. Biochem. Physiol. B, 97: 275-278.

Crews, D., Bergeron, J.M., Bull, J.J., Flores, D., Tousignant, A., Skipper, J.K., and Wibbels, T. 1994. Temperature-dependent sex determination in reptiles: proximate mechanisms, ultimate outcomes, and practical applications. Dev. Genet. 15: 297–312.

Dalrymple, G.H., Hampp, J.C., and Wellins, D.J. 1985. Male-biased sex ratio in a cold nest of a hawksbill sea turtle *(Eretmochelys imbricata)*. J. Herpetol. 19: 158-159.

D'Amato, A.F., and Marczwski, M. 1993. Aspectos da biologia de tartarugas marinhas (Cheloniidae) na região de Praia do Forte, muncípio de Mata de Sao João, Bahia, Brasil, durante o peri'odo reprodutivo 1990–1991. Arq. Biol. Technol. (Curitiba), 36: 513-519.

Diamond, A.W. 1976. Breeding biology and conservation of hawksbill turtles, *Eretmochelys imbricata* L., on Cousin Island, Seychelles. Biol. Conserv. 9: 199-215.

Dutton, P.H., Whitmore, C.P., and Mrosovsky, N. 1985. Masculinisation of leatherback turtle *Dermochelys coriacea* hatchlings from eggs incubated in Styrofoam boxes. Biol. Conserv. 31: 249-264.

Ewert, M.A., and Nelson, C.E. 1991. Sex determination in turtles: diverse patterns and some possible adaptive values. Copeia, 1991: 50-69.

Fisher, R.A. 1930. The genetical theory of natural selection. Clarendon Press, Oxford.

Girondot, M. 1999. Statistical description of temperature-dependent sex determination using maximum likelihood. Evol. Ecol. Res. 1: 479–486.

Girondot, M., Fouillet, H., and Pieau, C. 1998. Feminizing turtle embryos as a conservation tool. Conserv. Biol. 12: 353-362.

Godfrey, M.H. 1997. Sex ratios of sea turtle hatchlings: direct and indirect estimates. Ph.D. dissertation, University of Toronto, Toronto, Ont.

Godfrey, M.H., and Mrosovsky, N. 1997. Estimating the time between hatching of sea turtles and their emergence from the nest. Chelonian Conserv. Biol. 2: 581-585.

Groombridge, B., and Luxmoore, R. 1989. The green turtle and hawksbill (Reptilia: Cheloniidae): world status, exploitation, and trade. C.I.T.E.S. Secretariat, Lausanne, Switzerland.

Horrocks, J.A., and Scott, N.M. 1991. Nest site location and nest success in the hawksbill turtle *Eretmochelys imbricata* in Barbados, West Indies. Mar. Ecol. Prog. Ser. 69: 1-8.

Hoyle, M., and Richardson, J.I. 1993. The Jumby Bay Hawksbill Project 1987-1992. Georgia Sea Turtle Cooperative, University of Georgia, Athens.

Loop, K.A., Miller, J.D., and Limpus, C.L. 1995. Nesting by the hawksbill turtle *(Eretmochelys imbricata)* on Milman Island, Great Barrier Reef, Australia. Wildl. Res. 22: 241-252.

Lovich, J.E. 1996. Possible demographic and ecologic consequences of sex ratio manipulation in turtles. Chelonian Conserv. Biol. 2: 114-117.

Maciel, E.N., Mrosovsky, N., and Marcovaldi, M.Â. 1999. Thermal profiles of marine turtle hatcheries and nesting areas at Praia do Forte, Brazil. Chelonian Conserv. Biol. In press.

Marcovaldi, M.Â., and Filippini, A. 1991. Trans-Atlantic movement by a juvenile hawksbill turtle. Mar. Turtle Newsl. 52: 3.

Marcovaldi, M.Â., and Laurent, A. 1996. A six season study of marine turtles nesting at Praia do Forte, Bahia, Brazil, with implications for conservation and management. Chelonian Conserv. Biol. 2: 55-59.

Marcovaldi, M.Â., Godfrey, M.H., and Mrosovsky, N. 1997. Estimating sex ratios of loggerhead turtles in Brazil from pivotal incubation durations. Can. J. Zool. 75: 755-770.

Morreale, S.J., Ruiz, G.J., Spotila, J.R., and Standora, E.A. 1982. Temperature-dependent sex determination: current practices threaten conservation of sea turtles. Science (Washington, D.C.), 216: 1245-1247.

Mortimer, J.A., Ahmad, Z., and Kaslan, S. 1993. The status of the hawksbill *Eretmochelys imbricata* and green turtle *Chelonia mydas* of Melaka and Negeri Sembilan. Malayan Nat. J. 46: 243-253.

Mrosovsky, N. 1982. Sex ratio bias in hatchling sea turtles from artificially incubated eggs. Biol. Conserv. 23: 309-314.

Mrosovsky, N. 1988. Pivotal temperatures for loggerhead turtles *(Caretta caretta)* from northern and southern nesting beaches. Can. J. Zool. 66: 661-669.

Mrosovsky, N. 1994. Sex ratios of sea turtles. J. Exp. Zool. 270: 16-27.

Mrosovsky, N., and Godfrey, M.H. 1995. Manipulating sex ratios: turtle speed ahead! Chelonian Conserv. Biol. 1: 238-240.

Mrosovsky, N., and Pieau, C, 1991, Transitional range of temperature, pivotal temperatures and thermosensitive stages for sex determination in reptiles. Amphib.-Reptilia, 12: 169-179.

Mrosovsky, N., and Provancha, J. 1992. Sex ratio of hatchling loggerhead sea turtles: data and estimates from a 5-year study. Can. J. Zool. 70: 530-538.

Mrosovsky, N., Bapistotte, C, and Godfrey, M.H. 1999. Validation of incubation duration as an index of the sex ratio of hatchling sea turtles. Can. J. Zool. 77: 831-835.

Mrosovsky, N., Bass, A., Corliss, L.A., Richardson, J.I., and Richardson, T.H. 1992. Pivotal and beach temperatures for hawksbill turtles nesting in Antigua. Can. J. Zool. 70: 1920-1925.

Mrosovsky, N., Dutton, PH., and Whitmore, C.P. 1984. Sex ratios of two species of sea turtle nesting in Suriname. Can. J. Zool. 62: 2227-2239.

Raynaud, A., and Pieau, C. 1985. Embryonic development of the genital system. *In* Biology of the Reptilia. Vol. 15. B. Development. *Edited by* C. Gans and F. Billett. John Wiley and Sons, New York. pp. 149-300.

Ryder, C., Richardson, J.I., Corliss, and Bell, R. 1989. Habitat preference and beach management for nesting hawksbills, Jumby Bay, Antigua, West Indies. NOAA Tech. Mem. NMFS-SEFSC-232. pp. 263-266.

Silveira, A.M.R., and Patiri, V.J.A. 1995. Sobre a biologia de *Caretta caretta* (tartaruga cabe9uda) e *Eretmochelys imbricata* (tartaruga de pente) no litoral do município de Camaçari, Bahia. Resumos do XI Encontro de Zoologia do Nordeste, João Pessoa, Paraíba. p. 54. [Abstr.]

Schulz, J.P. 1975. Sea turtles nesting in Surinam. Zool. Verh. (Leiden), **143:** 1-143.

Vogt, R.C. 1994. Temperature controlled sex determination as a tool for turtle conservation. Chelonian Conserv. Biol. 1: 159-162.

Wibbels, T., and Hillis, Z.-M. 1995. Preliminary examination of hatchling sex ratios of hawksbill sea turtles from Buck Island Reef National Monument. Am. Zool. 35: 38A.

Wood, J.R., and Wood, RE. 1980. Reproductive biology of captive green sea turtles, *Chelonia mydas*. Am. Zool. **20:** 499-505.

Yntema, C.L., and Mrosovsky, N. 1980. Sexual differentiation in hatchling loggerheads *(Caretta caretta)* incubated at different controlled temperatures. Herpetologica, **36:** 33-36.

Zar, J.H. 1996. Biostatistical analysis. Prentice Hall, Upper Saddle River, N.J.