ELSEVIER

0006-3207(95)00027-5

Biological Conservation **74** (1995) 169–174

0006-3207/95/$09.50+.00

INCUBATION TEMPERATURES AND SEX-RATIOS IN THE MALAYSIAN LEATHERBACK TURTLE *Dermochelys coriacea*

E. H. Chan & H. C. Liew

Fisheries and Marine Science Centre, Universiti Pertanian Malaysia, Mengabang Telipot, 21030 Kuala Terengganu, Malaysia

(Received 19 April 1994; revised version received 14 November 1994; accepted 12 January 1995)

Abstract

Sex-ratio studies on the leatherback turtles of the Rantau Abang Turtle Sanctuary reveal that incubation of eggs in an open-air beach hatchery produced predominantly female hatchlings, while indoor incubation at average temperatures not exceeding 29·21°C and at 30·4°C produced 100% male and female hatchlings respectively. Production of 100% female hatchlings from the beach hatchery was maintained for nest sizes of 25, 50, 75 and 100 eggs. Sand temperatures at nest depth throughout the nesting beaches of the Sanctuary, as well as within the three beach hatcheries currently operational in the Sanctuary during the nesting season, exceeded values for production of 100% female hatchlings.

Keywords: Malaysian leatherback turtle, sex-ratio studies.

INTRODUCTION

It has been demonstrated irrevocably that sex determination in six species of sea turtles, *Caretta caretta, Eretmochelys imbricata, Chelonia mydas, Lepidochelys olivacea, L. kempii* and *Dermochelys coriacea*, is temperature-dependent (Janzen & Paukstis, 1991). Cool incubation temperatures are known to produce males while warm temperatures produce females. For each species, there is a narrow transitional range of temperature between the male- and female-producing temperatures in which both sexes may be differentiated (Mrosovsky & Pieau, 1991). Within the transitional range, the temperature variously referred to as the 'threshold' by Bull (1980), 'pivotal' by Mrosovsky and Yntema (1980), 'critical' by Pieau (1976) and 'SDT_{50}' by Limpus *et al.* (1983) produces a 1 : 1 sex ratio. Many studies have been conducted to determine temperature regimes affecting sexual differentiation in sea turtles as well as natural sex ratios produced from natural incubation of eggs in several well-known nesting beaches.

Studies on sex-ratio of the leatherback turtle *Dermochelys coriacea* (Mrosovsky *et al.*, 1984; Dutton *et al.*, 1985, Lescure *et al.*, 1985; Rimblot *et al.*, 1985; Rimblot-Baly *et al.*, 1987) have shown that incubation temperatures below 29·25°C produce 100% male hatchlings while temperatures exceeding 29·75°C produce 100% females. At 29·5°C, both sexes are produced, although not in a 1 : 1 ratio (Rimblot-Baly *et al.*, 1987). Some reports have provided evidence that a number of natural nesting beaches are producing female-biased sex-ratios (Standora & Spotila, 1985; Mrosovsky & Provancha, 1989) while others are able to maintain an approximate 1 : 1 sex-ratio output (Mrosovsky *et al.*, 1984; Rimblot-Baly *et al.*, 1987). A 1 : 1 sex ratio is usually maintained through seasonal temperature changes in the environment, or equal distribution of natural nests in both exposed and shaded portions of a nesting beach.

The phenomenon of temperature-dependent sex determination has important and far-reaching implications in conservation programmes where eggs are incubated in styrofoam boxes in an above-ground hatchery (Fretey & Lescure, 1982), in centralised protected beach hatcheries (Chan, 1991), or reburied in batches of *c.* 50 eggs instead of whole natural clutches (Chan, 1989). It is argued that small-sized nests experience reduced metabolic heating, and will thus have a masculinising effect on the hatchlings (Mrosovsky & Yntema, 1980). Because of the shift in incubation temperatures away from ambient sand temperatures at nest depth in naturally selected nests, artificial hatching practices face the danger of producing either all-male or all-female hatchlings.

The present study was initiated in order to provide information on temperature effects on sex determination of leatherback turtles over a wider geographical range. Sex ratios of leatherback hatchlings incubated in a beach hatchery in Rantau Abang (4° 51·9' N, 103° 23·8' E), in styrofoam boxes kept indoors, and in a temperature-controlled oven, were analysed. The effects of nest sizes of 25, 50, 75 and 100 eggs incubated in the beach hatchery relative to hatching success and hatchling sex ratios were also investigated. To supplement the sex ratio studies, sand temperatures at nest depth (40, 60 and 80 cm) along the 14 km of leatherback nesting beaches in the Rantau Abang Turtle Sanctuary, as well as in the three beach hatcheries currently operational within the sanctuary, were determined over the period during which eggs were in incubation, to reveal possible temperature profiles as well as seasonal trends.

MATERIALS AND METHODS

The hatchlings

Hatchlings sampled for sex determination were obtained from two experiments conducted from 20 July

Table 1. Data showing effects of incubation in the beach hatchery (A treatments), in styrofoam boxes (C treatments) and in an oven on the sex ratios of leatherback hatchlings

Treatment	No. of eggs incubated	No. of hatchlings emerged	No. of hatchlings sexed[a]	No. of female hatchlings	% Female hatchlings	Incubation temperature (°C)		Incubation duration (days)	Comments
						Mean ± SE	Range		
1A	25	8	5	5	100	Temperatures not monitored in the beach hatchery		58	One indeterminate gonad
2A	23	3	2	2	100			58–59	
3A	25	18	11(2)	10	91			56	
4A	25	23	4	4	100			55–56	
1C	25	16	11(3)	0	0	27·04 ± 0·58	23·5–28·8	76–79	
3C	25	25	9(1)	0	0	29·21 ± 1·01	27·3–32·0	63–65	
4C	25	24	9(1)	0	0	28·95 ± 1·34	26·0–31·8		
Oven	40	—	5	5	100	30·42 ± 0·80	28·35–33·0	58	No. of hatchlings emerged not indicated because embryos were progressively sampled for embryological studies

[a]In some trials gonads from further specimens were lost during histological preparation; these numbers are indicated in parentheses and are not used in set proportion calculations.

to 12 October, 1986. The first set was taken from experiments described in Chan (1989). Hatchlings were sampled from treatments 1A, 2A, 3A, 4A (incubated in the beach hatchery in Rantau Abang) and from styrofoam boxes 1C, 3C and 4C kept indoors (Table 1). Hatchlings were also sampled from eggs incubated in the oven. Temperatures in boxes 1C, 3C and 4C were monitored throughout the incubation period by means of mercury-in-glass thermometers (range −10–50°C, readability ± 0·5°) inserted horizontally through a hole made in the side of the box. Temperatures were read manually at 0900, 1200 and 1500 h and the readings averaged to give the daily incubation temperature. Evening temperatures were not monitored. However, since the boxes were kept indoors and not subjected to solar heating, the effects of night cooling were probably not appreciable. The oven temperature was set at 30°C but actual temperatures were read three times a day as well from a mercury thermometer. Temperatures in the beach hatchery in this series of experiments were not recorded due to logistical problems.

The second set of hatchlings was sampled from an experiment designed to determine the effect of nest size (i.e. number of eggs per nest) on hatching rates and sex ratios. Four nest sizes each replicated three times were tested, 25 eggs, A1–A3; 50 eggs, B1–B3, 75 eggs, C1–C3; and 100 eggs, D1–D3. In order to reduce the effect of hatch variability among natural clutches (Chan *et al.*, 1985), eggs assigned to each treatment were from different natural clutches. For example, in replicate 1, where three natural clutches were used, eggs from each clutch were assigned to treatments A–D in the ratio of 1 : 2 : 3 : 4. A total of nine natural clutches were used in these experiments. The eggs were incubated in the beach hatchery following procedures described in Chan (1989). Incubation temperatures were not monitored due to reasons stated earlier.

Sex determination

The hatchlings were euthanised in chloroform, and the kidneys with the attached gonads dissected out whole and fixed in 10% buffered formalin. The kidneys were cut into three transverse segments with the middle segment prepared for histology using standard histological techniques. Transverse sections 4–8 μm thick were made and stained in haematoxylin and eosin. Criteria for sexing the hatchlings followed the methods described in Mrosovsky *et al.* (1984) and Dutton *et al.* (1985).

Temperature profiling of the nesting beaches and beach hatcheries

As a supplement to the sex-ratio studies, temperature profiles within the Rantau Abang Turtle Sanctuary as well as in the three beach hatcheries in the Sanctuary were determined. Temperature readings were taken by means of YSI Tele-thermometers (Yellow Springs Instrument Co., Inc., OH, U.S.A.) using YSI series 400 thermistor probes. Twelve sampling stations at approximately 1 km apart were established along the nesting beaches. Station 1, also the northern most, was located at Kampong Jambu Bongkok (4° 56·3' N, 103° 21·2'E) while the last and most southerly station, Station 12, was located at Kuala Abang (4° 49·7' N, 103° 25' E). At each station, sand temperatures at depths of 80, 60 and 40 cm were determined monthly from May to October 1990 (i.e. months when egg incubation was in progress) at two points, A and B. Point A was located mid-way between the vegetation level and current high tide mark, while point B was at the high tide level. Temperatures were taken by digging a vertical hole 80 cm deep into the sand. Thermistor probes were then inserted horizontally into the undisturbed sand by the side of the excavated hole at the appropriate depths. The hole was then quickly covered with the moist excavated sand. Temperature readings were taken once they reached equilibrium, usually within 2–3 min.

Temperature readings for the three beach hatcheries were taken during the period when they were operational, from June to October 1990, at intervals of 1–3 weeks. Hatchery A was located at 4° 54. 9' N, 103° 22' E, B at 4° 51·9' N, 103° 23·8' E and C at 4° 50·5' N, 103° 24·7' E. Three sampling points were established in each hatchery, points A and C at the upper and lower extreme diagonal ends, and point B located right in the middle of the hatchery. Temperature readings were taken as described above, at depths of 80, 60 and 40 cm.

RESULTS

Effects of beach hatchery and styrofoam box incubation on sex ratios

Data showing the effects of beach hatchery and styrofoam box incubation are given in Table 1. Hatchlings produced from the beach hatchery showed a strong female bias. In fact, nests 1A, 2A and 4A produced 100% female hatchlings. The 91% female ratio recorded for nest 3A was due to the presence of one indeterminate gonad. Poor preparatory techniques resulted in gonads being lost from a number of slides. While there is no reason to suspect that the histological process might preferentially fail on testicular compared to ovarian tissue, for completeness, these numbers have been reported in Tables 1 and 2.

All eggs incubated in styrofoam boxes (1C, 3C and 4C) produced males (Table 1). Mean incubation temperatures in the boxes did not exceed 29·21°C (Table 1). Boxes 3C and 4C, which were placed next to each other in an enclosed laboratory, showed almost identical temperature conditions. Here, metabolic heating commenced on the 42nd day of incubation and reached the upper temperature limits of 32 and 33°C, respectively, about 3–5 days before emergence (Fig. 1). The five hatchlings incubated in the oven were all females. This is expected because of the high incubation temperatures maintained (mean of 30·42 ± 0·80°C and range from 28·5 to 33°C, Table 1, Fig. 1).

Effects of nest size on sex ratios and hatchling success

All hatchery nests, regardless of size, produced totally female hatchlings (Table 2), reinforcing the previous

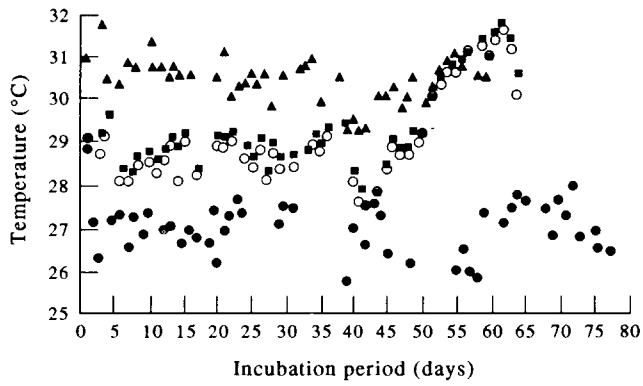

Fig. 1. Incubation temperature in styrofoam boxes 1C, 3C, 4C and in the oven. Box 1C (●); 3C (■); 4C (○); and oven (▲).

data showing the strongly female-biased ratios from the beach hatchery. Although incubation temperatures in these beach hatchery nests were not monitored, high temperatures would be expected since the hatchery is totally exposed to solar radiation. Hatch rates for nest sizes of 25, 50, 75 and 100 eggs are shown in Table 2. A one-way analysis of variance showed no significant difference between treatments as the variance within treatment was high (Table 3).

Temperature profiling of nesting beaches and beach hatcheries

The monthly temperature readings at the 12 stations along the Rantau Abang Sanctuary taken from May to October 1990 were pooled and averaged by stations, zones, depth and month as shown in Table 4. The average temperature throughout the nesting season, inclusive of egg incubation periods which extend to October, were high and exceeded 30·72°C for all the stations. Temperatures averaged for all the stations by zone and depth were similarly high and exceeded 30·97°C. Average temperatures by month ranged from 30·06°C in August to 33·53°C in May. However, no seasonal changes in temperature were observed.

Table 5 shows the pooled average temperatures for the beach hatcheries according to hatchery, zone, depth

Table 3. One-way analysis of variance for hatch rates (arcsine-transformed values) from different nest sizes

Source of variation	Sum of squares	d.f.	Mean square	F-ratio	*p*
Between groups	0·0418	3	0·0139	0·537	0·67
Within groups	0·2077	8	0·0259		
Total (corrected)	0·2495	11			

Table 4. Sand temperatures at the Rantau Abang Turtle Sanctuary in 1990 (refer to text for description of stations and zones)

Station	Total no. of readings	Average temperature (°C)	Standard error
1	36	31·23	0·116
2	36	30·96	0·116
3	36	31·01	0·116
4	36	31·15	0·116
5	36	31·00	0·116
6	36	31·04	0·116
7	36	30·95	0·116
8	36	31·36	0·116
9	36	31·12	0·116
10	36	31·16	0·116
11	36	31·24	0·116
12	33	30·72	0·121
Zones			
A	213	31·17	0·048
B	216	30·98	0·047
Depth (cm)			
40	143	31·10	0·059
60	143	31·16	0·059
80	143	30·97	0·059
Month			
May	72	33·53	0·082
June	72	30·67	0·082
July	72	31·21	0·082
August	72	30·06	0·082
September	72	30·75	0·082
October	69	30·24	0·084

Table 2. Effects of nest size on the sex ratio of leatherback hatchlings in the beach hatchery at Rantau Abang

Treatment	No. of eggs incubated	No. of hatchlings emerged	Incubation period (days)	No. of hatchlings sampled for sexing[a]	No. of females	% Females
A1	25	12 (48%)	56–58	9 (1)	9	100
A2	24	10 (42%)	54–56	9	9	100
A3	25	11 (44%)	55–57	9 (1)	9	100
B1	50	11 (22%)	56–57	10	10	100
B2	48	11 (23%)	55–56	8	8	100
B3	50	22 (44%)	55–57	8 (2)	8	100
C1	75	7 (9%)	55–57	6	6	100
C2	72	30 (42%)	54–57	20	20	100
C3	75	38 (51%)	55–58	17	17	100
D1	100	15 (15%)	56–69	14 (1)	14	100
D2	96	37 (39%)	54–58	17	17	100
D3	100	47 (47%)	55–56	20	20	100

[a]Values in parentheses as in Table 1.

Table 5. Sand temperatures at beach hatchery A, B and C in the Rantau Abang Turtle Sanctuary from June to October 1990 (refer to text for description of points)

	Total no. of readings	Average temperature (°C)	Standard error
Hatchery			
A	99	30·81	0·073
B	117	30·52	0·074
C	177	30·68	0·075
Point			
A	111	30·70	0·073
B	111	30·77	0·072
C	111	30·51	0·078
Depth (cm)			
40	111	30·63	0·093
60	111	30·72	0·075
80	111	30·64	0·055
Date			
12 June	27	31·02	0·045
20 June	27	30·70	0·112
28 June	18	30·25	0·093
5 July	27	32·11	0·139
12 July	27	31·31	0·177
19 July	18	30·14	0·048
31 July	27	30·33	0·067
7 August	27	30·87	0·135
18 August	27	29·94	0·074
12 September	18	30·94	0·078
13 September	9	30·57	0·089
20 September	27	30·46	0·055
4 October	27	29·87	0·059
14 October	27	30·48	0·128

and date. As in the nesting beaches, all the temperatures obtained were high. Pooled averages for Hatcheries A, B and C according to position in the hatchery and depth all exceeded 30·5°C. Temperatures for all the hatcheries averaged according to date of sampling exceeded 29·87°C. Pooled averages for readings taken on 18 August and 4 October were lower (29·94 and 29·87°C respectively) than the other sampling dates. However, no seasonal trends in temperature were observed.

DISCUSSION

Eggs of the leatherback turtle incubated in the beach hatchery at Rantau Abang produced hatchlings which were strongly female-biased. Eggs incubated at average incubation temperatures not exceeding 29·21°C, i.e. at 27·04°C (Box 1C), 28·95°C (Box 4C) and 29·21°C (Box 3C), produced a preponderance of male hatchlings. At an average incubation temperature of 30·42°C, 100% female hatchlings were produced. Thus it can be inferred that the pivotal temperature (temperature producing a 1 : 1 sex ratio) for leatherback turtles in Rantau Abang lies between 29·2 and 30·4°C. Dutton *et al.* (1985) found that the pivotal temperature for leatherback turtles in Suriname fell somewhere between 28 and 30·5°C. A more precise pivotal temperature of 29·5°C was reported by Rimblot-Baly *et al.* (1987) for the leatherback turtles of French Guiana. Lescure *et al.* (1985) found that at incubation temperatures lower than 29·25°C, 100% male leatherback hatchlings were produced while at 29·75°C and above, 100% females were produced. These data, in concert with the data obtained from the present study, confirm that 100% male leatherback hatchlings are produced at incubation temperatures not exceeding 29·2°C.

At this juncture, it is not desirable to conduct further experiments to determine the lower threshold temperature for production of 100% female leatherback hatchlings or the exact pivotal temperature. This is because the population in Rantau Abang has become so depleted (Chan, 1991) that it can ill afford to sacrifice hatchlings for sex determination. Temperature trends indicate that the three beach hatcheries, although spaced out over the sanctuary, will invariably produce strongly female-biased hatchlings. Therefore, current management techniques must be modified to ensure that some males are being introduced into the population with each recruitment.

Sand temperatures at nest depth from May to October 1990 throughout the 12 stations at the Rantau Abang Sanctuary, as well as in the three beach hatcheries currently operational, had average values all exceeding 29·87°C. This exceeds the lower temperature limit of 29·75°C reported by Rimblot-Baly *et al.* (1987) for the production of 100% female hatchlings. Indeed, all hatchlings produced from the Rantau Abang Beach Hatchery in 1986 which were sampled for sexing in this study were in effect 100% females. It is estimated that in 1990, the temperature regimes experienced in all the three beach hatcheries throughout the period in which they were operational would have produced 100% female hatchlings. Further, had there been any *in-situ* incubation of eggs in the sanctuary, a similar sex bias would have resulted.

The phenomenon of strong female bias in hatchling production is not peculiar to the Rantau Abang population. A number of sea turtle rookeries have been reported to produce predominantly female hatchlings under natural incubation conditions. Mrosovsky and Provancha (1989) found that more than 93% of the loggerhead *Caretta caretta* hatchlings produced on Cape Canaveral, Florida, USA were females; Standora and Spotila (1985) estimated that female-biased hatchling populations were also produced in the green turtle *Chelonia mydas* rookeries at Tortuguero, Seychelles Islands and South Yemen and the Kemp's ridley turtles *Lepidochelys kempii* at Rancho Nuevo, Mexico. Additionally, Wibbels *et al.* (1987) reported that the sex ratio of immature loggerheads along the Atlantic coast of the United States was significantly skewed toward female (66·3%). Other populations have been found to produce a more balanced sex-ratio approaching 1 : 1, e.g. in green and leatherback turtles in Suriname (Mrosovsky *et al.*, 1984) and leatherback turtles of French Guiana (Rimblot-Baly *et al.*, 1987). The question of what is the best sex-ratio for sea turtles remains unanswered. It is only possible to argue that what occurs naturally within each population must be the best.

All nest sizes (25, 50, 75 and 100 eggs) incubated in the beach hatchery produced predominantly female hatchlings. Reduced nest size therefore had no masculinising effect on the hatchlings. This is probably due to the fact that sand temperatures at nest depth in the beach hatchery had exceeded female-producing ranges throughout the incubation duration, therefore obliterating the effects of reduced metabolic heating. However, it is interesting to note that metabolic heating for the leatherback hatchlings observed in the present study occurred at the start of the third trimester of incubation. This is obviously outside the thermo-sensitive period, which has been identified to occur at the end of the first trimester and the beginning of the second trimester of embryonic life (Mrosovsky & Pieau, 1991). It is therefore apparent that for leatherback turtles, metabolic heating does not have any consequence on sex determination, particularly so when incubation temperatures already exceed pivotal levels. In contrast, Standora and Spotila (1985) found that for green turtles incubating at pivotal temperatures, metabolic heating resulted in female hatchlings at the centre of the clutch and male hatchlings along the periphery. The timing of metabolic heating may therefore differ for different species and could overlap with critical periods, and, in the words of Mrosovsky and Provancha (1989), 'variations in metabolic warming between clutches, and the degree of overlap of such warming with critical periods, require assessment'.

ACKNOWLEDGEMENTS

This study, which is part of the Sea Turtle Research and Conservation Project conducted by Universiti Pertanian Malaysia (UPM), was funded by ESSO Production Malaysia Inc., and excerpted from the senior author's doctoral dissertation entitled 'The conservation-related biology and ecology of the leatherback turtle, *Dermochelys coriacea* in Rantau Abang, Terengganu, Malaysia', Kagoshima University, supervised by Profs H. Kawatsu and G. Kawamura. The authors are indebted to the Fisheries Department of Terengganu for supplying leatherback eggs for the experiments and allowing the use of hatchery facilities. Research assistants and laboratory technicians from UPM, Kuala Terengganu assisted in field and laboratory work.

REFERENCES

Bull, J. J. (1980). Sex determination in reptiles. *Quart. Rev. Biol.*, **55**, 3–21.

Chan, E. H. (1989). White spot development, incubation and hatching success of leatherback turtle (*Dermochelys coriacea*) eggs from Rantau Abang, Malaysia. *Copeia*, **1989**, 42–7.

Chan, E. H. (1991). Sea turtles. In *The state of nature conservation in Malaysia*, ed. R. Kiew. Malayan Nature Society, Kuala Lumpur, Malaysia, pp. 120–35.

Chan, E. H., Salleh, H. U. & Liew, H. C. (1985). Effects of handling on hatchability of eggs of the leatherback turtle, *Dermochelys coriacea* (L.). *Pertanika*, **8**, 265–71.

Dutton, P. H., Whitmore, C. P. & Mrosovsky, N. (1985). Masculinization of leatherback turtle *Dermochelys coriacea* hatchlings from eggs incubated in styrofoam boxes. *Biol. Conserv.*, **31**, 249–64.

Fretey, J. & Lescure, J. (1982). A leatherback hatchery in French Guiana. *Mar. Turt. Newsl.*, **23**, 4–5.

Janzen, F. J. & Paukstis, G. L. (1991). Environmental sex determination in reptiles: ecology, evolution, and experimental design. *Quart. Rev. Biol.*, **66**, 149–79.

Lescure, J., Rimblot, F., Fretey, J., Renous, S. & Pieau, C. (1985). Influence of the incubation temperature of eggs on the hatchling sex-ratio in the leatherback, *Dermochelys coriacea*. *Bull. Soc. Zool. France*, **110**, 355–9.

Limpus, C. J., Reed, P. & Miller, J. D. (1983). Islands and turtles. The influence of choice of nesting beach on sex ratio. In *Proc. Inaugural Great Barrier Reef Conference*, ed. J. T. Baker, R. M. Carter, P W. Sammarco & K. P. Stark. James Cook University Press, North Queensland, pp. 397–402.

Mrosovsky, N., Dutton, P. H. & Whitmore, C. P. (1984). Sex ratios of two species of sea turtle nesting in Suriname. *Can. J. Zool.*, **62**, 2227–39.

Mrosovsky, N. & Pieau, C. (1991). Transitional range of temperature, pivotal temperatures and thermosensitive stages for sex determination in reptiles. *Amphibia–Reptilia*, **12**, 169–79.

Mrosovsky, N. & Provancha, J. (1989). Sex ratio of loggerhead sea turtles hatching on a Florida beach. *Can. J. Zool.*, **67**, 2533–9.

Mrosovsky, N. & Yntema, C. L. (1980). Temperature dependence of sexual differentiation in sea turtles: implications for conservation practices. *Biol. Conserv.*, **18**, 271–80.

Pieau, C. (1976). Données récentes sur la différenciation sexuelle en fonction de la température chez les embryons *d'Emys orbicularis* L. (Chelonian). *Bull. Soc. Zool. France*, **101** (suppl. 4), 46–53.

Rimblot, F., Fretey, J., Mrosovsky, N., Lescure, J. & Pieau, C. (1985). Sexual differentiation as a function of the incubation temperature of eggs in the sea turtle *Dermochelys coriacea* (Vandelli, 1761). *Amphibia–Reptilia*, **6**, 83–92.

Rimblot-Baly, F., Lescure, J., Fretey, J. & Pieau, C. (1987). Temperature sensitivity of sexual differentiation in the leatherback, *Dermochelys coriacea* (Vandelli, 1761). Data from artificial incubation applied to the study of sex-ratio in nature. *Ann. Sci. Nat., Zool. Paris*, **8**, 277–90.

Standora, E. A. & Spotila, J. R. (1985). Temperature dependent sex determination in sea turtles. *Copeia*, **1985**, 711–22.

Wibbels, T., Owens, D. W., Morris, Y. A. & Amoss, M. S. (1987). Sexing techniques and sex ratios for immature loggerhead sea turtles captured along the Atlantic Coast of the United States. In *Ecology of East Florida sea turtles*, ed W. N. Witzell. *U. S. Dep. Comm., NOAA Tech. Rep.*, NMFS 53, pp. 65–73.