

Significant difference of temperature-dependent sex determination between French Guiana (Atlantic) and Playa Grande (Costa-Rica, Pacific) leatherbacks (*Dermochelys coriacea*)

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Abstract – Temperature-dependent sex determination has been recently characterized for leatherbacks (*Dermochelys coriacea*) from Playa Grande, on the Pacific coast of Costa Rica. The authors concluded that the pattern of TSD in leatherbacks from Pacific Coastal Rica is the same as for leatherbacks from French Guiana, in the Atlantic. However, no statistical tests were performed to validate their conclusion. Here, we use a maximum-likelihood test to look for a possible difference between the populations. We found that the pattern of temperature-dependent sex determination in Atlantic and Pacific leatherbacks was significantly different. The temperature producing 50 % of each sex was not significantly different in both groups, but the range of temperatures producing both sexes was significantly narrower for the French Guiana population. We hypothesize that this difference could reflect a lower genetic polymorphism for temperature-dependent sex determination in this population. A low genetic diversity in the Guiana population compared to the Playa Grande population has been already observed for mitochondrial haplotypes. Our results emphasize the importance of statistical analyses in studies of temperature-dependent sex determination. © 1999 Éditions scientifiques et médicales Elsevier SAS

temperature-dependent sex determination / environmental sex determination / reptile / turtle / leatherback / *Dermochelys coriacea*

Résumé – Différence significative pour la sensibilité de la détermination du sexe à la température chez les tortues luths (*Dermochelys coriacea*) de Guyane française (Atlantique) et de Playa Grande (Costa-Rica, Pacifique). La détermination du sexe sensible à la température (TSD) a été récemment caractérisée pour les tortues luths (*Dermochelys coriacea*) de Playa Grande, sur la côte Pacifique du Costa Rica. Les auteurs concluaient que le profil de réponse à la température des tortues luths du Pacifique était le même que celui de cette même espèce pendant en Guyane française, côté Atlantique. Cependant aucun test statistique n'a été effectué permettant de valider cette conclusion. Un test par le maximum de vraisemblance a été utilisé

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pour rechercher une différence possible dans les profils de réponse à la température. Nous trouvons que les profils globaux sont en fait significativement différents entre les tortues luths de l'Atlantique et du Pacifique. La température produisant 50 % de chaque sexe n'est pas significativement différente entre les deux groupes, par contre l'intervalle de température produisant les deux sexes est significativement plus étroit pour les tortues luths pondant en Guyane française. Cet intervalle de température plus étroit pourrait refléter une variabilité génétique plus faible pour ce caractère dans cette population. Une diversité génétique plus faible pour les femelles pondant en Guyane française en comparaison de celles pondant à Playa Grande a d'ailleurs déjà été démontrée pour l'ADN mitochondrial. Nos résultats renforcent la nécessité de l'utilisation de tests statistiques pour l'étude de la détermination du sexe sensible à la température. © 1999 Éditions scientifiques et médicales Elsevier SAS

détermination du sexe sensible à la température / détermination du sexe sensible à des facteurs de l'environnement / reptile / tortue / tortue luth / *Dermochelys coriacea*

1. INTRODUCTION

Temperature-dependent sex determination (TSD) occurs in all marine turtle species (*Dermochelys coriacea*, *Chelonia mydas*, *Eretmochelys imbricata*, *Lepidochelys olivacea*, *Lepidochelys kempii*, *Caretta caretta*, *Natator depressus*) [14, 17, S. Hewavisenthi, pers. comm.]. However, the exact patterns of sex ratio response to different temperatures and differences among populations are not well known. The best analyzed species to date is *Caretta caretta*, in which six different populations have been analyzed [11–13] and exhibit some degree of differentiation among populations [6]. Recently the pattern of TSD for *Dermochelys coriacea* nesting at Playa Grande (Pacific coast of Costa Rica) was described by Binckley et al. [1] and the authors proposed that Pacific leatherbacks exhibit a pattern of TSD similar to the pattern observed for French Guiana (Atlantic) turtles [19, 20]. This information is particularly interesting because genetic differences between Atlantic and Pacific females for mitochondrial DNA have been observed [4]. Moreover, initial results obtained using nuclear microsatellites also indicate a genetic differentiation between Atlantic and Pacific leatherbacks [3]. However, a rigorous comparison of TSD pattern has not been performed. Therefore, we conducted a maximum-likelihood statistical procedure to derive the pivotal temperature (the constant temperature producing both sexes in equal amounts) and the transitional range of temperature (TRT, range of incubation temperatures producing both sexes) for Playa Grande leatherbacks [6]. These values were used to evaluate whether the observed distribution of males and females for each incubation temperature from French Guiana leatherbacks is compatible with the values expected if TSD distributions for both sites were the same.

2. MATERIALS AND METHODS

Data for sex determination in French Guiana leatherbacks were obtained from Rimblot et al. [19] and Rimblot-Baly et al. [20]. Data for sex determination in Playa Grande leatherbacks were obtained from Binckley et al. [1]. In the latter case, the raw numbers of males and females produced for each temperature were not published. Therefore, we calculated these values based on the following information: (1) the description that 8 to 19 eggs were sexed for each tested temperature, (2) the hatchling success at each temperature and (3) the sex ratio at each temperature. Based on the hatchling success (hs) the product $ns = hs \cdot ne$ has been established for ne equal 1 to 30 (ne being the number of eggs incubated at the analyzed temperature and ns being the number of sexed juveniles). This product gives an integer value of ns only for ne equal to the number of eggs incubated at the analyzed temperature. For example, the hatchling success is 73.9 % at 28 °C [1]. The ns value is an integer value equal to 17 for $ne = 23$ and $17/23 = 73.9$ %. Full data are shown in *table I*.

The statistical methodology we used is fully described in Girondot [6] and is summarized here briefly. It is based on the best fit curve of the sex ratio on the incubation temperatures to the function:

$$sr(t) = \frac{1}{1 + e^{\left(\frac{1}{S}(P-t)\right)}} \quad (1)$$

This equation has all the prerequisites for describing TSD: sr goes from 0 to 1 for S being positive or from 1 to 0 for S being negative with a transition more or less sharp according to the S parameter and with an sr value equal to 0.5 for $t = P$. The adjustment of the P and S parameters for a particular set of incubation temperatures and sex ratio is performed using maximum-likelihood analysis and the variance of the parameters is obtained by the values of the second order derivative at the maximum-likelihood S and P values [6].

Parameters (P_P ; S_P) were produced for the Playa Grande data (*table I*). The probability (p_i) of the observed

Table I. Incubation temperatures in °C (t_i), number of sexed embryos (N_i), number of males (M_i) and females (F_i), observed and expected male frequency (Osr_i and Esr_i) among sexed embryos for French Guiana and Playa Grande (Costa Rica) leatherbacks.

Tableau I. Températures d'incubation en °C (t_i), nombre d'embryons sexés (N_i), nombre de mâles (M_i) et de femelles (F_i) et fréquence observée et attendue des mâles parmi les embryons sexés (Osr_i et Esr_i) pour les tortues luths de Guyane française et de Playa Grande (Costa Rica).

Playa Grande (Pacific)							French Guiana (Atlantic)						
t_i	N_i	M_i	F_i	Osr_i	Esr_i	p_i	t_i	N_i	M_i	F_i	Osr_i	Esr_i	p_i
							27	33	33	0	1	1.00	–
							27.25	5	5	0	1	1.00	–
28	17	17	0	1	1.00	–	28	4	4	0	1	1.00	–
							28.25	1	1	0	1	1.00	–
							28.75	15	15	0	1	1.00	–
29	10	9	1	0.90	0.90	–							
							29.25	9	9	0	1	0.71	0.20
29.5	10	4	6	0.40	0.41	> 0.95	29.5	18	13	5	0.72	0.41	< 0.01
							29.75	36	0	36	0	0.16	< 0.01
30	14	1	13	0.07	0.05	–							
30.5	16	0	16	0	0.00	–	30.5	18	0	18	0	0.00	–
31	10	0	10	0	0.00	–							
31.5	12	0	12	0	0.00	–							
32	8	0	8	0	0.00	–	32	3	0	3	0	0.00	–

$$L_P = 3.246 \cdot 10^{-2}$$

$$P_P = 29.43 \text{ (SD 0.10)}$$

$$S_P = -0.200 \text{ (SD 0.007)}$$

(P_P ; S_P) are the parameters that maximized likelihood (L_P) for Pacific data; (p_i) are the probabilities of the observed number of males and females based on χ^2 test for each incubation temperature using P_P and S_P parameter values (dash indicates that the expected number of males or females is lower than 3 and the test cannot be performed). Values in grey shading indicate significant difference compared to the H_0 hypothesis, i.e. no difference between French Guiana and Playa Grande leatherbacks for TSD. SD indicates standard deviation.

(P_P ; S_P) sont les paramètres maximisant la vraisemblance (L_P) pour les données du Pacifique ; (p_i) sont les probabilités d'obtenir le nombre de mâles et de femelles basées sur un test de χ^2 et ce pour chaque température d'incubation en utilisant les valeurs P_P et S_P (un tiret indique que le nombre attendu de mâles ou de femelles est inférieur à 3 ce qui empêche la réalisation du test). Les valeurs sur fond grisé indiquent une différence significative par rapport à l'hypothèse H_0 , i.e. pas de différence entre le profil de détermination du sexe par la température des tortues luths de Playa Grande et de Guyane française. SD est l'écart type.

distribution of males and females for each n incubation temperature was obtained using a binomial distribution with M_i , F_i , and N_i being the observed number of males, females, and total of sexed embryos, respectively, for a particular temperature t_i and the theoretical sex ratio at this temperature, $sr(t_i)$ based on equation (1):

$$p_i = C_{N_i}^{M_i} (sr_i(t_i))^{M_i} (1 - sr_i(t_i))^{F_i} \quad (2)$$

The quality of the adjustment of equation 1 to the observed data for Playa Grande is based on the transformation of theoretical sex ratios to p -values using an χ^2 test for each incubation temperature. When the expected number of males or females falls between 3 and 5, a Yates' correction is applied and when it is lower than 3, the χ^2 test cannot be performed.

The values (P_A ; S_A) for French Guiana cannot be estimated because only one incubation temperature produced a

mixed sex ratio. The null hypothesis (H_0) is that no difference exists between the responses to incubation temperatures of the populations. Based on this hypothesis, we used (P_P ; S_P) values as estimates of (P_A ; S_A) and established the probability of the observed number of males and females for each incubation temperature using the χ^2 test. If some probabilities were lower than 0.05, we rejected H_0 and then concluded that temperature-dependent sex determination was significantly different in both locations.

To determine more precisely if pivotal temperatures, transitional range of temperatures or both are significantly different in both geographic groups, we estimated the range of possible values for P_A and S_A as being the values for which no p_i is lower than 0.05. The range of possible values of P_A and S_A were then tested against P_P and S_P respectively using Student's t -test.

3. RESULTS

The observed numbers of males and females for Playa Grande leatherbacks were adjusted to equation 1. The fit was very good and we could not reject its concordance with observed data at any incubation temperature (see *table I*). We confirm that both distributions are very similar (*figure 1*). The pivotal temperature established by maximum-likelihood method was similar to that estimated using a graphical method [1] (29.43 vs. 29.4 °C). However, contrary to the previous conclusion [1] both distributions were significantly different as the probabilities of obtaining the observed number of males and females for Atlantic data using P_P and S_P is lower than 5 % for incubation at 29.5 and 29.75 °C (*table I*).

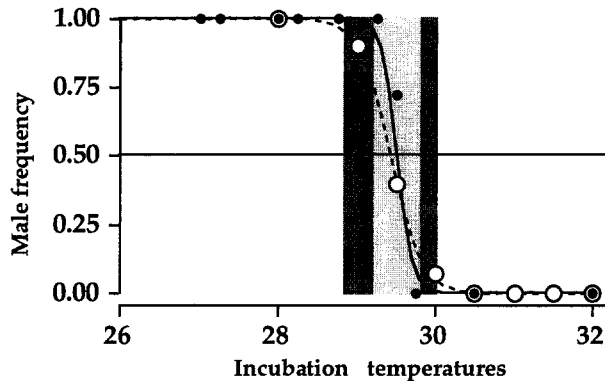


Figure 1. Relationship between incubation temperature and sex ratio for eggs from French Guiana, Atlantic (●) and from Playa Grande, Pacific (○). The curve (---) is the theoretical function of sex ratio upon incubation temperatures maximizing the likelihood for Playa Grande data. For French Guiana, the theoretical curve shown (—) is the curve that is the most similar to Playa Grande curve but still compatible with observed males and females number for each incubation temperature ($\chi^2 < 3.84$; $p_i > 0.05$). TRT_{5%} (range of temperature producing between 5 % and 95 % of each sex) is shown as a grey range of temperatures for Playa Grande (Pacific) and a lighter one inside for French Guiana (Atlantic).

Figure 1. Relation entre la température d'incubation et la *sex-ratio* pour les œufs de Guyane française, Atlantique (●) ou Playa Grande, Pacifique (○). La courbe (---) correspond à la fonction maximisant la vraisemblance pour les données de Playa Grande. Pour la Guyane française, la courbe présentée (—) est celle qui est la plus semblable à la courbe de Playa Grande mais qui est quand même compatible avec le nombre de mâles et de femelles à chaque température d'incubation ($\chi^2 < 3.84$; $p_i > 0.05$). La TRT_{5%} (intervalle de températures produisant entre 5 % et 95 % de chaque sexe) pour Playa Grande (Pacifique) est en grisé foncé et, à l'intérieur de celle-ci, la TRT_{5%} pour la Guyane française (Atlantique) est en grisé plus clair.

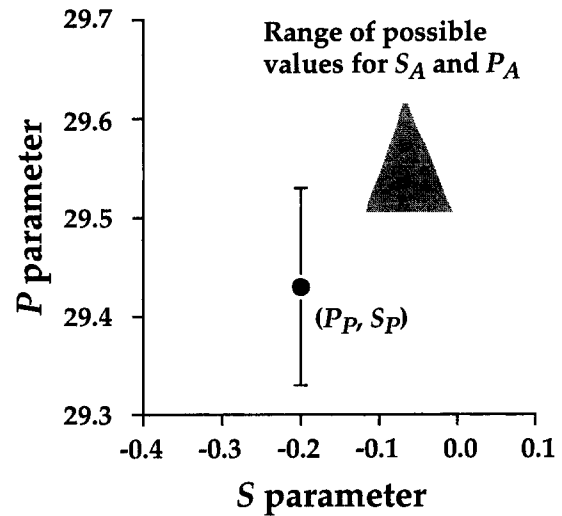


Figure 2. Range of values of S_A and P_A (French Guiana, Atlantic) that are compatible with the observed data of males and females number for each incubation temperature ($\chi^2 < 3.84$; $p_i > 0.05$) and values of P_P and S_P (Playa Grande, Pacific) that maximized likelihood. Error bars represent standard deviation (for the S axis, the standard deviation is too small to be shown).

Figure 2. Intervalles possibles pour les valeurs de S_A et P_A (Guyane française, Atlantique) qui sont compatibles avec les données observées de nombre de mâles et femelles pour chaque température d'incubation ($\chi^2 < 3,84$; $p_i > 0,05$). Les valeurs de P_P et S_P présentées sur le graphique correspondent aux valeurs maximisant la vraisemblance pour les données de Playa Grande, Pacifique. Les barres d'erreurs représentent l'écart type mais celui correspondant à l'axe S est trop faible pour être visible.

We established the range of possible values for P_A and S_A , i.e., the range of P and S values for Atlantic data that did not permit us to reject the observed distribution of males and females as being consistent with equation 1 ($p > 0.05$) for each incubation temperature (*figure 2*). The statistical difference between P_A and P_P on one side and S_A and S_P on the other were tested. For this test, we used the S_A and P_A values that were the most similar to the values for Playa Grande as a conservative approach ($P_A = 29.51$ and $S_A = -0.1$). We also needed a value for standard deviation of P_A and S_A but this value was not available because only one temperature produced a mixed sex ratio for French Guiana data. As an alternative, we used the value of standard deviation of S_P as a rough estimate for standard deviation of S_A (idem for P_P and P_A) because the number of incubation temperatures for each experiment was not very different (8 and 10).

With this assumption, the pivotal temperatures for both populations were not significantly different (t^* Student = 0.56, $p > 0.05$), a feature that was also noted by Binckley et al. [1]. On the other hand, the difference between S values for both groups was very significantly different (t^* Student = 10.10, $p < 0.001$). The difference was so great that the caveat of the use of standard deviation of S_P for S_A has no consequence on the final result that the S values were significantly different for both geographic groups.

4. DISCUSSION

Binckley et al. [1] concluded from their data that, “The two temperature versus percent female curves for Atlantic and Pacific leatherback populations are virtually identical in pivotal temperature and overall shape”. We show that in fact a significant difference exists between the sex ratio responses to incubation temperature between French Guiana and Playa Grande *Dermodochelys coriacea* (the Pacific and Atlantic oceans possess other nesting populations and thus are not solely represented by turtles from French Guiana and Playa Grande). The significant difference was due to changes not in the pivotal temperature (P parameter) but instead in the value of transitional range of temperature (TRT, proportional to S parameter). Our analysis used all the information available for each incubation temperature, particularly the number of sexed juveniles contrary to the previously published analysis that used simply the sex ratio as a comparative index, independent of the number of eggs used at each incubation temperature [1]. Standard deviation for P_A and S_A were not available and instead we used the standard deviation of P_P and S_P . However, the number of incubation temperatures was higher for Atlantic data than for Pacific ones (10 vs. 8) and the total number of eggs was also higher for the French Guiana data than for Playa Grande (142 vs. 97). Thus, we expect that the standard deviation of P_A and S_A would be lower than the standard deviation of P_P and S_P . Consequently, the option used here is conservative and the difference measured in S values should be even greater than reported here. These results emphasize the importance of the use of statistical tests when describing TSD, especially when comparing different populations.

Most studies of TSD have highlighted the importance of pivotal temperature as a key parameter [5, 18] and generally have neglected the TRT. Binckley et al.

[1] also discussed only the significance of quasi-identical pivotal temperatures for French Guiana and Playa Grande nesting populations. However, change in TRT can produce a very large effect for global sex ratio output of the beach. For example, in Suriname, green and leatherback turtles have almost the same pivots and nest at roughly the same time of year, but have different sex ratios, because of the differences in TRT [8]. The shape and extent of TRT is an index of the polymorphism of the character determining the response of sex ratio to incubation temperature [15]. This value is important because the larger it is, the more rapidly the population can adapt to sudden large temperature changes (e.g. global warming due to the greenhouse effect). A species with narrow TRT (which suggests no polymorphism) could not adapt its primary sex ratio because the genetic basis for the selection does not exist. If the TRT is wide (indicating that polymorphism exists), the primary sex ratio will change rapidly to compensate for the initial change [7]. A recent study of the TSD responses of three species of reptiles showed significant differences among clutches of individual species [18]. These results suggest underlying genetic polymorphisms of TSD, and also suggest that TSD species have the capacity to adapt to rapid environmental changes, in contrast to what has been previously suggested [9, 16].

Leatherbacks from French Guiana and Suriname have a very narrow TRT (low value of $|S|$ parameter) which indicates a low level of polymorphism for this character. This lower level of polymorphism for the French Guiana population could be more general than simply for the TSD character. Indeed, it is consistent with the lack of mitochondrial polymorphism (haplotype diversity = 0) for this population while the Playa Grande population exhibits six different haplotypes (haplotype diversity = 0.66 ± 0.09) [4]. The lack of polymorphism for Suriname and French Guiana could be the result of a recent genetic bottleneck. Leatherbacks were only sporadically described from French Guiana or Suriname, first in 1661 [cited in 2] and then in 1881 [10] whereas other marine turtles were more commonly cited. Village elders in Ya:lima:po in French Guiana indicated to one of us (M.G.) that leatherbacks were seen only exceptionally during the 1950s. Moreover, the reconstruction of the trend of the number of nests per year during the last 30 years shows a large increase of the frequentation in the three main nesting beaches in the region (Ya:lima:po, Matapica and Galibi) during the

1960s and 1970s [2]. The origin of these females is unknown but a global migration from another nesting beach is unlikely as no other population has shown a parallel massive decrease at the same time. We hypothesize that the Guianan population of leatherbacks originated from a very small number of females, a factor that would result in lower genetic diversity in this population as a result of genetic drift. Further studies of the TSD response of other leatherback populations in Atlantic and Pacific oceans could shed further light on this subject.

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REFERENCES

- [1] Binckley C.A., Spotila J.R., Wilson K.S., Paladino F.V., Sex determination and sex ratios of Pacific Leatherback Turtles, *Dermochelys coriacea*, *Copeia* 1998 (1998) 291–300.
- [2] Chevalier J., Cazelles B., Girondot M., Apports scientifiques à la conservation des tortues luths en Guyane française, *JATBA, Rev. d'Ethnobiol.* XL (1998) 485–507.
- [3] Dutton P.H., Use of molecular markers for stock identification, fingerprinting, and the study of mating behavior in leatherbacks, in: Bowen B.W., Witzell W.N. (Eds.), *Proceedings of the International Symposium on Sea Turtle Conservation Genetics*, NOAA Technical Memorandum NMFS-SEFSC-396, Miami, Florida, 1996, pp. 79–86.
- [4] Dutton P.H., Bowen B.W., Owens D.W., Barragan A., Davies S.K., Global phylogeography of the leatherback turtle (*Dermochelys coriacea*), *J. Zool., Lond.* 247 (1999) 397–410.
- [5] Ewert M.A., Jackson D.R., Nelson C.E., Patterns of temperature-dependent sex determination in turtles, *J. Exp. Zool.* 270 (1994) 3–15.
- [6] Girondot M., Statistical description of temperature-dependent sex determination using maximum likelihood, *Evol. Ecol. Res.* 1 (1999) 479–486.
- [7] Girondot M., Fouillet H., Pieau C., Feminizing turtle embryos as a conservation tool, *Conserv. Biol.* 12 (1998) 353–362.
- [8] Godfrey M.H., Sex ratios of sea turtle hatchlings: direct and indirect estimates, PhD Thesis (unpublished), University of Toronto, Toronto, Ontario, Canada, 1997.
- [9] Janzen F.J., Climate change and temperature-dependent sex determination in reptiles, *Proc. Nat. Acad. Sci. USA* 91 (1994) 7487–7490.
- [10] Kappler A., *Holländisch-Guiana, Erlebnisse und Erfahrungen während eines 43-jährigen Aufenthalts in der Kolonie Surinam*, Stuttgart, Germany, 1881.
- [11] Limpus C.J., Reed P.C., Miller J.D., Temperature dependent sex determination in Queensland sea turtles: Intraspecific variation in *Caretta caretta*, in: Grigg G., Shine R., Ehmann H. (Eds.), *Biology of Australian frogs and reptiles*, Royal Zoological Society, New South Wales, Australia, 1985, pp. 343–351.
- [12] Marcovaldi M.Â., Godfrey M.H., Mrosovsky N., Estimating sex ratios of loggerhead turtles in Brazil from pivotal incubation temperatures, *Can. J. Zool.* 75 (1997) 755–770.
- [13] Mrosovsky N., Pivotal temperatures for loggerhead turtles *Caretta caretta* from northern and southern nesting beaches, *Can. J. Zool.* 66 (1988) 661–669.
- [14] Mrosovsky N., Bass A., Corliss L.A., Richardson J.I., Richardson T.H., Pivotal and beach temperature for hawksbill turtles nesting in Antigua, *Can. J. Zool.* 70 (1992) 1920–1925.
- [15] Mrosovsky N., Pieau C., Transitional range of temperature, pivotal temperatures and thermosensitive stages for sex determination in reptiles, *Amphibia-Reptilia* 12 (1991) 169–179.
- [16] Mrosovsky N., Dutton P.H., Whitmore C.P., Sex ratios of two species of sea turtle nesting in Suriname, *Can. J. Zool.* 62 (1984) 2227–2239.
- [17] Paukstis G.L., Janzen F.J., Sex determination in reptiles: summary of effects of constant temperatures of incubation on sex ratios of offspring, *Smithsonian Herpetological Information Service*, New York, 1990.
- [18] Rhen T., Lang J.W., Among-family variation for environmental sex determination in reptiles, *Evolution* 52 (1998) 1514–1520.
- [19] Rimblot F., Fretey J., Mrosovsky N., Lescure J., Pieau C., Sexual differentiation as a function of the incubation temperature of eggs in the sea-turtle *Dermochelys coriacea* Vandelli, 1761, *Amphibia-Reptilia* 85 (1985) 83–92.
- [20] Rimblot-Baly F., Lescure J., Fretey J., Pieau C., Sensibilité à la température de la différenciation sexuelle chez la tortue Luth, *Dermochelys coriacea* Vandelli, 1761; application des données de l'incubation artificielle à l'étude de la sex-ratio dans la nature, *Ann. Sci. Nat.* 13 (1986) 277–290.