



Maternal transfer of trace elements in leatherback turtles (*Dermochelys coriacea*) of French Guiana

Elodie Guirlet^{a,*}, Krishna Das^b, Marc Girondot^{a,c}

^a Laboratoire d'Ecologie, Systématique et Evolution, Bâtiment 362, Université Paris Sud, 91405 Orsay Cedex, France

^b Centre de recherche MARE, Laboratoire d'Océanologie, Université de Liège, B6, B-4000 Liège, Belgium

^c Département Evolution et Systématique, Muséum National d'Histoire Naturelle de Paris, 25 rue Cuvier, 75005 Paris, France

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ABSTRACT

In sea turtles, parental investment is limited to the nutrients and energy invested in eggs that will support embryonic development. Leatherback females have the largest clutches with the biggest eggs of the sea turtles and the highest reproductive output in reptiles. The migration between foraging sites and nesting beaches also represents high energy expenditure. The toxicokinetic of pollutants in the tissues is thus expected to vary during those periods but there is a lack of information in reptiles. Concentrations of essential (Copper, Zinc, Selenium) and non-essentials elements (Cadmium, Lead, Mercury) were determined in blood ($n=78$) and eggs ($n=76$) of 46 free-ranging leatherback females collected in French Guiana. Maternal transfer to eggs and relationships between blood and eggs concentrations during the nesting season were investigated. All trace elements were detectable in both tissues. Levels of toxic metals were lower than essential elements likely due to the high pelagic nature of leatherbacks that seems to limit exposure to toxic elements. Significant relationships between blood and egg concentrations were observed for Se and Cd. Se could have an important role in embryonic development of leatherback turtles and Cd transfer could be linked to similar carrier proteins as Se. Finally, as multiple clutches were sampled from each female, trends in trace elements were investigated along the nesting season. No change was observed in eggs but changes were recorded in blood concentrations of Cu. Cu level decreased while blood Pb levels increased through the nesting season. The high demand on the body during the breeding season seems to affect blood Cu concentrations. Calcium requirement for egg production with concomitant Pb mobilization could explain the increase in blood Pb concentrations along the nesting season.

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1. Introduction

Levels and effects of pollutants in the tissues of marine vertebrates depend on several factors including the exposure to environmental contamination (through the diet mainly) and the biotic phase of life in which they are (Wolfe et al., 1998; Damstra et al., 2002; Das et al., 2003). Migrations, breeding and fasting represent a high energy demand on the body, and the toxicokinetics of pollutants in the tissues may vary during these periods. While these processes have been described for fishes and marine mammals (Nicoletto and Hendricks, 1988; Hammerschmidt et al., 1999; Debier et al., 2003a,b, 2006; Van de Vijver et al., 2004; Greig et al., 2007), little is known for marine turtles. Indeed, like the marine mammals, the marine turtles also go through phases,

namely migration, breeding, fasting and the laying of eggs. One extreme case is the leatherback turtle (*Dermochelys coriacea*), the largest and most pelagic of the sea turtles.

The population of leatherback turtles has experienced a serious decline over the past twenty years mainly due to them being an accidental fisheries bycatch. Also, their eggs and females may be harvested (Kaplan, 2005; Martinez et al., 2007). In the Atlantic Ocean, the leatherback turtle displays a marked migratory cycle of several thousand kilometres between pelagic feeding grounds in the Ocean and nesting sites primarily located in French Guiana, Suriname, and Gabon (Ferraroli et al., 2004; Hays et al., 2004; Fretey et al., 2007). One of the major Atlantic nesting sites for this species is the French Guiana where approximately 40% of the world's leatherback turtles come to nest (Spotila et al., 1996). Leatherback females come ashore at night around the high tide to lay an average of 7 clutches with an interval between nesting events of 10 days (Girondot and Fretey, 1996). They have the biggest eggs (~80 g) and the largest clutches by weight (~5–10 kg) among sea turtles; egg production represents the highest reproductive out-

* Corresponding author. Tel.: +33 169155688; fax: +33 169155696.

E-mail addresses: elodie.guirlet@u-psud.fr (E. Guirlet), krishna.das@ulg.ac.be (K. Das), marc.girondot@u-psud.fr (M. Girondot).

put in reptiles (Miller, 1997; Wallace et al., 2007). Finally, at the end of the breeding season leatherbacks undertake long migrations to feed upon gelatinous zooplankton in rich North Atlantic waters (Davenport, 1998). The foraging activity of females during the nesting season remains unclear and females may go through this period of high metabolic requirement with little or no food intake (Fossette et al., 2007; Caut et al., 2008). They survive mainly on energy reserves (Wallace et al., 2005) accumulated during their long migratory cycle of generally more than two years (Rivalan et al., 2005). The high mobility of females of the Atlantic population during their migration in the Atlantic Ocean could facilitate exposure to environmental toxicants. Indeed, during this feeding phase, leatherbacks consume a great quantity of prey equivalent to at least 50% of their body mass per day (Davenport, 1998). Leatherback females could also be confronted with pollution by ingesting contaminated water and/or preys in the neritic waters of the nesting beaches of French Guiana. Indeed, French Guiana coastal zones are subjected to natural metal contamination amplified by mining activities (Richard et al., 2000; Mol et al., 2001; Marchand et al., 2006). Non-essential metals include Mercury (Hg), Lead (Pb) and Cadmium (Cd) although several essential metals, notably Zinc (Zn) and Copper (Cu), can act as toxicants at elevated concentrations in organisms (Devkota and Schmidt, 1999; Kobayashi and Okamura, 2004).

Toxicokinetic and potential effects of trace elements during these key periods of life have been to date poorly investigated in sea turtles (Sakai et al., 1995). Moreover, metabolism and mobilization of elements, in periods during which protein and lipid mobilization are high, are likely to differ according to biochemical properties of respective elements. Methylmercury (MeHg) displays some lipophilic properties and thus may be detected in the adipose tissues but shows high binding affinities for blood for thiol ligand in the amino acid cysteine, haemoglobin and albumin, resulting in the high mobility of the organic form in the body (Clarkson et al., 2007). Other toxic metals such as Cd and Pb might compete with essential metals for binding site of metalloenzymes and metallothioneins (Klaassen et al., 1999). Among vertebrates of wildlife, the organic form of Hg (MeHg) has been shown to be immunotoxic, genotoxic and neurotoxic (Wolfe et al., 1998); Pb has been shown to produce adverse teratogenic and reproductive effects while Cd is teratogenic, carcinogenic and highly nephrotoxic (Eisler, 1985, 1988; Noonan et al., 2002). However while the toxicity of non-essential elements is well described for marine fishes, birds and mammals, relatively little is known about metal homeostasis and toxicity in reptiles (Eisler, 1988; Wolfe et al., 1998).

In birds, amphibians and reptiles, eggs receive their initial burden with maternal transfer during egg formation (Nagle et al., 2001; Kubota et al., 2002; Roe et al., 2004; Hopkins et al., 2006). Early life stages of oviparous organisms seem to exhibit higher sensitivity to chemical contaminants than adults (Russell et al., 1999). Indeed, in reptiles, ovo-exposure to toxic elements (cadmium, arsenic) has been shown to affect hatchling growth, foraging efficiency, mortality, thyroid function or later reproduction (Hopkins et al., 1999; Brasfield et al., 2004; Marco et al., 2004). Surprisingly, hatching success in leatherback turtles is lower than for other sea turtles and the reason of this high embryonic mortality remains unclear (Bell et al., 2003). Moreover, among leatherback nesting beaches, hatching success on Yalimapo beach is low compared to other nesting sites (Caut et al., 2006). In mammals, numerous studies showed that embryos and growing individuals are particularly sensitive to deficiencies in nutrients (Keen et al., 1997). Deficiencies lead to a dysfunction of their immune and endocrine systems (Kelleher and Lonnerdal, 2005). In reptiles, while Cu and Zn have a paramount role in the growth and the tissue development of embryo, Cd, Hg and Pb

are particularly toxic at this key period of development (Wolfe et al., 1998). Few studies have reported data on trace elements in sea turtle blood and eggs (see Table 1) because most of the available data involve stranded turtles and consequently maternal transfer of trace elements to eggs in sea turtles is poorly known and should be examined to assess risk for incubation success.

Overall, there is a clear need to improve knowledge on levels, toxicokinetic and effects of essential and non-essential elements in leatherback turtle. In the present study we investigate (i) the concentrations of Cu, Zn, Se, Cd, Pb, and Hg in blood and eggs of free-ranging leatherback females during their nesting season in French Guiana and (ii) the toxicokinetics of essential and non-essential elements by assessing the variations of trace element concentrations according to the number of nesting events and their transfer from females to their eggs.

2. Materials and methods

2.1. Study site and sample collection

The study was conducted at Yalimapo beach, situated within the Amana Natural Reserve, between the Maroni and Amana Rivers on the northwest coast of the French Guiana (Fig. 1), from March to July 2006. This beach is monitored regularly and nesting leatherback females encountered are tagged with an internal permanent marker (Passive Integrated Transponder [PIT] tags) located in the turtle's right shoulder muscle. These coded microchips are used to identify leatherback turtles and thus researchers working on this beach can have a temporal monitoring of the females during and between nesting seasons. Samples were obtained from leatherback females nesting on Yalimapo beach. During two months, from 16th March to 14th May 2006, we patrolled the beach each night around the high tide. Nesting females encountered during patrols were scanned for PIT tags. If females did not have a tag, then one was inserted. At the beginning of the nesting season (mid March), all the females were sampled; then progressively, sampling focused on the females already captured once or more. While the female laid its eggs, measurements and blood ($n=78$) and eggs ($n=76$) samples were taken. The curved carapace length (CCL) was measured using a flexible tape measure. The number of yolked-eggs of the clutch was recorded and, around the 20th egg, one yolked-egg was collected. Blood sampling was carried out in the venous sinus of the rear flipper using single use needles, plastic syringes and blood collection tubes containing heparin to prevent clotting. Whole blood and whole eggs samples were frozen at -20°C until analyses.

2.2. Sample preparation

After being weighed and dried at 60°C to a constant weight, 100 mg of blood and 500 mg of homogenized egg content (yolk and albumin) samples were digested in Teflon tubes with concentrated nitric acid, deionised water and H_2O_2 in a microwave oven (20 min between 0 and 600 W). After cooling, samples were diluted to 50 ml with deionised water in a volumetric flask. Samples for Cd, Cu, Se, Pb, and Zn were analysed by Inductively Coupled Plasma-Mass Spectrometer (ICPMS)(Elan DRC II). Samples for Hg were analysed by Direct Mercury Analyzer (DMA Milestones). A mean water content of $80.4 \pm 2.4\%$ and $80.8 \pm 1.8\%$ for blood and egg, respectively, was calculated in our samples to convert data on wet weight (WW) basis. Concentrations are expressed in $\mu\text{g g}^{-1}$ WW. Parallel to samples, a set of certified control material samples (DOLT-3 liver, National Research Council Canada and Whole

Table 1
Trace element concentrations ($\mu\text{g/g}$ wet weight) in sea turtle eggs and blood from literature and this study; mean \pm S.D. or range.

Species/location	Year	n	Tissue	Copper	Zinc	Selenium	Lead	Cadmium	Mercury	Reference
Green turtle (<i>Chelonia Mydas</i>)										
Cyprus	1994–1996	24	Egg content ^a				BDL ^d 0.403	0.013–0.305	BDL ^d -0.048	1
China	2001	30	Yolk ^b	0.340 \pm 0.036	45 \pm 3.6	3.5 \pm 0.6	0.49 \pm 0.008	BDL ^d	0.002 \pm 0.0001	2
China	2001	30	Albumen ^b	0.063 \pm 0.012	0.3 \pm 0.059	0.270 \pm 0.058	0.005 \pm 0.001	BDL ^d	<0.001	2
Japan	1990	1	Yolk	0.634	47.2		<0.03	<0.03	2.51	3
Japan	1990	1	Albumen	0.157	1.29		<0.03	<0.03	0.05	3
Loggerhead turtle (<i>Caretta caretta</i>)										
USA	2001	34	Blood ^b						0.029 \pm 0.008	4
Cyprus	1994–1996	3	Egg content				BDL ^d 0.983	0.058–0.14	0.04–0.143	1
Turkey		22	Yolk ^c	0.928 \pm 0.102	57.21 \pm 2.23		1.307 \pm 0.228	0.359 \pm 0.135	BDL ^d	5
Japan	1990	5	Egg content	1.05 \pm 0.199	14.7 \pm 1.44			0.013 \pm 0.004	0.0055 \pm 0.0016	6
Japan	1990	6	Yolk	1.57 \pm 0.073	34.4 \pm 3.18		<0.03	0.026 \pm 0.007	12.1 \pm 3.41	3
Japan	1990	6	Albumen	0.129 \pm 0.083	0.59 \pm 0.58		<0.03		0.49 \pm 0.24	3
Kemp's Ridley turtle (<i>Lepidochelys kempii</i>)										
USA	1994	106	Blood	0.215–1.3	3.28–18.9		0–0.034		0.0005–0.0673	7
Leatherback turtle (<i>Dermochelys coriacea</i>)										
French Guiana	2006	78	Blood	1.34 \pm 0.28	11.10 \pm 0.28	9.98 \pm 0.05	0.18 \pm 0.05	0.08 \pm 0.03	0.011 \pm 0.003	This study
French Guiana	2006	76	Egg	0.63 \pm 0.10	14.16 \pm 2.23	1.44 \pm 0.38	0.036 \pm 0.001	0.024 \pm 0.001	0.012 \pm 0.003	This study

1: Godley et al. (1999), 2: Lam et al. (2006), 3: Sakai et al. (2000); 4: Day et al. (2005); 5: Kaska and Furness (2001); 6: Sakai et al. (1995); 7: Kenyon et al. (2001).

^a Data from Godley et al. (1999) originally presented in dry weight basis were converted in weight wet using mean water content of 75%.

^b Mean \pm S.E.

^c Not stated in publication wet or dry weight specific.

^d BDL = Below detection limit.



Fig. 1. Map of Awala Yalimapo nesting beach, the study site in French Guiana.

Egg Powder Standard Reference Material 8415, National Institute of Standards and Technology) went through each set of analyses to ensure the accuracy and precision of the method. Recoveries for control materials ranged from 94% to 101% for Cu, Zn and Se and from 93% to 104% for Cd, Pb and Hg. Instrumental detection limits were: Cu, 0.020 ppb; Zn, 0.042 ppb; Se, 0.166 ppb; Cd, 0.005 ppb; Pb, 0.002 ppb; Hg, 1 ppb, respectively. All samples were above the detection limit except for Cd in the Whole Egg Powder (Table 2).

2.3. Statistical analysis

Factors affecting trace element concentration in blood and eggs in the history of each female during the nesting season (the variation of trace element concentrations of the same female between the different clutches laid) were examined. General linear models with repeated measures (repeated measures GLM) were carried out in which the dependent variable was each trace element concentration and the independent variable was the time in days of each clutch after the first clutch was observed (the time 0 corresponded to the day when we observed the first clutch for each female). Repeated measures were used to compare data from the

same female at different nesting events; we introduced the individual female as a repeated measure into the GLM. Two matrices were distinguished: blood and egg. The number of eggs in each clutch was added as a covariate. Repeated measures enabled comparison of data coming from the same female at different laying events. The normality of the dependent variables was confirmed prior to the analyses. The relationship between trace element concentration in egg and blood was then tested for each corresponding female using independent general linear mixed models (GLMM). Mixed models were used, because data coming from the same female at different time (representing different nesting) were correlated; this covariance structure was handled by introducing the individual female as a random effect into the GLMM. We performed GLMM in which the dependent variable was the trace element concentration in egg and the independent variables were the trace element concentrations in the blood of the corresponding female. Simple regression models were applied to look at potential correlations between concentrations of trace elements in eggs and in blood. The normality of the dependent variables was confirmed prior to the analyses. Computations were performed with STATISTICA 6.0 (StatSoft Inc., 2001) and SAS package (procedure MIXED, v. 9.1.3, SAS Institute Inc., 2004).

Table 2
Quality control results ($\mu\text{g/g}$ dry weight) acquired with certified materials

Certified material	Element	Assigned value	Measured value	<i>n</i>
DOLT-3 liver	Cu	31.2 ± 1	31.5 ± 0.6	10
	Zn	86.6 ± 2.4	87.3 ± 2.0	10
	Se	7.06 ± 0.5	6.61 ± 0.1	10
	Cd	19.4 ± 0.6	18.0 ± 0.2	10
	Pb	0.3 ± 0.1	0.3 ± 0.0	10
	Hg	3.4 ± 0.1	3.2 ± 0.3	5
Whole Egg Powder Standard Reference Material 8415	Cu	2.7 ± 0.4	2.9 ± 0.3	8
	Zn	67.5 ± 7.6	60.6 ± 1.4	8
	Se	1.4 ± 0.2	1.3 ± 0.0	8
	Cd	0.005	<LD (0.002)	8
	Pb	0.1 ± 0.0	0.1 ± 0.0	8

Limit of detection (LD) are indicated in italic.

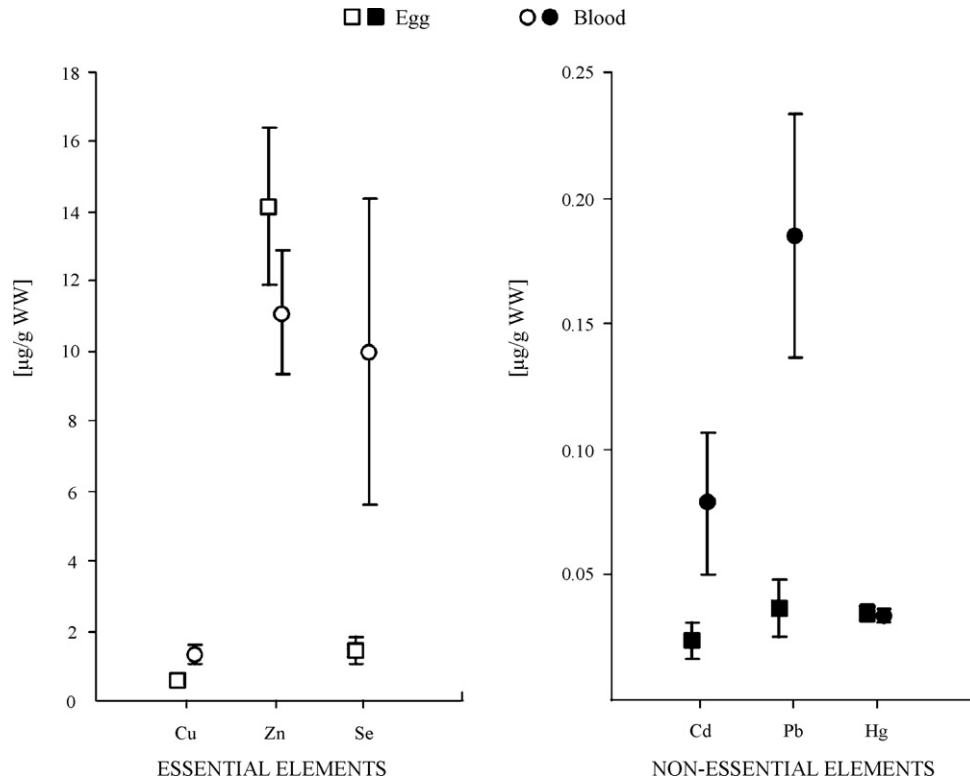


Fig. 2. Trace element concentrations in $\mu\text{g/g}$ wet weight (mean \pm S.D.) according to matrices (circles for blood and squares for egg) and to essential (in white) and non-essential (in black) elements in the leatherback turtle.

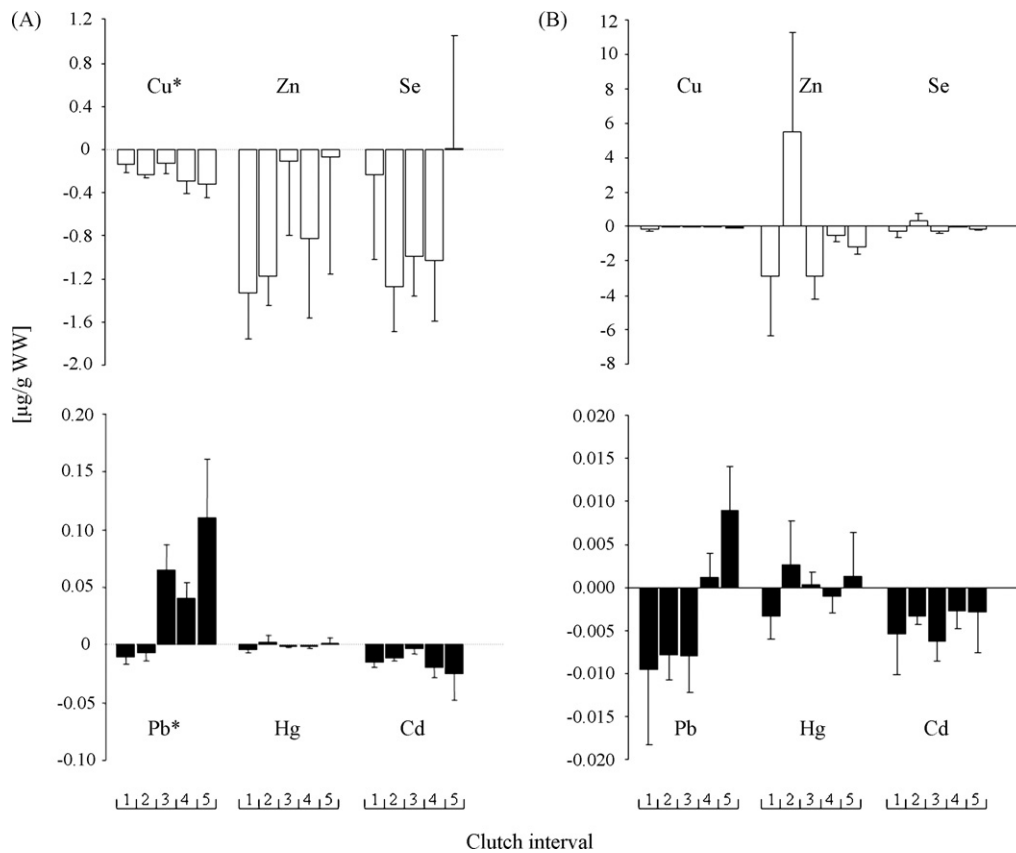


Fig. 3. Trends in trace element concentrations for (A) blood and (B) eggs during the nesting season. Difference in trace element concentrations (in $\mu\text{g/g}$ wet weight, mean \pm S.E.) are calculated for each clutch interval (1 = mean difference between the first and the second clutch, $n=8$; 2 = mean difference between the first clutch and the third clutch, $n=3$; 3 = mean difference between the first clutch and the fourth clutch, $n=15$; 4 = mean difference between the first clutch and the fifth clutch, $n=8$; 5 = mean difference between the first clutch and the sixth clutch, $n=2$). * Indicates significant variation ($P < 0.05$) along the nesting season.

3. Results

During the field study, we sampled 46 different leatherback females. Among these females, we sampled 5 females for 4 clutches, 4 females for 3 clutches, 19 females for 2 clutches and 19 females for 1 clutch. The different clutches (10 days between two clutches) could be consecutive or not and the maximum interval between the first and the last clutch laid by a female is 52 days corresponding to the sixth clutch. The CCL of sampled females ranged from 143 to 170 cm (mean \pm S.D. = 160 ± 6 cm). Trace element concentrations in the blood were not correlated to CCL ($P > 0.5$). The number of yolkeggs laid by the females varied between 37 and 114 eggs (mean \pm S.D. = 87 ± 16 eggs). The concentrations of Hg, Pb, Cd, Se, Cu and Zn, detected in the leatherback eggs and blood samples are summarized in Table 1 and Fig. 2.

3.1. Inter-clutch variation in trace element concentrations

The number of eggs in each clutch was included as a covariate, but had no effect on trace element concentrations neither in blood nor in eggs (GLMM, $P > 0.05$). The time in days had no effect on any trace element concentration in egg ($P > 0.05$). In blood, the time in days had no effect on Hg, Cd, Se and Zn concentrations indicating that concentrations remain constant along the nesting season ($P > 0.05$). Cu concentrations in blood decreased significantly with time ($F_{74} = 6.06$, $P = 0.014$) and Pb concentrations increased significantly with time ($F_{71} = 27.42$, $P < 0.0001$). Fig. 3 illustrates these variations but time in days used in statistical analyses has been replaced by clutch interval in order to improve visual representation.

3.2. Maternal transfer

No significant relationship between concentration in blood and egg was observed for Hg, Pb, Cu, and Zn (GLMM, $P > 0.05$). For Se and Cd, egg concentrations were positively correlated with their corresponding concentrations in blood (Se: $F_{1,32} = 63.07$, $P < 0.001$; Cd: $F_{1,32} = 8.5$, $P = 0.0064$). Simple linear regressions of trace element concentration in blood against concentration in eggs confirmed the statistically significant relationship for Se and Cd (Fig. 4).

3.3. Element excretion via eggs

The total amount of trace element excreted by females via eggs was calculated from burdens in egg content, weight of the egg and the total number of yolkeggs in the clutch. As leatherback females nest an average of 7.5 times (Girondot and Fretey, 1996) in a nesting season, we could also estimate the total amount excreted in a nesting season by multiplying these values by 7.5. The amount of elements eliminated were in the order of $Zn > Se > Cu > Pb > Cd > Hg$. During a nesting season, females transfer a higher burden of essential elements (mean \pm S.D. in mg; Zn: 691.27 ± 163.20 ; Se: 69.42 ± 22.61 ; Cu: 30.95 ± 7.80) than toxic elements (mean \pm S.D. in mg; Pb: 1.737 ± 0.641 ; Cd: 1.169 ± 0.395 ; Hg: 0.591 ± 0.198).

4. Discussion

Female sea turtles do not attend their nest nor protect eggs or hatchlings. After migration to the nesting site, parental investment is limited to the nutrients and energy invested in the yolk that will support embryonic development and the post natal period of the hatchlings (Hewavisenthi and Parmenter, 2002). Maternal transfer of energy, nutrients and trace elements during egg production

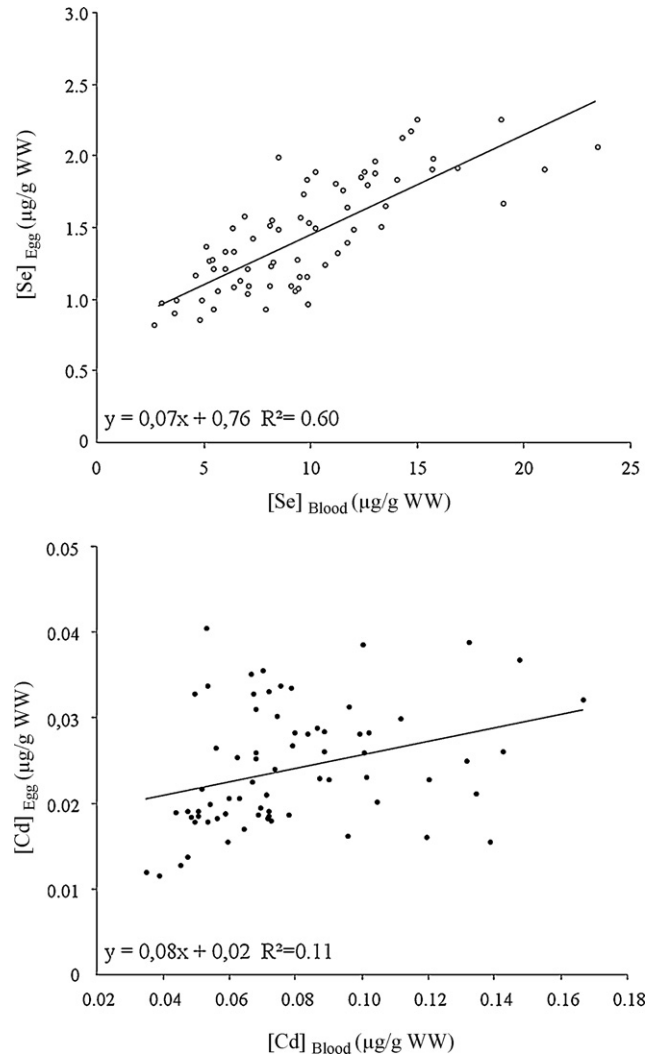


Fig. 4. Linear regressions between trace element concentrations in egg and blood of the corresponding leatherback females (Se = Selenium; Cd = Cadmium; WW = Wet weight). Equations and associated regressions statistics are shown.

could be especially high in leatherback turtles because they have the highest reproductive output among reptiles (Miller, 1997).

This study provides the first data on Cu, Zn, Se, Cd, Pb, and Hg levels in blood and eggs of free-ranging leatherback turtles. Generally, non-essential elements are well known to accumulate in organs, such as kidney, liver and pancreas of sea turtles (Caurant et al., 1999; Anan et al., 2001) but other tissues such as blood and eggs could be used to investigate recent exposure. Indeed, blood is known to be more indicative of recent exposure than others tissue (Blanvillain et al., 2007). Concerning eggs, their production begins with vitellogenesis, the process by which follicles are provisioned with lipids that is complete prior to the arrival of the females at the nesting beach (Rostal et al., 1996, 2001). Then, at the beginning of the breeding season, around the nesting site, mating with males occur and mature follicles will be ovulated and fertilised (Miller, 1997; Miller et al., 2003). The fertilised eggs continue into the oviduct where albumin is secreted around the yolk and finally the eggshell is secreted and is fully finished one week after ovulation (Miller, 1985) ending the egg production process in sea turtles. In reptiles, the ovulation and the supply of albumin, and eggshell for all the eggs to be laid during the season happen progressively all along the nesting season (Palmer et al., 1993). Thus, samples

of blood and eggs allow a further insight in the toxicokinetics of non-essential and essential elements of leatherback turtles during their breeding season, a period of high energy expenditure and investment for females (Wallace et al., 2005). Trace element concentrations measured in leatherbacks are generally similar or lower than concentrations reported in other marine turtle species such as the green turtle, the loggerhead turtle and the Kemp's ridley (Table 1). Food and water are the main sources of exposure to metals for marine vertebrates including sea turtles (Caurant et al., 1999). As leatherback turtles feed mainly upon gelatinous zooplankton (Davenport, 1998), biomagnification might be limited because of this low trophic level diet (Godley et al., 1999; Maffucci et al., 2005). Ecological factors such as variation in feeding locations may lead to variations in contaminant levels among female leatherbacks. The foraging grounds of leatherback turtles are located in a different part of the North Atlantic Ocean (James et al., 2005; Doyle et al., 2007); the high mobility of leatherback across the Atlantic could enhance exposure to environmental toxicants. Movement patterns as well as the availability of food resources could help to explain variations in trace element concentrations among nesting females. However, the high pelagic nature of the leatherbacks seems to limit exposure to pollutants as shown by the low concentrations found in tissues in this study.

As females acquire trace elements through the food chain, they can store or eliminate them (Burger and Gochfeld, 1991). A possible way to excrete trace elements in sea turtles is through reproduction by deposition in their eggs (Sakai et al., 1995). Cu, Zn and Se are essential for normal growth, metabolism for living cells and structure and function of many proteins vital for cell function (Eisler, 1998; Pappas et al., 2006). Thus, a maternal transfer to eggs is necessary for successful development of the embryos (Keen et al., 1997). In our study, concentrations of essential elements are higher in eggs and blood compared to non-essential elements, reflecting the lower exposure of leatherback to these toxic metals. The few studies concerning direct effects of Pb toxicity on behaviour, growth or hatching success in turtles reported higher concentrations than those in our study (Burger et al., 1998; Ozdilek and Ozdilek, 2007). For Cd, a recent study on freshwater turtles (*Trachemys scripta* and *Chrysemis picta*) showed that low Cd levels in yolk could impact on gonadal development (Kitana and Callard, 2008) and were in the same order as in egg content in our study (0.007 and 0.024 ng/g, respectively). However, these low Cd concentrations are not likely to threaten leatherbacks at the embryo stage but could impact the animals later in life by disrupting reproductive processes and lowering fertility (Kitana and Callard, 2008). Concerning Hg, blood concentrations for leatherback turtles from French Guiana are smaller than any other values previously reported for sea turtles (Table 1). The highest Hg blood concentrations were reported for *Chelydra serpentina*, a freshwater turtle inhabiting a polluted river in the USA, with values up to 3500 µg/kg (Bergeron et al., 2007). It has been suggested that maternal transfer to eggs of toxic elements could be advantageous: females could regularly get rid of a part of their metal burden during nesting events (Burger and Gibbons, 1998). However, the question arises about the impact of these compounds on embryos and their putative physiological systems to prevent toxicity (Roe et al., 2004).

In leatherback turtles from French Guiana, each clutch mass represents about 5–10 kg and all clutches from the whole nesting season account for 20% of the females body mass (Miller, 1997). However, eggs contain a high percentage of water (Hewavisenthi and Parmenter, 2002; Wallace et al., 2006). This huge reproductive output could represent a sink in which gravid leatherbacks could get rid of toxic elements along the nesting season.

Metals are linked with protein transport, called metalloproteins, which have been shown to bind metals and transport them into

oocytes or into eggs in oviparous species. In fishes and amphibians, binding of elements such as Cd or Zn to vitellogenin is an important mechanism for ovarian uptake of the metals (Ghosh and Thomas, 1995; Falchuk and Montorzi, 2001). In reptiles, the copper transporter protein (CRT) has been suggested to function in Cu acquisition and transport into growing oocytes and eggs (Riggio et al., 2002) and a study by Unrine et al. (2006) showed that Se was also incorporated in the lipovitellin, another egg protein (Unrine et al., 2006). Because Se is also an essential nutrient, this element may have been transferred to eggs as a constituent of important selenoproteins (Hopkins et al., 2006). Several studies have also investigated the possible role that metallothioneins (MTs), a family of small stable metalloproteins, play in metal homeostasis, transfer during oogenesis and detoxification (Hamer, 1986; Palminter, 1998). MTs are known to show affinity for Zn, Cu, Cd and Hg. However their role in maternal transfer to offspring remain unclear in mammals and reptiles (Palminter, 1998; Riggio et al., 2003).

All essential and non-essential elements were detectable in blood and in eggs of the leatherback, reflecting a maternal transfer. However, expected correlations between females and their eggs were only observed for Se and Cd (Fig. 4). These results suggest that toxicokinetics of Se and Cd differed from that of Zn, Cu, Pb and Hg when focusing on maternal transfer. Se is a key nutrient in the activation of the thyroid gland. This endocrine gland secretes thyroid hormones (T4, T3), important for the development and growth of vertebrates. Se is a cofactor of several selenoenzymes (deiodinases) that can then convert thyroid hormones in more active or inactive molecules (Sutija and Joss, 2006). The role of thyroid gland has been poorly described in reptiles. However, Se, selenoproteins and thyroid hormones appear to play an important role in the development of reptiles (Shepherdley et al., 2002a,b) as in other classes of vertebrates. In leatherback turtles, we suspect similarly a pivotal role of the thyroid gland at the beginning of embryonic development. Se might activate the synthesis and release of thyroid hormones from embryo's thyroid. However, thyroid hormones might also be transferred from the mother to egg yolk as already documented in birds (McNabb and Wilson, 1997; Wilson and McNabb, 1997). Positive correlation for Cd between blood and eggs could be linked to similar carrier proteins such as albumin and vitellogenin or other selenoproteins. Indeed Se is also known to interact with Cd to reduce toxicity (Sasakura and Suzuki, 1998). The reason for the different toxicokinetics for Se on the one hand and for Cu and Zn on the other hand is likely to be linked to two processes (1) homeostatic regulation of Zn and Cu and (2) importance of Se in developing embryos. Zn and Cu are closely regulated through homeostasis and transfer to offspring appeared independent of the levels encountered in mothers.

In the literature, studies on the maternal transfer of Se for reptiles are well documented (Nagle et al., 2001; Hopkins et al., 2004; Roe et al., 2004; Unrine et al., 2006), and present a strong relation between concentration in eggs and concentrations accumulated in female tissue, that is consistent with the results of the present study. Reptilian studies on the remaining elements examined in this study are generally lacking; the only available data come from monitoring studies that only provide contamination in freshly laid eggs, reflecting a potential contamination coming from a maternal transfer (Table 1). However, Nagle et al. (2001) found that slider turtles (*T. scripta*) inhabiting contaminated basins accumulated multiple contaminants, including Cd, without transferring it to eggs, while our results clearly show a maternal transfer of cadmium to eggs. Therefore, maternal transfer is likely to depend on the species, the level of contamination and the nature of the element considered.

In this study, maternal transfer has been investigated to assess egg contamination at the moment of the nesting event. But later, during incubation, contaminants could also be transferred from

the nest environment into the eggs. Indeed, during incubation, the number of open pores of the permeable eggshell of turtles increases due to water or gas exchange between eggs and nest environment, facilitating the transfer of contaminants from nest material into eggs (Hewavisenthi and Parmenter, 2001; Canas and Anderson, 2002). Permeability of eggshells to soil contaminants should also be considered as a way of contamination that could affect hatching success of the nest for reptile species with permeable eggshells deposited in contaminated substrate (Marco et al., 2004).

Hg but also Pb, Zn and Cu displayed different kinetics in blood and eggs of leatherbacks turtles compared to Se and Cd through the nesting season (Fig. 3). Cu decreased throughout the nesting season. This trend for Cu is difficult to explain but could be the result of an important maternal transfer to albumin combined with (1) a low dietary intake (little or no food intake during the nesting season to supply enough of this essential elements for females and eggs), (2) insufficient Cu reserves in liver and kidney where storage occurs (Andreani et al., 2008). Indeed, compared to Se and Zn, females' reserves for Cu could be low considering Cu concentrations in blood (Fig. 2).

In contrast, an increasing trend occurred in Pb blood concentrations. This variation could be explained, first, by external contamination. As blood is more indicative of recent exposure than others tissue (Blanvillain et al., 2007), it is potentially a good matrix to investigate recent contamination. Rivers and coastal environment of French Guiana are exposed to environmental pollution via anthropogenic (industries, gold mining activities) or natural sources (naturally enriched soil and sediment, run-off and atmospheric deposition) (Richard et al., 2000; Mol et al., 2001; Marchand et al., 2006). The neritic waters near Yalimapo beach could be one source of contamination for leatherback turtles by ingestion of either contaminated preys or great quantities of polluted water during each nesting season (Nendza et al., 1997). But no data are available on the contamination of potential prey for leatherbacks in this area, and the question of leatherback foraging activity during nesting season in French Guiana remains unclear (Fossette et al., 2007; Caut et al., 2008). However, females seem to ingest at least a significant volume of water (1) to decrease their body temperature in warm waters of nesting tropical beaches (Southwood et al., 2005) and (2) to ensure egg production (albumin is mainly composed of water; Wallace et al., 2006). But environmental measurements of Pb on sediment (12 mg/kg dry weight) and water (<1 µg/l) in the study area seem to reveal moderate pollution (Creocean, 2006; Marchand et al., 2006), which would not explain the Pb increase in blood.

A second hypothesis to explain the increase in blood Pb concentrations would rely on calcium (Ca) mobilization during egg formation. Ca is required for the ossification of the embryonic turtle skeleton and is maternally obtained and stored in shell and yolk (Bilinski et al., 2001). Therefore, during egg formation, females have to provide an effective amount of Ca, particularly for eggshell secretion, that occurs on the days following the ovulation (Miller, 1985). In vertebrates, Ca requirements could be met by an increase in absorption of Ca from diet, but if females do not feed at all or only little during this period (as is opposed for females of our studies (Fossette et al., 2007; Caut et al., 2008), Ca would come from resorption of female bones (Silbergeld, 1991). However, the kinetics of Pb follow those of Ca in bones and Ca and Pb mobilization from bones are concomitant, as Pb uptake and storage is related to calciotropic factors (Silbergeld, 1991; Pattee and Pain, 2003). The result of a massive Ca mobilization from female bone to provide Ca for more than 500 eggs nested during the nesting season (Girondot and Fretey, 1996) would therefore lead to a significant increase in blood Pb concentrations along the nesting season.

Concerning the other toxic metals, the lack of variation in concentrations for Cd and Hg in blood through time could be the results

of limited pollution in the waters near nesting beaches or a period of time spent in these polluted water is too small so that females could not bioaccumulate the metals.

Finally, in egg, no fluctuation had been observed for trace elements concentrations between the different clutches laid suggesting a constant maternal transfer to egg along the nesting season.

5. Conclusion

The present study provides the first data on baseline trace element concentrations in wild leatherback turtles. Whole blood has proven useful for measuring trace element levels in turtles. Levels of toxic metals such as Hg, Cd and Pb were low but always detectable in blood and eggs suggesting a maternal transfer. Pb increase in the blood of females throughout the nesting season is likely to suggest Pb mobilization from bones associated with Ca requirement for egg formation and eggshell secretion. In contrast, Cu levels decreased in blood of females raising the question of Cu limitation at the end of the nesting period. Metal levels in eggs were not correlated to levels in blood with the exception of Se and Cd. Further investigations are obviously needed to better understand the exact role of trace elements in sea turtle development as well as their potential relationship with adverse effects on hatching success due to maternal and environmental contamination.

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