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Etude des facteurs écologiques et écotoxicologiques impliqués dans la réussite d'incubation chez la tortue luth, *Dermochelys coriacea*, de Guyane Française

Soutenue le 10 septembre 2008 devant les membres du jury :

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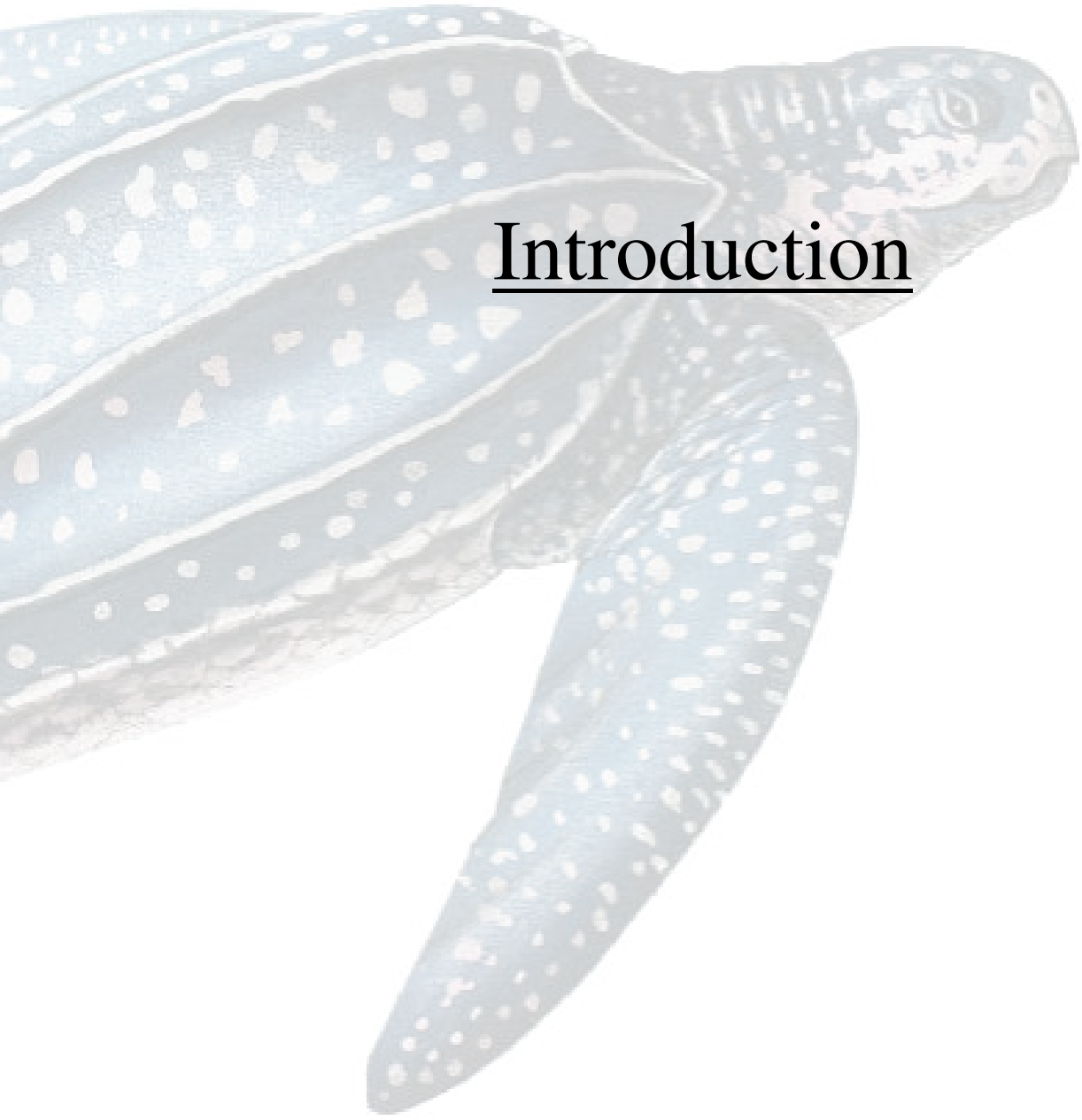
Introduction



Introduction

Les reptiles forment l'une des classes de vertébrés les plus menacées actuellement. Les populations de reptiles connaissent effectivement un déclin global dont les principales menaces identifiées sont la perte et la dégradation de l'habitat, l'introduction d'espèces envahissantes, la pollution environnementale, les maladies et les changements globaux (Gibbons et al., 2000). Devant la prise de conscience générale de ce déclin, l'importance des études portant sur l'identification précise des menaces et la quantification de leur impact s'est accrue. Chez les tortues marines, les études se sont d'abord intéressées aux facteurs écologiques naturels impliqués dans le succès de la reproduction (Allen et al., 2001; Girondot et al., 2002; Baskale and Kaska, 2005). Les études portant sur les effets anthropiques comme la pollution environnementale ont émergé plus récemment ; en effet, les reptiles forment l'un des taxons de vertébrés les moins étudiés en terme d'écotoxicologie (Gardner, 2006).

Les populations de tortues luths (*Dermochelys coriacea*), considérées comme en danger critique d'extinction (IUCN 2006), ont connu un déclin rapide principalement du aux impacts anthropiques (prises accidentelles par les bateaux de pêche et récolte excessive des œufs sur les plages de ponte (Kaplan, 2005; Martinez et al., 2007; Peckham et al., 2007)). Les populations du Pacifique semblent plus touchées que celles d'Atlantique dont les effectifs présentent une tendance plus stable (Spotila et al., 1996; Spotila et al., 2000). Cependant, détecter un déclin dans les populations longévives peut être retardé jusqu'à ce que, par exemple, le manque des classes reproductives devienne évident. La tendance d'une population est donc un élément fondamental pour définir le statut de conservation d'une espèce mais difficile à établir (Mace and Lande, 1991).

Le site de ponte de Yalimapo en Guyane Française, l'un des sites majeurs de la population atlantique, présente la plus faible réussite d'incubation pour cette espèce (Torres 2002, Maros et al., 2003), entrainant des interrogations sur les facteurs impliqués et les conséquences du faible niveau de recrutement au niveau de la population sur le long terme. L'identification de ces facteurs et la quantification de leurs effets sur le succès de la reproduction permettraient d'augmenter les connaissances sur le fonctionnement de la plage de Yalimapo en tant que site de ponte majeur des tortues luths d'Atlantique, et comprendre les risques auxquels les tortues sont exposées. C'est dans ce contexte que s'est inscrite ma thèse.

L'introduction est composée de deux parties principales : une partie très générale pour introduire le contexte de l'étude (espèce, site d'étude et contexte environnemental) suivie d'une partie expliquant les différentes questions abordées et la démarche scientifique suivie au cours de mes trois années de thèse.

1. Contexte général

1.1. Tortue luth

La tortue luth (*Dermochelys coriacea* Vandelli 1761) est l'unique représentante actuelle de la famille des Dermochélidés. Elle se distingue sans ambiguïté des autres tortues marines grâce à son absence d'écaille. En effet, elle possède à la place une pseudo carapace (composée d'ostéodermes juxtaposés) recouverte d'une peau lisse brillante, de couleur bleu nuit avec de nombreux points blanchâtres : cette apparence de « cuir » épais et souple, lui a valu son nom latin *Dermochelys coriacea* et son nom anglais leatherback turtle (Figure 1).

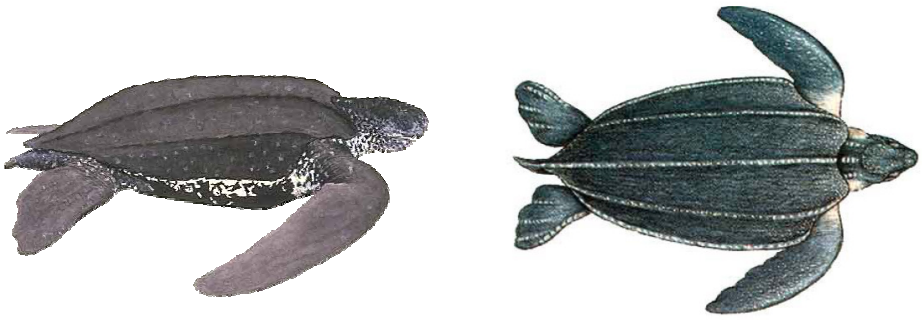


Figure 1 : Tortue luth, *Dermochelys coriacea*

Elle est la plus grosse des tortues marines avec une taille atteignant jusqu'à 2 mètres et un poids moyen de 400 kg. Elle diffère des autres tortues marines aussi bien par sa morphologie que par son métabolisme (Davenport, 1998). La tortue luth se nourrit principalement de méduses, salpes et autres organismes gélatineux (Bjorndal, 1997) qu'elle absorbe en quantité importante (Davenport, 1998). Son mode de vie pélagique lui permet d'effectuer de grands déplacements à travers l'océan (Ferraroli et al., 2004). En effet, c'est le reptile qui a la plus grande aire de répartition puisqu'on la retrouve dans tous les océans jusqu'au cercle polaire (Figure 2).

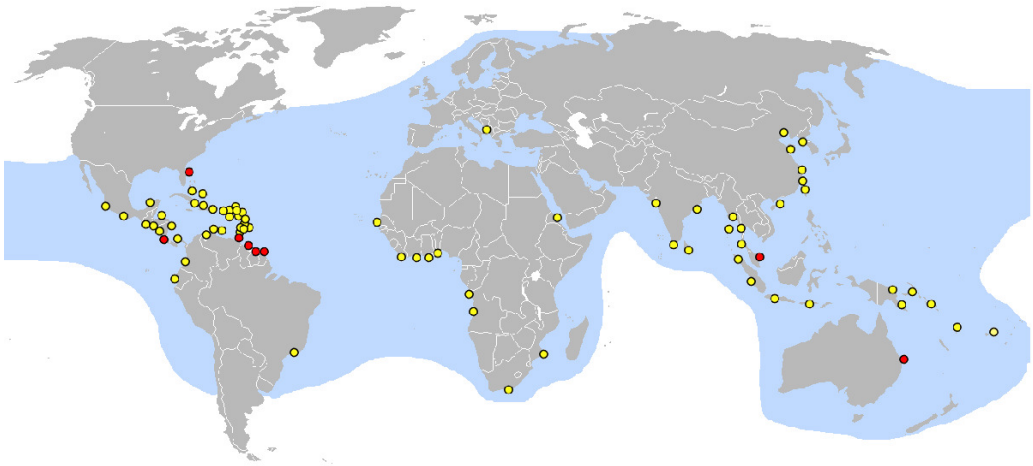


Figure 2 : Aire de répartition de la tortue luth (zone bleue). Les plages de pontes sont représentées par des points rouges pour les sites principaux ou jaunes pour des sites moins fréquentés. http://fr.wikipedia.org/wiki/Tortue_luth

Ainsi, la tortue luth passe la majorité de son cycle de vie en mer mais les femelles restent dépendantes de la terre ferme pour leur reproduction (Figure 3). Les femelles viennent ainsi pondre leur œufs dans des nids qu'elles creusent dans le sable de la plage et retournent à l'eau dès le processus de ponte achevé. Au cours de la saison de ponte, les femelles pondent tous les 10 jours (Girondot and Fretey, 1996) et jusqu'à 13 fois (moyenne 5 à 7 selon les années)(Rivalan et al., 2006). Entre chaque ponte, elles se dispersent autour de la plage jusqu'à 140 km des côtes et restent au niveau du plateau continental. La tortue luth présente les pontes les plus importantes en poids (5-10kg), les œufs les plus gros (80g) et la fréquence de ponte la plus élevée de toutes les tortues marines, ce qui en fait le reptile ayant l'investissement reproductif le plus important (Miller, 1997). En plus de ces traits caractéristiques, les femelles pondent les nids les plus profonds (70-100cm), dans lesquels elles déposent des œufs fécondés ainsi que des sortes d'œufs plus petits composés uniquement d'albumen et d'une coquille (souvent appelés à tort œufs infertiles et que l'on retrouve sous le nom de Shelled Albumen Globs -SAGs- en anglais)(Wallace et al., 2004). La fonction des SAGs reste à ce jour peu comprise.

Les tortues luths sont observables dans tous les océans du monde et il existe des populations distinctes entre les océans pacifique et atlantique ; ces populations diffèrent dans leurs zones d'alimentation ainsi que vraisemblablement dans certaines caractéristiques morphologiques (taille) et reproductives (durée de l'intervalle de remigration, quantité d'œufs produits (Wallace et al., 2006)).

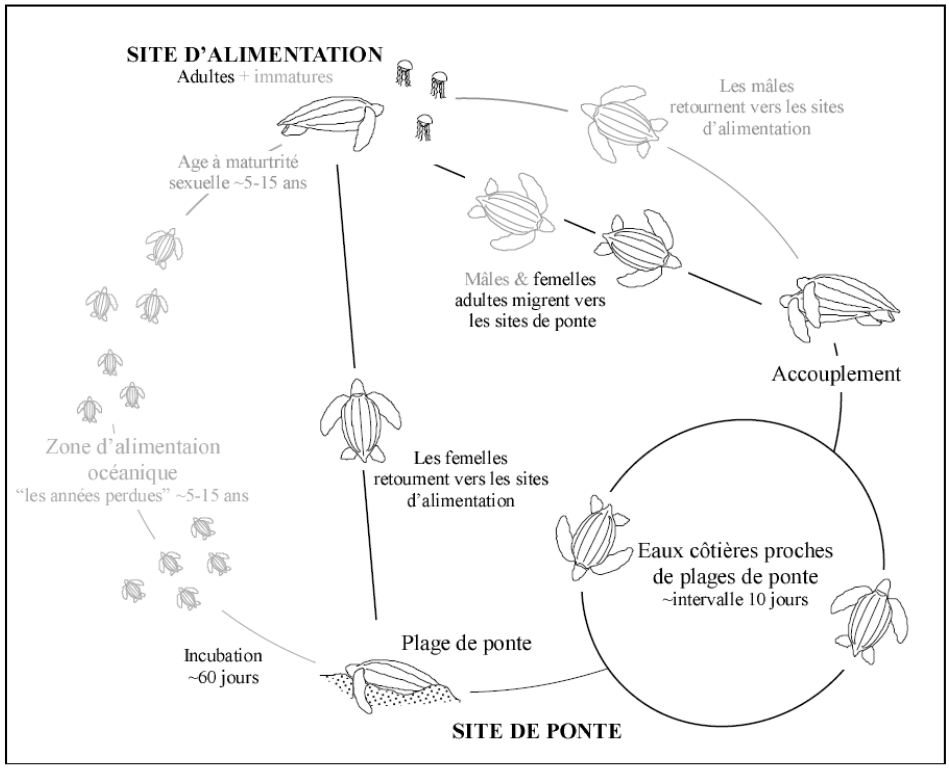


Figure 3 : Cycle de vie simplifié de la tortue luth ; les parties inconnues ou mal connues sont indiquées en gris

Dans la population atlantique, les femelles qui viennent pondre en Guyane française, repartent s'alimenter en Atlantique Nord entre deux saisons de ponte. Contrairement à leurs parentes du Pacifique, les tortues luths de l'Atlantique ne suivent pas des couloirs étroits de migration mais se dispersent largement en traversant les courants comme ils se présentent à elles. Lorsque les femelles quittent la région des Guyanes, elles empruntent deux principales voies à peu près en ligne droite soit vers le nord dans la zone du Gulf Stream, soit vers l'Afrique au niveau de la ceinture équatoriale (Ferraroli et al., 2004; Hays et al., 2004). Les tortues ralentissent leur course et suivent les fronts océaniques associées avec des systèmes de courants marins locaux, qui sont généralement riches en faune et flore marines. Les zones d'alimentation utilisées entre deux saisons de ponte se répartissent dans tous l'océan Atlantique Nord. Ces zones se situent du coté ouest de l'Atlantique Nord (Nouvelle Ecosse et côtes Est des Etats-Unis (James and Herman, 2001; James et al., 2005a; James et al., 2005b)) ou du côté est de l'Atlantique Nord (Irlande, Golf de Gascogne, Les Açores, Cap vert (Eckert, 2006; Houghton et al., 2006; Doyle et al., 2007)).

Les tortues luths sont des « capital breeders » c'est-à-dire qu'elles vont stocker de l'énergie au niveau des zones d'alimentation qu'elles utiliseront plus tard pour la reproduction. Cependant, les femelles ne se reproduisent pas tous les ans ; en dessous d'un certain seuil de ressources, elle n'entrera pas en vitellogénèse (production du jaune des

œufs)(Hamann et al., 2003). L'intervalle entre deux saisons de ponte, correspond à la périodicité des saisons de reproduction et est donc dépendant des ressources disponibles sur les sites d'alimentation. L'intervalle de retour sur les sites de ponte varie généralement entre 1 et 4 années, avec une majorité de femelles revenant après 2 ou 3 ans (Rivalan et al., 2005). Durant une saison de reproduction, l'allocation des ressources (limitantes) est un compromis entre (trade-off) entre la survie des parents et le succès de la reproduction. Ce compromis physiologique est connu sous le terme de coût de la reproduction (Stearns, 1989). L'existence d'un coût de reproduction pourrait conduire à une baisse dans le taux de survie adulte, et aurait favorisé l'apparition de la reproduction intermittente. En effet, un investissement reproductif important une année pourrait être compensé par une saison non reproductive l'année d'après, entraînant une alternance entre une saison reproductive et non-reproductive (Clutton-Brock, 1998).

Les tortues luths figurent sur la liste rouge de l'UICN (dans la catégorie « en danger critique » depuis 2000) et fait l'objet de conventions et de programmes internationaux de protection et de conservation. En effet, les effectifs ont connu un dramatique déclin depuis quelques années (Spotila et al., 1996; Spotila et al., 2000). Le déclin des populations peut être expliqué principalement par la récolte excessive des œufs sur les sites de ponte et le massacre des femelles pour leur viande (Kaplan, 2005; Martinez et al., 2007), la destruction des sites de ponte (Clarke et al., 2000), la pollution environnementale (Aguirre et al., 1994; Lutcavage et al., 1997; Godley et al., 1999; Tomas et al., 2002; Keller et al., 2004a) mais surtout par les prises accidentelles des adultes dans les filets (Hall et al., 2000; Pradhan and Leung, 2006). Ainsi l'un des objectifs majeurs en termes de conservation réside actuellement dans la meilleure compréhension de leurs trajets de migration, permettant ainsi de diminuer ces prises accidentelles en déterminant des zones de protection hautement utilisées par les individus. Chez les espèces longévives, la dynamique des populations est influencée principalement par la survie des stades adultes ; d'importantes fluctuations du taux de survie des stades adultes vont avoir un impact majeur sur la stabilité des populations. Cependant, il est également important de s'intéresser aux œufs en tant que stade à protéger car la stabilité de la population ne peut être assurée si, pendant de nombreuses années, le recrutement de juvéniles est insuffisant ou leur mortalité trop élevée avant d'atteindre l'âge de la maturité. La protection des œufs seule ne permettra pas de rétablir une population qui diminue mais limitera la vitesse de ce déclin. Les œufs ne peuvent donc pas être ignorés d'autant plus que les menaces sur les sites de ponte ne cessent d'augmenter (croissance démographique, destruction de site de ponte, pollution, érosion, gêne par la lumière artificielle)(Heppell, 1997).

1.2. Réussite d'incubation

Une fois l'ensemble de la ponte déposée dans le nid creusé dans le sable par la femelle, les œufs sont laissés à eux-mêmes et dépendent d'une part des conditions maternelles (réserves allouées par la femelle dans l'œuf) et d'autre part des conditions environnementales pendant toute la durée passée dans le nid. Au fur et à mesure de l'incubation qui dure environ 60 jours dans le sable de la plage, l'embryon va se développer en absorbant le jaune de l'œuf comme source d'énergie et en utilisant également de l'eau provenant de l'albumen et du sable environnant. La température, l'humidité et les échanges de gaz vont créer un microclimat plus ou moins favorable pour le développement embryonnaire des œufs. L'interaction entre les caractéristiques physiques du substrat de la plage, le climat local, et les œufs de la ponte vont générer un microclimat spécifique et influencer l'incubation. Ce microclimat est cependant dynamique et varie avec l'activité biologique dans la ponte et sur la plage (Ackerman, 1997). Les facteurs environnementaux qui peuvent affecter le bon déroulement du développement embryonnaire comprennent des facteurs écologiques (biotiques et abiotiques) directement liés à la plage ou à des facteurs écotoxicologiques intrinsèques (présence de contaminants dans l'œuf) ou extrinsèques (présence de contamination dans le substrat du nid).

Facteurs écologiques abiotiques

Le développement des œufs, amassés au fond du nid, va largement dépendre de certains facteurs écologiques abiotiques qui ont été très étudiés chez les reptiles. Il s'agit de la température, de l'humidité du nid et des échanges gazeux.

Température. La période d'incubation va dépendre de la température du nid avec une durée d'incubation plus longue pour des températures basses. Les tortues marines présentent également une détermination du sexe dépendante de la température (Temperature-dependant Sex Determination TSD) ; de faibles températures produiront des mâles tandis que des températures élevées produiront des femelles (Ciofi and Swingland, 1997; Pieau et al., 1999). Cependant les œufs ne peuvent se développer à de trop faibles ou trop fortes températures. La tolérance thermale pour le développement des embryons incubés à température constante se situe entre 25-27°C et 33-35°C (Ackerman, 1997; Lopez-Castro et al., 2004). Dans des conditions de température variable, les œufs peuvent supporter de plus grands écarts de température pendant un temps limité.

Granulométrie et Teneur en eau du nid. La composition du sable, et plus particulièrement la taille des grains, est un facteur important pour un environnement optimal pour le développement des œufs. La taille des grains va en effet jouer sur les échanges d'O_2 et

de CO_2 entre le nid et l'extérieur et peut également affecter le niveau d'humidité du nid. L'humidité du sable est favorisée par la taille des grains qui permet au substrat de retenir l'eau. Selon Mortimer (1990), un sable sec et grossier est instable et passible d'effondrement. Pour ces raisons, la réussite d'incubation est diminuée lorsque la taille des grains augmente. La teneur en eau du nid semble devoir se trouver dans un intervalle de valeurs optimales nécessaire pour assurer le développement embryonnaire. En effet, la réussite d'incubation diminue lorsque la teneur en eau du nid augmente (Lopez-Castro et al., 2004; Foley et al., 2006) ou que la teneur en eau est trop faible (Mortimer, 1990). Par exemple, chez la tortue d'eau douce *Chelydra serpentina*, les embryons se développant dans un environnement sec présentent un cœur plus gros reflétant une demande importante de la pompe circulatoire pour maintenir une circulation suffisante du sang plus visqueux et en quantité moins importante que chez les embryons provenant de milieu plus humide. Les conséquences de cette circulation sanguine peuvent aller jusqu'à l'hypoxie des tissus et une taille des embryons réduite (Packard and Packard, 2002).

Erosion, inondation des nids. Si l'érosion marine est à l'origine de la destruction de nids, il n'est pas rare qu'ils soient simplement inondés. Un grand nombre de nids sont ainsi perdus car une proportion appréciable des nids de luths est pondue tout près de la mer (le nid est complètement détruit, emporté par les vagues). Les inondations, provoquées par la régulière immersion à marée haute ou par la table de l'eau de la plage, provoqueraient l'infiltration d'eau de mer jusque dans le nid asphyxiant ainsi les œufs (Milton et al., 1994; Foley et al., 2006). L'asphyxie dépendrait de la durée et de la fréquence de l'immersion et du stade de développement des œufs (Losos et al., 2003).

Salinité. La réussite d'incubation semble aussi être affectée par la salinité ; alors que la salinité augmente la réussite d'incubation diminuerait (Mortimer, 1990; Foley et al., 2006).

pH. Le pH du sol peut affecter le développement embryonnaire et la réussite d'incubation des espèces déposant leur ponte dans le sol et d'autant plus celle qui ont une coquille d'œuf très perméable, ce qui favorise les échanges d'eau et de gaz entre l'œuf et l'environnement du nid (Marco et al., 2005). Le pH peut modifier ces échanges d'eau et de gaz ou modifier directement le pH de l'œuf et affecter le développement embryonnaire. Marco et al. (2005) ont démontré chez le lézard *Lacerta monticola* que le pH n'avait pas d'effet direct sur le temps ou la réussite d'incubation ; en revanche un pH trop acide au cours de l'incubation avait un effet néfaste sur les échanges d'eau, sur la taille des nouveaux nés et sur leur vitesse de fuite, ce qui affecte directement leur survie. Les pluies acides ou

l'acidification des sols pourraient donc contribuer au déclin des espèces de reptiles ayant des coquilles présentant une perméabilité importante.

Facteurs écologiques biotiques

Les facteurs écologiques biotiques ont également été bien étudiés et concernent essentiellement l'impact des prédateurs présents sur les plages de ponte et les effets de densité de ponte provoqués directement par les femelles.

Prédation. Les principales menaces naturelles pour les œufs de tortues marines sont causées par les prédateurs naturels. Sur les plages de ponte de Guyane, ces prédateurs peuvent être des crabes (crabes fantômes, *Ocypode quadrata*), des insectes (courtilières (Maros et al., 2003), des oiseaux (urubus, bihoreau, grand duc de virginie), ou encore des chiens errants (Chevalier et al., 1998).

Densité-dépendance. A la fin de la saison de ponte, sur une plage utilisée de manière aussi forte que Yalimapo, les nids sont distribués sur la plage de façon très dense. Lorsque une femelle vient alors pondre un nouveau nid, il arrive souvent qu'elle en détruise un en creusant le sien (Girondot et al., 2002; Caut et al., 2006).

Facteurs écotoxicologiques

Aux facteurs écologiques viennent s'ajouter les facteurs parentaux qui peuvent également affecter le développement et la survie de l'embryon. Parmi ces facteurs parentaux, les réserves nutritionnelles stockées dans le jaune de l'œuf peuvent être associées à des contaminants qui perturbent le développement de l'embryon allant parfois jusqu'à provoquer la mort de celui-ci.

L'impact écotoxicologique des polluants environnementaux dépend de leur forme chimique (nommé "espèce chimique"), de leur concentration, du contexte environnemental et de la possibilité de passage dans la chaîne du vivant (Snedaker et al., 1999). On distingue entre autres les éléments traces métalliques essentiels ou non essentiels (aucun rôle positif pour l'activité biologique) toxiques à certaines doses ou encore les composés organochlorés (pesticides, polychlorobiphényles) qui sont largement présents dans l'environnement du à leur utilisation excessive dans les activités agricoles et industrielles.

Eléments traces métalliques. La notion d'élément-traces métalliques, ou ETM (anciennement métaux lourds), est actuellement une notion relativement floue, sans définition scientifique, technique ou juridique qui soit unanimement reconnue. Dans mon étude, les ETM concerneront les éléments métalliques compris entre le cuivre et le plomb dans le

tableau périodique des éléments. Beaucoup d'ETM ont une utilité dans le processus biologique, par exemple le zinc et le cuivre qui sont des oligo-éléments indispensables. Tous les ETM sont présents naturellement à l'état de traces dans le sol. L'activité humaine peut cependant avoir renforcé cette présence naturelle.

Polluants organiques persistants (POPs) : pesticides organochlorés et polychlorobiphényles (PCBs). Les pesticides et PCBs (usages agricoles et industriels) ont largement été utilisés au XXe siècle mais voient leur utilisation de plus en plus réglementée et même interdite dès les années 70 pour certains. Le premier pas pour la lutte contre la production massive et l'utilisation incontrôlée se fait avec la Convention de Stockholm sur les POPs (2001). Cependant ces molécules ont été largement dispersées dans l'environnement du fait de leurs propriétés spécifiques: faible biodégradabilité (persistance), omniprésence due au transport via les courants aquatiques ou atmosphériques, effets toxiques à faible dose, aptitude à s'accumuler dans les organismes (bioaccumulation) et le long des chaines trophiques (bioamplification).

La contamination des œufs par ces composés peut entrainer des perturbations de la reproduction et une diminution de la valeur adaptative (fitness) des femelles en augmentant par exemple la mortalité embryonnaire ((alligator commun, Rauschenberger et al., 2004b). Ce phénomène s'explique par un transfert des polluants de la femelle vers les œufs, qui se retrouvent contaminés, pouvant entrainer une diminution de la réussite d'incubation. Le transfert maternel des polluants vers les œufs apparaît comme l'exposition principale des embryons aux polluants POPs chez les reptiles (Rauschenberger et al., 2004a). La contamination des œufs peut également provenir de l'environnement du nid (Wu et al., 2000b). En effet, dans les régions tropicales, les températures élevées pourraient faciliter la diffusion des composés volatiles présents dans le substrat du nid à travers les pores de la coquille souple et donc l'absorption de ces composés toxiques par les œufs (Wu et al., 2000a). L'exposition des œufs à des sols contaminés contribue également à l'augmentation de la charge en polluants et au risque de diminution de la réussite d'incubation par la perturbation du développement embryonnaire (Canas and Anderson, 2002). L'augmentation des niveaux de contaminants à la fois sur les sites d'alimentation et sur les sites de ponte pourrait affecter les systèmes physiologiques des tortues marines en général et contribuer ainsi au déclin de ces populations. Cependant, dans ce manuscrit, seuls les facteurs écotoxicologiques intrinsèques, c'est-à-dire la contamination des œufs issus du transfert de la mère, seront envisagés. L'étude des effets de la pollution du nid sur les œufs pendant leur incubation étaient initialement prévu dans l'étude mais n'a finalement pas pu être réalisée pour des raisons logistiques.

1.3. Guyane Française, plage d'Awala Yalimapo

Pontes de tortues marines

La Guyane française est un site majeur de pontes de 3 tortues marines, la tortue verte (*Chelonia mydas*), la tortue luth (*Dermochelys coriacea*) et la tortue olivâtre (*Lepidochelys olivacea*). Les pontes sont réparties sur tout le littoral avec des concentrations importantes à l'Ouest pour la verte et la luth et à l'Est pour l'olivâtre. Long de près de 350 km, le littoral guyanais se situe entre les latitudes 4°N et 6°N. Il est constitué d'une succession de mangroves et de plages de sable évoluant rapidement en raison d'une dynamique côtière particulière. La Guyane étant située à 400km au NO de l'estuaire du fleuve Amazone (Figure 4), une grande partie des sédiments charriés par ce fleuve est ramenée le long du littoral guyanais sous forme de bancs de vase par le courant des Guyanes (Pujos and Froidefond, 1995; Froidefond et al., 2004). Ces bancs de vase généralement longs de 30 à 60 km, longent la côte. Cette dynamique engendre l'apparition et la disparition régulière des plages, suite à l'arrivée puis au départ des bancs de vase, à l'érosion et aux dépôts de sable provenant des fleuves locaux. Ces évolutions du littoral entraînent une rapide modification des plages de nidification.

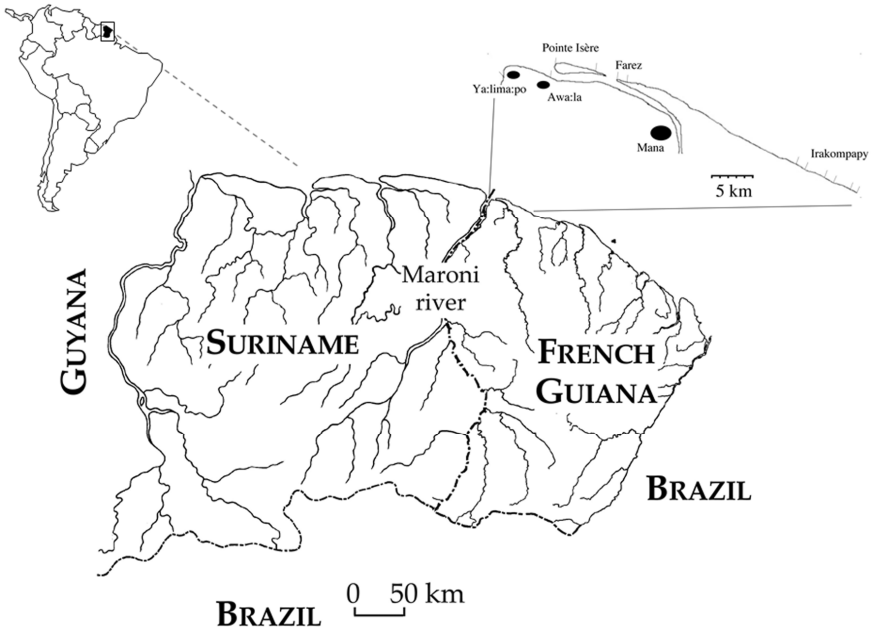


Figure 4 : Situation de la Guyane Française en Amérique du sud et de la plage de Yalimapo dans l'ouest guyanais

Dans l'ouest guyanais, ou se trouvent la majorité des pontes de tortues luths, les plages changent très rapidement et certaines disparaissent ou apparaissent d'une saison de ponte à l'autre. Située entre les fleuves Maroni et Mana, la plage de Yalimapo est depuis plus de 20

ans la plus stable de la région (Figure 4). Cette stabilité est certainement due au dégagement de la vase par le fort débit des deux fleuves. Depuis la fin des années 70, cette plage concentre la grande majorité des pontes de tortues luth du plateau des Guyanes (Girondot and Fretey, 1996).

En raison du grand nombre de plages utilisées par les tortues et de leur accessibilité plus ou moins facile, la démarche adoptée en Guyane Française concernant les tortues luths a été de focaliser les efforts sur cette plage. Elle se situe dans la réserve naturelle de l'Amana (Figure 5) et fait l'objet de comptages de pontes relativement régulièrement depuis 1979 et d'un protocole d'identification des femelles depuis 1985. La plage d'Awala-Yalimapo est située entre les deux villages amérindiens du même nom et entre l'embouchure du Maroni et de la Mana (57°57 W / 5°45 N). Elle mesure environ 4 km de long pour une largeur qui varie de quelques mètres à 20 mètres en fonction de la marée.

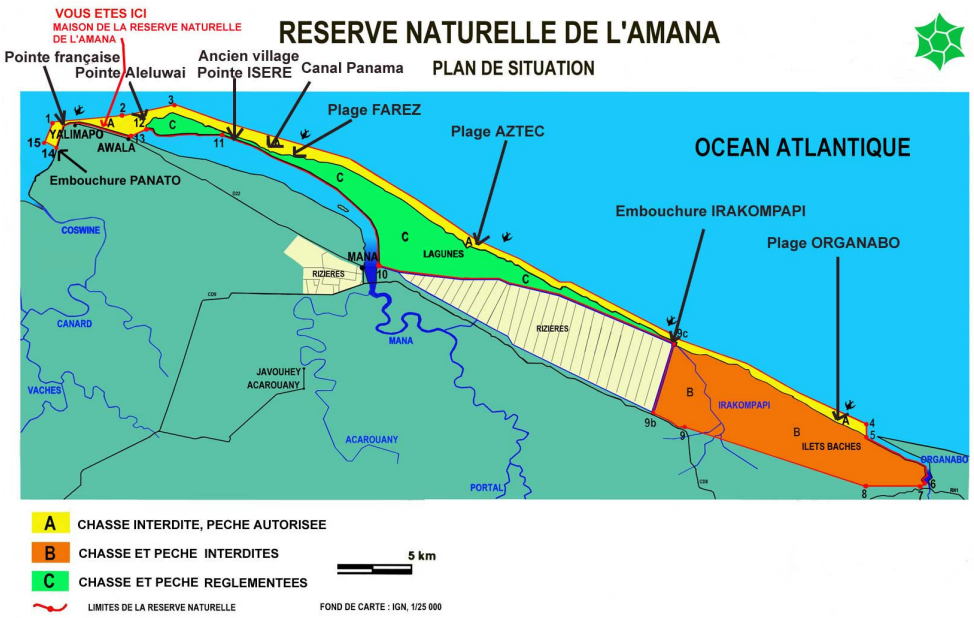


Figure 5 : Schéma de la situation de la plage de ponte de Yalimapo et de la proximité des rizières par rapport à la réserve naturelle de l'Amana

Pollution environnementale

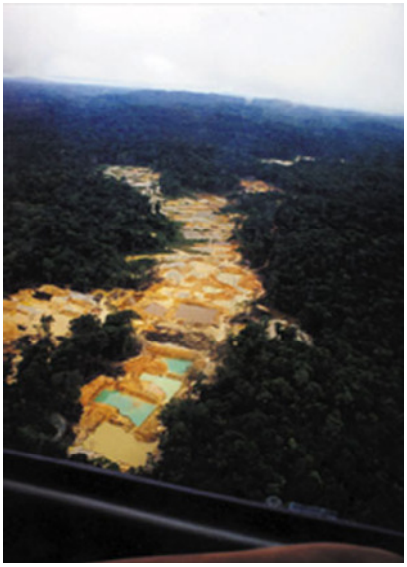
La plage de ponte de Yalimapo est l'un des sites majeurs de ponte pour les tortues luths (Spotila et al., 1996). Bien que faisant partie d'une réserve naturelle, elle est malheureusement soumise à différentes sources de pollution.

En effet, la Guyane Française présente des sous sols naturellement riches en métaux et, de plus, a été et est encore largement soumise à la menace environnementale que représente l'orpaillage, associé à l'usage du mercure (Figure 6). La contamination des

populations autochtones dans les régions des sites d'orpaillage est désormais connue et reliée au mercure, notamment au travers de la consommation des poissons carnivores contaminés par ce métal qui s'accumule progressivement le long de la chaine alimentaire (Malm et al., 1997). Le long du Maroni, la contamination des poissons en mercure et autres métaux est bien présente mais va de façon décroissante vers l'aval et l'estuaire, atteignant des concentrations relativement basses au niveau de l'embouchure et du large (Mol et al., 2001). Cependant, les seuils de toxicité pour les reptiles sont encore très peu connus et une exposition, même faible, peut représenter une menace pour les tortues évoluant dans la région du Maroni.

Les pesticides présentent également une source potentielle de contamination et de pollution environnementale en Guyane. Synthétisés à partir des années 40, les pesticides organochlorés ont été massivement utilisés dans l'agriculture pour le traitement des cultures et la prévention de la détérioration des récoltes après moisson et lors du stockage.

A



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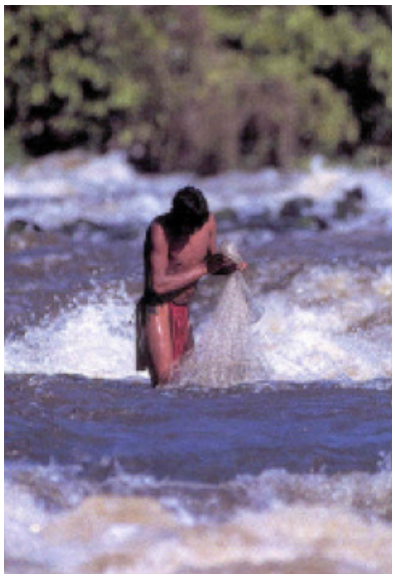

Figure 6 : (A) Vue aérienne d'un site d'orpaillage dans la région de Saint-Elie. © CNRS-LTHE, Photo : Jean-Paul Gaudet. (B) Les populations amérindiennes du Haut-Maroni sont fortement consommatrices de poissons contaminés par le mercure.

En Guyane, le développement de la riziculture à Mana dans les années 1980 constitue le seul projet abouti de mise en culture de grandes surfaces en Guyane. Ce projet entre malheureusement en concurrence avec la protection des espaces mis en réserve, avec l'exemple de la juxtaposition de la réserve naturelle de l'Amana et des rizières de part et d'autre du fleuve Mana (Figures 5 et 7). Des traitements intensifs contre les ravageurs du riz, principalement contre les punaises, se succèdent selon un calendrier préétabli, avec des interventions supplémentaires en cas de pullulations locales (Klein, 2003). Cette lutte

chimique a évidemment des conséquences sur l'environnement qui sont encore peu évaluées. Les tortues marines mais aussi les poissons et crustacés, pour lesquels le marais à mangrove est une nurserie, sont menacés, et par conséquent l'activité de pêche dans la zone. L'accumulation progressive des POPs dans l'environnement a entraîné leur interdiction dans la plupart des pays. Ils restent cependant largement utilisés dans certaines régions tropicales pour le contrôle des insectes, vecteurs de maladies (Bidlan and Manonmani, 2002). Des doses considérables ont été et sont encore largement déversées dans l'environnement. Fortement persistants, ils sont aujourd'hui de redoutables polluants des sols et des milieux aquatiques.

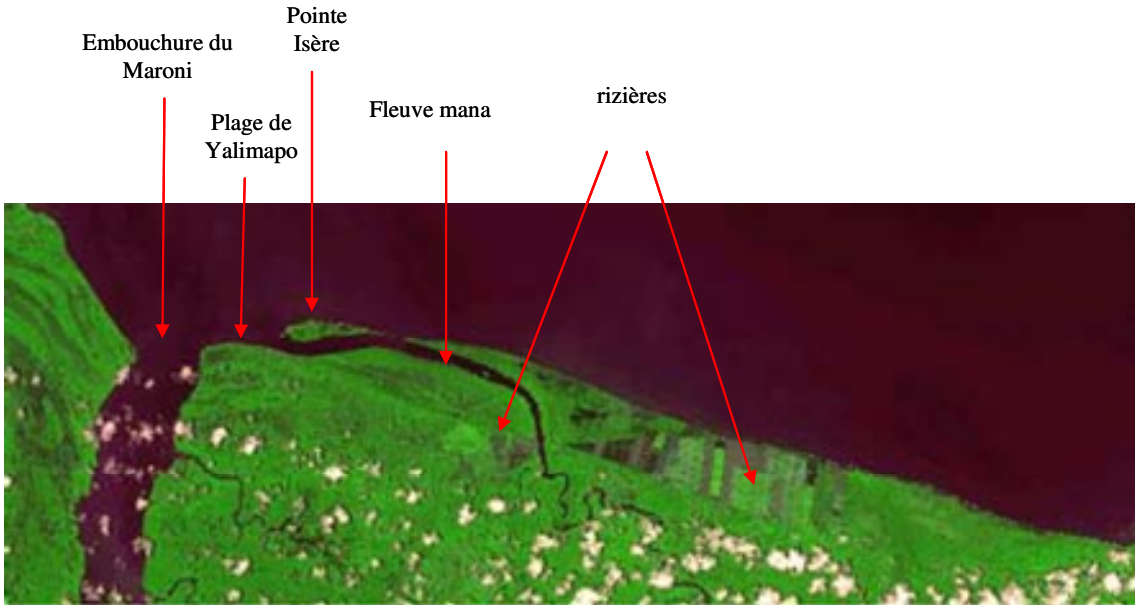

Figure 7 : Image du satellite Spot 4 du 23 septembre 2001 (Composition colorée Copyright CNES / distribution Spot Image) permettant de voir la proximité des rizières par rapport au site de ponte.

Du fait de leurs caractéristiques (persistance, faible solubilité dans l'eau), les POPs en général (pesticides organochlorés et PCBs) sont adsorbés sur les particules organiques, que l'on trouve en grande quantité dans les fleuves, et finalement s'accumulent dans les estuaires et les sédiments créant ainsi une source de contamination secondaire. Puis, les POPs s'accumulent dans les organismes et contaminent ainsi toute la chaîne alimentaire. Des études ont montré qu'ils affectaient la fonction endocrine en se comportant comme des antagonistes d'hormones sur les récepteurs ou en inhibant les enzymes responsables de la synthèse des hormones ou de leur dégradation (Vonier et al., 1996). Ces substances perturbatrices du système endocrinien sont des substances qui peuvent causer des effets néfastes en interférant d'une manière ou d'une autre avec les hormones du corps ou les messagers chimiques. Comme les hormones jouent un rôle crucial dans la différenciation des cellules à des stades précoces, une exposition à ces substances dans l'œuf peut altérer le

processus normal de développement (Lyons, 1999). Un dérèglement de la fonction endocrine n'est cependant pas une surprise étant donné qu'un certain nombre de pesticides ont été synthétisés pour fonctionner comme des régulateurs d'hormones et de croissance pour contrôler les populations d'espèces nuisibles (Crisp et al., 1998).

Comme les tortues marines utilisent les eaux côtières et de l'estuaire pendant la saison de ponte, il est intéressant de se demander si l'activité humaine de cette région peut menacer le cycle biologique de ces espèces (Kaska et al., 2004).

Site de ponte majeur et faible taux de réussite observé

Chez les tortues luth, la réussite d'incubation est plus faible que chez les autres tortues marines sans que les causes de ce faible taux d'incubation soient réellement bien compris (Girondot et al., 1990; Bilinski et al., 2001; Bell et al., 2004). De plus, les quelques études qui se sont intéressées au taux de réussite des nids de luths sur la plage de Yalimapo ont révélé des taux de réussite très faibles (Tableau 1) mais ces estimations se basent sur un échantillonnage dont les méthodes sont peu standardisées. Il est généralement plus élevé sur d'autres plages de pontes (Table 2). Les œufs de luths semblent donc être plus sensibles à certains facteurs et la qualité de la plage de Yalimapo semble aussi influencer très nettement l'incubation des œufs et donc le nombre de nouveau-nés. Il est donc important de s'intéresser aux facteurs spécifiques de cette plage et des femelles qui y pondent, afin de mieux comprendre la faible réussite d'incubation sur ce site, et d'envisager l'impact que pourrait avoir un faible recrutement sur de nombreuses années au niveau d'une plage de ponte fortement fréquentée.

Les exigences générales pour une plage de nidification sont multiples. La pente de la plage doit être suffisamment élevée pour ne pas être submergée entièrement à marée haute et que la table de l'eau n'atteigne pas les nids. Le substrat de la plage doit permettre à l'O_2 et au CO_2 de diffuser dans et hors du nid, et enfin le substrat doit être humide et assez fin pour que le nid ne s'effondre pas au cours de l'émergence des petites tortues. La réussite d'incubation des œufs dans le nid va dépendre de ces conditions environnementales dans le sable de la plage mais les œufs seront également soumis à d'autres facteurs environnementaux tels que la prédation.

Cette plage fait partie d'une réserve naturelle mais est soumise à différentes sources de pollution (pesticides avec la proximité des rizières qui bordent la réserve (Figure 5)), métaux lourds présents naturellement dans les sédiments dont le rejet dans l'environnement

est accentué par les activités d'orpaillage (Marchand et al., 2006) ou encore les hydrocarbures pétroliers avec les dégazages et les rejets naturels au large des côtes.

Tableau 1 : Réussite d'incubation sur les plages de Guyane Française et du Surinam

Pays	Plage	Année	Réussite d'incubation	Référence
Guyane Française	Yalimapo	2001	33,3-39,0%	(Torres 2002)
Guyane Française	Yalimapo	2001-2002	35,9%	(Maros 2003)
Surinam	Samsamto	2000	37,5%	(Hilterman 2001)
Surinam	Matapica	2000	40,6%	(Hilterman 2001)
Surinam	Galibi	2001	10,0%	(Hilterman & Goverse 2002)
Surinam	Matapica	2001	38,9-52,7%	(Hilterman & Goverse 2002)
Surinam	Matapica	2002	56,0%	(Hilterman & Goverse 2003)
Surinam	Babunsanti	2002	25,8%	(Hilterman & Goverse 2003)

Tableau 2 : Réussite d'incubation sur d'autres sites de ponte

Pays	Plages	Réussite d'incubation	Référence
Porto Rico	Culebra	75%	(Tucker 1986)
Costa Rica	Tortuguero	40%	(Leslie et al. 1996)
Iles Vierges, USA	Sainte Croix	67%	(Boulon et al, 1996)

Le réseau hydrographique très dense en Guyane favorise la collecte des polluants et leur transport jusqu'aux embouchures. Les estuaires représentent finalement de véritables pièges à polluants chimiques (Richard et al., 2000; Marchand et al., 2006). La plage de Yalimapo est d'autant plus exposée qu'elle se situe à l'embouchure du fleuve Maroni et de la rivière Mana, lieu de convergence et d'accumulation de ces polluants. La pollution environnementale a en effet été suggérée comme étant une des principales causes du déclin de reptiles (Lutcavage et al., 1997; Gibbons et al., 2000) et elle est d'autant plus inquiétante qu'elle est présente aussi bien au niveau du site de ponte que sur les sites d'alimentation des tortues marines. Devant le faible taux de réussite d'incubation de ce site et la pollution environnementale potentielle de la région, la contribution de ces polluants pourrait constituer l'un des facteurs affectant le développement embryonnaire.

2. Objectifs et démarche scientifique de ma thèse

L'objectif général de ma thèse a donc été de rechercher les facteurs qui peuvent affecter le développement embryonnaire et la réussite d'incubation des œufs pondus sur la plage de Yalimapo. J'ai voulu envisager ces facteurs en deux temps (Figure 8).

Dans un premier temps, je me suis focalisée sur les facteurs écologiques qui pouvaient jouer sur le développement des œufs une fois qu'ils sont en incubation dans le nid. Les données de la littérature sur l'étude de la réussite d'incubation chez les tortues portaient en effet sur ce genre de facteurs (prédation, inondation, température, salinité…) mais très peu de données étaient disponibles pour la plage de ponte de Yalimapo.

Cette partie a été réalisée grâce à une collaboration qui m'a permis d'analyser un jeu de données récolté à partir d'une campagne de terrain en Guyane en 2002. L'analyse de ces données a permis de mieux comprendre une partie des facteurs qui pouvaient affecter le développement de l'embryon.

Puis, je me suis assez vite interrogée sur ce qui pouvait également jouer sur la réussite d'incubation avant que les œufs ne soient déposés dans le substrat du nid. Ainsi, dans un deuxième temps je me suis donc focalisée sur la « qualité » des œufs déposés par les femelles c'est-à-dire, voir si la composition de ces œufs, en termes de contaminants environnementaux, ne pouvait pas également jouer sur le développement de l'embryon pendant l'incubation. Cette partie s'intéresse donc aux facteurs écotoxicologiques intrinsèques, c'est-à-dire à l'influence de la mère sur la qualité de ses œufs.

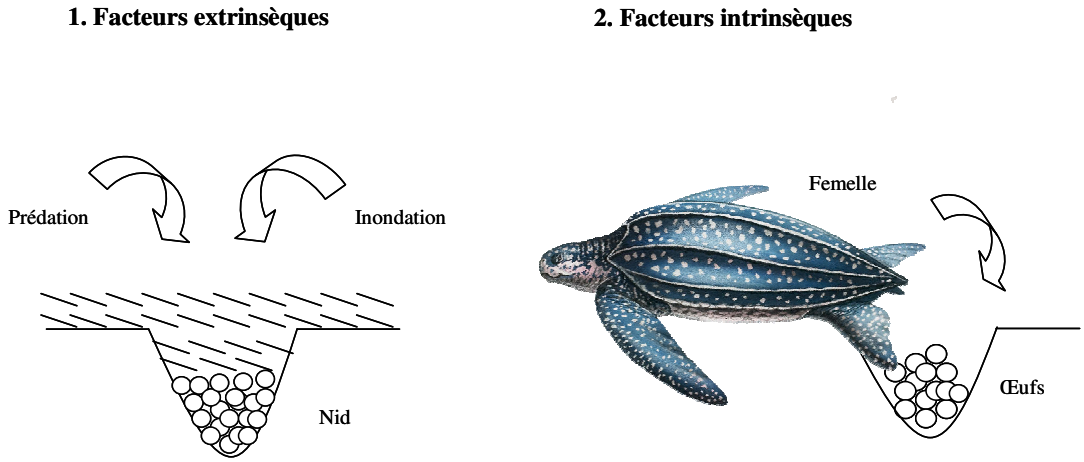

Figure 8: schéma de l'objectif général de l'étude

2.1. Facteurs écologiques

Après le processus de ponte terminé, les femelles ne dispensent aucun soin parental à leurs œufs puisque une fois les œufs déposés dans le nid et celui recouvert et balayé, elles retournent à l'eau directement. L'œuf fonctionne comme un système fermé en ce qui concerne les nutriments et l'énergie que la femelle a investis dans ses œufs. Il dispose au moment de la ponte de tous les éléments nécessaires pour assurer son développement jusqu'à l'éclosion deux mois plus tard. Cependant la coquille souple et perméable des œufs va permettre des échanges d'eau et de gaz entre l'œuf et son environnement. La réussite d'incubation des œufs dans le nid va donc dépendre en partie des conditions favorables dans le sable de la plage (température, humidité, échanges de gaz). Les facteurs écologiques qui peuvent affecter le bon déroulement du développement embryonnaire sont d'origines biotiques (prédation, microorganismes, densité-dépendance) ou abiotiques (température, granulométrie, teneur en eau du nid, érosion ou inondation des nids, pH, pollution) et directement liés à la plage.

Dans un premier temps, j'ai considéré les facteurs extrinsèques, c'est-à-dire les facteurs écologiques qui affectent le développement de l'œuf une fois que celui se trouve dans le nid. Je me suis concentrée sur certains facteurs biotiques (prédation) et abiotiques (immersion, inondation) affectant le développement des œufs et du nid dans son ensemble; ces différents paramètres sont susceptibles de ralentir ou de stopper le développement.

Pour cette partie de ma thèse, j'ai travaillé en collaboration avec Stéphane Caut qui disposait d'un jeu de données important récolté sur la plage d'Awala Yalimapo en 2002. Nous nous sommes donc intéressés à la position du nid sur la plage (par rapport à la végétation en haut de plage et la ligne de marée haute en bas de plage) et voir en quoi elle pouvait jouer sur la réussite d'incubation des œufs. En effet, en haut de plage, les conditions sont plus sèches, avec une végétation plus importante, donc une densité de racines dans le sable également plus élevée et enfin, une densité accrue d'insectes prédateurs (les courtilières). En bas de plage, il y a en revanche plus de risques d'érosion pour les nids proches de la ligne de marée haute.

Les premiers résultats de cette partie sont présentés dans le Chapitre 1: Caut S, **Guirlet E**, Jouquet P & Girondot M. 2006. Influence of nest location and yolkless eggs on the hatching success of leatherback turtle clutches in French Guiana. *Canadian Journal of Zoology*, 84: 908-915.

L'un des résultats majeurs de cette partie, correspond au rôle que peut jouer le site de sélection, donc la position du nid, sur la réussite d'incubation, avec notamment l'influence des distances aux lignes de marée haute et ligne de végétation. Nous avons donc voulu pousser

l'étude plus loin en testant l'influence de l'inondation des nids (durée, fréquence, période du développement embryonnaire à laquelle l'inondation survient) sur la réussite d'incubation. En effet, le développement des embryons pendant l'incubation dépend en partie de l'humidité, de la température et des échanges gazeux dans le nid et ces facteurs vont être modifiés lors des épisodes de submersion et il se pourrait qu'à force d'inondations trop fréquentes ou trop longues, le développement de l'embryon soit stoppé. Cette idée nous est venue lors d'observations pendant la fouille des nids en fin d'incubation. Nous avons observé de nombreux embryons morts à différents stades du développement embryonnaire. Nous nous sommes donc demandés s'il y avait un rapport entre les inondations, et ce blocage embryonnaire observé.

Les résultats de cette partie sont présentés dans le chapitre 2: **Guirlet E**, Caut S & Girondot M. Effect of inundation on the embryonic development of Leatherback turtles (*Dermochelys coriacea*). En préparation.

2.2. Facteurs intrinsèques ou effet maternel

Après avoir étudié l'effet de différents facteurs écologiques sur le taux de réussite, je me suis intéressée aux facteurs intrinsèques à l'œuf. Aux différents constituants présents dans l'œuf (lipides et protéines dans le jaune et l'albumen) au moment de la ponte, peuvent s'ajouter d'autres composés (contaminants) qui pourraient être néfastes au développement de l'embryon pendant la période d'incubation. L'œuf est ici considéré comme un produit de fabrication issu des réserves de la mère. Les œufs pondus n'étant pas tous identiques selon les femelles, on peut considérer ces œufs comme ayant une « qualité » variable (Packard et al., 1993) qui peut jouer sur le développement et la réussite d'incubation. Cette « qualité » des œufs peut être définie dans ce contexte comme l'ensemble des nutriments et de l'énergie permettant à l'embryon de se développer, altéré par d'autres constituants neutres ou néfastes qui ont été également transmis à l'œuf par la femelle et qui pourront avoir un effet négatif sur le développement et la croissance.

Cette partie correspond à l'étude des facteurs écotoxicologiques pouvant altérer le développement et la réussite d'incubation des œufs. L'objectif était d'étudier qualitativement et quantitativement les polluants (éléments traces métalliques et composés organochlorés) présents dans le sang et les œufs au moment de la ponte, c'est-à-dire transmis par la mère. Cette approche est très novatrice dans le domaine des tortues marines. Contrairement aux effets des différents facteurs écologiques sur le taux de réussite plus fréquemment étudiés chez les tortues marines, l'approche écotoxicologique sur le développement embryonnaire est

très récente chez les tortues marines et complètement nouvelle pour les tortues luth. Je me suis donc lancée dans cette approche écotoxicologique sur les tortues luths de Guyane Française en essayant d'adopter la démarche la plus logique et progressive possible pour répondre à mes objectifs. Cette démarche a consisté à identifier les contaminants environnementaux présents dans les œufs, puis à évaluer les effets de ces contaminants sur la réussite d'incubation (Figure 9).

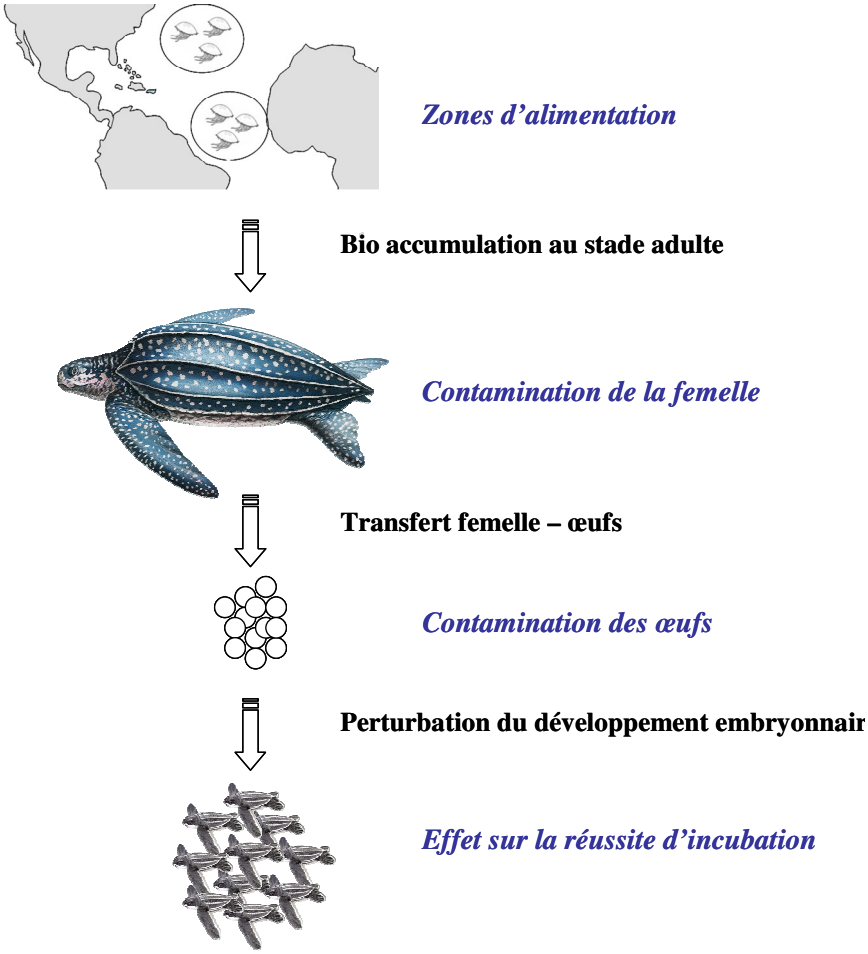

Figure 9 : Représentation des différentes étapes de la partie Ecotoxicologie

Ecologie trophique des femelles

Afin d'étudier la contamination des œufs, il est utile de s'intéresser à la contamination des femelles car c'est le transfert maternel qui va être responsable de la présence ou non de polluants dans les œufs au moment de la ponte. Or, ce transfert maternel des polluants aux œufs va dépendre en premier lieu de la contamination de la femelle qui dépend elle-même de la nourriture ingérée par celle-ci. En effet, chez les tortues marines, la principale voie d'exposition aux polluants est la nourriture (Caurant et al., 1999). La quantité bio accumulée

par la femelle va dépendre de son âge, de la contamination de ses proies qui va elle-même varier en fonction des espèces consommées et de la zone d'alimentation.

Un des points importants dans l'analyse des concentrations en polluants est donc de tenir compte de la composition du régime alimentaire et des zones d'alimentation. Chez les tortues luths, l'alimentation est essentiellement basée sur le macro-zooplancton gélatineux (méduses, salpes et autres organismes gélatineux). Cependant les connaissances sur l'utilisation des différentes zones d'alimentation restent limitées aux études télémétriques qui fournissent des renseignements sur un nombre très limité d'individus. Les suivis satellites permettent de voir dans quelles directions les tortues partent après la saison de ponte mais les données sont disponibles en général pour la première année de migration et non pour la totalité des 2 ou 3 années qui vont séparer deux saisons de ponte (en raison du matériel qui cesse d'émettre en général au bout d'une année maximum).

Néanmoins, il a été démontré que les tortues luths de l'Atlantique avaient plusieurs zones d'alimentation, parfois distantes de plusieurs milliers de kilomètres. Cependant, on ne sait pas si les femelles utilisent toujours les mêmes sites d'alimentation ou si elles varient en fonction des années. Il semble que le nombre d'année qui s'écoule entre deux saisons de ponte, dépend de la vitesse à laquelle les femelles accumulent les réserves nécessaires pour entamer une nouvelle saison de reproduction c'est-à-dire que cela va dépendre de la zone d'alimentation (qualité et quantité de proies disponibles) que les femelles vont utiliser et la vitesse à laquelle elles vont parvenir à ces zones favorables. La quantité de ressources disponibles sur les sites d'alimentation semble être un facteur important dans l'investissement reproducteur des femelles. Si les femelles diffèrent dans les zones d'alimentation utilisées, elles vont donc potentiellement différer dans la charge corporelle de contaminants. Il est donc intéressant de se demander si une différence peut être observée au moment d'une saison de ponte entre des femelles ayant des intervalles de retour sur les sites de ponte différents et si cette différence pourrait être liée à une différence de zone d'alimentation. Pour étudier l'utilisation des différentes zones d'alimentation par les femelles, il a été fait appel à la méthode des isotopes stables.

Cette méthode se base sur les ratios en isotopes (en général, le carbone $^{13}C/^{12}C$ et l'azote $^{15}N/^{14}N$) présents naturellement chez les individus, et qui entrent dans les réseaux au niveau de la production primaire. Cette signature caractéristique dépend de nombreux processus biogéochimiques (Peterson and Fry, 1987). Le $\delta^{15}N$ montre une augmentation marquée par palier avec chaque niveau trophique, il semble donc être un bon indicateur du niveau trophique d'un consommateur (DeNiro and Epstein, 1981; Wada, 1987; Fry, 1988;

Hobson and Welch, 1992; Hobson et al., 1994). L'enrichissement est d'environ +4‰ à chaque niveau trophique (Schoeninger and Deniro, 1984), ce qui peut s'expliquer par une excrétion préférentielle des isotopes légers (Peterson and Fry, 1987). Contrairement à l'azote, l'abondance relative des isotopes stables en carbone semble peu changer avec chaque niveau trophique (Rau et al., 1983; Fry and Sherr, 1984; Hobson and Welch, 1992). Le rapport isotopique du carbone est plutôt utilisé pour étudier les différentes origines des nutriments d'un individu (Hesslein et al., 1993). En effet, le $\delta^{13}C$ est distinctement différent entre les animaux terrestres, marins et d'eau douce. Les signatures des animaux marins sont enrichies en ^{13}C par rapport à ceux d'eau douce (Fry, 1983). Beaucoup d'études ont utilisé les mesures en carbone et en azote stable, pour tracer la relative contribution des ressources marine et/ou terrestre d'un individu (Hobson, 1987; Hobson and Stirling, 1997). Ainsi, la mesure des différentes signatures isotopiques peut être extrêmement utile dans l'évaluation du régime des consommateurs en réseau trophique complexe (Fry, 1988; Harrigan et al., 1989; Hobson and Stirling, 1997).

De plus, l'analyse des isotopes stables dans différents tissus d'un même individu, apporte des informations sur le régime à plus ou moins long terme (Hobson and Clark, 1993). Chaque tissu est caractérisé par son propre renouvellement (*turnover*). L'analyse isotopique du plasma intègre l'assimilation de la nourriture sur une courte durée, alors que l'analyse des hématies (RBC) apporte une information sur une période plus longue (Seminoff et al., 2007). L'utilisation des isotopes stables permet ainsi d'éviter certains biais associés aux méthodes conventionnelles d'analyse du régime alimentaire (l'analyse des estomacs, des fèces, des régurgitas ou des observations directes) qui se basent sur la nourriture ingérée et non l'assimilation, et reflètent le régime à court terme.

Les résultats de cette partie sont présentés dans le chapitre 3: **Guirlet E**, Caut S, Angulo E, Das K & Girondot M. 2008. Isotope analysis reveals two feeding areas for the Atlantic leatherbactk turtles. *PLoS ONE* 3(3):e1845. doi:10.1371/journal.pone.0001845.

Transfert maternel

Chez la tortue luth, la vitellogénèse (phase d'accumulation de protéines et de lipides dans les follicules, futurs jaune de l'œuf) pourrait commencer plus d'un an avant la ponte (Rostal et al., 1996; Miller, 1997). Le vitellus constitue les réserves énergétiques utilisées par les embryons durant le développement embryonnaire. Il est produit par l'organisme maternel et s'accumule dans l'ovocyte au cours de l'ovogenèse. Le vitellus est constitué principalement de protéines, lipides et ARN. La protéine majeure du vitellus est la vitellogénine, une glyco-

lipo-protéine, synthétisée au niveau du foie en réponse à la présence d'œstrogènes et transportée jusqu'à l'ovaire par voie sanguine. Alors que certaines données tendent à monter que la vitellogénèse serait complète avant l'arrivée des femelles sur les plages de ponte (Rostal et al., 1996; Hamann et al., 2002) d'autres données suggèrent au contraire, pour les espèces ayant un investissement reproductif intra-annuel important (nombres de ponte et d'œufs élevés dans une saison de ponte), que la vitellogénèse pourrait se poursuivre au cours de la saison de ponte (existence d'ovocytes de toutes tailles au niveau de l'ovaire, Girondot, obs. Pers). Avant chaque ponte, une phase de croissance rapide de ces follicules va s'engager afin de compléter les œufs proprement dits avec l'albumen et la coquille et constituer une ponte d'environ 80 œufs de 80g (Miller, 1997).

Lorsque la ponte est prête, la femelle vient sur la terre ferme creuser un nid et y déposer ses œufs. Une fois la ponte accomplie, la femelle retourne directement à la mer ne dispensant aucun soin parental à sa progéniture. Cependant, pour assurer le maximum de chance au bon déroulement du développement embryonnaire de ses œufs, la femelle fournit tous les éléments essentiels dont l'embryon aura besoin pour son développement. Chez les tortues marines, l'investissement parental est ainsi limité à la fabrication des œufs (transfert d'énergie et de réserves). Au moment où il est pondu, l'œuf contient en théorie les éléments dont il a besoin pour assurer la croissance de l'embryon jusqu'à l'émergence. L'œuf peut être considéré comme un système clos en ce qui concerne les nutriments, l'énergie et les éléments essentiels, fournis par la femelle et présents dans l'œuf au moment de la ponte (Hewavisenthi and Parmenter, 2002).

Lors du transfert de nutriments et d'énergie, il est possible qu'un certain nombre d'autres composés passe dans les œufs. Ce transfert concomitant concerne certains types de molécules qui vont profiter du passage de lipides ou de protéines pour passer dans les œufs (Sasakura and Suzuki, 1998; Chen et al., 2006; Stavros et al., 2008). Il est donc envisageable de retrouver dans les œufs des molécules lipophiles tels que des pesticides ou des PCBs, ou des ETM toxiques, qui ont le potentiel de perturber le développement de l'œuf pendant l'incubation (Eisler, 1985, 1988; Wolfe et al., 1998; Noonan et al., 2002).

Les POPs sont généralement recherchés dans les tissus adipeux, car ils sont hautement lipophiles, mais aussi dans le sang. En effet, les polluants atteignent les tissus adipeux via le flux sanguin, s'y accumulent jusqu'à ce que la graisse soit mobilisée, processus qui entraine la recirculation des polluants dans le sang. La concentration en contaminants au niveau du sang peut donc fluctuer au cours du temps en fonction de la mobilisation des lipides et l'état nutritionnel (dépense énergétique, jeûne, …)(Lydersen et al., 2002; Keller et al., 2004b). Ces

cycles récurrents d'accumulation et de mobilisation peuvent augmenter les risques de toxicité pour les organismes (Corsolini et al., 2000).

J'ai donc voulu définir qualitativement et quantitativement les molécules qui pouvaient être transmises aux œufs par la mère au moment de la production des œufs et qui de plus, pourraient par la suite affecter le développement de l'embryon lors de l'incubation (ce que j'appelle facteurs écotoxicologiques).

Le transfert maternel a été d'abord étudié pour les ETM puis pour les pesticides et les PCBs dans le sang de la femelle et dans ces œufs au cours de la saison de ponte. J'ai ainsi pu définir le niveau de contamination de cette espèce en Guyane Française, quantifier le transfert maternel et étudier leurs cinétiques au cours la saison de ponte.

Les résultats de cette partie sont présentés dans le chapitre 4 pour les éléments traces métalliques: **Guirlet E**, Das K & Girondot M. 2008. Maternal transfer of trace elements in the leatherback turtles of French. *Aquatic Toxicology*. 88 (4): 267–276.

Ainsi que dans le chapitre 5, pour les organochlorés: **Guirlet E**, E, Das K & Girondot M. Toxicokinetic and maternal transfer of organochlorine compounds in the leatherback turtles during the nesting season in French Guiana. En préparation.

Réussite d'incubation

Afin de finir la boucle de ma réflexion, j'ai commencé à m'intéresser à l'effet des facteurs écotoxicologiques sur la réussite d'incubation et le développement embryonnaire. Plusieurs études ont déjà démontré les effets néfastes de certains polluants environnementaux sur le développement embryonnaire, la survie des jeunes, ou encore la réussite d'incubation des œufs dans un environnement contaminé pour certaines espèces de reptiles de l'ordre des crocodiliens et des chéloniens (Guillette et al., 1994; Crain et al., 1997; Portelli et al., 1999; Willingham and Crews, 1999; Rauschenberger et al., 2003).

J'ai ainsi voulu finir par l'étude de l'effet des différentes contaminations présentes dans les œufs sur le taux de réussite sur la plage de Yalimapo. Pour cela j'ai suivi les nids pour lesquels un œuf et du sang de la femelle avaient été analysés, afin d'évaluer la réussite d'incubation de ces nids et d'étudier les différentes relations possibles entre la contamination des œufs au moment de la ponte et la réussite d'incubation.

Les résultats de cette partie sont en cours d'analyse et seront discutés dans la section *discussion&perspective* de ce manuscrit.

L'objectif de cette introduction était de présenter l'organisation de ce manuscrit qui est une thèse sur articles. Chaque chapitre correspond à un article publié ou en cours de préparation, qui a apporté son lot de réponses et de questions sur la problématique de départ concernant les raisons de la faible réussite d'incubation à Yalimapo (Figure 10).

C'est dans ce contexte d'évaluation des facteurs ayant un impact sur le développement des embryons pendant l'incubation qu'a eu lieu ma recherche Cette étude revêt un aspect novateur du fait qu'elle rassemble pour la première fois un grand nombre de données écotoxicologiques sur une espèce de tortues, chez qui les études écotoxicologiques restent sous-représentées en comparaison à d'autres taxons. De plus pour cette espèce, aucune donnée écotoxicologique sur individus vivants n'était disponible. Enfin, cette étude concerne un écosystème naturel d'importance, au niveau international, car c'est l'un des plus grands sites de ponte pour cette espèce, au niveau national par son aspect patrimonial, et au niveau régional du fait de son inclusion dans une réserve naturelle protégée.

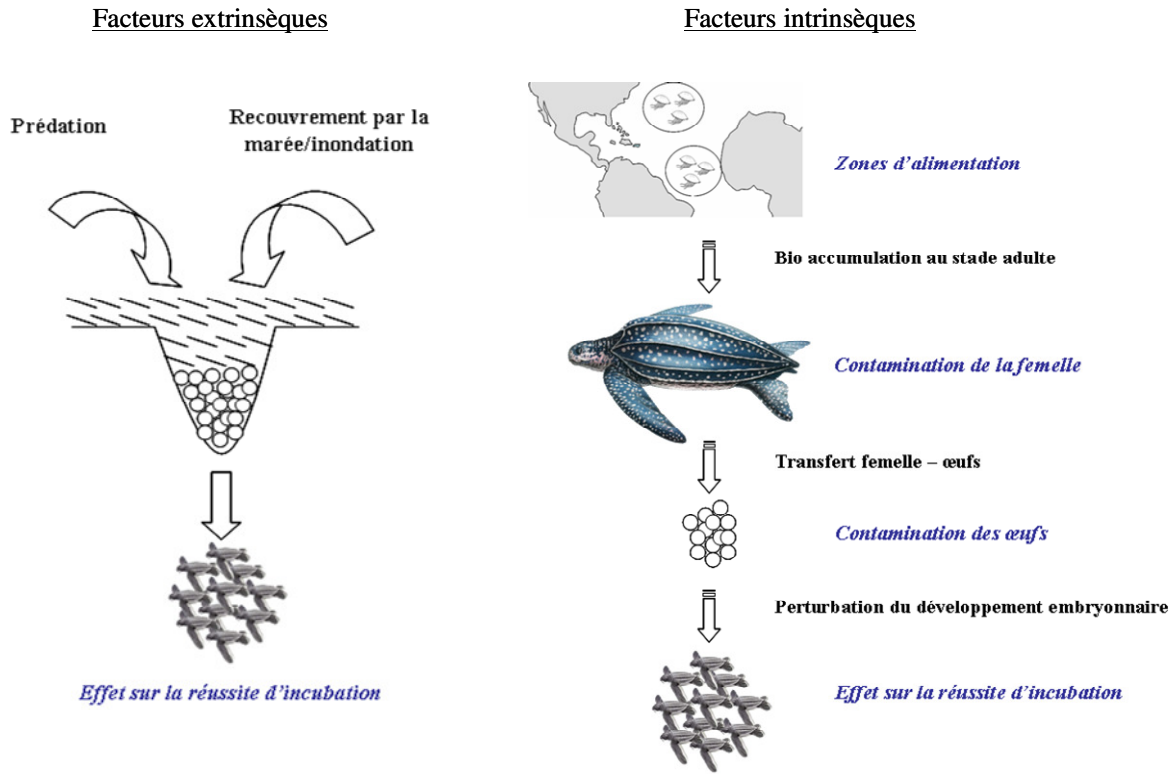

Figure 10 : Récapitulatif de la démarche d'analyse des facteurs écologiques et écotoxicologiques jouant sur la réussite d'incubation des nids pondus sur les plages de Guyane.

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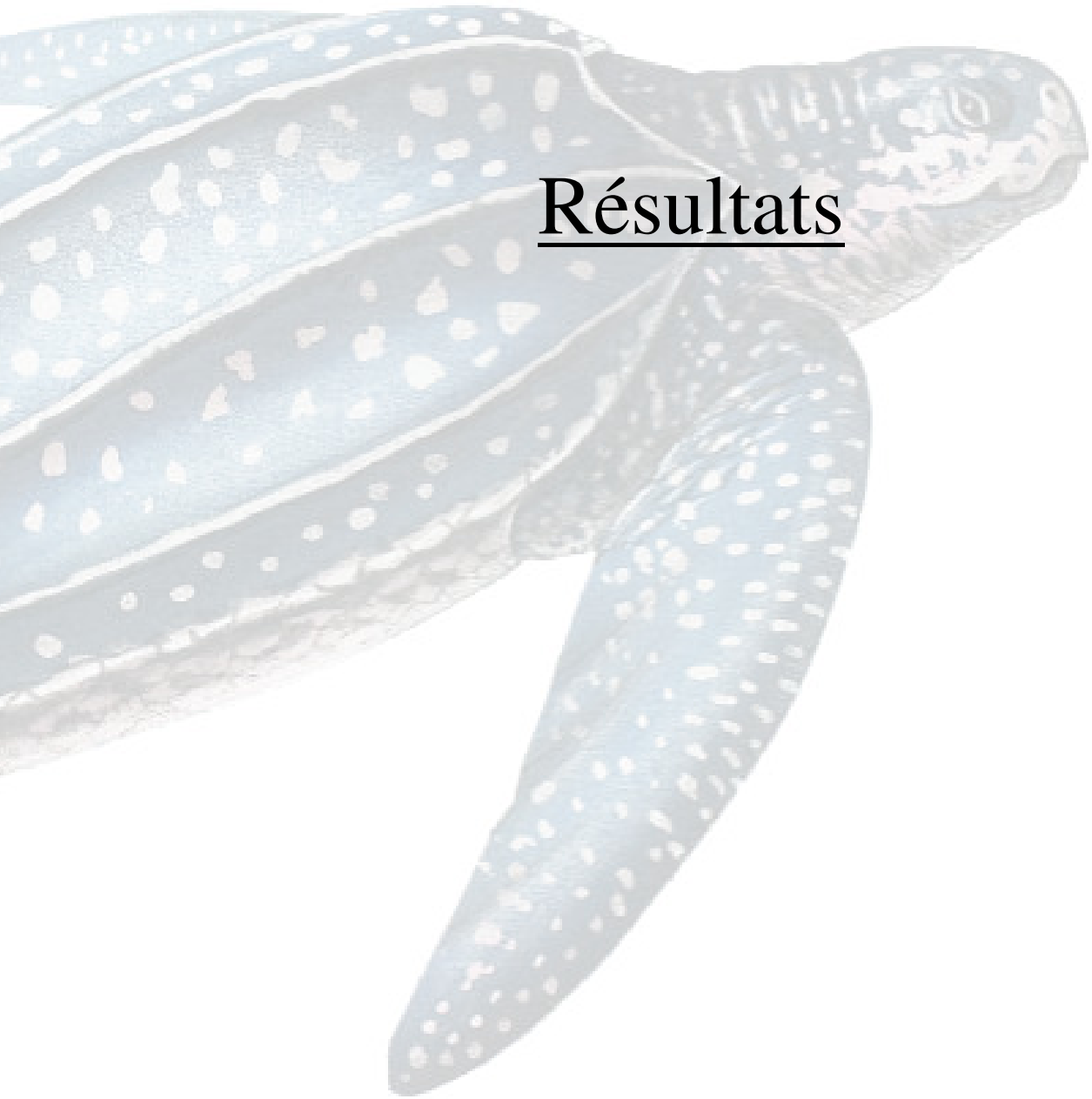
Partie II:

Résultats



Chapitre 1

Influence of nest location and yolkless eggs on the hatching success of leatherback turtle clutches in French Guiana

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Influence of nest location and yolkless eggs on the hatching success of leatherback turtle clutches hatch in French Guiana.

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Abstract

The hatching success of leatherback turtles, *Dermochelys coriacea* (Vandelli, 1761), is influenced by heterogeneously distributed ecological factors. However, the hatching success according to the nest site selection has rarely been studied and little is known about the role of nest site selection and infertile (yolkless) egg production on the predation rate and the development of fertile eggs in leatherback turtle nests laid in French Guiana. A field study of 99 leatherback turtle nests was conducted to quantify the relationships between hatching success and (1) the nest site selection (*i.e.*, vegetation line, sea tide line) and (2) the infertile eggs, especially their state after incubation (*i.e.*, hydrated or dehydrated) and their effects on predation rate. We found that the hatching success on this beach was very low (38.2%). This study illustrates that nest site selection influences the predation rate and the dehydration of yolkless eggs, while the production of yolkless by leatherback turtles have consequences on nest success. The proportion of yolkless eggs in the clutch, as well as their status at the end of incubation (hydrated or not, preyed upon) correlates with the predation rate, the development of yolked eggs, and hatching success. There was a significant relationship between nest location (relative to high tide line and vegetation line) and both the predation rates of eggs and the percentage of dehydrated yolkless eggs within a clutch. The production of yolkless eggs was related to hatching success and supports the hypothesis that yolkless eggs have a positive effect on the future of the clutch.

Résumé

Le succès d'émergence chez la Tortue Luth, *Dermochelys coriacea* (Vandelli, 1761), est influencé par la distribution hétérogène des facteurs écologiques. Cependant, la sélection du site de ponte et le succès d'émergence ont rarement été étudiés et peu de choses sont connues sur le rôle du choix du site de ponte et des œufs infertiles sur le taux de prédation et le développement des œufs fertiles dans les nids de Tortue Luth pondus en Guyane Française. Une étude de terrain s'est déroulée sur 99 nids de Tortue Luth pour quantifier les relations entre le succès d'émergence et la sélection du site de ponte (e.g. ligne de végétation, ligne de marée), les œufs infertiles, spécialement leur état après incubation (e.g. hydraté ou déshydraté) et leurs effets sur le taux de prédation. Nous avons trouvé un très faible taux de réussite sur cette plage (38%). Notre étude montre que la sélection du site de ponte influence le taux de prédation et la déshydratation des œufs infertiles, bien que la production de tels œufs infertiles chez la Tortue Luth a des conséquences sur le taux de réussite du nid. La proportion d'œufs infertiles dans la ponte, comme leur état à la fin de l'incubation (hydraté ou non, détruits par prédation), est corrélée avec le taux de prédation et de développement des œufs fertiles et le succès d'émergence. Il y avait une relation significative entre la position du nid (en rapport avec la ligne de marée haute et la ligne de végétation) et le taux de prédation des œufs et le pourcentage des œufs infertiles déshydratés à l'intérieur de la ponte. La proportion des œufs infertiles a été reliée au succès d'émergence et est en accord avec l'hypothèse que les œufs infertiles ont un effet positif sur le futur de la ponte.

Introduction

For organisms that lay eggs in a nest, hatching success is believed to be influenced by a number of interacting ecological factors including temperature, moisture and water salinity. To improve the nest microclimate, animals can select particular locations thereby modifying the properties of the nest (*e.g.*, gecko, Bragg et al. 2000; insects, Korb and Linsenmair 2000; Mallon, Pratt and Franks 2001; birds, Reid et al. 2000; Giese and Cuthbert 2003; and spiders, Bilde et al. 2002). Nest site selection has been studied in various reptiles, particularly sea turtles. A wide range of biological, chemical and physical factors have been reported as influencing the location of successful sea turtle nests, including oxygen and salinity level (Ackerman 1980), moisture content (McGehee 1990; Mortimer 1982; Bjorndal and Bolten 1992), temperature (Stoneburner and Richardson 1981; Mrosovsky et al. 1984), sand texture, type and density (Mortimer 1982; Miller 1985; Horrocks and Scott 1991; Cardinal et al. 1998), artificial lights on the beach (Salmon et al. 1995) or beach topography (Hays et al. 1995). The distance of the nest to the supra-littoral vegetation and to the high tide line may also be important ecological factors influencing nest site selection (Hays et al. 1995; Wang and Cheng 1999; for more information, see Bjorndal and Bolten 1992).

The forces governing nest site selection by sea turtles are still not fully understood. Nest site selection may have consequences on the nest success (*i.e.*, development and predation rate of embryos) or on the emergence and survival of newborn turtles. For example, flooding of nests by sea water leads to egg mortality from suffocation (Whitmore and Dutton 1985) and/or chloride toxicity (Bustard and Greenham 1969), alternatively hatchlings that emerge far above the high tide line may take longer to reach the sea, may be unable to find the sea at all, or may be more susceptible to predation on the beach (Mrovosky 1983; Horrocks and Scott 1991). Moreover, nests excavated amongst supra-littoral vegetation may suffer high egg mortality as a result of roots invading the egg chamber (Leslie et al. 1996). However, Horrocks and Scott (1991) showed that the hawksbill turtle (*Eretmochelys imbricata*

(Linnaeus, 1766)) may prefer to nest amongst the vegetation, a strategy tentatively accounted for by the fact that vegetated nest sites were less compacted than non-vegetated ones and had a higher hatchling escape success rate. Predators of sea turtle nests include dogs, birds, rats, lizards, frogs, crabs and insects (Brown and Macdonald 1995; Baran and Turkozan 1996; Broderick and Godley 1996; Leslie et al. 1996; Broderick and Hancock 1997; Yerli et al. 1997; Baran et al. 2001; Maros et al. 2003), and predation rates may also vary according to the location of the nest.

Many leatherback turtle nests are often prone to erosion and flooding by the tide (Mrosovsky 1983; Eckert 1987). Consequently, mean nest success of leatherback turtles ranges from 20 to 70% depending on the year, the beach and the method used to evaluate nest success (Whitmore and Dutton 1985; Girondot et al. 1990; Leslie et al. 1996; Sarti et al.1996; Spotila et al. 2000; Bilinski et al. 2001; Bell et al. 2003). Moreover, leatherback turtle clutches have a higher frequency than other sea turtles of eggs without yolks, commonly referred to as "yolkless eggs". Leatherback turtle clutches contain an average of 58-114 yolked eggs and 21-56 yolkless eggs (Leslie et al. 1996; Bell et al. 2003; Maros et al. 2003). These yolkless eggs are smaller than fertile yolked eggs and almost exclusively contain albumin (Pritchard 1971), are thin-shelled and irregular in shape, and are generally the last of the eggs laid in a clutch. The role or function of yolkless eggs in leatherback turtle nests is not well understood, but they may provide some selective advantage by improving the hatching success of the viable eggs (Whitmore and Dutton 1985).

In this paper, we investigated the possible relationship between nest site selection of leatherback turtles and the hatching success. Additionally, we investigated the possible influence of yolkless eggs on hatching success and on the protection of yolked eggs from predation.

Materials and methods

Study site and species

Research was carried out on the Awala Yalimapo beach in French Guiana (53°57'W-5°45'N), located within the Amana Nature Reserve, on the inshore plain of coastline between the Mana and Maroni Rivers. The beach is 4 km in length and varies in width from a few meters to 20 m, depending on the tide line. For this study, we chose a 300-m long section of beach that was sufficiently frequented by turtles but also sufficiently distant from sites visited by tourists.

The beach of Awala Yalimapo has one of the highest nesting densities of the leatherback turtle with about 30-40% of the world's population of nesting females and approximately 50% of all leatherback turtles nesting in the region of French Guiana and Suriname from March to August (Girondot and Fretey 1996; Fretey and Lescure 1998; it should be noted that the exact proportion varies according to the year). The high concentration of eggs on Awala Yalimapo beach could attract animals such as dogs, black vultures (*Coragyps atratus* (Bechstein, 1793)), ghost crabs (*Ocypode quadrata* (J.C. Fabricius, 1787)) and mole crickets (*Scapteriscus didactylus* (Latreille, 1804)) that prey on both leatherback turtle eggs and hatchlings (Fretey and Lescure 1980; Maros et al. 2003).

Data collection

The nests analyzed in the study were laid from 20 May to 4 June 2002. For each freshly laid nest encountered, we used a plastic measuring tape to measure the minimum distance from the nest to (1) the last high tide line ("sea distance"), to (2) the vegetation line (slight vegetation (*Ipomea*), sand still visible), and (3) the width of the beach (high tide line – vegetation line). At the time of oviposition or later during nest covering, we measured the minimum straight carapace length of the nesting female (SCLmin) (Bolten 1999) and the female identity was recorded (Passive Integrated Transponder (PIT) tags). All nest locations were localized to within 1 m by triangulation to numbered stakes placed every 10 m along the vegetation of the beach. To locate specific nests after incubation, we placed a ring of plastic isolated copper in

the sand above the clutch when the female was covering the nest and then used a metal detector to locate nests at the end of incubation. After 50 days of incubation, we monitored nests daily for signs of emergence. All nests were excavated 48 hours after signs of emergence. Nest contents were examined and divided into categories (Table 1). The number of shell fragments (*S*), live hatchlings (*L*), dead hatchlings (*D*), emerged (*E=S-(L+D)*), undeveloped yolked eggs (*UD*), yolkless eggs (*Y*) and the number of yolked and yolkless eggs preyed upon by ghost crabs (described in Viseux 2001) and mole crickets (described in Maros et al. 2003) were counted. Yolked and yolkless eggs are included in estimate of predation rate. We defined three categories of yolkless eggs; yolkless eggs still filled with albumin (hydrated eggs, *YH*), yolkless eggs intact without albumin (dehydrated eggs, *YD*) and yolkless eggs without albumin because they had been preyed upon (preyed upon eggs, *YP*). Hatching success (*HS*) was determined by dividing the number of eggs that successfully produced hatchlings that left the nest (estimated by number of shell fragments, *S*) by the total number of yolked eggs originally laid by the female (*YE=S+UD*).

Statistical analysis

To determine the consistency of nest site selection relative to distance from the sea and distance from the vegetation, we tested the normal distribution with the Shapiro-Wilk W test because of its good power properties when compared to a wide range of alternative tests (Shapiro and Wilk 1965). An important aspect of the "description" of a variable is the shape of its distribution, which tells us the frequency of values from different ranges of the variable. We tested the skewness (which measures the deviation of the distribution from symmetry); if it is clearly different from zero, then the distribution is asymmetrical, whereas normal distributions are perfectly symmetrical. If the kurtosis (which measures "peakedness" of the distribution) is clearly different from zero, then the distribution is either flatter or more peaked than normal; the kurtosis of the normal distribution is zero. Analyses were done using Statistica 6.0 (Statsoft, lnc.).

Table 1. Generalized linear model (GLM) for the hatching success of leatherback turtles in Guiana.

	Estimate	Deviance	d.f.	F Ratio	Prob>F	%
Hatching success model						
Model		**516.8**	**6**	**5.40**	*0.0001*	**25.7**
Constant	**0.5808**					
Yolkless dehydrated eggs	**1.615e-2**	**173.1**	**1**	**10.86**	*0.0015*	**33.5**
Yolkless hydrated eggs	**-5.658e-2**	**6.46**	**1**	**0.41**	**0.5261**	**1.6**
Sea distance	**-0.1505**	**9.04**	**1**	**0.57**	**0.4538**	**1.7**
Vegetation distance	**-2.439**	**1.65**	**1**	**0.10**	**0.7482**	**0.3**
Sea distance x vegetation distance	**0.2823**	**231.1**	**1**	**14.5**	*0.0003*	**44.7**
Yolkless dehydrated x yolkless hydrated	**5.583e-3**	**95.33**	**1**	**5.98**	*0.0167*	**18.4**

Generalised linear model (GLM) was used for the analysis of hatching success. One model with first-order interactions was fitted:

G(hatch) = adult size + sea distance + vegetation distance + number of yolkless eggs (in the clutch, dehydrated, preyed upon, hydrated) + number of yolked eggs + interactions + error

Where G is a link function and hatch represent hatching success. Adult size, sea distance, vegetation distance, number of yolkless eggs (in the clutch, dehydrated, preyed upon, hydrated) and number of yolked eggs were treated as continuous independent variables. As dependent variables were percentages, we used binomial error distribution and logit link function (GLMStat). The significance of factors and interactions were tested and only significant terms were left in the final model. However, a non-significant term was kept if its interaction with another factor was significant. We started from a complete model on which we applied a backward elimination procedure to obtain the final model, using the following criteria: the variable with the maximum non-significant probability was excluded at each step (Dobson 1990). The final model was attained when all variables retained were statistically significant ($P<0.05$). Moreover, we used this model to obtain the percentage of deviance accounted for by each variable when compared to the null model (Martinez et al. 2003).

GLM with binomial error distribution and logit link were performed to look for possible correlations of paired variables. Different parameters related to the hatching success,

yolkless eggs (in the clutch, hydrated, preyed upon, dehydrated) and yolked eggs (in the clutch, undeveloped, preyed upon) were dependent variables and distance from the sea and the vegetation were continuous independent variables. A similar GLM was used to test for correlation between yolkless eggs and yolked eggs predated by mole crickets.

All calculations were performed using GLMStat, version 5.5. For all statistical tests, α=0.05.

Results

Nests

Over a period of 15 days, we marked all the nests laid (n = 99) along a 300m stretch of beach. At the end of the study, five nests were lost to erosion, six nests to dog predation and two nests to human poaching. Therefore, 99 nests were analyzed for nest site selection, and 86 nests were analyzed for hatching success and the role of yolkless eggs (because the 13 lost nests were not excavated).

Leatherback turtle nests (n = 86) had on average 87.8 (SE 2.3) yolked eggs and 24.7 (SE 1.1) yolkless eggs per clutch. All nests had some yolkless eggs. The overall hatching success of all nests was 38.2% (SE 2.4) with 74/86 nests hatched. Mole cricket predation was recorded in 94.2% of the nests (81/86 nests), with an egg predation rate of 16.6% (SE 1.0) and a mean 17.7 (SE 1.2) eggs predated per nest. Mole crickets preyed upon yolkless eggs significantly more often than yolked eggs ($F = 9.180$, $p = 0.003$, n = 86, Figure 1), with a yolked predation rate of 14.3% predation rate was 48.9% of nests (42/86 nests), with an egg predation rate of 4.9% (SE 0.5) and a mean of 4.1 (SE 0.4) eggs predated per nest. Ghost crabs predated only the yolked eggs. At the end of nest incubation, yolkless eggs were found to be intact but dehydrated (mean: 59.1%, SE 2.4), still filled with albumin (mean: 17.8%, SE 2.5), preyed upon by mole crickets (mean: 22.5%, SE 2.0), or indeterminate (mean: 0.6, SE 0.8).

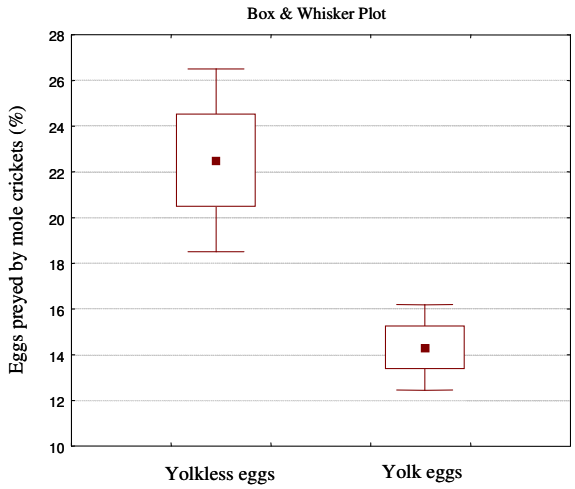

Figure 1. Proportion of yolkless and yolked eggs of leatherback (*Dermochelys coriacea*, (Vandelli, 1761)) preyed upon by mole crickets (*Scapteriscus didactylus* (Latreille, 1804)) (n=86, means, vertical line for SE).

(SE 0.9) and a yolkless predation rate of 22.5% (SE 2.0). In the case of ghost crabs, the

Nest site choice

Leatherback turtles tended to lay their clutches away from the sea and near the vegetation behind the beach (n = 99) (Figure 2). The distribution of the distance from the sea was normal (W = 0.99, p = 0.94, skewness = -0.08, kurtosis = -0.14). In contrast, the distribution of the distance from the vegetation was leptokurtic (W = 0.91, p<0.001, skewness = 1.08, kurtosis = 0.81). Correlations of paired variables with different parameters relating to hatching success and nest site showed that the distance to the vegetation line and predation rate by mole crickets on eggs (F = 5.980, p = 0.0166) and especially on yolked eggs (F = 4.726, p = 0.0325) was negatively significant and the number of yolkless dehydrated (F = 4.482, p = 0.0372) was positively significant. All other possible correlations of paired variables were non-significant. Clutch size (yolked plus yolkless eggs) did not vary from the tide line to the vegetation line. The quantities of yolked and yolkless eggs were not correlated, and clutch proportions were similar across the nesting zone. Finally, the proportion of undeveloped yolked eggs (*UD*) and the proportion of yolked eggs preyed upon by ghost crabs were not

significantly correlated with the distance between the nests and the vegetation or between the nests and the high tide line.

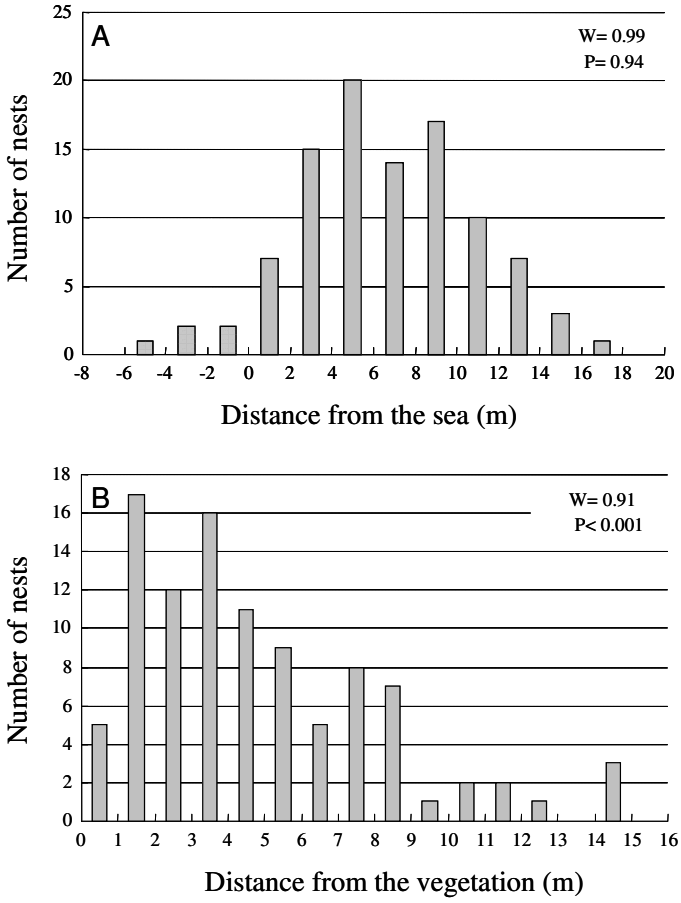

Figure 2. Frequency distribution of the distance between nest sites and (A) the sea tide and (B) the vegetation for all nests measured (N= 99). Negative values in (A) indicate nests that were laid below the sea tide. We tested the normal distribution with the Shapiro-Wilk W test (W).

GLM analysis models

The model for hatching success accounted for 25.69% of the original deviance (Table 2). Some factors that are directly involved in hatching success estimation were not used. For example, number of undeveloped yolked eggs (%) directly influences hatching success. Sea distance and vegetation distance were not significant alone, but their interaction was highly significant ($p = 0.003$) and accounted for 44.72% of model deviance.

The interaction term between yolkless dehydrated eggs x yolkless hydrated eggs was found to be significant ($p = 0.0167$), however only the factor of dehydrated yolkless eggs was significant ($p = 0.0015$) and represented 33.49% of the model deviance. Hatching success

increased with yolkless dehydrated eggs and the interaction between sea distance x vegetation distance. We used a simple GLM model for hatching rate to account for the 'sea distance x vegetation distance' interactions (*G(hatch) = sea distance + vegetation distance + interaction + error*). The hatching rate was higher near the vegetation line if the width of the beach was small and greatest in the middle of the beach if the width of the beach was large (Figure 3). It appears as if actual hatching rates remain relatively constant near the vegetation line, even as the beach width increases.

Table 2. Generalized linear model (GLM) for the hatching success of leatherback turtles in Guiana. The percentage of deviance accounted for by each variable and by the model (compared with a null model) are shown (%).Only significant terms were left in the final model; adult size, number of yolkless eggs (in the clutch and preyed on) and number of yolked eggs were dropped.

	Estimate	Deviance	d.f.	F Ratio	Prob>F	%
Hatching success model						
Model		516.8	6	5.40	***0.0001***	25.7
Constant	0.5808					
Yolkless dehydrated eggs	1.615e-2	173.1	1	10.86	***0.0015***	33.5
Yolkless hydrated eggs	-5.658e-2	6.46	1	0.41	0.5261	1.6
Sea distance	-0.1505	9.04	1	0.57	0.4538	1.7
Vegetation distance	-2.439	1.65	1	0.10	0.7482	0.3
Sea distance x vegetation distance	0.2823	231.1	1	14.5	***0.0003***	44.7
Yolkless dehydrated x yolkless hydrated	5.583e-3	95.33	1	5.98	***0.0167***	18.4

Discussion

Impact of yolkless eggs on hatching

Several studies report the presence of larvae of two polyphagous fly families (Phoridae and Sarcophagidae) in the nests of sea turtles (Trauth and Mullen 1990; Acuna-Mesen and Hanson 1990; Disney 1994; Broderick and Hancock 1997). Three genera of flying Sarcophagidae are known to attack the supple shell of sea turtle eggs (Lopes 1982; Lopez Barbosa 1989; Andrade et al. 1992; Vasquez 1994; McGowan et al. 2001). These studies indicate that the

eggs are vulnerable to predation by insects. However, the level of this impact is unknown and we have no idea whether eggs that show signs of predation are actually viable at the time when predation inferred occurs Moulis (1997) describes a reduction of 15% of hatchling emergence for some *Caretta caretta* (Linnaeus, 1758) nests infested by the invasive red fire ant (*Solenopsis invicta* Buren, 1972), compared to non-infested nests. Although the level of egg predation by insects is suspected to be very high, it has rarely been documented for the leatherback turtle.

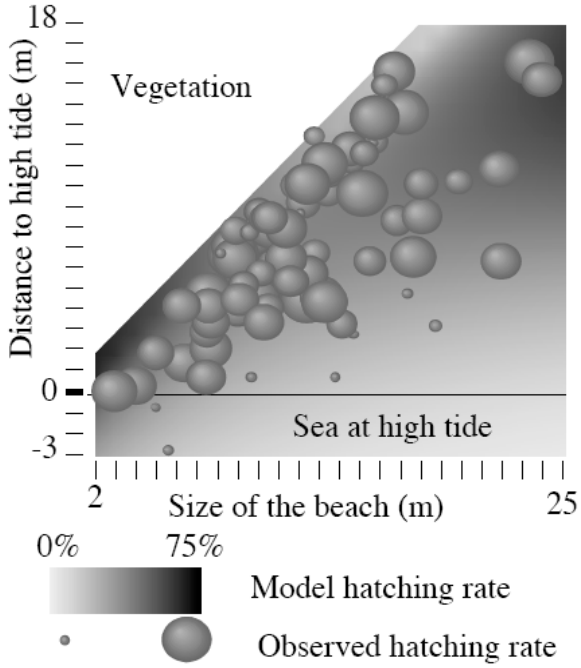

Figure 3. Hatching rate according to the size of the beach and the distance to high tide. Surface color represents model hatching rate (GLM analysis) and spheres represent observed hatching rates.

During this study, predation occurred mainly by ghost crabs and mole crickets. Sea turtle eggs are rarely the principle food source of ghost crabs (Arndt 1994; Loegering et al. 1995; Watts and Bradshaw 1995; for more information, see Hill and Green 1971) and we found that their impact on leatherback turtle eggs may be relatively small (predation rate 2.4%, n=86). In terms of predation by mole crickets, our results are consistent with those of Maros et al. (2003) who found that about 18% of leatherback yolked eggs laid on this beach were preyed upon by mole crickets (10 nests).

As suggested by Leslie et al. (1996), we found that the predation rate was related to the position of the nests. In particular, we found that the proportion of eggs preyed upon by mole crickets increased near the vegetation zone, especially for the yolkless eggs. This is consistent with Maros et al. (2003) who found a greater density of mole crickets near the vegetation line.

Most of the yolkless eggs were dehydrated at the end of incubation (approximately 59%). The proportion of eggs preyed upon by mole crickets was negatively correlated with the vegetation distance, whereas the proportion of dehydrated yolkless eggs was positively correlated. As yolkless eggs dehydrate, they might release substances into the surrounding egg chamber that act as deterrents to mole crickets (Dutton and Mc Donald 1995). Most of the yolkless eggs are laid at the top of the egg mass, therefore yolkless eggs may be the first eggs encountered by mole crickets if they dig straight down from the surface of the sand. Our data showed that hydrated yolkless eggs were more often preyed upon than yolked eggs.

Consequently, yolkless eggs could act, in part, as an efficient means of protection against the mole cricket predation of yolked eggs in French Guiana. As such, 3 scenarios may occur when mole crickets find a leatherback turtle clutch: (1) mole crickets encounter yolkless eggs with a greater probability than yolked eggs; (2) if yolkless eggs are hydrated, the mole crickets preferentially feed on them compared to yolked eggs; and (3) if yolkless eggs are dehydrated, some substances from the eggs may inhibit the actions of mole crickets.

Though we have evidence of the role of yolkless eggs on the development of yolked eggs, they may have several positive impacts. The more hydrated yolkless eggs there are at the end of the incubation period, the less likely that yolked egg successfully finishes their development. These results support the hypothesis that yolkless eggs might help maintain physicochemical conditions (for example, moisture by dehydrated eggs) (Dutton and McDonald 1995). Dutton and McDonald (1995) did not find a significant difference in hatching success between clutches with or without yolkless eggs, probably because few predators are present in St. Croix (especially mole crickets) and clutches were collected as

they were laid and reburied at sites on stable areas of the high beach platform. Altogether, these results suggest that yolkless eggs could play an important role in the successful incubation of the nest; some may act as decoys for predators while others may be important for successful incubation of yolked eggs and the escape of hatchlings from the nest (Dutton and McDonald 1994).

Nest site selection and hatching success

Nest-site selection of leatherback turtles has been described as being highly variable and widely dispersed (Weishampel et al. 2003), with some clutches suffering a near complete mortality following wash over by high tides on the day of laying (Mrosovsky 1983; Eckert 1987). In contrast, we found that few leatherback turtle nests (5%) of were laid below the high tide line and most nests were strongly aggregated in the upper part of the beach.

The beach of Awala Yalimapo has one of the lowest hatching success rates of leatherback turtles in the world (Girondot et al. 2002). The hatching success of sea turtles is thought to be strongly related to the distance that the nest is laid from the sea and from the vegetation behind the beach (Hays and Speakman 1993; Godley et al. 2002). Our data indicates a strong relationship between hatching success and the interaction of sea distance x vegetation distance, except for the predation by mole crickets which increases significantly toward the vegetation line. It is likely that various micro-environmental factors such as sand content and compaction, oxygen, chloride and moisture levels, and temperature varied from the vegetation to the sea level. Nests placed closer to the vegetation are likely to encounter a substrate with reduced moisture, temperature and compaction (Spotila et al. 1987; Weisrock and Janzen 1999; for more information, see Godfrey et al. 1996). It is also likely that the low overall hatching success rate on this beach made it difficult to determine the interactions among the nest location, relative proportions of yolked and yolkless eggs and hatching success. It should be noted however, that when eggs from French Guiana were incubated

under laboratory conditions, the hatching rate was close to 80% (Girondot et al. 1990). The principal factors that affect hatching success may be different from those we considered in this study. We suggest that other ecological factors in the beach of Awala Yalimapo (*i.e.*, organic matter, oxygen exchange and moisture contents) may limit the development of eggs (Ackerman 1980; McGehee 1990). Another probable important variable related to egg mortality is bacterial and fungal attacks (Girondot et al. 1990).

Nest site selection as integrative response

The correlation between hatching success and nest location leads to the question of how and why leatherback turtles choose their nest site. There are probably many factors influencing nest success, (*i.e.*, predation rate, the physiological state of yolkless eggs, and soil properties), which may vary along the beach and interact with each other. Moreover, as suggested by Horrocks and Scott (1991), the length of beach crawl may be an important factor influencing the nesting behavior of turtles. The large size of leatherback turtles and the slow speed of their hatchlings may make the length of the crawl on land critical, and may partly explain the tendency for leatherback turtles to nest closer to the sea compared to green turtles (*chelonia mydas* (Linnaeus, 1758)) which also nest on beaches in the Guianas (Whitmore and Dutton 1985). Mrosovsky (1983), at the population level, and Eckert (1987), at the individual level, suggested that leatherback turtles have developed a scattered nesting strategy, whereby nests are randomly distributed throughout a beach to maximize clutch survival over an unpredictable nesting area. But Nordmoe et al. (2004) showed that the leatherback turtle preference for open sand at Playa Grande is consistent with that seen in Malaysia (Chua 1988). On Krofajapasi beach in Suriname, Whitmore and Dutton (1985) found a general preference for open sand nest sites among leatherback turtles, as opposed to the green turtle preference for vegetative cover. In a recent study, Kamel and Mrosovsky (2004) suggested that nesting patterns cannot be characterized in a simple manner, and there appears to be two main aspects of this behavior: one aspect emphasizes non-random repeatable choices and the

other reflects scatter in the actual nesting pattern. During our study, we saw four adult turtles attacked by packs of stray dogs on the beach, illustrating that nesting can be dangerous for females. Nest site also influences the proportion of dehydrated yolkless eggs which, in turn, may influence the predation rate by mole crickets. Our results on nest site selection showed that leatherback turtle females nest at intermediate distances from the tide and near the vegetation, and these results correspond with those of Kamel and Mrosovsky (2004). Nest site selection may be a trade-off between decreasing the loss of nests from tidal flooding or erosion when laying at a short distance from the sea, with the increase of possible predation rates on females and hatchlings when nests are laid near the vegetation (Mrosovsky 1983).

Conclusion

Leatherback turtles that nest in French Guiana represent about 30%-40% of the world's breeding population (Girondot et al. 2002). The serious decline in female populations at many of the world's leatherback turtle rookeries (Spotila et al. 2000) increases the urgency to obtain a better understanding of the status of the species at Awala Yalimapo beach. Previous workers at the site have proposed that the nesting success was a significant factor governing the population dynamics (Fretey and Lescure 1979). We found that the hatching success on this beach was very low (38.2%) compared to the values observed for this species at Culebra (75%, Tucker 1989) and at St Croix (67%, Boulon et al. 1996). Several factors affecting incubation success are known for Surinamese and French Guianan beaches. Our results indicate that the nest site may affect hatching success, especially with regard to mole cricket predation. In Awala Yalimapo beach the predation rate was 16.6%, but preliminary results indicate that 40% of leatherback turtle eggs in Galibi beaches are predated by mole crickets (Hoekert at al. 1998). We find that yolkless eggs have a positive effect on the future of the clutch. This is an important result as yolkless eggs are often ignored in hatchery studies

with relocated nests and laboratory incubators, and as such, highlights the fact that they should be included in such studies.

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Chapitre 2

Effect of inundation on the hatching success of Leatherback turtles

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Effect of inundation on the hatching success of Leatherback turtles

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Introduction

For animals that lay their eggs in a nest, the selection of a nest site may strongly influence offspring survival and therefore have important consequences for the reproductive fitness of the adult. Selection of a nest site is an adaptative trade off between the cost of searching for a site (both in terms of energy and predation risk) and the reproductive benefits of selecting a site suitable for successful incubation (Wood & Bjorndal 2000). In many oviparous reptiles, environmental factors influence embryo survivorship (Horrocks & Scoot 1991; Resetarits 1996), hatchling size (Packard & Packard 1988), performance (Janzen 1993), growth (Bobyn & Brooks 1994), behaviour (Burger 1991), and sex determination (Spotila *et al.* 1994). Selection of nest sites can also influence probability of nest predation (Fowler 1979). For sea turtles the survival of the offspring may be strongly related to the distance that the nest is laid from the sea and from supra-littoral vegetation behind the beach (Mrosovsky 1983). Placement of nests close to the sea increases the likelihood of inundation and egg loss to erosion whereas placement of nests farther inland increases the likelihood of desiccation, hatchling misorientation, and predation on nesting females, eggs, and hatchlings (Bustard & Greenham 1968; Whitmore & Dutton 1985).

In many species without protracted parental care, a female's nesting behaviour and preferences can affect the development and survival of her offspring. Sea turtle eggs are deposited in a large clutch in a nest excavated by the female in sandy marine beaches. Incubation requires several months during which the sea turtle embryos grow from a gastrula stage to a fully formed organism capable of independent existence. During this process yolk stored in the egg by the female is transformed into embryonic tissue. The developing embryo

is coupled to the nesting beach through the exchange of the O_2, CO_2, H_2O, and heat that is either required or produced by the energy transformation process (Ackerman 1997). The embryonic period of sea turtles is a critical period of their life cycle because they are exposed to a diversity of biotic and abiotic factors. Parameters considered important to the chance of survival include: salinity, moisture, gas exchange, temperature, rainfall, tidal inundation, erosion, and predation (Bustard & Greenham 1968; Yntema & Mrosovsky 1980; McGehee 1990; Ackerman 1997).

The leatherback turtle (*Dermochelus coriacea*), the largest of seven species of sea turtle, is classified as critically endangered by the Species Survival Commission (IUCN 2006). This unique reptile is distributed worldwide and migrates to tropical or subtropical nesting beach (Ferraroli *et al.* 2004, Hays *et al.* 2004, Caut *et al.* 2008). After reaching sexual maturity, females typically nest every 2-3 years. During nesting seasons, females oviposit cluches of about 65 eggs that can be more than 5 kg in mass and the bottom of the nest ~75-100 cm below the sand surface. Leatherback turtles can lay up to 14 clutches in a season (Briane *et al.* 2007) and often place their nests in the open sand near the water, but rarely in the vegetation (Whitmore & Dutton 1985). However, leatherbacks have relatively low hatching success (~50%) while hatching success of other marine turtle species is ~85% or more (Bell *et al.* 2004). This low hatching success of leatherbacks is largely a result of embryonic mortality rather than egg infertility (Whitmore & Dutton 1985; Bell *et al.* 2004), but the specific cause remains unknown.

Favourable nest placement is critical to the survival of sea turtle populations. The location of oviposition determines the reproductive success of the species by influencing both the sex of hatchlings and their probability of survival (Bjorndal & Bolten 1992). For this reason, researchers have investigated possible strategies and cues that might influence nest placement. Mrosovsky (1983), at the population level, and Eckert (1987), at the individual

level, suggested that leatherbacks have developed a scatter nesting strategy whereby nests are randomly distributed throughout a beach to maximize clutch survival on an unpredictable nesting area. However, Leatherback turtles often nest in places where their eggs are destroyed by high tides. Poor nest site selection ranges from <2.5% in Malaysia to around 40% in the Guianas and appears to be related to beach topography (Mrosovsky 1983).

Many of nesting beaches are particularly susceptible to coastal hazards such as storm surges and coastal erosion and, with the projected rise in sea level resulting from anthropogenic global warming, threats to coastal areas are increasing (Huang 1997). Indeed, the increase in the number and concentration of greenhouse gases in the atmosphere has the potential to cause an elevation in the global mean air temperature and mean sea level of 1-4.5°C and 31-150cm, respectively by the year 2100 (Houghton & Woodwell 1989; Warrick & Oerlemans 1990). Changes in air temperature and sea levels of this magnitude are capable of causing shifts in the population structure of reptiles and would cause the northward movement of climatic zones (Daniels & White 1993). Turtles nest on a variety of beach types, which may be affected in different ways by sea-level rise. The mechanisms by which nesting females choose a beach or site on a beach are poorly understood (Mortimer 1995). These include a number of physical features, such as beach length, width, height, slope, orientation and vegetation (Mortimer 1995; Kikukawa *et al.* 1999). All these features are likely to be affected by beach-front development and sea level rise.

The inundation of nests close to the sea by seawater and predation of nests farther inland both affect the hatching success, and therefore the natural sex ratio. Discussion of the effects of global warming on sea turtle population have focused on the loss of nesting beach habitat as a result of an increase in sea level and on the changes in sex ratio (White & Daniels 1993; Davenport 1997; Nicholls *et al.* 1999; Fish *et al.* 2005). The effect of inundation and indirectely the sea level rise on leatherback hatching success have not been study yet but

should have been because of the particularity of this species to lay its clutches nearer to the high tide line than other sea turtle species.

The beach of Awala Yalimapo has one of the highest nesting densities of leatherback females with about 30% of the world's population of nesting females and approximately 50% of all leatherbacks nesting in the region of French Guiana and Suriname (Girondot & Fretey 1996; Fretey & Lescure 1998; it should be noted that the exact proportion varies according to the year). However, hatching success on this beach has been proved to be smaller than on others leatherback nesting sites (Tucker 1989; Boulon *et al.* 1996, Girondot *et al.* 2007). Low hatching success compounds the problem of population decline that results from adult mortality caused by incidental capture fisheries (Kaplan 2005; Martinez *et al.* 2007) and understanding causes of low hatching success would therefore be an important conservation step towards preventing extinction in a species (Ralph *et al.* 2005). In the present study, we have determined level and frequency of tidal inondation, and hatching success to investigate the tolerance of nest inundation in the context of sea level rise. We have assessed the rate of leatherback turtle embryonic mortality and the factors affecting the embryo development in relation to specific embryological stages and incubation time at Awala Yalimapo beach to find out causes of poor hatching success.

Materials and Methods

Study site and data collection

Research was carried out on the Awala Yalimapo beach in French Guiana (53°57W-5°45'N). The beach is located within the Amana Nature Reserve, on the inshore plain of coastline between the Mana and Maroni Rivers. The beach is 4 km in length and the width varies from a few meters to 20 m, depending on the tide line. For this study, we chose a 300-m long

section of beach that was sufficiently frequented by turtles but also sufficiently distant from sites frequented by tourists (Figure 1).

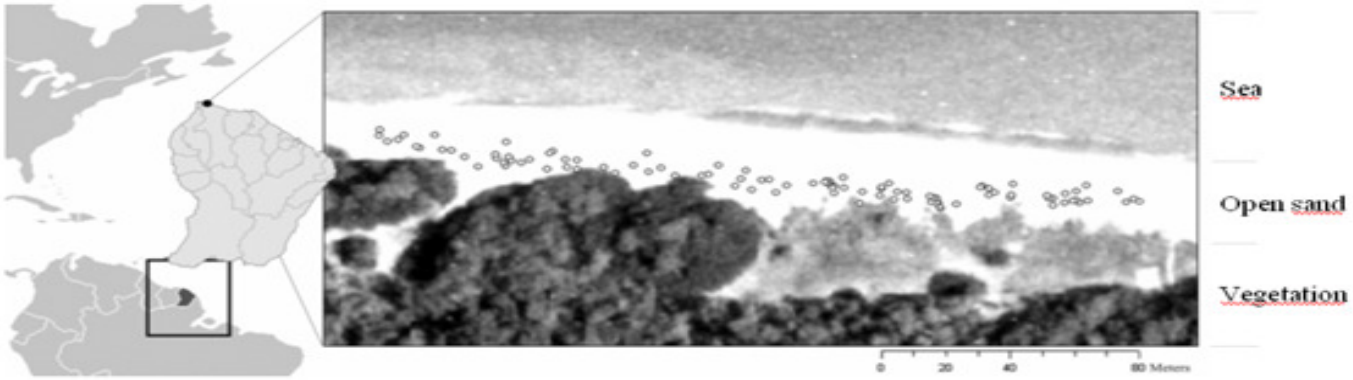

Figure 1. Location of the study site in French Guiana and pattern of distribution of the studied nests. The 97 nests (symbol circle) are distributed along a 300m section of the Yalimapo beach, between the vegetation line and the sea.

The nests analyzed in the study were laid from 20 May to 4 June 2002. For each freshly laid nest encountered, we measured the minimum distance from the nest to the last high tide line, to the vegetation line (sparse creeping vegetation (*Ipomoea pes-caprae*), sand still visible) with a plastic meter. All nest locations were localized to within 1 m by triangulation to numbered stakes placed every 10 m along the vegetation of the beach. To locate specific nests after incubation, we placed a numbered ring of plastic isolated copper in the sand above the clutch during the covering of the nest by the female. We used a metal detector that captured signals from probes in the copper at the end of incubation.

Hatching success and stage of death of embryos

All nests were excavated 48 hours after first signs of emergence, and we recorded the total number of yolked (including 'hatched' eggs) and shelled albumin globs (SAGs). We dissected unhatched eggs in situ and staged them using different criterias:

- Category $E_{<10}$: Egg with no visible signs of development or dead embryo < 10 mm in length.

- Category $E_{10\text{-}60}$: Egg with dead embryo ≥ 10 mm and < 60 mm in length.

- Category $E_{>60}$: Egg with dead embryo > 60 mm.

We estimated the percentage of embryos for each category as the number of embryos in the category divided by the total number of yolked eggs. Hatching success was estimated by the number of large fragment of shells divided by total number of yolked eggs.

Embryonic size and time of incubation

Embryonic growth has been described for a number of marine species (Cratz 1982; Miller 1985) and freshwater (Yntema 1968). Miller (1985) reviewed the embryology of sea turtles and presented a stage-by-stage description. Evidence of a great homogeneity in the embryonic development stages of Chelonians is shown. But, there is still, some particuliarities in the leatherback. Renous *et al.* (1989) presented a stage-by-stage description of leatherback development and reported that many measures of embryonic growth could be related to developmental time (incubation period). We used these data of embryonic growth of leatherback turtle (Renous et al. 1989). We fitted these data using a exponential function: $y = 2.85 \exp(0.05x)$ $R^2 = 0.99$, with y the embryonic size (mm, with maximum 110 mm) and x the time of incubation.

To analyze the effect of inundation on the embryonic development arrests, we determined for each category a corresponding time of incubation. The first category $E_{<10}$ corresponded to the period 1 (0 to 24 days), the second category $E_{10\text{-}60}$ to the period 2 (24 to 57 days) and the third categorie $E_{>60}$ to the period 3 (57 to 68 days).

Measures of inundation

During each day of incubation time, we measured the minimum distance from the nest to the day high tide line for each nest (represented by the high-water mark, see figure 2). To define the characteristics of inundation for each of the three periods (0 to 24 days, 24 to 57 days, 57 to 68 days), we define two parameters:

- Level of inundation: the sum, in meters, of daily distances between the nest and the high tide line for inundated days (negative values in figure 2),

- The first day inundated corresponding to the first day of the incubation for which an inundation was recorded for each specific period.

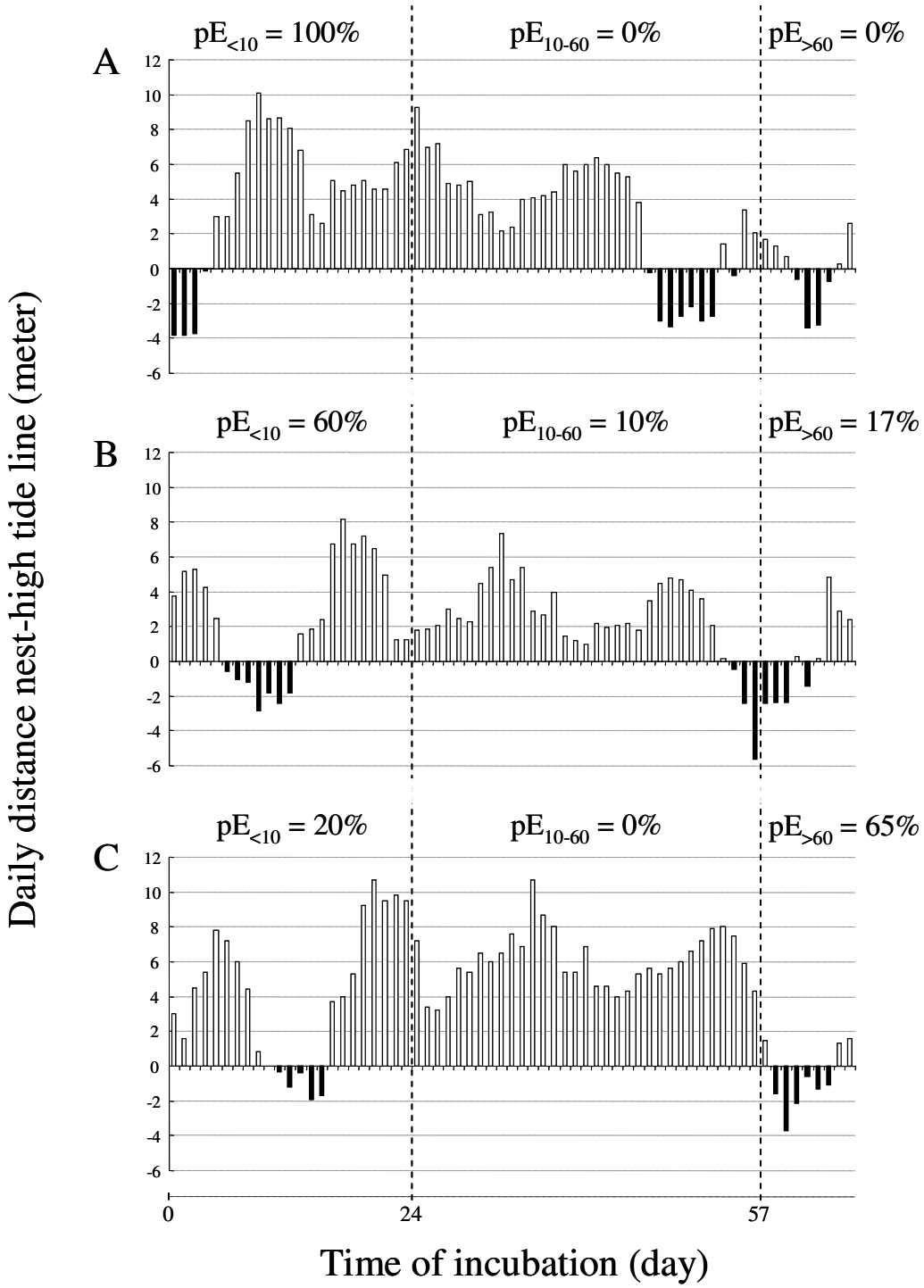

Figure 2. Pattern of inundation in three nests during the 65 days of the study. Daily distances of tide line to the nest are shown for 3 nests having a null hatching success rate but differing in their percentage of the three embryos categories size. These percentages ($pE_{<10}$, pE_{10-60} and $pE_{>60}$) are shown above the time of incubation corresponding to the category. Negative distance nest-high tide line represents inundation events and is highlighted in black.

Statistical analysis

First, generalized linear models (GLM) with binomial error distribution and logit link were performed to assess the effect of inundation on (1) hatching success, (2) percentage of unhatched eggs category $E_{10\text{-}60} + E_{>60}$, and (3) percentage of unhatched eggs $E_{<10}$. Nests were considered as inundated when they were covered by tide at least once during the incubation time.

Second, GLM were performed among inundated nests only to investigate variables responsible for developmental arrest through incubation process. Incubation period was therefore divided in three periods (1 ($E_{<10}$), 2 ($E_{10\text{-}60}$) and 3 ($E_{>60}$), see before). Factors affecting embryo development were examined during each period separately. One model with first-order interactions was fitted for each three period:

G^1 ($E_{<10}$ / number of total eggs) = Level of inundation^1 + first day inundated^1 + interaction + error

G^2 ($E_{10\text{-}60}$ / (number of total eggs- $E_{<10}$) = Level of inundation^2 + first day inundated^2 + interaction + error

G^3 ($E_{>60}$ / (number of total eggs- ($E_{<10}$ + $E_{10\text{-}60}$) = Level of inundation^3 + first day inundated^3 + interaction + error,

where G is a link function and ^{1}, ^{2} and ^{3} corresponding to the different period defined above. Level of inundation and first day inundated were treated as continuous independent variables. As dependent variables were percentages, we used binomial error distribution and logit link function (SAS). The significance of factors and interactions were tested and only significant terms were left in the final model. However, a non-significant term was kept if its interaction with another factor was significant. We started from a complete model on which we applied a backward elimination procedure to obtain the final model, using the following criteria: the variable with the maximum non-significant probability was excluded at each step (Dobson

1990). The final model was attained when all variables retained were statistically significant ($P<0.05$).

The normality of the dependent variables was confirmed prior to the analyses. Computations were performed with SAS package (procedure MIXED, v. 9.1.3, SAS Institute Inc., 1999).

Results

Nests

Over a period of 15 days, we marked and followed 97 nests along a 300 m stretch of beach. At the end of the study, five nests were lost by erosion, six nests to dog predation and two nests to human poaching. Therefore, the remaining 84 nests were analyzed for hatching success and the effect of inundation on embryonic mortality.

On 84 nests, 23 have been inundated at least one day, 55 nests were not inundated and 6 were nests in vegetation. The percentage of nests covered by sea was 31.46% (28/84 nests). We noted a total of 257 embryos > 10 mm (mean 11.17, SE 3.4) and 16 nests with embryos > 10 mm. On 55 nests not inundated, we noted a total number of 150 embryos > 10 mm (mean 2.72, SE 0.34) and 39 nests with embryos > 10 mm. On 6 nests laid in vegetation, we noted 31 embryos (mean 5.17, SE 1.66) and 4 nests with embryos > 10 mm.

Embryonic mortality

We calculated the frequency of occurrence of embryonic size for nest inundated, not inundated and laid in vegetation (Figure 3). We have not shown the embryos < 10 mm because we choose to group them in the category $E_{<10}$. Then we observed different pattern of embryos ditribution:

- The first between 24 and 44 days (corresponding at embryo size 10 to 30 mm) in which there was only one embryo. This was probably due to the fact that we hadn't any nest inundated between the times of incubation 16 to 44 days.

- The second between 44 days and 57 days (corresponding at embryo size 30 to 60 mm), in which there was a majority of embryos from inundated nest.

These two phases corresponds to category of embryos $E_{10\text{-}60}$.

- The last phase between 57 to 68 days (corresponding at category $E_{>60}$), in which we observed an important frequency of occurrence of embryo from all tree categories of nests. We decided to eliminate nest laid in vegetation, because the sample size was small (~5%) and when nests are laid too near the supra-littoral vegetation, the roots may penetrate into the nest chambers and destroy the eggs (Figure 4A).

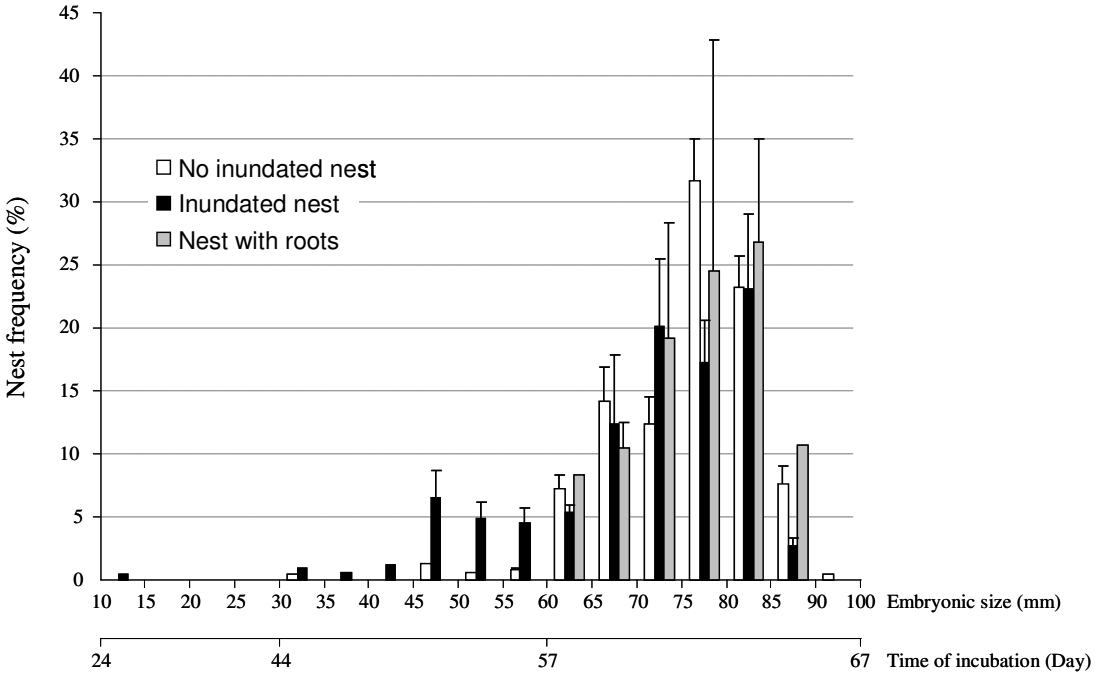

Figure 3. Distribution of embryos size according to the category of nest in which they were encountered (nest inundated or not, nest with roots, mean SE). The x-axis shows both embryonic size and corresponding time of incubation.

Effect of inundation

The hatching success, the percentage of unhatched eggs ($E_{10\text{-}60}$ + $E_{>60}$) and the percentage of unhatched eggs ($E_{<10}$) were significantly different between nest inundated or not (Figure 5). Hatchling success was significantly smaller for nest inundated (GLM, $\chi^2 = 189.08$, $P<0.0001$).

The percentage of unhatched eggs were significantly higher in inundated nests ($E_{10\text{-}60}$ + $E_{>60}$: GLM, $\chi^2 = 198.02$, $P<0.0001$; $E_{<10}$: GLM, $\chi^2 = 121.71$, $P<0.0001$).

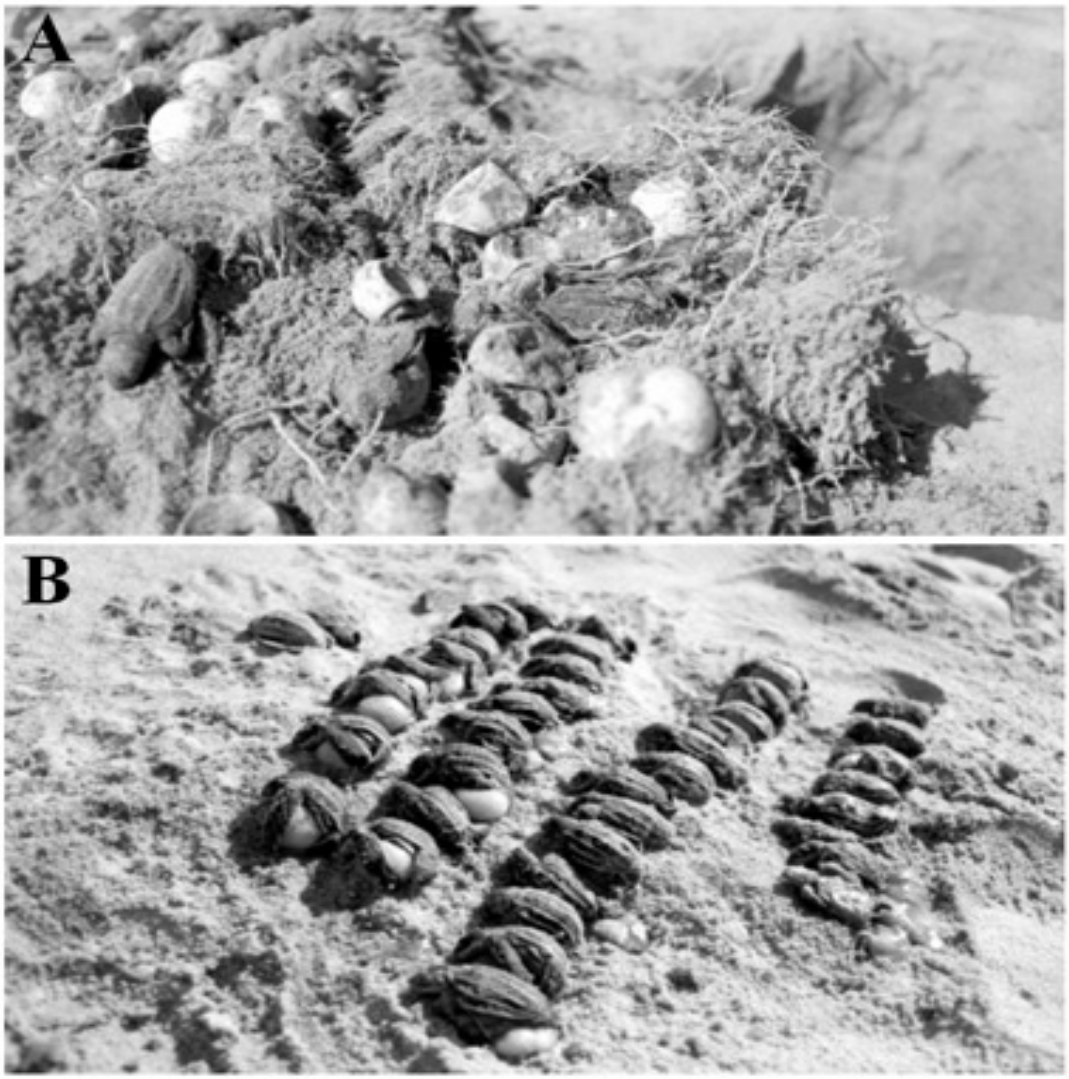

Figure 4. For sea turtles, the survival of the offspring may be strongly related to the distance from the vegetation and the sea. (A) Nest excavated among supra-littoral vegetation may suffer high egg mortality resulting of roots invading the nest chamber. (B) Nests laid too near the water are at higher risk to be inundated and embryonic arrest.

Embryonic arrest vs time of inundation (Table 1)

The GLM model for percentage of unhatched eggs $E_{<10}$: The interaction term between level of inundation x first day inundated was found to be significant ($P < 0.0001$). However, when taken alone, only the level of inundation was significant ($P < 0.0001$). The percentage of unhatched eggs $E_{<10}$ increased with level of inundation and the interaction between level of inundation x first day inundated.

The GLM model for percentage of unhatched eggs $E_{10\text{-}60}$: The interaction term between level of inundation x first day inundated was found to be significant ($P < 0.0001$). Level of inundation and first day inundated were also significant alone ($P < 0.0001$ and $P = 0.0044$ respectively). The percentage of unhatched eggs $E_{10\text{-}60}$ increased with level of inundation and the interaction between level of inundation x first day inundated.

The GLM model for percentage of unhatched eggs $E_{>60}$: The interaction term between level of inundation x first day inundated was found to be significant ($P = 0.0181$). However, when taken alone, only the level of inundation was significant ($P = 0.0452$). The percentage of unhatched eggs $E_{>60}$ increased with level of inundation during this period.

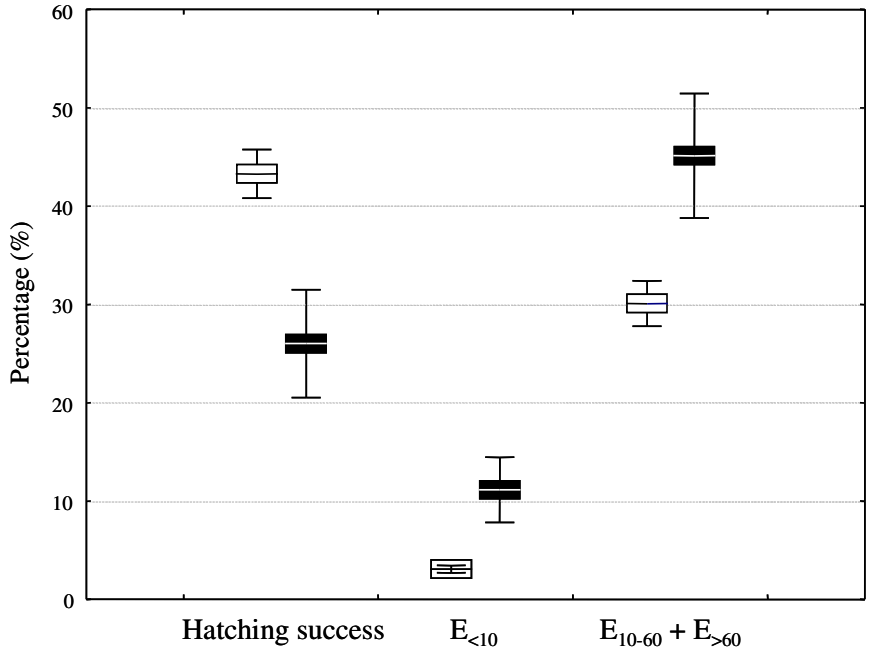

Figure 5. Effect of inundation on hatching success, percentage of embryos $E_{<10}$ and $E_{10\text{-}60} + E_{>60}$ (mean, SE). Inundated nest are represented in black and no inundated nests in white.

Discussion

In marine turtles, the physical conditions experienced by the egg during incubation in the nest have been shown to strongly affect embryonic development and hatching rate (Mortimer 1990; Ackerman 1997; Marco *et al.* 2005). Temperature and moisture are the most general factors in this regard and thermal and hydric conditions in the nest are highly linked to

the nest location on the beach (Wood & Bjorndal 2000). Nest site selection have already been correlated with egg developmental success (Caut *et al.* 2006; Foley *et al.* 2006). As female marine turtles do not provide parental care to eggs nor offspring, female's nesting behaviour and nest site selection are important factors that can largely affect the survival of embryos and juveniles (Kamel & Mrosovsky 2004).

Table 1. Variations of the percentage of dead embryos found in nests at the end of the incubation time. Three models were tested according to the size of embryos related to the period in which an inundation event occured.

Models	**dF**	**F/χ^2**	**P**
E<10			
Level of inundation	1	175.15	*<0.0001*
Fisrt day inundated	1	1.91	0.1668
Interaction	1	70.46	*<0.0001*
E10–60			
Level of inundation	1	23,37	*<0.0001*
Fisrt day inundated	1	8,12	*0,0044*
Interaction	1	24,2	*<0.0001*
E>60			
Level of inundation	1	4,01	*0,0452*
Fisrt day inundated	1	0,39	0,5325
Interaction	1	5,58	*0,0181*

Leatherback turtles have been shown to nest closely to the tide line than other marine turtles; the reason for this behaviour could be their large size and the lower speed of the hatchling making the distance travelled on the beach more difficult than for green turtles and may partly explain the tendency for leatherbacks to nest closer to the sea (Whitmore & Dutton 1985). Consequently, if beaches are relatively narrow, most of the clutches will be nest to close to the tide line and inevitably a large proportion will be loose by erosion or inundation.

Our results first showed that a lot of nests were inundated at least once during the incubation periods. Those nests were not automatically associated with null hatching success but presented a higher proportion of dead embryos that in non inundated nests or nests with roots. This result highlights the existence of the effect of inundation on developmental

embryonic arrest. We showed that embryonic mortality (stage and proportion) was linked with the time of the immersion event, the frequency and the level of immersion.

Effects of inundation

The effects of the nest hydric environment and immersion during incubation have been largely studied in reptiles (Losos *et al.* 2003) but not much in marine turtle, for which studies concerns generally *Caretta caretta* or *Chelonia mydas* (Mortimer 1990; Foley *et al.* 2006; Ozdemir et al. 2008). The effect of moisture and/or immersion on eggs of leatherback turtles has for example never been studied while their nests are the most likely to be inundated. According to Ackerman *et al.* (1974), the main reason for the failure of development during flooding appears to be due to gas exchange limited or stopped when the eggs are in an environment saturated with moisture. Indeed, hatching success of loggerhead turtle has been shown to be decreased when water content increased this way (Foley *et al.* 2006).

Hydric environment includes water salinity, moisture content, stage and frequency of immersion. Salinity of the nest may be related to rainfall and occasional tidal inundation but immersion in salt water hasn't been shown to have effect on young eggs of lizard (Losos *et al.* 2003). Moreover, direct inundation of nest could also cause embryonic mortality by asphyxiation (Foley *et al.* 2006). Mortality during inundation may rely on the stage of development at which inundation occurs. Our results showed a relationship between time of incubation and embryonic arrest. Early and late embryonic stages were identified as the critical periods in the embryonic development of turtles (Girondot *et al.* 1990, Ozdemir *et al.* 2008). The present study confirmed and is in agreement with these results, since embryonic mortality was higher in both of these stages than during the middle stage. Bell *et al.* (2003) studied the development of leatherback embryos in the laboratory and showed that early stages appeared to be critical. Whitmore & Dutton (1985) and Wyneken *et al.* (1988) also found a high proportion of early embryonic mortality in leatherbacks, and Blanck & Sawyer

(1981) found that 52.1% of unhatched loggerhead eggs in their hatchery contained embryos in the precarapace stage. Moreover the decomposition of early stage embryos to an unrecognizable state, which resulted in underestimate and classification of eggs as "unknown or undeveloped" (it was the explication of your category $E_{<10}$). For the late stage, the sensitive period for temperature dependent sex determination begins and corresponds to the time of most rapid growth and organogenesis. The development of an embryo involves the synchronization of complex physiological processes. If one or more of these processes are slowed or disrupted by fluctuating temperature or hydric environment, the embryo may become deformed or not develop at all (Packard & Packard 1988; Morris *et al.* 1983). This stage seems sensible for all nest in general, we found this stage in each nest (inundated or not, nest with roots), but higher for inundated nest. We found also that mortality during inundation could also be largely dependent of the frequency and the length of inundation. However, inundated nest have already been seen to show high hatching rate suggesting that nests in marine turtles have a certain tolerance to immersion and can survive a substantial period of time in saltwater. The figure 2 provides an interesting basis to understand visually what occurred during the incubation period for three nests differing in their proportion of embryo categories. When nests are strongly inundated at the very beginning of the incubation (as nest A in figure 2), hatching success is null with $pE_{<10}$=100%; this period of immersion is so important that it caused the death of all eggs. The embryos at this stage are well known to be very sensitive in the leatherback (Girondot *et al.* 1990). When the inundation event occurs with a lower level and later in the incubation, eggs will begin to develop, and $pE_{<10}$ will be lower. But if a second inundation event occurs, hatching success will be strongly impacted and high proportion of $pE_{10\text{-}60}$ and $pE_{>60}$ observed (as nest B in figure 2). Finally, a nest with a light inundation at the beginning but a very strong one at the end, will also present a null hatching success, but dead embryos will be large corresponding to a late developmental arrest,

i.e. a high proportion of $pE_{>60}$ (as nest C in figure 2). Indeed, late stage has also been described as a sensitive stage for leatherback embryos (Girondot *et al.* 1990).

Calcium is another factor linked to inundation and that has already been shown to affect development. Calcium is a major component of the eggshell (Ewert et al. 1984; Packard et al. 1984*a*, 1984*b*) and is leached from the shell during immersion (Seymour *et al.* 1997). Eggshell is an important source of calcium for the developing embryo (Packard 1994), and reduced calcium availability may limit embryonic growth. The loss of calcium potentially alters eggshell permeability too and leads to higher rates of water exchange. Concomitant with decreased sources of Calcium and higher permeability of eggshell to water (leading to water uptake by the yolk and increases in yolk mass and size), immersion also represents an extended period of low oxygen levels for the embryo (Kennett *et al.* 1998). Therefore, prolonged immersion of eggs has been hypothesized to have consequences for the developing embryo that are measurable in terms of hatching success and hatchling size. Specifically, hatching success have been predicted to decrease with an increased in the immersion period (Kennett et *al.* 1998).

In our study, only nests covered by tide once or more were included in the analysis of effect of inundation on embryonic development. However, nests that have not been covered by tide could also suffer from adverse effect of inundation because of immersion due to the water table below the surface of the beach. The water table is the level at which the ground water pressure is equal to atmospheric pressure, and is above the mean sea level (Li et al. 1997). Our investigation didn't assess the height of the water table and therefore underestimated the number of nests concerned with deleterious effects of immersion.

Nesting beaches threatened

The nest placement has important consequences for offspring survival, it is likely that this behaviour is or has been under strong selection. If nest site selection has a genetic basis,

then individual females should be consistent in their particular choice of nesting sites; that is, their choices should be repeatable (Boake 1989). The significant repeatability of nest site choice with respect to distance from the high tide line suggests that this behaviour may show evolutionary potential. In this case, it appears that leatherbacks may have the opportunity for further evolution of nest site choice in response to selection. This is particularly important in the context of current environmental changes and habitat destruction and alteration. Many of these areas are particularly susceptible to coastal hazards such as storm surges and coastal erosion and, with the projected rise in sea level resulting from anthropogenic global warming, threats to coastal areas are increasing (Huang 1997). In addition to nest location, choice of beach is also important for nesting females. Recent genetic studies support the suggestion that females of some turtle species return to nest on their natal beaches (Lahanas *et al.* 1994; Dutton *et al.* 1999; Hatase *et al.* 2002). If natal beaches and those in the surrounding area are altered so that they no longer offer suitable nesting areas, this could reduce the reproductive success, and ultimately the size, of local nesting populations. Although it is possible that other beaches in the area could be used, extensive coastal development is occurring regionally, and alternatives are likely to become increasingly scarce. Until critical habitat can be more clearly identified, it is essential from a conservation standpoint that choice of beach and new site are not limited because reproductive success could be compromised.

Acknowledgments

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Chapitre 3

Isotope Analysis Reveals Foraging Area Dichotomy for Atlantic Leatherback Turtles

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Isotope Analysis Reveals Foraging Area Dichotomy for Atlantic Leatherback Turtles.

S. Caut*, E. Guirlet*, E. Angulo, K. Das, M. Girondot

Abstract

Background. The leatherback turtle (*Dermochelys coriacea*) has undergone a dramatic decline over the last 25 years, and this is believed to be primarily the result of mortality associated with fisheries bycatch followed by egg and nesting female harvest. Atlantic leatherback turtles undertake long migrations across ocean basins from subtropical and tropical nesting beaches to productive frontal areas. Migration between two nesting seasons can last 2 or 3 years, a time period termed the remigration interval (RI). Recent satellite transmitter data revealed that Atlantic leatherbacks follow two major dispersion patterns after nesting season, through the North Gulf Stream area or more eastward across the North Equatorial Current. However, information on the whole RI is lacking, precluding the accurate identification of feeding areas where conservation measures may need to be applied.

Methodology/Principal Findings. Using stable isotopes as dietary tracers we determined the characteristics of feeding grounds of leatherback females nesting in French Guiana. During migration, 3-year RI females differed from 2-year RI females in their isotope values, implying differences in their choice of feeding habitats (offshore *vs.* more coastal) and foraging latitude (North Atlantic *vs.* West African coasts, respectively). Egg-yolk and blood isotope values are correlated in nesting females, indicating that egg analysis is a useful tool for assessing isotope values in these turtles, including adults when not available.

Conclusions/Significance. Our results complement previous data on turtle movements during the first year following the nesting season, integrating the diet consumed during the year before nesting. We suggest that the French Guiana leatherback population segregates into two

distinct isotopic groupings, and highlight the urgent need to determine the feeding habitats of the turtle in the Atlantic in order to protect this species from incidental take by commercial fisheries. Our results also emphasize the use of eggs, a less-invasive sampling material than blood, to assess isotopic data and feeding habits for adult female leatherbacks.

INTRODUCTION

Yalimapo beach in French Guiana, South America, is one of the main nesting grounds for the largest of the sea turtles, the leatherback (*Dermochelys coriacea*), visited by up to 30–40% of the world's population of nesting females [1]. The major nesting season at Yalimapo extends from March to the end of August, including a peak in June, with some sporadic nesting outside this period [2]. During the nesting season a female may lay as many as 14 clutches, with an average interval between nesting events of 10 days [1]. Due to the segregation of breeding and foraging sites, at the end of the reproductive season leatherbacks migrate several thousand kilometers [3,4] across ocean basins to pelagic foraging areas where their prey, large jellyfish and other gelatinous organisms, is more abundant than on tropical coasts [5–7]. The leatherback is the only marine turtle that feeds on gelatinous zooplankton throughout its life. This diet is extremely poor in lipids and energy, and consequently very little energy and nutrient is extracted from a given mass of prey [5]. Davenport [5] estimated that leatherbacks in cold sea water (e.g. North Atlantic) consume a quantity of prey equivalent to at least 50% of their body mass per day.

During the breeding season, energy requirements are high because of egg production, nest construction, and swimming activity between nesting events [8]. However, leatherbacks appear to adopt different strategies during their inter-nesting intervals, depending on their nesting site [9]. In the Eastern Pacific Ocean (e.g. Costa Rica and Mexico rookeries) leatherbacks may minimize energy expenditure to maximize the amount of energy allocated

to ovipositing and egg production [8,10]. In the Atlantic Ocean and Western Pacific Ocean (Indonesia rookery) leatherbacks cover great distances at high speed and disperse extensively, probably for feeding [9,11-14]. However, the majority of studies on the feeding ecology of leatherback turtles have been based on dive patterns during inter-nesting intervals, and not on diet analysis. Diving activity during the migration cycle is closely related to foraging activity on pelagic prey [15], whereas diving during inter-nesting intervals is less well understood [16]. Hays *et al.* [6] showed that dive duration varied with foraging success; dives were much longer in feeding areas, where foraging success was higher, than along tropical coasts, which provided limited foraging opportunities.

The time between successive nesting seasons for a female is called the remigration interval (RI). The RI for leatherback turtles is variable, but is most commonly 2 or 3 years [17]. The reasons for variation in the RI are unknown [18]. It seems that female turtles require a specific level of energy reserves prior to migration to nesting beaches, in order to undergo vitellogenesis and nest successfully [19-21]. They may delay reproductive migration until a reproductive energy reserve threshold is reached [22]. Availability of nutrients, particularly in aquatic systems, has been shown to be affected by climatic oscillations such as the El Nino Southern Oscillation (ENSO) and the North Atlantic Oscillation (NAO) [18,21]. This implies that abundance and distribution of gelatinous prey for leatherbacks are spatially and temporally unpredictable [18]. Thus, the RI would depend on the capacity of breeding turtles to find favorable foraging areas.

Recent satellite transmitter data has revealed that Atlantic leatherbacks follow two major dispersion patterns after nesting, either through the North Atlantic area or more easterly at low latitudes across the North Equatorial Current [3,4,23]. It has been suggested that the RI could be heavily influenced by ecological conditions in turtle foraging areas, such as surface temperature or trophic status [24,25]. It may be that dispersion patterns are linked to the RI of

female turtles, with varying ecological conditions and varying proximity of foraging areas to nesting beaches determining the specific RI for each foraging area.

Individuals that exploit geochemically different habitats, or feed on different resources, can be differentiated using stable isotope measurements, as the isotope profile of consumers reflects that of their prey. This approach is based on the fact that stable isotope ratios of nitrogen ($^{15}N/^{14}N$, noted $\delta^{15}N$) and carbon ($^{13}C/^{12}C$, noted $\delta^{13}C$) in the consumer tissues reflect those in their resources in a predictable manner due to selectivity for lighter isotopes during a consumer's metabolic processes [26,27]. The difference between isotopic values of consumer and their preys, called discrimination factor, vary among tissues and taxa, but is often between 0-1‰ for $\delta^{13}C$, and 3-4‰ for $\delta^{15}N$ [26-28]. Also, isotope analysis offers advantages over traditional methods (e.g. direct observation of feeding behavior, gut content analysis) because it provides time-integrated information on foods assimilated. The period of time over which the tissue isotope values of a consumer reflect the values of their diet is called the turnover rate. Tissues such as liver and plasma have high turnover rates that reflect recent diet, whereas tissues with slower turnover rates, such as blood cells and muscle, reflect diet over longer periods [29,30]. Stable isotope measurements have also been used to infer estimates of trophic level and animal movement patterns for both invertebrates and higher vertebrates [31]. Consumers are typically enriched in ^{15}N relative to their food and consequently $\delta^{15}N$ measurements serve as indicators of a consumer's trophic position [32,33]. By contrast, $\delta^{13}C$ values vary little along the food chain and are mainly used to determine primary sources in a trophic network [32,34]. In the marine environment, the $\delta^{13}C$ values can also indicate inshore versus offshore, or pelagic versus benthic contribution to food intake [35-39]. This difference may be related to the tendency of $\delta^{13}C$ values to decrease from low to high latitudes, due to oceanographic factors such as CO_2 concentration effects on carbon fixation by phytoplankton [40,41].

The use of stable isotope has become a powerful tool for clarifying questions about nutritional ecology and migration of marine vertebrates [42]. However, relative to other taxa, there is a paucity of stable isotope studies in marine turtles. The first was Godley *et al.* [43] that determined trophic status of marines turtles from the Mediterranean Sea and the European Atlantic Ocean. Since then, analyses of isotopes were used to study respiratory physiology [44], feeding habitat in Japan [45,46], western Mediterranean [47,48] and bahamas [49] and trophic dichotomy between ocean basins [41].

In this paper we present the first isotopic analysis of leatherback turtle nesting in French Guiana and inferred from isotopic leatherback data collected several times on the same females during a nesting season. We hypothesized that females with different RIs forage at different locations, and differ in the isotopic values of the tissues subject to a low turnover (Red Blood Cells - RBC). Our hypothesis predicted that females with 3 and 2 year RI exploit geochemically different habitats varying in latitude (North Atlantic *vs.* West African coast) and/or origin of carbon source (offshore *vs*. coastal). We also tried to investigate whether females forage in the breeding areas. Finally, we examined the relationship between stable isotope values of eggs and female tissues to assess egg analysis as a less-invasive method of sample collection for isotope measurements in this endangered marine turtle.

RESULTS

From March to May 2006, we sampled 52 leatherback females at Yalimapo beach in French Guiana: 1 female sampled for 5 different clutches (interval between first and last clutch: 44 d), 7 females for 4 clutches (minimum and maximum interval between the first and the last clutch: 27-49 d), 1 female for 3 clutches (40 d), 14 females for 2 clutches (9-43 d) and 29 females for 1 clutch. The different clutches were not necessarily consecutive; For example, a female that was sampled for 4 clutches during 49 days laid 6 clutches during this period, but

2 were not sampled. Among the turtles 23 had been tagged and recorded in a passive integrated transponder tags (PIT) database, which showed that 7 had a 3-year RI and 16 had a 2-year RI.

The curved carapace length (CCL) of sampled females ranged from 142–172 cm (mean ± SE = 160 ± 1 cm, n = 48). A preliminary analysis showed that CCL had no effect on $\delta^{13}C$ and $\delta^{15}N$ in any tissue ($P > 0.5$).

Two eggs were sampled from each of three clutches to assess intra-clutch variability in isotope values. Differences between the values for the 2 eggs within a clutch were of the same order as the measurement error, ranging from 0.10–0.15‰ for $\delta^{13}C$ and 0.07–0.15‰ for $\delta^{15}N$. Despite the small sample size, we assumed that a single egg-yolk reflected the $\delta^{13}C$ and $\delta^{15}N$ of the whole clutch.

Do females forage during the breeding season? We tested whether tissue isotope ratios varied over the nesting period by performing general linear models, with repeated measures of $\delta^{13}C$ and $\delta^{15}N$ for each tissue in relation to the time (in days) of each clutch after the first clutch was observed. The number of eggs in each clutch was included as a covariable, but had no effect on $\delta^{13}C$ and $\delta^{15}N$ for any tissues ($P > 0.2$ in all cases). The time of each laying event had no effect on $\delta^{15}N$ values for any tissue (Table 1A). Time also had no effect on the $\delta^{13}C$ value for RBC and plasma, but it had a significant effect on the $\delta^{13}C$ value for egg-yolk (Table 1A). $\delta^{13}C$ values in egg-yolk decreased significantly from one clutch to the next. Thus, except for egg-yolk $\delta^{13}C$ values, isotope values of females did not change significantly over the breeding season (Fig. 1). The mean (± SD) isotope values of the whole body of the jellyfish sampled at the beach during the breeding season were very high for carbon and similar for nitrogen (−15.8‰ ± 1 and 9.3‰ ± 1.4, *n*=5, respectively) compared with the values for blood (RBC: −18.8‰ ± 0.1 and 9.5‰ ± 0.2, plasma: −21.1‰ ± 0.1 and 10.2‰ ± 0.2, *n*=63, respectively) and for egg-yolk (-18.83‰ ± 0.42 and 11.16‰ ± 1.26, *n*=81).

Table 1. Variations of isotopic values of three different tissues. Variations with (A) the time in days between nesting events and the number of yolked-eggs and (B) the remigration interval. (C) Relationships between egg-yolk isotopic ratios and RBC and plasma isotopic ratios. Significant results are marked in bold.

	Dependent variables →		$\delta^{13}C$		$\delta^{15}N$	
	Independent Variables↓	*N*	F/χ2	P	F/χ2	P
A	Egg-yolk	*81*				
	Number of eggs		0.60	0.437	0.15	0.702
	Days between nesting		6.15	***0.013***	0.17	0.681
	Plasma	*63*				
	Number of eggs		0.11	0.736	0.87	0.352
	Days between nesting		0.06	0.800	1.51	0.219
	RBC	*63*				
	Number of eggs		1.29	0.256	0.20	0.653
	Days between nesting		0.00	0.990	2.95	0.086
B	Egg-yolk	40				
	Remigration interval		2.10	0.156	0.57	0.453
	Plasma	*31*				
	Remigration interval		4.25	0.054	<0.01	0.989
	RBC	*31*				
	Remigration interval		10.22	***<0.01***	<0.01	0.973
C	Egg-yolk	*50*				
	Plasma		122.27	***<0.001***	178.60	***<0.001***
	Regression equation		$\delta^{13}C_P=4.66+1.37\delta^{13}C_{Egg}$ ($R^2=0.84$)		$\delta^{15}N_P=1.58+0.81\delta^{15}N_{Egg}$ ($R^2=0.89$)	
	RBC		138,3	***<0.001***	24.54	***<0.001***
	Regression equation		$\delta^{13}C_{RBC}=3.72+1.19\delta^{13}C_{Egg}$ ($R^2=0.86$)		$\delta^{15}N_{RBC}=2.76+0.64\delta^{15}N_{Egg}$ ($R^2=0.64$)	

Do females with 2-year and 3-year RIs differ in their isotope values? Females with 2-year and 3-year RIs differed significantly in the $\delta^{13}C$ values in RBC. In plasma the difference between the two groups was nearly significant for $\delta^{13}C$ (Table 1B). However, $\delta^{15}N$ values in RBC and plasma remained similar between turtles with 2-year and 3-year RIs (Table 1B, Fig. 2). Isotopic values ($\delta^{13}C$ and $\delta^{15}N$) of egg-yolk were not different between females with 2-year and 3-year RIs (Table 1B).

Relationship between egg-yolk and female blood isotope values. Egg-yolk $\delta^{13}C$ and $\delta^{15}N$ were positively correlated with the corresponding RBC and plasma $\delta^{13}C$ and $\delta^{15}N$ values for females (Table 1C).

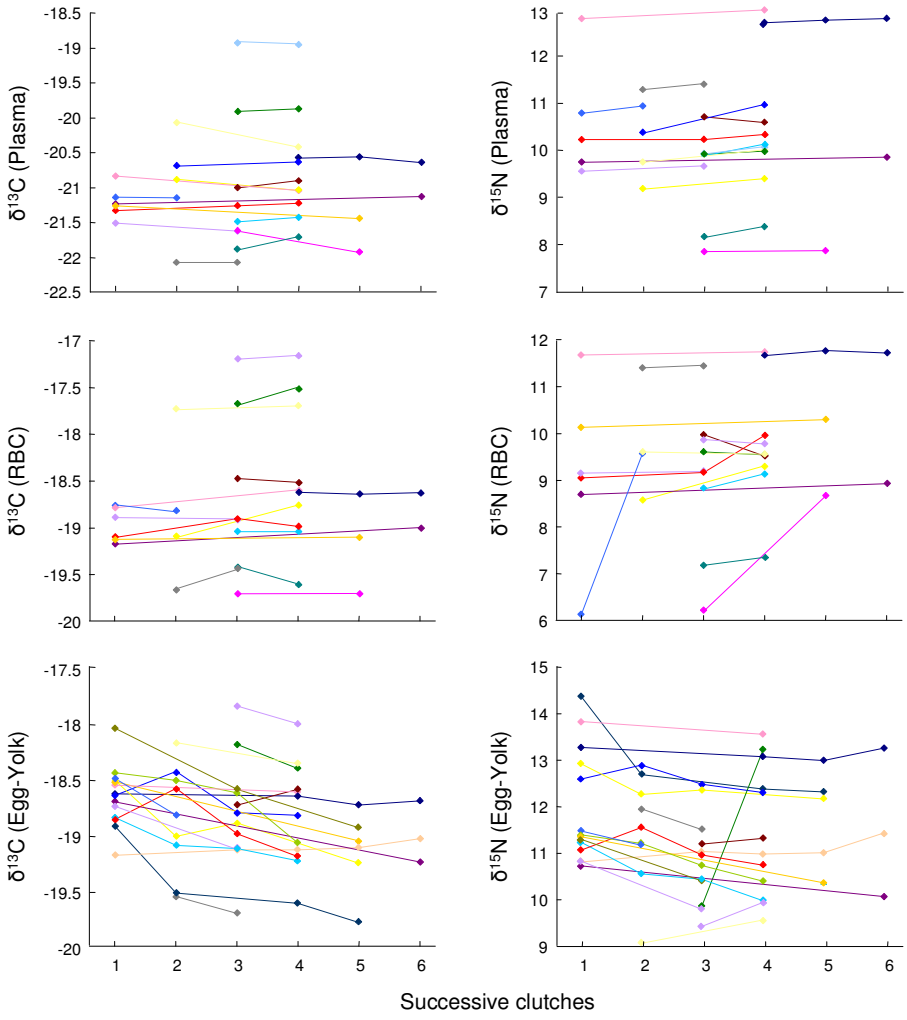

Figure 1. Trends in isotopic values for $\delta^{13}C$ and $\delta^{15}N$ in plasma, RBC, and egg-yolk of nesting leatherback turtles. Each colour represents data for the successive clutches laid by one female; each sampled clutch is represented by a point.

DISCUSSION

Do females forage during the breeding season? Several studies using techniques such as video recording, mouth sensors and depth recorders have failed to adequately elucidate the feeding habits of leatherback turtles during the nesting season [10,12,16]. Diving patterns

tended to show that Atlantic leatherback females feed during internesting intervals [9,13], and foraging was suggested in southern Caribbean females (although with low success, [12]). Conversely, video recording showed no foraging during the breeding season in Costa Rica [10]. However, with one exception in a foraging area (in the north-west Atlantic [50]), no study has involved diet analysis because this species is difficult to study.

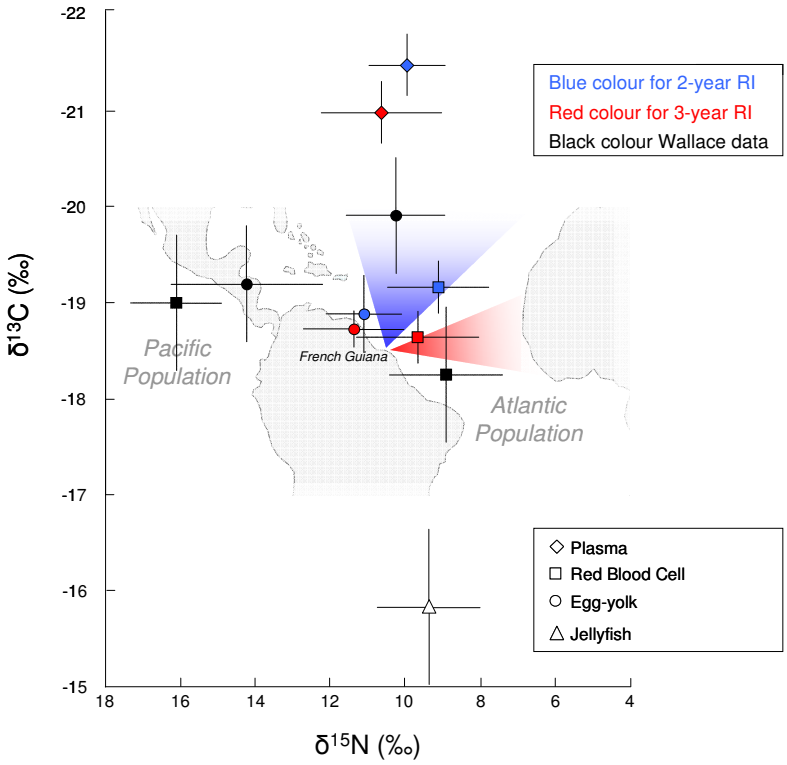

Figure 2. Effect of remigration interval (RI) on $\delta^{13}C$ and $\delta^{15}N$ leatherback tissus (plasma, RBC and egg-yolk). Values are mean ± SD. The black symbols represent the values of Wallace *et al.* [41] for Atlantic and Pacific populations of leatherback turtles, provided for comparison. The map shows the two major patterns of migration of Atlantic turtles, from nesting beaches to foraging areas, following Ferraroli *et al.* [3] and Hays *et al.* [23].

The use of stable isotope ratios has become a powerful tool to estimate and clarify the feeding ecology of many vertebrate, especially when species are particularly difficult to study by traditional methods [31]. The stable isotope ratios can provide new insights into the foraging activity of females during the breeding season, but measuring turnover rate and discrimination factor (the isotopic difference between the animal and its food source) in turtles'tissue is a necessary process to enable a correct interpretation. Stable isotope values for

plasma have been shown to represent more recent dietary resources in bears [51]. However, there is a lack of information on plasma turnover rates for turtles. Seminoff *et al.* [52] compared plasma values in green sea turtles (*Chelonia mydas*) at 371 and 614 days in a captivity study, and found that turnover was at least 371 days. However they did not show the dynamic of the turnover. Seminoff *et al.* [53] estimated that nitrogen turnover rate in plasma of freshwater turtles, *Trachemys scripta*, was 142 d increasing linearly, with a half-life (the time in which half of the stable isotopes were exchanged in the tissue) of 35.6 days. Based on these unique data on turtles and knowing that the longest nesting season observed during our study for a leatherback female was 49 days, we can suppose that isotopic turnover of plasma in the leatherback turtles sampled may have not been completed, but that a change in isotopic values could have been detected. However, no significant variation in plasma $\delta^{13}C$ and $\delta^{15}N$ throughout the breeding season was detected.

Parallel measures of isotope values in jellyfish sampled on the nesting beach did not indicate food intake near the beach. Massive strandings of two jellyfish genera (*Rhizostoma* and *Aurelia*) are regularly observed on Yalimapo beach, and they are known to be a common prey of leatherback turtles [50]. If turtles had eaten and begun to assimilate this prey, the isotopic value of plasma would have been -15.9‰ and 12.2‰ for carbon and nitrogen respectively (based on discrimination factors estimated on green turtle [52]). Even if the turnover was not reached, the time frame of our study (a maximum interval of 49 d) should have allowed a change in isotopic values as it represents the half-time of turnover in plasma of emydid turtle [53]. During this interval, emydid turtles increased 0.9‰ and 2.4‰ for carbon and nitrogen respectively, while leatherbacks increased only 0.1‰ and 0.11‰; this result seems to indicate that leatherbacks did not feed during this period.

A further complicating factor to interpret isotopic data is the nutritional stress [34,54]. It is generally accepted that tissues of fasting animals become enriched in $\delta^{15}N$ following

nutritional restriction [54-56]. Hobson and Clark [57] explained that increases in diet-tissue fractionation values are due to mobilization and redeposition of proteins elsewhere in the body or amino acid composition changes in tissues. Following this argument, it could be expected $\delta^{15}N$ in mobilized organic molecules to be higher in comparison with the initial state of the organic molecules in the body reserves. Voigt and Matt [58] gave another possible explanation: the different extent of metabolic processing of two nitrogen sources, i.e. internal body reserves (fat) and external food sources (carbohydrates), may lead to different nitrogen enrichments. However, our inability to detect such an increase may also be due to the species of study. Hobson and Clark [57], as well as Hobson *et al.* [54], documented an increase in $\delta^{15}N$ values with nutritional stress in several avian species. Kempster *et al.* [59] found that a 35% reduction in food intake had no effect on $\delta^{15}N$ values of tissues in song sparrows (*Melospiza melodia*). Williams *et al.* [60] found whole blood and blood cells from nutritionally restricted tufted puffin (*Fratercula cirrhata*) nestlings were significantly depleted in ^{15}N compared to well-fed conspecifics. Thus, the effect of nutritional restriction could be dependent of species-specific differences in physiological response [60] or largely a function of the level of nutritional stress [59].

While past experiments on mammals, birds, fishes, and insects have shown changes in stable isotope ratios due to nutritional stress, there has been no research on this topic in reptiles. The detection of nutritional stress in wild population is very difficult compared to experimental studies. Indeed, the short timeframe of our samples in relation to the slow turnover rate of turtle blood, the particular physiological characteristic of the endothermic capacity of leatherbacks [5], the relatively low metabolic rates between nesting events [8] and the few data on foraging at this period [9,10] make it difficult to establish a conclusion concerning fasting or foraging activity of females during nesting season [53]. Based on these

considerations, it is obvious that this point would benefit from further studies on the isotopic values of sea turtles.

Remigration intervals and foraging sites. Leatherback turtles undertake long migrations between nesting sites and foraging grounds. The causes of variation in the RI of female leatherbacks are unknown, but may be linked to the availability of food sources and thus the ability to store enough energy resources to undertake migration to breeding areas [25]. Stable isotope analyses can offer additional information on feeding strategies or movement patterns of migratory species [31,34,61]. The stable isotope composition of an organism depends on the diet source and also on the isotopic signature at the base of the food web [26,27,62]. Indeed, different oceanic processes affect isotopic baselines of $\delta^{15}N$ and $\delta^{13}C$ [61]; spatial and temporal variability has been shown to be incorporated and conserved through several trophic levels across ocean basins or within a region of a single basin [41,56,63]. Hobson [42] illustrated this approach by the new maxim ‘‘you are what you swim in’’ that complements the well-known dogma of stable isotope ecology ‘‘you are what you eat’’ [64]. Consequently, the stable isotope ratios of animal tissues have the potential of characterizing isotopically distinct regions.

Leatherback females in French Guiana (2-year and 3-year RI) did not differ in their $\delta^{15}N$ values. Wallace *et al.* [41] showed that $\delta^{15}N$ in leatherback turtles indicates oceanographic differences, as the distinct nitrogen cycling regimes among oceans influences the baseline $\delta^{15}N$ signatures of marine food webs [41]. Thus, the $\delta^{15}N$ values of egg yolk and RBC were significantly different between an Atlantic population (St Croix) and a Pacific population (Costa Rica, more enriched by 4.5 and 7.2‰, respectively). In this sense the $\delta^{15}N$ values for French Guiana turtles were more similar to the St Croix population (Fig. 2). The turtles in our study had a broad range of $\delta^{15}N$ values (e.g. $\delta^{15}N$ for RBC = 6.1−12.3‰), which probably reflects different feeding strategies on gelatinous zooplankton: leatherbacks are

known to feed on planktivorous jellyfish (*Rhizostoma octopus*, *R. pulmo*, *Aurelia aurita, Stomolophus meleagris*) or jellyfish that forage on fish or crustaceans (*Cyanea capillata*, *C. lamarckii*, *Pelagia noctiluca*) [65].

Although individual females may show a degree of subregional fidelity to areas such as northward to the Gulf Stream area or eastward to tropical waters [3,23], Atlantic leatherbacks could disperse widely across most of the ocean basin but have not yet been tracked across the equator [66]. During their remigration interval, several foraging grounds have been identified: North Atlantic ocean as Scotia Nova, East Costs of USA, Ireland costs, Bay of Biscay or more southern in West African and Iberian coasts [9,11,19,50,67-69]. However, two main migration patterns have been showed: northward to the Gulf Stream area or eastward to tropical waters [3]. We hypothesized that these patterns could be linked to different ecological foraging areas, and that this could explain differences in RI between females foraging in those areas. C isotope ratios are conservative from phytoplankton up to top consumers with less than 1‰ enrichment per trophic level [62] and hence are ideal to trace gradients in the marine environment, as the signature of a consumer should reflect the sources of C at the base of the food chain. The $\delta^{13}C$ values in the tissue of marine animals have been shown to vary with latitude (e.g. [34,63]), reflecting the depletion of ^{13}C in phytoplankton towards higher latitudes [61,70]. Moreover, the general pattern of inshore, benthically linked food webs being more enriched in ^{13}C compared with onshore, pelagic food webs presents a potentially useful tool for marine biologists. Also, stable isotope analysis has increasingly been used to infer animal movement patterns from invertebrate to higher vertebrate (see reviews, [31,34,42]). Differences in $\delta^{13}C$ blood values between 2-year and 3-year RI females suggest that the French Guiana leatherback population segregates into two distinct isotopic groupings, and seem to confirm that RI is linked to foraging areas. As isotope signatures could provide information on both the latitude, and the pelagic *vs.* neritic nature of

the foraging ground, it is possible to reveal foraging area dichotomy for 2-year and 3-year RIs. The low RBC $\delta^{13}C$ values in the 2-year RI turtles suggests that the C resource of these turtles is situated in a more northern and/or offshore region (high latitude in North Atlantic), and the high values of the 3-year RI turtles indicate a more southern and/or coastal foraging area (West African and Iberian coasts). Confirming these conclusions, maps of gelatinous organism distribution provide information on prey location and indicate that these foraging areas (central North Atlantic and west coasts of Africa) support several appreciable aggregations of potential prey, especially due to the presence of upwelling and frontal areas [11,71]. Moreover, leatherbacks are generally considered completely pelagic, but are also observed in coastal waters when food is available [11,72].

Use of egg yolk isotope signatures

Stable isotope ratios in the diet become incorporated into egg yolk in 8–15 days in birds [73], but could require more time in reptiles due to metabolic differences. Female leatherbacks arriving at the nesting beach carry a full complement of follicles to supply yolks for all the eggs that will be laid during the season [74]. Vitellogenesis probably lasts 3–6 months and is complete upon arrival at nesting sites before mating begins [75]. Thus, the energy and chemical components contained in the follicles were derived from food eaten in the foraging area [74]. Hobson [73] showed in birds that following a change of diet the isotope signature of yolk was proportional to the additional mass of yolk formed from the new diet. The situation in sea turtles is more complex. Before each nesting event yolk is formed from dietary sources during rapid follicle growth, and so could reflect the contribution of a new diet to isotope values. In our study, the $\delta^{15}N$ values in egg yolks were not significantly different among serial clutches from one turtle, but the $\delta^{13}C$ values were significantly different and tended to decrease from one clutch to another. No explanation for these different trends is apparent. If females had fed on jellyfish at Yalimapo beach and incorporated this C

source into egg yolk, the trend of egg yolk $\delta^{13}C$ values should have been reversed. Hatase *et al.* [46] found opposite results in green turtles (*Chelonia mydas*), with $\delta^{13}C$ not significantly different and a significant enrichment in $\delta^{15}N$ egg yolk values among five serial clutches from one turtle. This enrichment could not be attributed to nutritional stress from fasting [46]. Hatase *et al.* [45] found no significant difference in $\delta^{13}C$ and $\delta^{15}N$ in egg yolks among four serial clutches of a single loggerhead turtle.

We found a positive relationship between isotope values ($\delta^{13}C$ and $\delta^{15}N$) of the blood of females and their egg yolks. This indicates that stable isotope analysis of egg components is a viable method for assessing foraging ecological questions in marine turtles. Thus, to study the diet of adult sea turtles, stomach lavage or blood sampling during laying could be replaced by a procedure less-invasive, sampling the yolk of a single egg.

Management and conservation significance. Establishing patterns of movements of free-ranging animals is crucial for a better understanding of their feeding ecology and life history traits, and is a prerequisite for their conservation. Tracking animal movements can be done directly using remote-sensing techniques or indirectly using biochemical markers like naturally occurring stable isotopes. This method is less-invasive, repeatable and can be applied over different time scales to investigate migration or feeding ecology, and is very appropriate to the study of endangered species such as sea turtles. Stable isotope analysis in sea turtle can also be used to assess the feeding ecology and habits in inaccessible locations. For example, Reich *et al.* [49] identified habits and diet of the cryptic juvenile life stage of the green turtle. However, studies using stable isotope to infer foraging location are few. In the present study, stable isotopic values combined with telemetry data of literature were used to improve knowledge on the foraging areas used by the two remigration groups of females. Unfortunately, the theoretical and experimental basis of this method remains poorly validated for sea turtles, but is essential for the correct interpretation of field data. The first study, by

Seminoff *et al.* [52], of *Chelonia mydas* maintained in a controlled environment is very encouraging. However, although the use of stable isotope analysis has a number of advantages in food web and migration studies, the optimal approach is to combine it with direct observation of feeding behaviour (or stomach analysis on dead female), which provides a taxonomic resolution of resources, and satellite telemetry, which provides migration travels [42,45].

We underline two important points for the conservation of leatherback turtles. Firstly, two foraging area dichotomy for Atlantic leatherback turtles are used by 2-year and 3-year RI females. Foraging in the more southern and coastal area of West Africa seems to delay the return of females to breeding areas by one year. More research is needed to understand whether females always select the same foraging areas, and if so what the evolutionary benefits are in choosing a particular foraging site (e.g. it would be very interesting to compare isotopic values in tissues from the same individual between seasons). Fisheries bycatch is believed to be among the primary causes of leatherback turtle decline followed by egg and female harvest [76,77]. Recent research has shown that small-scale fisheries, which operate near coasts, may be a greater threat to leatherback turtles than industrial-scale fisheries [78], and the conservation measures proposed by these authors are clearly needed in coastal foraging areas. This study responds to an aforementioned need to delineate the feeding habitats of the leatherback turtle in the Atlantic Ocean, to protect this species from incidental take in commercial fisheries. In addition, isotope values of eggs have been shown to reflect the values of female tissues, providing an effective and non-invasive method for study of this endangered reptile.

MATERIALS & METHODS

Study site and collection of samples

Research was carried out within the Amana Nature Reserve at Yalimapo beach in French Guiana (53°57′W, 5°45′N), on the inshore plain of the coastline between the Mana and Maroni Rivers (Fig. 3A). Leatherback turtles nesting on this beach are tagged with internal permanent markers (passive integrated transponder [PIT] tags) placed in the turtle's right shoulder muscle, which enables temporal monitoring of the females during and between nesting seasons. Since 1985 conservation workers on this beach have surveyed turtles almost every night during the main nesting season (mid-April to mid-July).

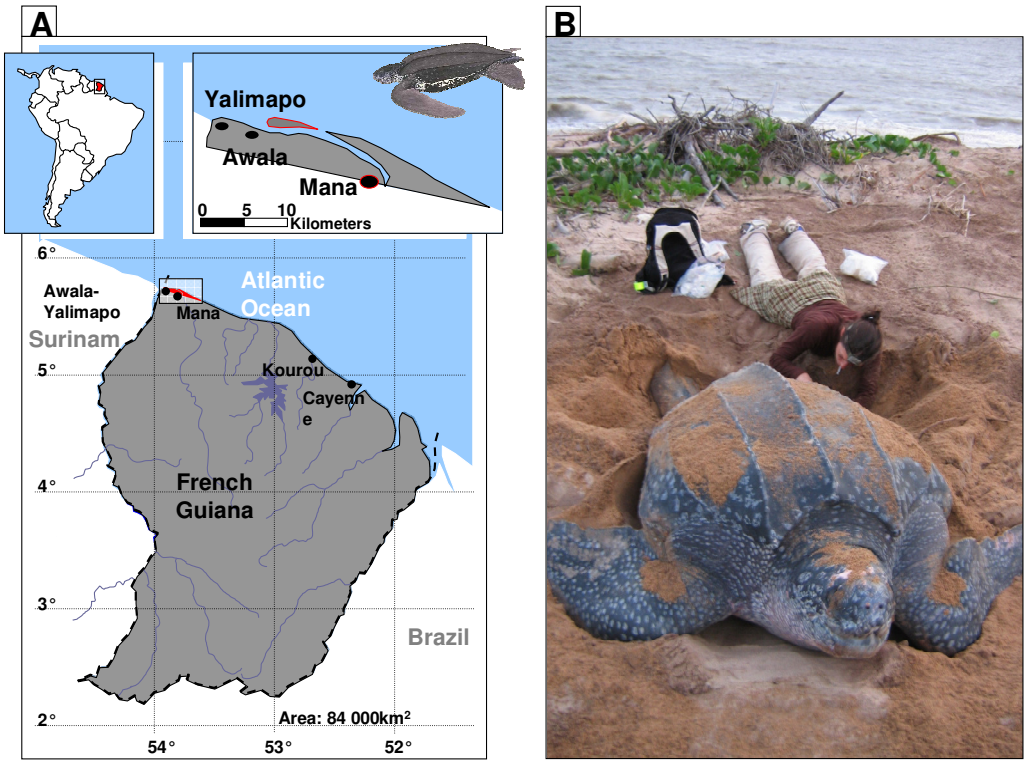

Figure 3. Location of the study site, and leatherback turtle blood sampling. A Map of Awala Yalimapo nesting beach in French Guiana. B Blood sampling of a large female leatherback turtle (*Dermochelis coriacea*) during a nesting event.

Eggs and blood were sampled nightly from turtles on Yalimapo nesting beaches, from 4 h before until 4 h after the high tide from the 16th March to 14th May, 2006. Nesting

females were scanned for PIT tags. At the beginning of the nesting season all females were sampled. Sampling then focused on females that had already been captured one or more times. The curved carapace length (CCL) was measured to the nearest cm as an index of size. The number of eggs in each clutch was recorded and one fertile egg was collected. For three females we collected two eggs laid successively, in order to examine intra-clutch variation in stable isotope ratios of the egg yolk. Blood was sampled in the venous sine of the rear flipper [79], using single-use syringes and blood collection tubes containing heparin to prevent clotting (Fig. 3B). In April and May we also collected jellyfish as potential prey of leatherbacks in the area; they strand regularly on Yalimapo beach. All samples were frozen at −20°C until analyzed.

Stable isotope analysis

Samples of egg yolk and turtle blood (plasma and red blood cells − RBC, centrifugalized at 5200 rpm for 5 min), and whole specimens of jellyfish were used for isotope analyses. Lipids were previously removed from egg yolk with a dichloromethane-methanol (2:1) solution. All samples were dried at 60°C for 48 h, ground to a fine powder, weighed in tin capsules and stored in a dessicator until isotope measurement. Isotope analyses were performed using an IsoPrime spectrometer (*MicroMass*, Service Central d'Analyse, Solaize, France) coupled to a EuroEA 3024 analyzer. Stable C and N isotope ratio are expressed as:

$$\delta^{13}C \text{ or } \delta^{15}N = [(R_{sample}/R_{standard}) - 1] \times 1000$$

Where R is $^{13}C/^{12}C$ or $^{15}N/^{14}N$ for $\delta^{13}C$ and $\delta^{15}N$, respectively. The standard for the C isotopic ratio is IAEA-NBS 21 (graphite −28.13‰), and for the N isotopic ratio is IAEA-N1 (+ 0.4‰) and IAEA-N2 (+ 20.3‰). Ten replicate assays of internal laboratory standards indicated measurement maximum errors (SD) of ± 0.15‰ and ± 0.2‰ for stable carbon and nitrogen isotope measurements, respectively.

Hypothesis tested and statistical analysis

We firstly examined variation in isotope ratios in the plasma, RBCs and egg yolk of each female throughout the nesting season (between different laying events) in order to establish whether females forage during the breeding season. We carried out general linear models with repeated measures (repeated measures GLM) in which the dependent variable was each isotope ratio ($\delta^{13}C$ and $\delta^{15}N$), and the main independent variable was the time in days of each clutch since the first clutch was observed (time 0 corresponded to the day when we observed the first clutch for each female). We added a covariable that was the number of eggs in each clutch. Repeated measurements enabled comparison of data from the same female at different laying events. The normality of the dependent variables was confirmed prior to the analyses.

We secondly searched for differences in RBC and plasma isotope values between 2-year and 3-year RI females. We carried out a general linear mixed model (GLMM) for each tissue and each isotopic ratio ($\delta^{13}C$ and $\delta^{15}N$; the dependent variables of each model). We used mixed models because values for the same female at different times (representing different laying events) were correlated; this covariance structure was handled by introducing the individual females as a random effect into the GLMM.

Finally, to assess if female isotope ratios could be estimated from egg samples only, we tested the relationship between isotope ratios of egg yolk, and RBC and plasma from the same female. We performed independent GLMM in which the dependent variable was $\delta^{13}C$ or $\delta^{15}N$ for egg yolk and the independent variables were $\delta^{13}C$ and $\delta^{15}N$ of RBC or plasma. Individual females were introduced in the model as a random effect, as explained above. Because one of the goals of this analysis was to establish a method to obtain isotope ratios of females when female tissue samples are not available, we determined regression equations between egg yolk isotope ratios and those for RBC and plasma, through simple regression models when GLMM were significant.

All analyses were performed with the STATISTICA package, version 6.0 [80], and the SAS package, version 9.1.3 [81].

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Chapter 4

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

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Elodie Guirlet, Krishna Das and Marc Girondot

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 represent also 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 that 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.

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; 2003b; Van de Vijver et al., 2004; Debier et al., 2006; 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 accidental fisheries by catch, or eggs and females harvest (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 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 (~ 80g) and the largest clutches by weight (~5 to 10 kg) among sea turtles; egg production represents the highest reproductive output 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 remain 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 subsist 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 Guinan coastal zones are subjected to natural metals 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 chemicals contaminants than adults (Russell et al., 1999). Indeed, in reptiles, ovo-exposure to toxic elements (cadmium, arsenic) have been shown to affect hatchling size 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) and 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 their 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 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.

Table 1. Trace element concentrations (µg/g wet weight) in sea turtle eggs and blood from literature and for this study; mean ± SD or range. 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.

Species/Location	Year	n	Tissue	Copper	Zinc	Selenium	Lead	Cadmium	Mercury	Reference
Green turtle (*Chelonia Mydas*)										
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±0.036	45±3.6	3.5±0.6	0.49±0.008	BDL[d]	0.002±0.0001	2
China	2001	30	albumen[b]	0.063±0.012	0.3±0.059	0.270±0.058	0.005±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 (*Caretta caretta*)										
USA	2001	34	blood[b]						0.029±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 ± 0.102	57.21 ± 2.23		1.307 ± 0.228	0.359 ± 0.135	BDL[d]	5
Japan	1990	5	egg content	1.05±0.199	14.7±1.44			0.013±0.004	0.0055±0.0016	6
Japan	1990	6	yolk	1.57±0.073	34.4±3.18		<0.03	0.026±0.007	12.1±3.41	3
Japan	1990	6	albumen	0.129±0.083	0.59±0.58		<0.03		0.49±0.24	3
Kemp's Ridley turtle (*Lepidochelys kempii*)										
USA	1994	106	blood	0.215-1.3	3.28-18.9		0-0.034		0.0005-0.0673	7
Leatherback turtle (*Dermochelys coriacea*)										
French Guiana	2006	78	blood	1.34±0.28	11.10±0.28	9.98±0.05	0.18±0.05	0.08±0.03	0.011±0.003	this study
French Guiana	2006	76	egg	0.63±0.10	14.16±2.23	1.44±0.38	0.036±0.001	0.024±0.001	0.012±0.003	this study

[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±SE

[c] Not stated in publication wet or dry weight specific

[d] BDL= Below detection limit

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 (Figure 1), from March to July 2006.

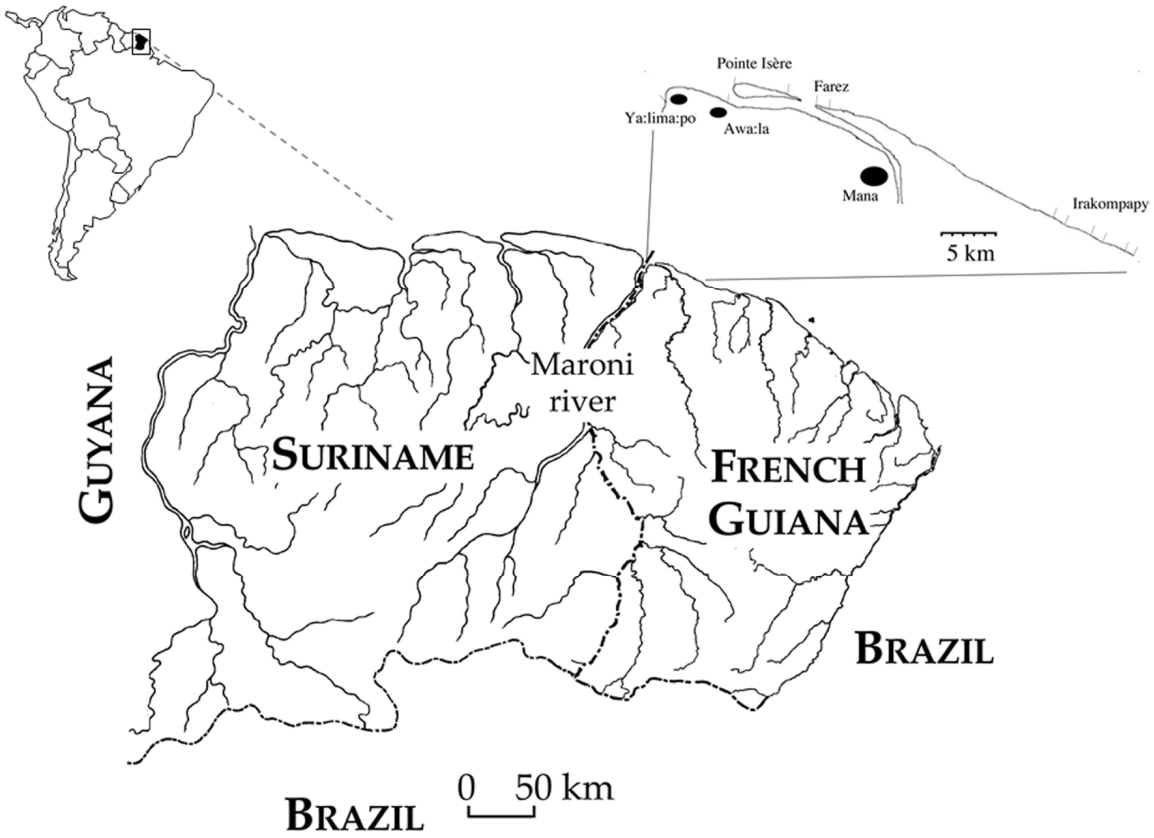

Figure 1. Map of Awala Yalimapo nesting beach, the study site in French Guiana.

This beach is monitored regularly and leatherback nesting females encountered are tagged with an internal permanent marker (Passive Integrated Transponder [PITs] 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 didn't 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 venosus 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 until constant weight, 100mg of blood and 500mg of homogenized egg content (yolk and albumen) samples were digested in Teflon tubes with concentrated nitric acid, deionised water and H_2O_2 in a microwave oven (20 minutes between 0 and 600 Watt). After cooling, samples were diluted to 50ml 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 ± 2.4 % and 80.8 ± 1.8 % for blood and egg respectively was calculated in our samples to convert data on wet weight (WW) basis. Concentrations are expressed in

µg.g^{-1} WW. Parallel to samples, a set of certified control material samples (DOLT-3 liver, National Research Council Canada and Whole Egg Powder Standard Reference Material 8415, National Institute of Standards and Technology) were repeated throughout each set of analyses to ensure method's accuracy and precision. Recoveries for control materials ranged from 94% and 101% for Cu, Zn and Se and from 93% and 104% for Cd, Pb and Hg. Instrumental detection limits were: Cu, 0.020; Zn, 0.042; Se, 0.166; Cd, 0.005; Pb, 0.002; 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

Table 2. Quality control results (µg/g dry weight) acquired with certified materials. Limit of detection (LD) are indicated in italic.

Certified material	Element	Assigned value	Measured value	n
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

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 covariable. 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 trace element concentrations 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., 1999).

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 cm to 170 cm (mean ± SD = 160±6 cm). Trace element concentrations in the blood was not correlated to CCL (P>0.5). The number of yolked-eggs laid by the females varied between 37 and 114 eggs (mean ± SD = 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 Figure 2.

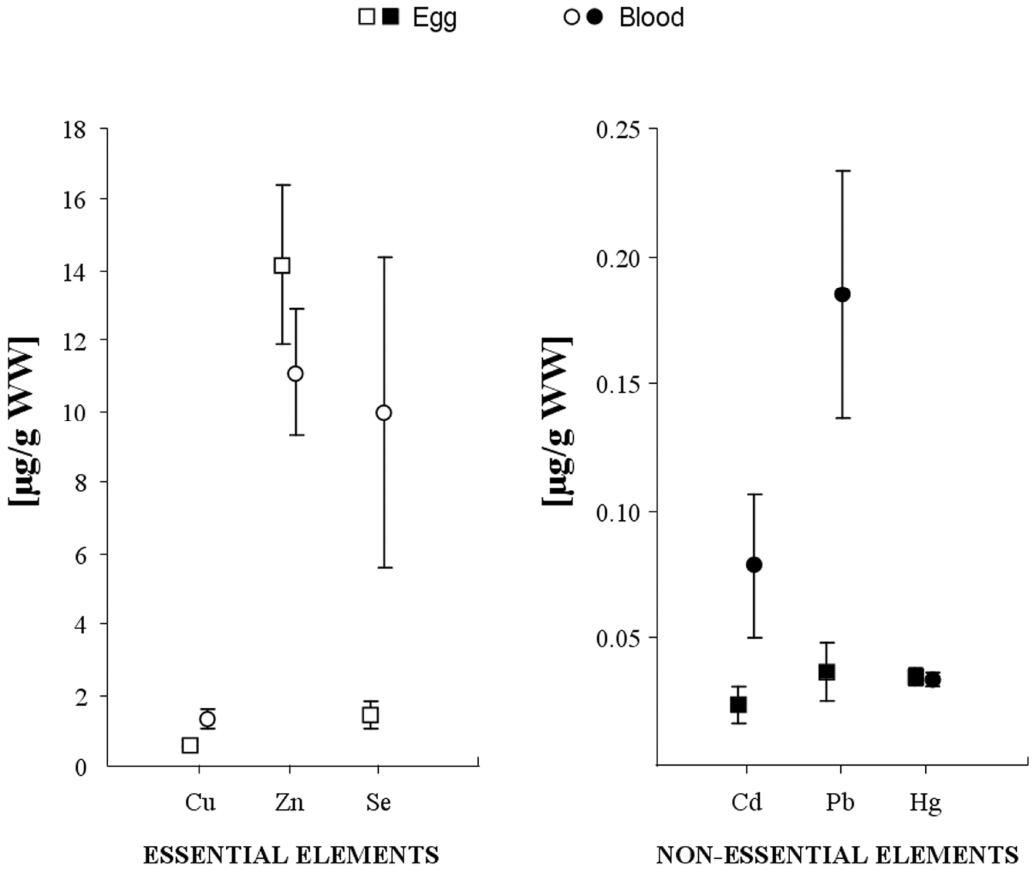

Figure 2. Trace elements concentrations in µg/g wet weight (mean ± SD) according to matrices (circles for blood and squares for egg) and to essential (in white) and non essential (in black) elements in the leatherback turtle.

3.1. Inter-clutch variation in trace element concentrations. The number of eggs in each clutch was included as a covariable, but had no effect on trace element concentrations neither in blood nor 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). The figure 3 illustrates these variations but time in days used in statistical analyses has been replaced by clutch interval in order to improve visual representation.

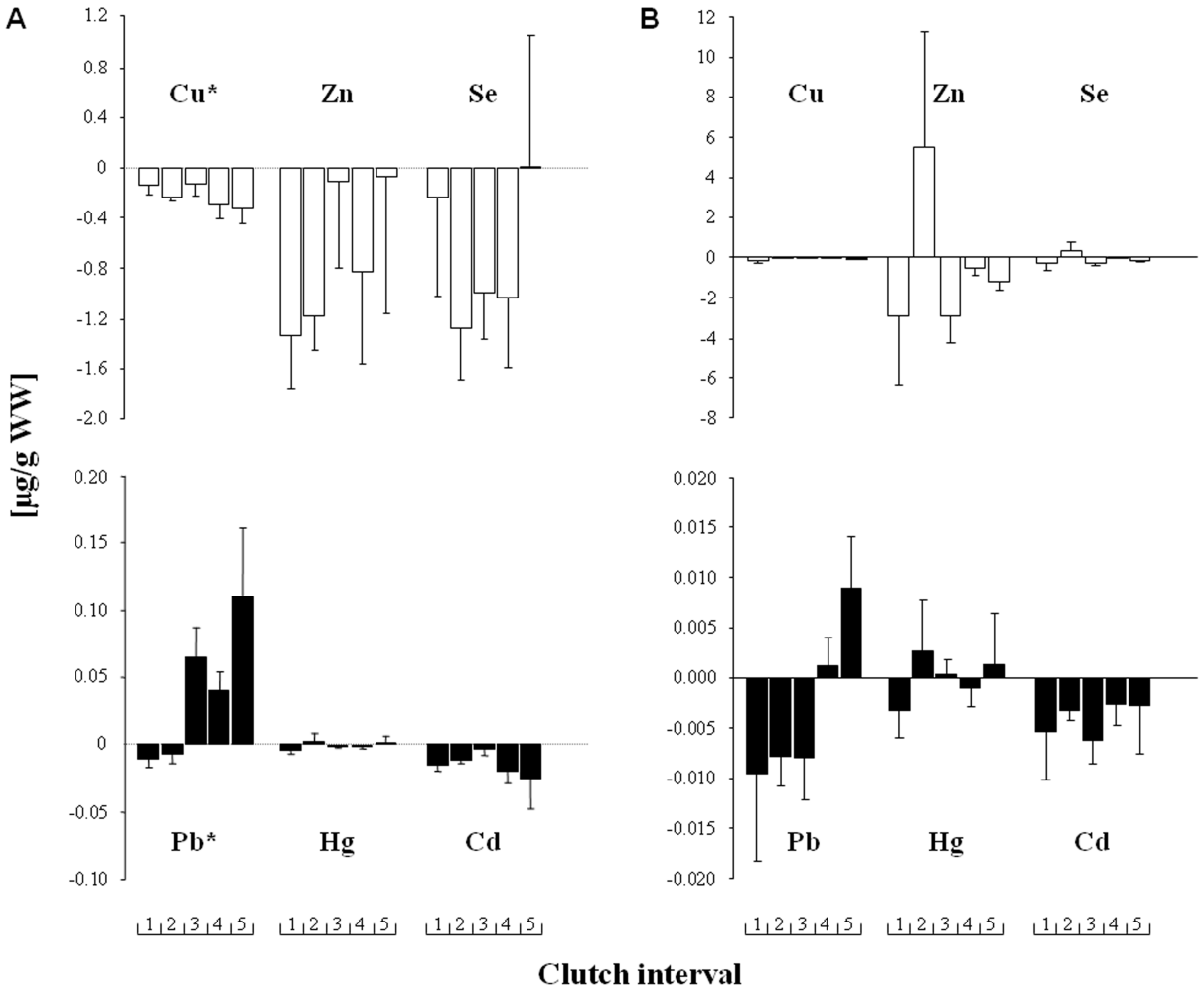

Figure 3. Trends in trace element concentrations for (A) blood and (B) eggs during the nesting season. Difference in trace element concentrations (in µg/g wet weight, mean ±SE)) are calculated for each clutch interval (1= mean difference between the first and the second clutch, n=8; 2= mean difference 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.

3.2. 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 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 (Figure 4).

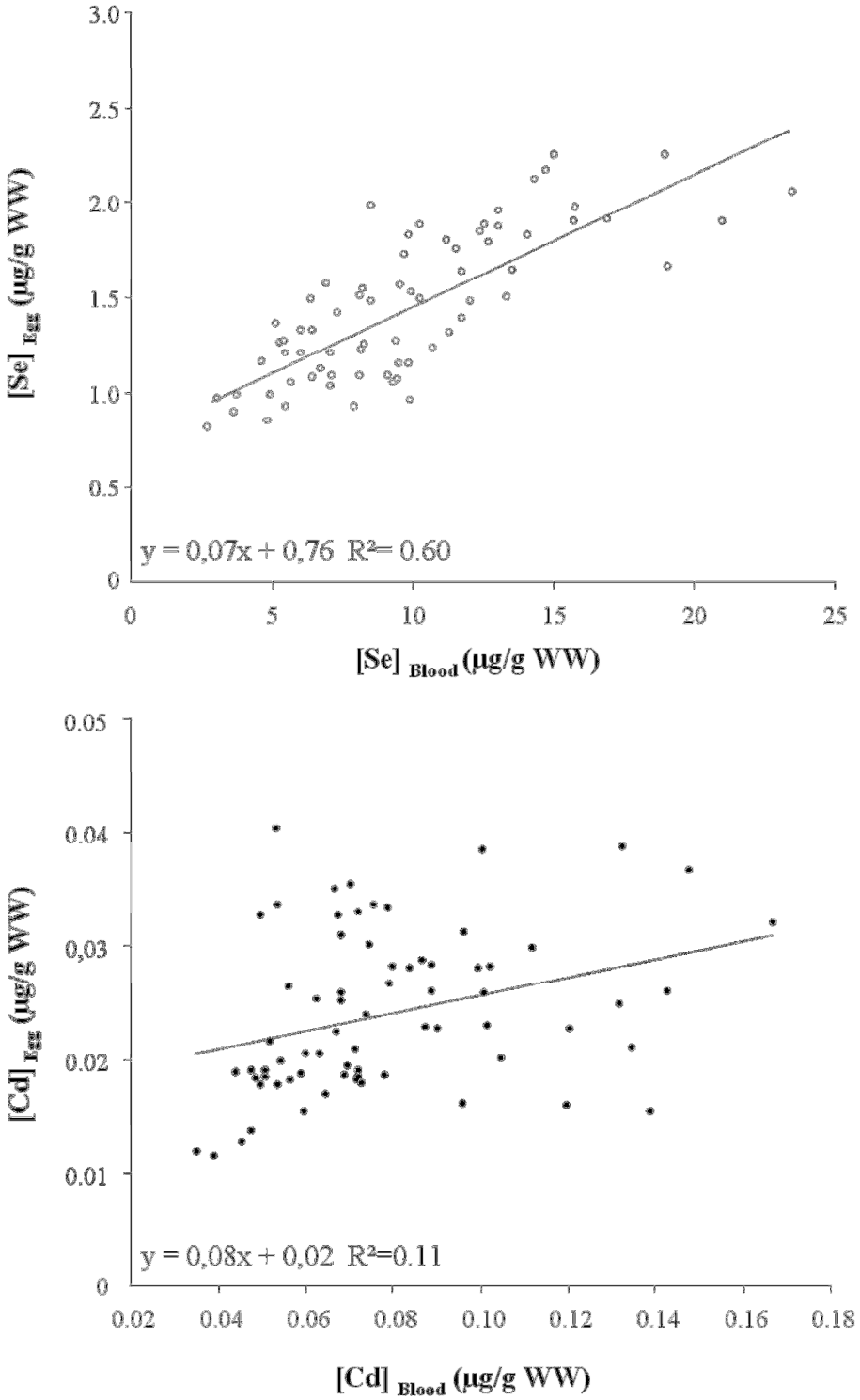

Figure 4. Linear regressions between trace elements concentrations in egg and blood of the corresponding leatherback females (Se=Selenium; Cd= Cadmium; WW= Wet Weight). Equations and associated regressions statistics are shown.

3.3. Element excretion via eggs. The total amount of trace element excreted by females via eggs, were calculated from burdens in egg content, weight of the egg and the total number of yolked-eggs 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 on the order of Zn>Se>Cu>>Pb>Cd>Hg. During a nesting season, females transfer a higher burden of essential elements (mean ± SD in mg; Zn: 691.27 ± 163.20; Se: 69.42 ± 22.61; Cu: 30.95 ± 7.80) than toxic elements (mean ± SD 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 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; Rostal et al., 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 continues into the oviduct were albumen is secreted around the yolk and finally the eggshell is secreted and is fully finished one week after ovulation (Miller, 1985) that ends the egg production process in sea turtles. In reptiles, the ovulation and the supply of albumen, and eggshell for all the eggs to be laid during the season happen progressively all along the nesting season (Palmer et al., 1993). Thus, 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 source 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 threatened leatherbacks at the stage of embryos but could impact 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 o 3500µg/kg (Bergeron et al., 2007). It has been suggested that maternal transfer to eggs of toxic elements could be advantageous: females could get rid regularly 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 seleno-proteins (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; Palmiter, 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 (Palmiter, 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 (Figure 4). These results suggest that toxicokinetics of Se and Cd differed from 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 the 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; Shepherdley et al., 2002b) 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) homeostasis 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 Se maternal transfer for reptiles are well documented (Nagle et al., 2001; Hopkins et al., 2004; Roe et al., 2004; Unrine et al., 2006), and present strong relation between concentration in eggs and concentrations accumulated in female tissue, that is consistent with the results of the present study. Reptiles studies on the remaining elements examined in this study are generally lacking; the only available data come from monitoring studies that only provides contamination in fleshly laid eggs, reflecting a potential contamination coming from a maternal transfer (Table 1). However, Nagle et al. (2001) found that slider turtles (*Trachemys scripta*) inhabiting contaminating 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 (Figure 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 albumen 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 (Figure 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 (albumen is mainly composed of water; Wallace et al., 2006). But Pb environmental measurements on sediment (12mg/kg dry weight) and water (<1µg/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 egg shell secretion, that occurs 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 or few during this period (as supposed for females of our studies (Fossette et al., 2007; Caut et al., 2008), Ca would come from female bones resorption (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 lead therefore 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.

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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Chapter 5

Maternal transfer and Toxicokinetic of PCBs and chlorinated pesticides in the leatherback turtles nesting in French Guiana

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Maternal transfer and Toxicokinetic of PCBs and chlorinated pesticides in the leatherback turtles nesting in French Guiana.

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Introduction

Pollution and pollution-related disease have been cited as presenting increasingly greater threats to populations of reptiles (Gibbons et al., 2000; Keller and McClellan-Green, 2004). Environmental contaminants such as organochlorine compounds (OCs), including polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs), are widely detected in biological and environmental samples since they were highly used in anthropogenic activities (Snedaker et al., 1999). Exposures to OCs are known to cause deleterious effects in reptiles on immune and endocrine function leading to developmental and reproductive effects such as sexually dimorphic morphology (Bishop et al., 1998), oestrogenic effect by reversing gonadal sex (Bergeron et al., 1994)), decreased hatching rate and increased hatchling deformities and disorientation (Bishop et al., 1991; Bishop et al., 1994; Bishop et al., 1998). But the exact role of chemical pollutants on sea turtle health is poorly known and no information on toxicological effects and detrimental threshold concentrations are available.

The leatherback turtle, *Dermochelys coriacea*, is the most pelagic of the sea turtles and migrate over thousands of kilometres between foraging and nesting grounds (Ferraroli et al., 2004; Hays et al., 2004). Contaminant exposure of leatherbacks may vary according to the time spent on foraging grounds and routes of migration (Keller et al., 2004). Indeed, turtles may forage two or three years (sometime more) between two reproduction periods and this feeding interval is defined as remigration interval (RI) (Rivalan et al., 2005). Consequently, while some turtles may feed repeatedly in contaminated sites others feed in more pristine areas. We showed previously that leatherback turtles nesting in French Guiana (one of the largest nesting populations) displayed detectable levels of non essential elements such as Hg,

Pb in blood and eggs (Guirlet et al. 2008). This raises concern about other organic contaminants such as polychlorinated biphenyls (PCBs) and chlorinated pesticides. These toxic organic compounds have been shown to affect offspring viability of several vertebrate species (Woodward et al., 1993; Bishop et al., 1998; Willingham, 2001). However, current levels, toxicokinetic and potential harmful effects have, to our knowledge, never been described in free-ranging marine turtles. The study of this toxicokinetic would help to assess risk on turtle's health as recurrent cycles of accumulation and mobilization can raise the risk of contamination by chlorinated toxic substances.

The toxicokinetic of OCs have been shown to be closely related to the mobilization of lipid reserves during biotic phases of high energy demand such as migration, breeding or fasting leading to OCs release in blood stream in seal and phocids (Lydersen et al., 2002; Debier et al., 2003b) and could be similar in marine turtles. The turtle breeding season includes vitellogenesis, migration to nesting sites, mating and nesting events (Owens, 1997) that represent high reproductive costs for female (Wallace et al., 2005). This leads to large variations in lipid reserves and body weight of females in few months (Eckert, 2006). Leatherbacks mobilized their lipid tissues during migrations, or when they are yolking eggs (i.e. vitellogenesis) (Keller et al., 2004). But studies reporting OCs concentrations in sea turtles are limited and most of studies concerns stranded turtles (Caurant et al., 1999). To monitor concentration in time in free-ranging organism, non-lethal sampling is required. Blood and egg samples can be collected from live sea turtles and could provide this adequate tool. The contents of sea turtles eggs represent the diet, nutrients and chemical compounds ingested by adult females on foraging grounds and during vitellogenesis (Miller, 1997). The concentrations in eggs reflect also the exposure of the developing embryo. Eggs have already been shown to represent the contaminant burden of certain compounds present in female tissues (Guirlet et al., 2008). Blood offers several benefits over traditional tissue sampling. It

can be collected easily and relatively non-destructively from free-ranging populations and facilitates the repeated collection of larger numbers of samples, which improves both the monitoring of OC levels and the assessment of toxicological effects.

PCB can reach measurable levels in nutrient system rich in lipids that represent egg-yolk. This may expose embryo to high levels during development, a time during which toxic effect may be more detrimental than during adulthood. Indeed, lab experiments and wildlife studies showed that OC maternally transferred were associated with reduced clutch success and increased embryonic mortality (Bishop et al., 1991; Rauschenberger et al., 2004b).

Our study investigates first the distribution and patterns of PCBs and OCPs in blood and egg samples of 38 nesting leatherback females. Then, the tokicokinetic of OCs linked to potential lipid mobilization during the nesting season was examined. Thirdly, we focused on potential contamination differences according to RI of females. Finally, egg contamination due to maternal transfer of OCs from female to eggs was investigated.

Materials and methods

Study site and sample collection. We conducted this study on the Yalimapo beach, in French Guiana. The sample collection was similar as described in (Guirlet et al., 2008). Blood and eggs samples were collected and frozen at – 20°C until analyses.

Sample preparation.

Extraction. Egg samples were thawed and homogenised with an Ultra-Turax (Ika-Werk 18/10 Janke & Kunfel). A 4g sample was then lyophilised over 16h and dry matter was determined gravimetrically. A 500mg sample of lyophilized egg with 500mg of anhydrous sodium sulphate and 100µl of PCB 112 (used as a surrogate marker) were extracted with a mixture of hexane, dichloromethane and methanol (5:2:1, v:v:v) at 80°C under a pressure of 1500 Psi using an accelerated solvent extractor (ASE) (Dionex ASE 2000, Dionex Corporation). The

solvent with the extracted fat was collected in pre-weighed vials and was evaporated at 40°C under nitrogen flow (Turbovap LV Zymark). The fat content of egg samples was determined gravimetrically. Lipids were then dissolved into 3 ml of hexane and collected into a tube. The mixture was homogenized by vortexing during 1 min. For blood samples, 4ml of whole blood were first deproteinised by adding 100 µl of triethylamine, 5ml of formic acid and 50µl of PCB 112. The mixture was stabilized for 30 min in an ultrasound bath (Julabo USR 05) and then centrifuged for 10 min. The organic phase was then transferred is a new tube.

Sample clean-up. After deproteinisation and lipid extraction, all prepared samples (egg and blood) were then purified by acid and Florisil clean-ups. A 2 ml volume of sulphuric acid mixture (fuming sulphuric acid 30% and concentrated sulphuric acid 98%, 1:3, v:v) was added to the sample and the mixture was homogenized by vortexing before being centrifuged for 3 min at 3000RPM at 10°C (Jouan-Vel). The organic phase was transferred to another tube and the acidic phase was extracted with 3 ml of hexane, vortexed and centrifuged for another 3 min. The organic phases were pooled and reduced to 1 ml under a nitrogen flow. The second clean-up was performed with Florisil® solid phase cartridges (Supelco, EnviFlorisil). The cartridges were first conditioned with 5 ml of acetone, 5 ml of an acetone-hexane mixture (50:50, v:v) and finally with 12 ml of hexane, successively. The sample was then deposited on the top of the column. Polar molecules were retained on the Florisil® (magnesiumsilicate mixture). The vials containing the sample were rinsed with 3 ml of hexane and added to the cartridge to elute PCBs and pesticides retained on the column.

Sample conditioning and analysis. The eluates were evaporated under a gentle stream of nitrogen just to dryness. Samples were finally conditioned with 50µl of mirex (100pg/µl) as an internal standard at a final concentration of 50pg/µl and 70µl of hexane were added to the almost dry samples. 120µl of the samples were transferred into a 2ml vial for injection. Blood and eggs purified extracts were then analysis by gas chromatography equipped with a 63 Ni

electron capture detector (GC-ECD Thermo Quest, Trace 2000) and an automatic injector. PCB congeners and pesticides were separated along a 30mX0.25mm column (Restec RXI-5ms) with 0.25µm film thickness, by gradual increase of temperature along successive stages: 60°C for 2 minutes, 60°C to 140°C at 20°C/min, 140°C for 3 minutes, 140°C to 270°C at a rate of 2.5°C/min and a final stage at 270°C for 12 min. The injector is at ambient temperature and the detector kept at 300°C. The carrier gas was hydrogen with a flow rate of 4ml/min and a pressure of 130kPa; the make-up gas was a mix Ar:CH4 (95:5) with a flow rate of 30ml/min. Identification of compounds was realised using Chromcard 2.2 software for windows. The chemicals identification was done according to the retention time of the compounds and their quantification was performed by comparison with the external standard in a certified calibration mixture using a linear calibration curve for each PCB and each pesticide whose concentration ranged from 1 to 75pg/µl. For quality assurance, a blank was run with each sample series to control the clean-up procedures. A quality control (QC) was also run and analysed in parallel, which consists in bovine blood and cream, for blood and egg series respectively, enriched with a defined concentration of PCBs and pesticides The recovery was calculated on the basis of the concentration of the surrogate 112 (50µg/µl), added before deprotenisation for blood samples and before rapid solvent extraction for egg samples. In egg samples, PCBs concentrations were calculated on a wet-mass basis and on a lipid-mass basis in order to minimize inter-individual variation and to allow comparison of results to other studies. In blood, PCBs are bound to lipoprotein and albumin but the lipid fraction in blood is very low (<1%) compared to eggs in marine turtles and expressing ΣPCBs concentrations in blood on a lipid weight basis would thus lead to an overestimation.

Statistical analysis. General linear models with repeated measures (repeated measures GLM) were first carried out in order to investigate the variations of pesticides (ΣDDT, ΣHCH) and ΣPCBs concentrations during the nesting season and secondly, the variation in lipid content in

eggs; the dependent variable was each organochlorine concentration and each percent of egg lipid content 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 normality of the dependent variables was confirmed prior to the analyses.

Second, differences in organochlorines concentration in eggs and blood between 2-year and 3-year RI females were investigated. General linear mixed model (GLMM) for each tissue (egg and blood) and each sum of class of organochlorine contaminant (the dependent variables of each model) were carried out. We used mixed models because values for the same female at different times (representing different laying events) were correlated; this covariance structure was handled by introducing the individual females as a random effect into the GLMM. This statistical test was also performed on groups of congeners, according to their degree of chlorination.

Finally, the relationship between organochlorine concentrations in eggs and blood was then tested for each corresponding female using independent general linear mixed models (GLMM). We performed GLMM in which the dependent variable was organochlorine concentrations in eggs and the independent variables were the organochlorine 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., 1999).

Result

During the field, 38 different leatherback turtles were sampled. As females nest several times on the same beach, 19 females could have been sampled at different clutch event. Among them, 15 females were sampled for 2 different clutches (interval of time maximum between the first and the last clutch sampled for a female, interval max: 41 days), 3 females for 3 clutches (interval max: 52 days), and 1 female for 4 clutches (interval max: 49 days). A total of 57 blood and 50 egg samples were analysed for 23 congeners (28, 44, 52, 66, 70, 87, 95, 101, 105, 118, 128, 138, 149, 153, 156, 170, 180, 183, 187, 194, 195, 206, 209) and 5 pesticides (*pp*'-DDT, *pp*'DDD, *pp*'DDE, α-HCH, γ-HCH). Results of the PCBs analysis were accepted when recoveries were between 50 and 150 % (Table 1). Recoveries range for the surrogate in QC ranged between 72.2 and 96.6% for cream and 50.7 and 98.5% for bovine blood and averaged 96.0 and 73.4% for egg and blood samples respectively. Instrumental detection limits (LD) were: 0.067 and 0.075 ppb for egg and blood samples respectively.

Table 1. Quality control results acquired with certified values and comparison with measured values (mean±SD).

compound	certified values (ng/g wet mass)	50%	150%	Quality control (cream) concentration (ng/g wet mass)
28	22,2	11,09	33,27	18,66 ± 7,87
52	15,1	7,53	22,59	8,89 ± 5,20
101	21,5	10,76	32,28	13,94 ± 4,77
118	16,1	8,04	24,13	9,72 ± 3,98
153	16,3	8,14	24,43	11,41 ± 4,14
138	16,6	8,28	24,84	10,11 ± 3,33
180	14,1	7,06	21,19	9,21 ± 3,82
α-HCH	11,7	5,83	17,50	8,37 ± 3,95
γ-HCH	12,2	6,10	18,30	8,36 ± 4,07
pp'-DDE	15,6	7,79	23,38	9,20 ± 3,35

OC concentrations and patterns. Total PCB concentrations (ΣPCBs) were calculated as the sum of all individual quantified congeners. Total DDT (ΣDDTs) was calculated as the sum of *pp*'-DDT, *pp'*-DDE and *pp'*-DDD concentrations and total HCH (ΣHCHs) was calculated as

the sum of α-HCH and γ-HCH concentrations. All these concentrations are presented in Table 2.

Table 2. Organochlorine compounds concentrations in turtle blood and egg

Matrices	OCs	mean	SD	n	min	max	%
Blood (ng/ml)	ΣPCB	0,76	0,75	52	0,13	3,22	56,6
	ΣDDT	0,28	0,21	44	0,08	0,95	20,5
	ΣHCH	0,31	0,21	13	0,11	0,86	22,9
Egg (ng/g wet mass)	ΣPCB	8,29	5,39	46	0,93	24,13	81,2
	ΣDDT	1,49	1,25	43	0,27	5,74	14,6
	ΣHCH	0,43	0,26	44	0,08	1,06	4,2
Egg (ng/g lipid)	ΣPCB	69,69	48,76	46	6,16	217,9	82,1
	ΣDDT	11,67	9,25	43	1,96	37,35	13,8
	ΣHCH	3,5	2,07	45	0,16	9,83	4,1

Of the 23 targeted congeners, 19 were detected in blood samples and 20 in egg content samples. PCBs 52, and 170 were detected in egg and blood samples but could not be correctly quantified due to interferences during extraction; they were therefore removed for further analysis. In blood, the ratio of the number of samples in which a substance was detected to the total number of samples were the highest for PCBs 28, 153+105, 180, 101 and 44 (19% contribution to total samples at least); PCBs 138, 118, 70, 195 and 95+66 were detected less frequently occurring in 17.5%, 15.8%, 15.8%, 14% and 10.5% respectively (Figure 1). In egg samples, PCBs 44, 153+105, 187, 101, 95+66, 70, 87 and 118 were detected the most frequently (70% contribution to total samples at least); PCBs 180, 149 and 195 were detected less frequently occurring in 64%, 42% and 20% respectively (Figure 1). Congener's patterns in blood (mean percent contribution to ΣPCBs) were dominated by PCBs 149, 138, 187, 44 and 28 with respectively 15.6%, 8.4%, 7.5%, 7.1%, and 7% mean contribution to ΣPCBs (Figure 2A). In eggs, congener's patterns were dominated by PCBs 28, 95+66, 44, 153+105, 138, and 70 with respectively 11.7%, 11.5%, 9.7%, 9.7%, 8.3% and 8% mean contribution to ΣPCBs (Figure 2A). When grouping congeners by degree of chlorination, 3-4 cl (group of

tri+tetra PCBs), 5 cl (penta PCBs), 6 cl (hexa PCBs) and 7-8-9 cl (septa+octa+nona PCBs), the 4 groups were equally represented in blood (between 20 and 30% contribution)(Figure 2B).

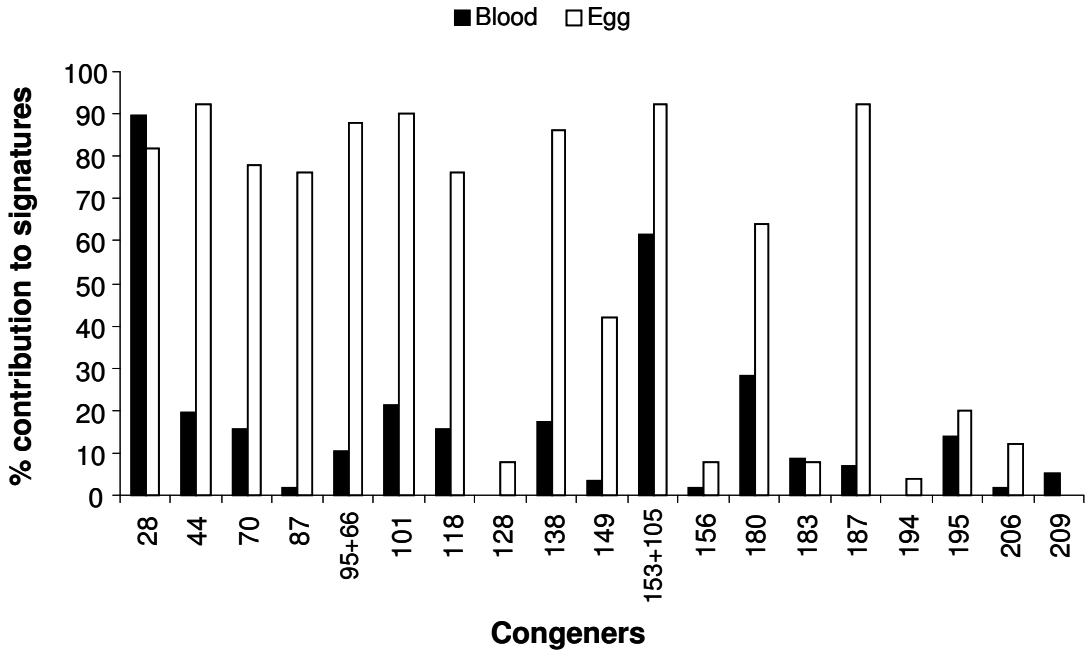

Figure 1. PCB congener signature (percent contribution to total samples) in leatherback turtle blood and egg samples.

In eggs, groups pattern presents a tendency for lower chlorinated group (3-4 cl and 5 cl groups with respectively 34.4% and 30.8%) than higher chlorinated group (6 cl and 7-8-9 cl groups with respectively 24.2% and 10.5%)(Figure 2B). In blood PCBs were the predominant OCs class found representing 56.6% of the total OCs concentrations. HCH compounds were the second class with 22.9% closely followed by DDT and metabolites with 20.5% (Table 2). DDE was detected in 74% of samples contrary to DDT and DDD occurring only in 1.8% and 5.3% respectively. In egg, PCBs were the predominant OCs class found, representing more than 80% of the sum of OCs analysed. ΣDDTs were the second most abundant OCs class measured, with 14%, represented mainly by the major metabolite *pp'*-DDE, which occurred in 86% of samples and accounted for 76% of the ΣDDT concentration.

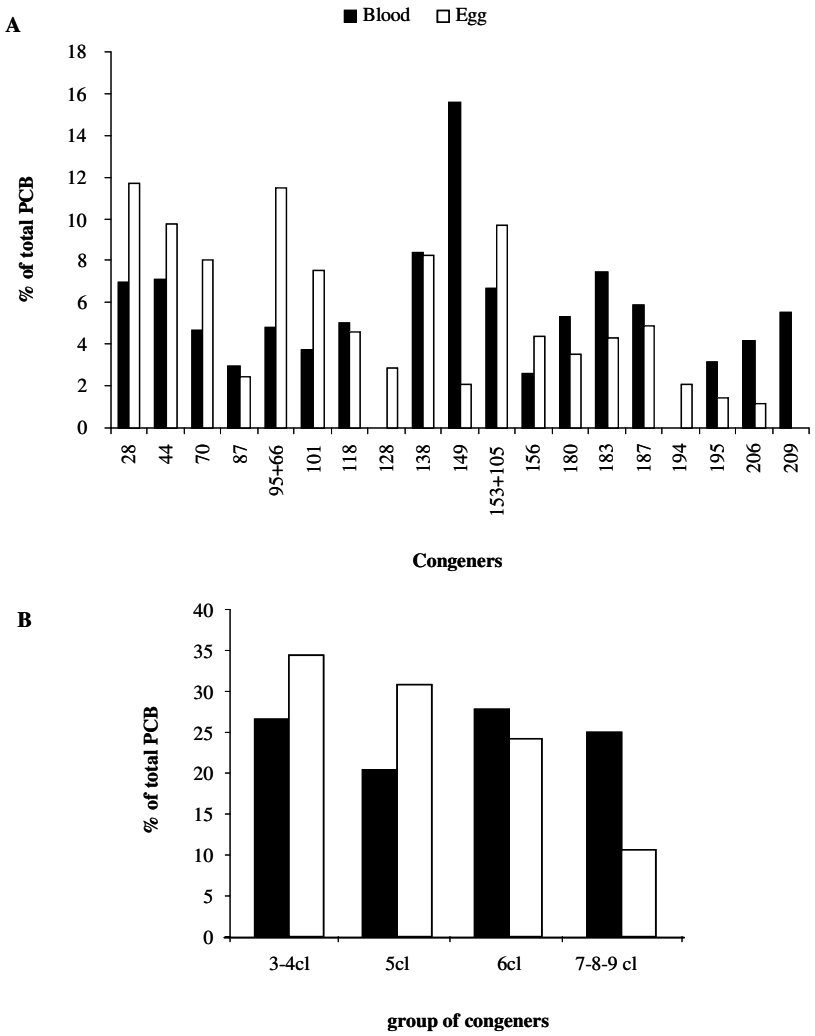

Figure 2. PCB patterns (of the ΣPCBs) in blood and egg samples (wet-mass basis): (A) for each congener, (B) by homologue group (according to chlorination of congeners).

Variations in concentrations and lipids along the nesting season. The time in days had no effect on OCs compounds concentrations in blood indicating that concentrations remain constant along the nesting season in female's blood (Table 3A). In contrast, the time in days had a significant effect for ΣPCBs, ΣDDTs, and ΣHCHs in eggs on a wet mass basis, for which concentrations decreased significantly along the nesting season from one clutch to the next (Table 3A). The percent of lipid (Plip) formed 12.90% of the egg content (SD = 5.13%, range 3.82 to 25.49%). A significant decrease in Plip (expressed as the percent of lipid relative to the wet-mass of the egg content) was observed for females samples along the nesting season (GLMrepeated , P= 0.011) (Figure 3).

Table 3. Variations of organochlorines compounds concentrations in blood and eggs. Variation with (A) the time in days between nesting events and (B) the remigration interval. (C) Relationships between eggs and blood organochlorine compounds concentrations.

	Dependent variables →		ΣPCB		ΣDDT		ΣHCH	
	Independent variables ↓	N	F/χ^2	P	F/χ^2	P	F/χ^2	P
A	Blood							
	Days between nesting		0.01	0.913	0.17	0.676	0.02	0.902
	Egg (wet mass basis)							
	Days between nesting		23.37	***<0.001***	4.24	***0.039***	10.90	***0.001***
	Egg (lipid normalized)							
	Days between nesting		5.41	***0.020***	1.63	0.202	1.00	0.318
B	Blood							
	Remigration interval		2.51	0.139	4.22	0.070	0.13	.
	Egg (lipid normalized)							
	Remigration interval		8.87	***0.017***	21.98	***0.003***	2.26	0.172
C	Egg (lipid normalized)							
	Blood		5.60	***0.042***	59.22	***<0.001***	1.85	0.124

As the percent of lipid in egg content decreases along the nesting season, concentrations in eggs sample have to be normalized to the lipid content to investigate variation in egg samples. In fact, when lipid normalized, the time in days had no longer significant effect for ΣDDTs and ΣHCHs but still for ΣPCBs in eggs (Table 3A).

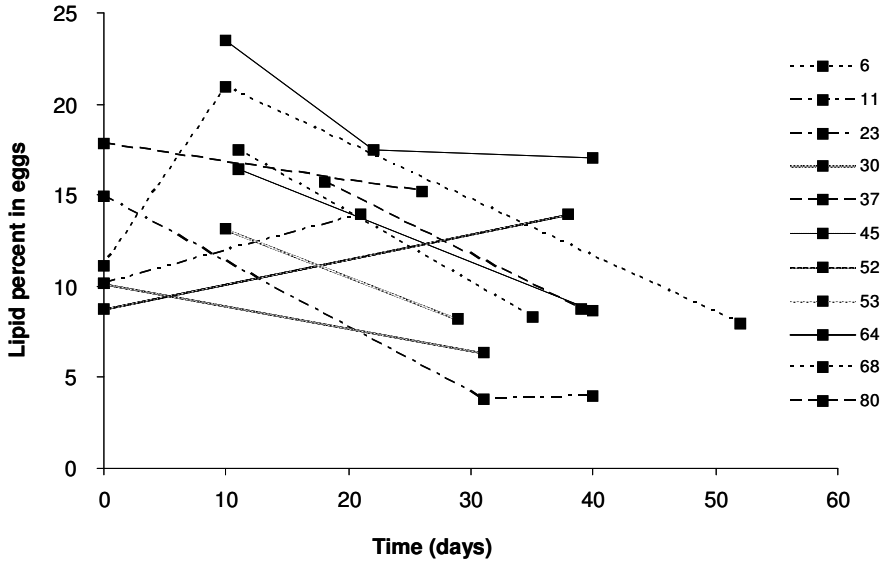

Figure 3. Changes through the nesting season in lipid egg content (expressed as the percent of lipid relative to the wet mass of the egg) from different clutches laid by 11 females. Day 0 represent the day where the first clutch for a female was observed.

Variation in concentration with Remigration interval. GLMM was used to test differences between OC concentrations in blood and eggs (lipid-normalized) for the 2 and 3-years remigrant females. No significant differences was observed between 2 and 3 years remigrant females blood concentrations (Table 3B) (Figure 4). Concerning eggs, remigration interval had a significant effect on ΣPCB and ΣDDT but not for ΣHCH (Table 3B) (Figure 4). When using group of chlorination (4 groups of PCBs: 3-4 cl, 5 cl, 6 cl, 7-8-9 cl) as dependent variables, higher chlorinated groups of congeners (6 cl and 7-8-9 cl groups) were significantly higher in the eggs of the 3 years remigrant females (Figure 4).

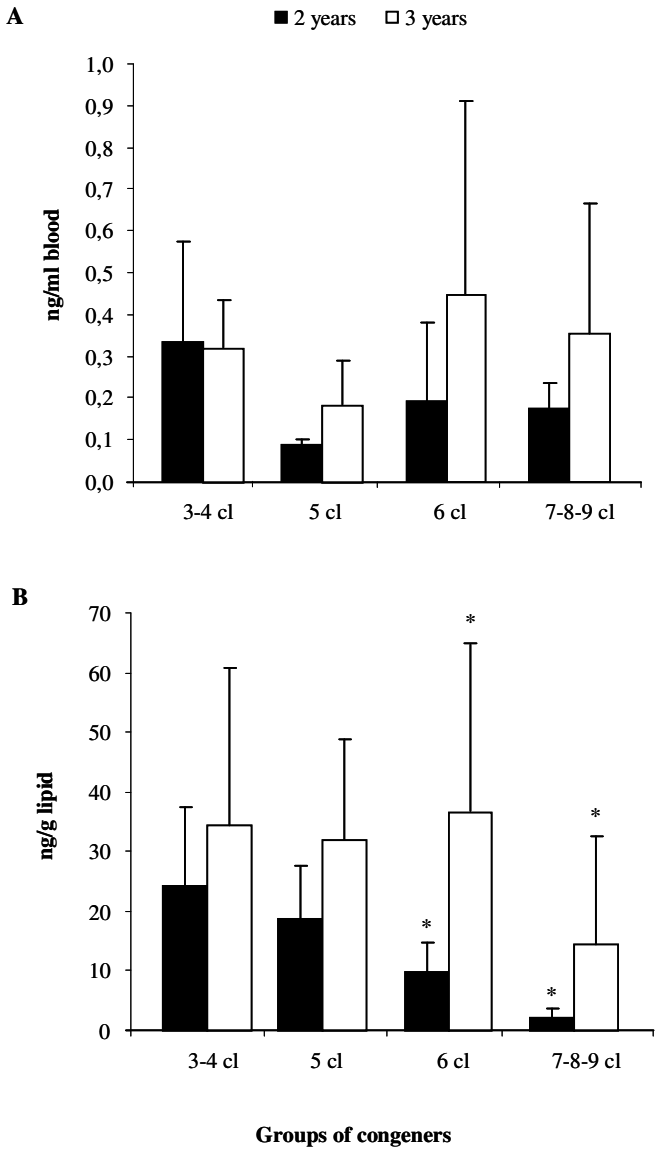

Figure 4. Variation in concentrations of OCs in blood and egg samples with remigration interval.

Maternal transfer. We used female blood-egg pairs to examine maternal transfer of OCs. GLMM was performed to test relationship between concentrations in eggs when regressed against concentrations in blood. Significant relationships were found for ΣPCBs and ΣDDTs (Table 3C) for which concentrations in eggs (lipid-normalized) were positively correlated with their corresponding concentrations in blood (wet mass basis). Concerning ΣHCHs, GLMM could not be performed because too many concentrations in blood were below the LD therefore there were not enough resources to display the test completely. Simple linear regressions for ΣPCBs and ΣDDTs in eggs against ΣPCBs and ΣDDTs in blood confirmed the statistically significant relationship for ΣPCBs and ΣDDTs (Figure 5).

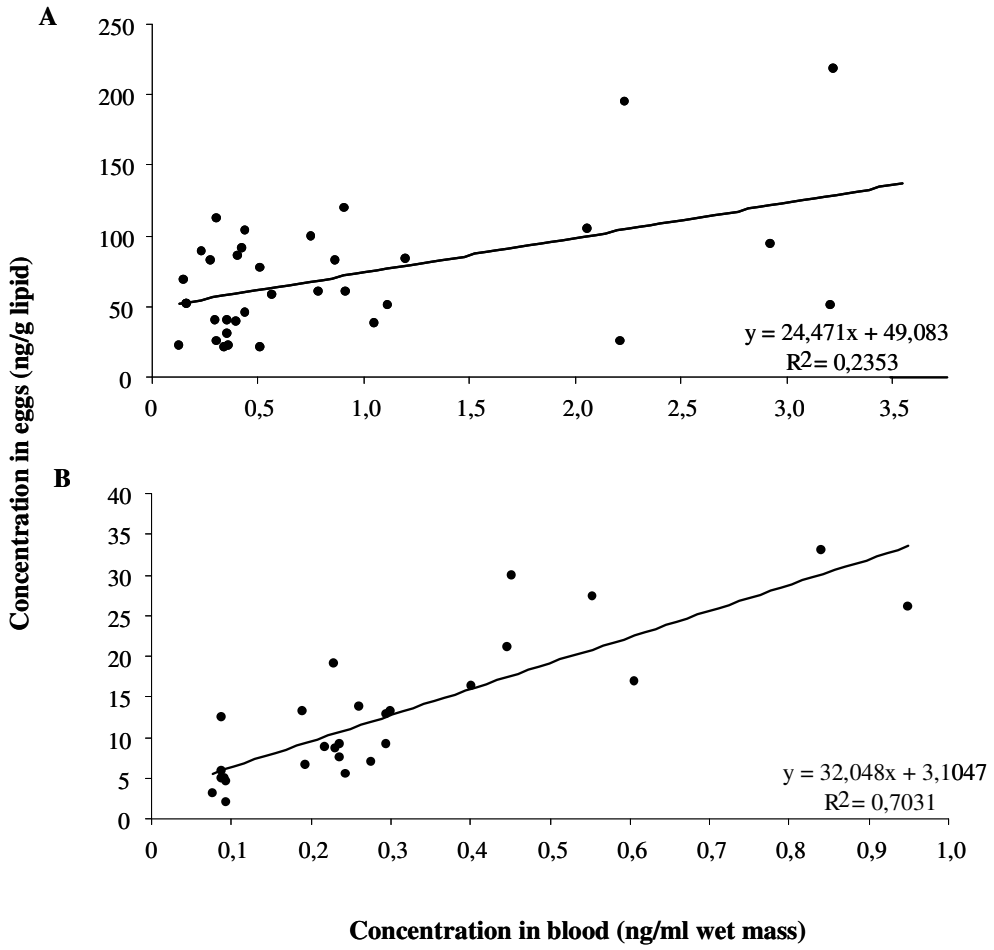

Figure 5. Relationship between concentrations in eggs (lipid-normalized) and in blood (wet mass basis) in leatherback turtles sampled in French Guiana for: (A) ΣPCB and (B) ΣDDT.

Discussion

While some areas and species have been widely studied, as the snapping turtles in Canada (Bishop et al., 1994; Bishop et al., 1998; de Solla and Fernie, 2004; Dabrowska et al., 2006), no previous data have focused on French Guiana and leatherback turtles. This study has dealt with contamination in blood, providing an easy and non destructive sampling method that can be repeated in time without affecting females, and eggs representing the diet, nutrients and chemical compounds ingested by adult females (Miller, 1997). Results indicate that leatherback turtles nesting in French Guiana are exposed to environmental organic contaminants, accumulate and transfer them to their eggs.

Concentration and Patterns of OCs in leatherback and comparison to other marine turtles. Leatherback samples in this study present relatively low OCs concentrations possibly resulting from low trophic level diet such as jellyfish (Davenport, 1998). These low levels may also result from oceanic grounds used by leatherbacks, remote from coastal anthropogenic sources (James et al., 2005b; Doyle et al., 2007). OCs concentrations in leatherback eggs (Table 2) are on the same order of magnitude than the herbivorous green turtle from Greece (6,1 and 4,3 ng/g wet weight for ΣPCBs and ΣDDTs respectively; (McKenzie et al., 1999)) and lower than those encountered in omnivorous loggerhead turtle from Florida or Greece (Florida: 144 ng/g wet mass and Greece 89 ng/g wet mass for loggerhead turtles eggs concentrations of ΣPCBs and ΣDDTs; (Cobb and Wood, 1997; McKenzie et al., 1999; Alava et al., 2006). In blood, OC concentrations in the leatherback are on the same order than the loggerhead and the Kemp's ridley turtles (Keller et al., 2004). Congeners patterns are dominated by lower chlorinated congeners as PCBs 28, 44, 70, 95+66, 101, and 118, present in high percent in samples, followed by PCBs 138, 153, 180 and 187 usually found in main proportion in marine turtles species and other marine vertebrate (Rybitski et al., 1995; McKenzie et al., 1999; Corsolini et al., 2000; Miao et al., 2001; Debier

et al., 2003a; Keller et al., 2004). These compounds are found in high proportions in industrial PCB formulation and are not susceptible to metabolic degradation. The discrepancy in concentrations and patterns of congeners found between leatherbacks and other marine turtles could be due to differences in contamination of feeding areas or to the species capacity of PCB bioaccumulation and biotransformation (trophic level, enzymatic metabolism of species) (Boon et al., 1997; Keller et al., 2004; Gomara, 2007). Indeed, PCB patterns dominated by lower chlorinated PCBs have been found in animals relying on lower trophic levels (Serrano et al., 2000). Concerning OCPs, the main *p,p'*-DDT metabolites, *p,p'*-DDE, was present in the greatest concentrations and contribution in eggs and blood samples. The low occurrence of DDT in samples suggests the absence of new contribution to the environment studied leading progressively to a relative increase of DDE (Aguilar and Borrell, 2005). The ratio DDE/DDT can thus be used for an estimate of the period of use of DDT (Kale et al., 1999). In most predators feeding far from the source of DDT contamination, *p,p'*-DDE is generally the most abundant among DDT isomers.

Toxicokinetic in blood during the nesting season. OCs concentrations are expected to vary during periods of high energy requirement, because of a release of contaminants stored in adipose tissue into blood stream after lipid reserves mobilization. OCs concentrations have been effectively shown to fluctuate in blood of turtles and seals, after fasting or low food intake leading to high mobilization of lipid stores and weight loss (Lydersen et al., 2002; Keller et al., 2004; Debier et al., 2006). In the leatherback females, lipid mobilization is also likely to occur during vitellogenesis (lipids accumulation in follicles, i.e. future egg-yolks), migrating or nesting season. Indeed, large seasonal variations occur in blubber layer of females in response to vitellogenesis and/or to their long migrations to nesting beaches; body mass is depleted by 33% between leatherbacks in foraging areas and leatherback nesting turtles of the same carapace length around nesting sites (James et al., 2005b). During the

nesting season, females will nest in average 7 massive clutches every 10 days (Girondot and Fretey, 1996) making this production of eggs the highest reproductive output in reptiles (Miller, 1997). Moreover, the foraging activity during the nesting season remains unclear and females may go through this period of high energy expenditure with little or no food intake (Fossette et al., 2007; Caut et al., 2008). Therefore, the vitellogenesis, the migration towards nesting sites and the nesting season are potential phases during which lipid reserves may be highly solicited leading to release of OCs from fat into blood stream. However, females in our study showed constant OCs concentrations in blood. Based on the discussion above, the most likely hypothesis to explain this finding could be that lipid reserves have already been mobilized during migration during which costs are larger in comparison to those of reproductive stages (Hamann et al., 2002a). James et al (2005b) showed important weight loss between foraging grounds and nesting sites that support our hypothesis. Moreover, although females rely on endogenous reserves from the time they live foraging grounds, Wallace et al (2005) showed that leatherback females have relatively low metabolic rates between nesting events, spending thus little energy during nesting season that also support our hypothesis. OC concentration in blood may thus have increased during migration while female body mass loss is derived from catabolism of lipid from their blubber and during which sampling is unfortunately unavailable.

Maternal transfer. In amphibians and reptiles, females often transfer a part of their burden to eggs as a result of OCs lipophilic nature (Kadokami et al., 2004; Rauschenberger et al., 2004a). Indeed, nearly all of the substances detected in leatherback blood were also detected in eggs indicating that compounds were maternally transferred during egg production. Moreover, significant relationships between blood and egg concentrations have been observed for ΣPCBs and ΣDDTs indicating that this maternal transfer to eggs was made as a function of ΣPCBs and ΣDDTs in female blood. Investigation of a potential relationship for ΣHCHs

wasn't possible because too many values were below the LD. In eggs, when grouping PCBs by degree of chlorination (Figure 3B), the percent of each group decreases when chlorination increase suggesting a preferential transfer of lower chlorinated groups from blood to eggs. This preferential reproductive transfer has been previously observed in frogs (Kadokami et al., 2004), turtles (McKenzie et al., 1999) and marine mammals such as pinnipeds and cetaceans (Green et al., 1996)(McKenzie et al., 1997) and is based on the lipophilic nature (assessed by the octanol-water partition coefficient, log K_{ow}) of the compound being transferred; the higher the log K_{ow} is, the more lipophilic a compound is and the more difficult the maternal transfer will be.

As female turtles do not attend their nest nor protect their eggs, parental investment is limited to the nutrients and energy invested by females in egg content (Hewavisenthi and Parmenter, 2002). Lipids and proteins in egg-yolk are the primary reserves that will provide energy and building materials to facilitate embryogenesis (Wallace et al., 2006). Lipids measurements would thus provide a reliable method for assessing a part of parental investments and it is of interest to investigate if maternal transfer is equal for all clutches to be laid. In turtles, the supply of yolk is simultaneous and equal in follicles forming one clutch, (Bowden et al., 2004). Among clutches, the timing and stages of follicular growth is not well known and vitellogenesis of all clutches to be laid is not likely to occur at the same time. Our results showed a significant decrease in lipid percent in egg across clutches and suggest thus that yolk deposition in follicles is not simultaneous in all future clutches and that a progressive diminution occurs in reproductive investment from females into their clutches. In contrast, data for the green turtle reported similar lipid content across clutches in one season (Hamann et al., 2002b). This difference between species could be linked to different timing of vitellogenesis; as it is thought to be complete prior the leatherbacks arrival on nesting beaches (Rostal et al., 1996), it may continue into early nesting season in green turtles (Wibbels et al.,

1990). But data on timing of vitellogenesis are very sparse and timing and stages of follicles are actually poorly known. The decrease in lipids in leatherback eggs also explains the decrease in OCs concentrations in eggs found in different clutches when reported on a wet mass basis and the constant concentration for ΣDDTs and ΣHCHs when lipid normalized (Table 3A). Surprisingly the decrease for ΣPCBs persists even after normalisation and the reason for this decrease remains unclear.

As early life of stage of oviparous organism often exhibit a greater sensitivity to environmental contaminant and because our data suggest that leatherback females do sequester OCs in their eggs, further studies need to focus on the relationships of OCs and embryonic mortality to assess risk for this population. The hatching success on Yalimapo beach is actually low and the causal risk between contaminants and embryonic development of exposed turtles is important to investigate. But no experimental data is available on the effect of contaminants for concentrations of PCBs or OCPs as low as those founds in eggs of this study. Even for higher concentrations in eggs, no study so far has been able to demonstrate a clear dose-response relationship between PCBs or OCPs and low hatching success.

Variation in concentration with Remigration interval. Sea turtles are capital breeders, often taking more than one year to prepare the next breeding season. When foraging, leatherbacks allocate during vitellogenesis energy resources into egg-yolk (proteins, lipids and OCs associated) (Rostal et al., 1996). Therefore egg content analysis could help to inform on contamination of females due to their diet on previous foraging grounds. Adult foraging sites, that differ in abundance and distribution of gelatinous prey (Saba et al., 2007), have indeed been suggested to greatly influence the levels and patterns of OCs transferred to eggs (Alava et al., 2006). Main foraging areas for leatherback are located all around the North Atlantic Ocean (James and Herman, 2001; James et al., 2005a; James et al., 2005b; Houghton et al.,

2006; Doyle et al., 2007) and feeding locations may be responsible for the inter-individual difference in OCs concentrations among females and their clutches (Bishop et al., 1994). Moreover, Caut et al (2008) showed that the remigration interval in North Atlantic influenced carbon isotopic signature of females as a result of different feeding areas used by the different remigrant females (Caut et al., 2008). Females with different remigration interval could therefore present different body and eggs burden if foraging grounds differed in contamination. Indeed, our results show significant difference in OCs concentration in eggs according to remigration interval (2 or 3 years). Eggs of three years remigrant females present higher OCs burden in eggs, leading to the hypothesis that foraging grounds used by this group of remigrant females are more contaminated. But no significant difference was observed in blood of the two groups of remigrant females. A hypothesis could be that contrary to egg-yolks that are produced before nesting season and represent thus diet of female on foraging ground, blood inform on recent changes and exposure (Blanvillain et al., 2007); therefore blood sampled during nesting season have integrated all changes that have occurred between foraging areas and nesting beaches. If mobilisation of lipid reserves occurred during migration as expected, and if levels in blood have changed, blood is not representative of levels on foraging grounds. Keller et al (2004) showed that turtles foraging in offshore areas may be less exposed than turtles closer to the coasts suggesting that 3 years remigrant females would have foraged in more contaminated sites probably closer from coastal waters. According to Caut et al (2008), the group of three years remigrant females would correspond to females foraging in lower latitude and/or coastal areas that support our findings. Data on jellyfish contamination could add information to help to confirm this hypothesis, but despite their ecological importance, data are extremely sparse on the accumulation of contaminants (Fowler et al., 2004).

Finally, identifying sources of contamination is difficult for the leatherback turtle as they forage in large geographic areas and because few data are available concerning their location. But OCs contamination has provided some data in this sense and further investigations need also to be realised.

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Partie III:

Discussion & Perspectives

Discussion & Perspectives

Discussion

Les populations naturelles présentant un déclin des effectifs ou un recrutement faible des juvéniles sont rarement exposées à un seul facteur responsable de cette tendance. Les chercheurs doivent rester conscients, lorsqu'ils s'intéressent à ces questions, du potentiel d'un maximum de facteurs susceptibles d'influencer ces paramètres. De plus, un faible succès d'éclosion ou une faible survie des juvéniles, associées à une mortalité accrue des adultes chez une espèce longévive, peuvent conduire à un déclin marqué de la population. Si la population est soumise à des pressions chroniques, alors le déclin peut être difficile à enrayer (Irwin and Irwin, 2006).

D'importants déclins de population ont été observés chez les tortues luths ces 25 dernières années (Spotila et al., 1996; Spotila et al., 2000). Ils résultent principalement des prises accidentelles des stades adultes (Peckham et al., 2007), sensibles pour les espèces longévives, dont les fluctuations vont avoir un impact majeur sur la dynamique de la population. De plus, le succès de ponte chez cette espèce est inférieur aux autres tortues marines, sans que les causes de cette mortalité embryonnaire accrue soient réellement comprises (Bell et al., 2004). Enfin, sur la plage de Yalimapo en Guyane Française, l'un des sites majeurs de ponte des tortues luths (Girondot and Fretey, 1996), le succès de ponte des tortues luth présente les taux de réussite d'incubation les plus faibles mesurés pour cette espèce (Torres 2002, Maros et al., 2003).

Les études composants ce manuscrit se sont donc intéressées à différents facteurs, naturels et anthropiques, impliqués dans la diminution de la réussite d'incubation des nids pondus sur cette plage. Différentes approches ont permis de mettre en évidence une prédation importante des œufs par les courtilières, un impact de l'inondation des nids par les marées sur le développement embryonnaire, et de déterminer des concentrations de polluants (ETM et OCs) dans le sang et les œufs des tortues luths de Guyane. L'approche écotoxicologique avait pour objectif de fournir un début de diagnostic sur l'impact potentiel de ces paramètres écotoxicologiques sur la réussite d'incubation des œufs.

1. Résultats majeurs

1.1. Impact des facteurs écologiques

Les deux premiers chapitres se sont intéressés au choix du site de ponte et à son importance sur la réussite d'incubation des nids. Whitmore & Dutton (1985) ont suggéré que la grande taille des tortues luths ainsi que la vitesse réduite des émergences sur la plage, pourraient expliquer la tendance des luths à pondre plus près de la mer en comparaison aux tortues vertes présentes sur les mêmes plages. Cependant l'effort que va fournir la femelle pour choisir l'emplacement de son nid va avoir un effet direct sur la densité de prédateurs et la probabilité d'inondation ou d'érosion au niveau de la localisation choisie pour le nid, et donc sur sa réussite d'incubation (Leslie et al., 1996). Le chapitre 1 a montré en effet que la sélection du site de ponte influençait le taux de prédation des œufs par les courtilières. Le taux de prédation augmente au niveau de la zone de végétation due à une densité accrue de courtilières. Le chapitre 1 a également montré que la sélection du site de ponte influençait l'hydratation des SAGs présents dans les pontes de luth. Etant donné que leur proportion dans la ponte et leur état d'hydratation sont corrélés positivement avec le développement des œufs et le succès d'éclosion, ils pourraient donc agir comme une sorte barrière de protection des œufs. Plusieurs autres hypothèses ont déjà été avancées pour expliquer la présence et le rôle des SAGs dans les pontes des luths : augmentation de l'air interstitiel dans la ponte pour augmenter la concentration en O_2 et faciliter les échanges de gaz ou tampon pour limiter les fluctuations de température dans le nid (Frazier and Salas, 1984; Wallace et al., 2004). Cependant, leur véritable rôle reste mal connu.

Le succès d'éclosion est également supposé être fortement relié à la distance à la ligne de marée haute. Le risque d'érosion est augmenté à cet endroit vers la ligne de marée haute (Leslie et al., 1996) et certains nids sont entièrement détruits par l'érosion des marées. D'autres sont simplement immergés à un moment de l'incubation sans pour autant présenter une réussite d'incubation nulle, suggérant que les œufs peuvent supporter des périodes d'immersion. Cependant, peu de données existent sur le seuil de tolérance des œufs face aux épisodes d'immersion (fréquence, durée, stade de développement des œufs soumis à l'inondation). Le chapitre 2 a donc abordé les effets de l'inondation des nids suivis en milieu naturels et soumis à différents patterns d'immersion par les marées.

Les résultats de ce chapitre ont permis de montrer l'importance de l'effet de l'inondation sur le développement des embryons. Les nids qui ont été inondés ne présentent

pas forcément une réussite d'incubation nulle, suggérant le fait que les œufs de luths peuvent supporter de courtes périodes d'immersion. En revanche, les nids inondés présentent une proportion d'embryons bloqués à différents stades de développement plus importante que les nids non inondés ou les nids pondus près de la végétation. Ces résultats suggèrent due les périodes d'inondation vont jouer sur la mortalité embryonnaire avec une mortalité croissante pour des inondations fréquentes, et importantes. Le moment de l'inondation pendant la période d'incubation va également jouer sur le stade de l'embryon auquel il va être bloqué.

1.2. Facteurs écotoxicologiques : Utilité des études de monitoring de la pollution environnementale.

La mise sur le marché chaque année de nouvelles molécules chimiques de plus en plus nombreuses entraine la nécessité d'évaluer les niveaux de concentration rencontrés dans les tissus des organismes en milieu naturel et d'évaluer leurs effets sur les écosystèmes. Le biomonitoring est une évaluation de l'exposition animale à des produits chimiques de l'environnement par la mesure de ces produits et de leurs métabolites dans différentes matrices. Les résultats de ces mesures donnent une idée de la charge corporelle de l'individu analysé. Ce biomonitoring permet alors :

- d'établir les taux de références et les données de bases pour une espèce et une région données,
- d'améliorer l'évaluation du risque et plus spécialement l'évaluation de l'exposition à différents stades du cycle de vie,
- de mieux réguler les actions en fournissant des données nouvelles sur les types de produits contaminants les individus et leurs niveaux de contamination,

Afin d'assurer ce monitoring, il convient de choisir les bonnes espèces et/ou les bons tissus. Dans le cas de mon étude, le biomonitoring des tortues luths nidifiant en Guyane implique des prélèvements sur des femelles vivantes donc, un prélèvement selon des méthodes non-létales et peu invasives, au moment du processus de ponte. Les tissus répondant à ces critères étaient le sang et les œufs. Le prélèvement de sang permet l'accès à une évaluation de l'exposition récente de la femelle (Blanvillain et al., 2007) ainsi qu'à un suivi temporel tout le long de la saison de ponte. Les prélèvements de sang sur le terrain ne sont pas toujours faciles à réaliser et pour certaines femelles le volume prélevé a été insuffisant pour pouvoir par la suite réaliser l'ensemble des analyses (isotopiques et écotoxicologiques). L'échantillonnage des œufs permet l'accès aux données de transfert maternel et au risque toxicologique pour le développement des embryons. Il est cependant

nécessaire de vérifier au préalable si un œuf est bien représentatif de la ponte entière. C'est généralement le cas chez les reptiles produisant simultanément tous les œufs d'une même ponte (Hopkins et al., 2004; Roe et al., 2004), et qui était effectivement vrai dans mes différentes études (résultats non montrés).

Les études de biomonitoring des luths présentent cependant plusieurs difficultés. L'accès aux échantillons est limité aux femelles pendant la saison de ponte lorsqu'elles viennent sur la terre ferme pour pondre. De plus, la ségrégation entre les sites d'alimentation et les sites de ponte entraine des migrations de plusieurs milliers de kilomètres (Ferraroli et al., 2004; Hays et al., 2004), pendant lesquelles, très peu de données écologiques ou comportementales sont disponibles. Ce manque d'informations concernant la majorité du cycle de vie des luths rend parfois difficile l'interprétation des données. Pendant la rédaction des discussions des articles de la partie écotoxicologie, j'ai en effet manqué d'information sur certaines précisions concernant la chronologie de la vitellogénèse et de la migration retour des femelles, ou pour la partie isotopes, les turnovers précis des tissus.

Selon D. Peakall (1992), l'approche classique en écotoxicologie pour établir le risque toxicologique pour une population naturelle est de déterminer la quantité de composés chimiques présents et de comparer les valeurs avec celles issues de données expérimentales connues pour avoir un effet. Etant donné l'absence de données écotoxicologiques sur les tortues luths (données expérimentales et naturelles), il a fallu commencer par l'identification et la quantification des polluants présents dans le sang et les œufs prélevés pendant la campagne d'échantillonnage de 2006. Les analyses réalisées sur les sangs des femelles ont permis d'établir les premières données pour la luth d'Atlantique. Puis les analyses réalisées sur les œufs ont permis de déterminer le transfert maternel des polluants et de réfléchir sur l'évaluation du risque de l'exposition des stades sensibles.

Contamination des femelles et transfert maternel

Les niveaux de contamination observés (ETM et OCs) dans les tissus (sang et œufs) se sont révélés relativement faibles par rapport aux données de la littérature pour d'autres espèces de tortues marines (Sakai et al., 1995; Cobb and Wood, 1997; Godley et al., 1999; McKenzie et al., 1999; Kenyon et al., 2001; Keller et al., 2004; Alava et al., 2006; Lam et al., 2006). Ces faibles concentrations et les patterns des polluants dans les tissus (signatures des PCB) reflètent le bas niveau trophique de la luth (Boon et al., 1997; Davenport, 1998; Godley et al., 1999; Serrano et al., 2000; Keller et al., 2004; Maffucci et al., 2005; Gomara, 2007), et les zones d'alimentation utilisées, généralement pélagiques et éloignées des côtes, donc des

principales sources de pollution (James et al., 2005; Doyle et al., 2007). Au sein des femelles, les différences rencontrées dans les concentrations peuvent être expliquées par des différences d'utilisation dans les zones d'alimentation (Bishop et al., 1994).

La seule source de contamination possible pour les œufs collectés pour cette étude, est le transfert maternel, puisque ces œufs ont été récupérés, directement au moment de la ponte ; aucun contact avec le sable n'a eu lieu. Ce transfert maternel est initié lors de la vitellogénèse quand les femelles commencent à synthétiser de grandes quantités de lipoprotéines pour fournir aux œufs les nutriments, les éléments essentiels ainsi que les hormones dont les embryons auront besoin pour leur développement (Hamann et al., 2003). Cependant, ce transfert peut également servir de transport pour les contaminants environnementaux. Les molécules lipophiles telles que les OCs vont donc passer de la femelle vers sa progéniture, ainsi que certains ETM toxiques, comme le Hg qui va se lier aux protéines du jaune de l'œuf (Hopkins, 2006).

Ce transfert maternel permet aux femelles d'éliminer une partie des polluants présents dans ses tissus entrainant une diminution significative de leur charge corporelle (Burger and Gochfeld, 1991; Sakai et al., 1995; Burger and Gibbons, 1998; Kadokami et al., 2004; Rauschenberger et al., 2004). Les mâles, lorsqu'ils peuvent être étudiés, ont souvent par conséquent, des concentrations plus élevées que chez les femelles. Au cours de mes analyses de polluants, il est apparu que la majorité des contaminants du sang de la mère étaient également retrouvée dans les œufs confirmant l'existence d'un transfert de la mère vers les œufs chez les luths. Concernant, les ETM essentiels ce transfert maternel, est nécessaire dans une certaine mesure pour assurer le bon déroulement du développement embryonnaire; pour les ETM non essentiels et les OCS, ce transfert maternel peut être néfaste et entrainer des perturbations du développement de l'embryon pouvant aller jusqu'à provoquer sa mort (Roe et al., 2004). En effet, les stades précoces de développement semblent plus sensibles que les stades adultes (Hamann et al., 2003) ; par conséquent, les niveaux de polluants rencontrés dans les œufs des luths reportés dans ce manuscrit, même faibles, peuvent avoir un effet pendant le développement embryonnaire. Cet effet, sans aller forcément jusqu'à la mort de l'embryon, peut altérer une fonction physiologique ou diminuer la fitness du juvénile produit (voir plus loin).

Toxicocinétique des polluants

Beaucoup de reptiles ont des périodes dans leur cycle de vie pendant lesquelles les contaminants stockés peuvent être remobilisés entrainant une toxicité potentielle. Pendant ces

périodes, les reptiles utilisent généralement leurs stocks de réserves, conduisant ainsi au relargage de contaminants associés à ces réserves (ex : OCs pour les réserves lipidiques). Chez les luths, la période de reproduction représente pour les femelles un investissement énorme, le plus important chez les reptiles, avec des pontes importantes en termes de masse, de nombre de nids pondus dans la saison, et d'intervalle de retour sur les sites de ponte (Miller, 1997). Un tel investissement énergétique (migration, production des œufs) a potentiellement d'importantes répercussions sur les femelles à commencer par une diminution importante des réserves lipidiques (James et al., 2005). En effet, pour produire ses œufs (plusieurs centaines pendant une saison de ponte (Girondot and Fretey, 1996)) et revenir jusqu'au niveau des sites de ponte, la femelle va largement utiliser ses réserves entrainant la remobilisation des polluants, stockés dans les réserves lipidiques (Keller et al., 2004). Au niveau de la cinétique des éléments analysés dans le sang des femelles, deux variations ont été enregistrées au cours de la saison de ponte, qui, contrairement aux attentes, ne concernent pas les OCs lipophiles mais les ETM (chapitres 4 et 5) :

- Une diminution des concentrations de cuivre, qui pourrait être la conséquence d'un transfert important vers les œufs, conjugué à des réserves maternelles faibles. Si la femelle ne se nourrit pas ou peu pendant la saison de ponte, la déplétion des réserves de cuivre peut être forte. En effet, la question de l'alimentation pendant la saison de ponte reste peu documentée. Il se pourrait que les femelles essaient de s'alimenter au niveau des côtes guyanaises mais vu l'absence de variation des signatures isotopiques (voir chapitre 3) et la perte de poids importante des femelles (James et al., 2005) entre les sites d'alimentation et les sites de ponte, les tentatives d'alimentation sont surement rares ou avec un succès faible (Fossette et al., 2007), ne permettant pas de rétablir le stock de cuivre de la femelle et donc de contrecarrer les diminutions occasionnées.

- Parallèlement, une augmentation en plomb est observée dans le sang des femelles. Cette augmentation a été attribuée à l'utilisation des réserves en calcium de la femelle pour la fabrication des coquilles qui provoquerait le relargage de plomb des os de la femelle, dont le métabolisme est associé à celui du calcium (Silbergeld, 1991; Pattee and Pain, 2003).

Au niveau des OCs aucune variation n'a donc été mise en évidence chez la femelle pendant la saison de ponte ; il se peut que le relargage de contaminants des lipides dans le sang ait eu lieu avant l'arrivée sur les plages de ponte, c'est-à-dire pendant la migration, au moment de laquelle l'accès aux échantillons est très limité.

Ainsi la période de reproduction chez les luths entrainerait une diminution de certains éléments essentiels (Cu) chez la femelle, une augmentation de certains ETM toxiques (Pb) et

hypothétiquement, une augmentation des polluants lipophiles (PCBs et OCPs) dans le sang des femelles pendant la migration vers les plages de ponte, suite à la mobilisation des réserves lipidiques dans lesquelles sont concentrés les polluants lipophiles. Un suivi des femelles sur toute la saison de ponte aurait permis d'apporter plus d'informations sur la cinétique des polluants chez la femelle, mais la collecte d'échantillons sur le terrain en 2006 a du être interrompue pour cause de problèmes logistiques et n'a duré que deux mois (15 mars 15 mai) au lieu de quatre (terrain prévu initialement jusqu'au 15 juillet).

1.3. Intervalle de remigration et sites d'alimentation

Les valeurs isotopiques en carbone sont de plus en plus utilisées dans les études sur les migrations d'animaux (Kelly, 2000; Rubenstein and Hobson, 2004) car elles sont connues pour varier avec les bassins océaniques, la latitude ou encore les habitats (océaniques vs benthiques) (Takai et al., 2000; Cherel et al., 2005; Wallace et al., 2006). L'analyse des signatures isotopiques des tissus des femelles différant dans leur intervalle de retour sur les sites de ponte a permis de mettre en évidence une différence significative de la valeur en carbone dans le sang des femelles (chapitre 3). Une différence en carbone entre deux groupes d'individu peut donc suggérer une ségrégation d'habitats entre les deux groupes concernés. L'utilisation des isotopes stables nous a alors permis d'avoir une information au niveau spatial. De plus, le tissu utilisé dans l'analyse isotopique, va également apporter des informations au niveau temporel. En effet, chaque tissu a un taux de renouvellement propre (appelé turnover). En ce qui concerne le sang, le turnover des cellules rouges (RBC) est plus long que celui du plasma, ce qui permet, à partir d'un prélèvement de sang, d'obtenir une information temporelle sur une période plus ou moins longue en analysant le plasma et les RBC séparément ; il existe cependant peu de données précises pour le turnover chez les reptiles, qui varie pour chaque espèce et chaque tissu. Chez les tortues, les études de Seminoff (Seminoff et al., 2006; Seminoff et al., 2007) ont permis d'obtenir les premières valeurs de turnovers donc d'utiliser l'analyse isotopique des RBC pour renseigner sur l'écologie alimentaire des tortues luths de mes analyses plusieurs mois avant les prises de sang, c'est-à-dire lorsqu'elles se trouvaient sur les zones d'alimentation. L'analyse isotopique du plasma a permis d'obtenir des informations sur une plus courte période précédant la saison de ponte. L'ensemble de ces données a permis de suggérer l'utilisation de différentes aires d'alimentation précédant l'arrivée des femelles sur les sites de ponte. La dispersion des femelles dans tout l'atlantique Nord à la fin de la saison de ponte avait déjà été révélée par les données télémétriques (Ferraroli et al., 2004; Hays et al., 2004). Mais ces données ne

renseignaient que sur une période d'une année après la saison de ponte alors que les données isotopiques permettent de renseigner sur une période d'une année avant la saison de ponte ; l'idéal serait de coupler les deux types d'approches sur les mêmes femelles. D'autres études concernant les turnovers des tissus et les valeurs isotopiques des proies des luths permettraient également d'augmenter la qualité des interprétations de mes analyses isotopiques.

Une importante conséquence de l'utilisation de zones d'alimentation distinctes par certains groupes de femelles pourrait également résider dans l'accumulation différentielle de polluants entre groupes. Il était donc intéressant de se demander si, tout comme les signatures isotopiques, les niveaux de contamination allaient également varier avec les zones d'alimentation utilisées, reliées à l'intervalle entre deux saisons de ponte. L'analyse de méduses issues de différentes zones de l'Atlantique Nord auraient pu être utile pour commencer à répondre à cette question, mais malgré l'importance écologique des méduses, aucune étude toxicologique n'est actuellement disponible (Fowler et al., 2004). Une différence entre les concentrations d'OCs a en effet été observée entre les œufs des femelles ayant un intervalle de retour de 2 ans et celles de 3 ans. L'étude des différences de concentrations dans les OCs dans les œufs apporte une information supplémentaire dans la qualité des zones d'alimentation utilisées par les femelles. D'après les valeurs isotopiques, les femelles ayant un intervalle de remigration de trois ans utiliseraient des zones d'alimentation dans les faibles latitudes et/ou les eaux plus côtières (chapitre 3), ce qui correspondraient d'après les données de télémétrie, aux zones d'alimentation proches de l'Afrique Occidentale (Eckert, 2006; Houghton et al., 2006; Doyle et al., 2007). Les femelles ayant un intervalle de remigration de 3 ans présentent des concentrations dans leurs œufs en OCs plus élevés suggérant que les sites d'alimentation utilisés pendant la production des œufs étaient plus contaminés (chapitre 5). Si les zones d'alimentation utilisées par les femelles de notre étude revenant après trois ans sont bien situées près de l'Afrique Occidentale, cela suggérerait également que cette zone géographique est plus polluée et donc représente une menace potentielle (intensité de la menace à quantifier) pour les luths. D'autres études écotoxicologiques seraient nécessaires pour confirmer cette hypothèse.

2. De l'exposition à des polluants environnementaux aux effets écotoxicologiques : Evaluation du risque pour les tortues luths de Guyane Française.

2.1. Comparaisons avec la littérature

Après avoir déterminé la quantité de composés chimiques présents pour une population naturelle, l'approche classique en écotoxicologie se poursuit par comparaison des valeurs avec des données expérimentales connues pour avoir un effet.

Les effets toxiques et biologiques des contaminants environnementaux vont dépendre de la concentration, la voie d'exposition, l'espèce, l'âge et le sexe. Un des aspects problématiques de la toxicologie est la diversité des réponses biologiques et toxiques observées avec divers modèles animaux qui rend difficile les extrapolations de risque pour une espèce qui n'a pas été étudiée. Les espèces de reptiles ayant généralement été sous représentées dans les études écotoxicologiques (Gardner, 2006), ils existent donc moins de données pour comparer et évaluer le risque pour les tortues luths de mon étude. Cependant, parmi les études écotoxicologiques chez les reptiles, les tortues représentent une partie non négligeable de ces études ; il existe donc des données expérimentales fournissant une base pour évaluer les concentrations à partir desquelles des effets sont observées, ainsi que des données issues de suivis en milieu naturel qui permettent d'évaluer les contaminants retrouvés le plus fréquemment.

Afin de voir si les luths de mes études présentaient des risques avec leurs niveaux de concentrations en polluants, j'ai recherché des données dans la littérature qui reportaient des effets chez d'autres espèces de tortues pour les mêmes concentrations mesurées. Le problème majeur de cette démarche est que la plupart des études sur les effets des contaminants chez les tortues utilisent des concentrations bien supérieures à celles mesurées chez les luths. Très peu d'études permettent donc d'avoir accès aux effets potentiels pour de faibles doses. Voici seulement deux exemples de résultats pour des tortues exposées aux mêmes concentrations de contaminants recherchés dans les chapitres 4 et 5.

● Fonction immunitaire : Keller et al (2006) se sont intéressés aux effets des OCs sur la fonction immunitaire chez la tortue caouanne (*Caretta caretta*) et ont trouvé une induction de la prolifération des lymphocytes pour des concentrations en *pp*'-DDE du même ordre de grandeur que dans les luths de mon étude. L'exposition à des OCs pourrait moduler les

réponses du système immunitaire des tortues marines, avec le risque d'une hypersensibilité et de troubles ou maladies auto-immunes (Keller et al., 2006).

● Fonction reproductive : une étude récente sur les tortues aquatiques *Trachemys scripta* et *Chrysemis scripta* a montré que de faibles concentrations de Cadmium dans les œufs, comparables a celles mesurées pour les luths, pouvaient avoir un effet sur le développement des gonades. Ces faibles concentrations ne menacent donc pas la survie des embryons mais pourraient avoir un effet au niveau des stades adultes en modifiant le processus de reproduction ou en diminuant la fertilité (Kitana and Callard, 2008).

2.2. Réussite d'incubation et contamination des œufs à Yalimapo

Cependant, les données de la littérature permettent seulement de comparer les concentrations de certains polluants testés seuls ou par groupe, mais ne donnent pas une vision réaliste des effets potentiels toxiques. L'analyse des résidus dans les tissus, seule, ne fournit que peu d'informations sur la source de l'exposition, la date, la durée ou encore la fréquence et pour évaluer les risques écotoxicologiques et les efforts de conservation à fournir. Les résultats de biomonitoring seuls ne donnent pas non plus d'idées sur les effets au niveau cellulaire, physiologique ou pathologique. Le biomonitoring mesure seulement des concentrations d'un produit dans un tissu à un moment donné ; des efforts considérables sont donc à fournir pour interpréter les risques potentiels ou les sources d'exposition. Dans tous les cas, la présence d'une substance n'indique pas forcément un risque pour l'organisme. Le risque, ou toxicité, va dépendre de l'ensemble des substances présentes, de leurs concentrations et des effets additifs ou synergiques potentiels. Le biomonitoring doit donc être associé à d'autres données de biomonitoring environnemental pour évaluer ces risques en reliant les concentrations analysées à d'autres paramètres disponibles lors des études de terrain. En effet, chez les espèces protégées, les possibilités d'expérimentions sont limitées. Il faut donc identifier des paramètres de terrain exploitables pour tester l'effet des contaminants.

La complexité des systèmes naturels rend cependant difficile la distinction entre les variables d'intérêt (ici les polluants) et les autres variables non toxicologiques. Les études de terrain permettent souvent d'établir seulement des corrélations plutôt que l'identification même des facteurs provoquant l'effet. Seules, elles ne permettent donc pas d'évaluer correctement les risques mais constituent la première approche nécessaire pour évaluer les bases des risques. J'ai donc choisi de mesurer la réussite d'incubation des nids des tortues pour lesquelles j'avais des données pour les ETM et les OCs d'un œuf issu de la ponte, afin de voir une éventuelle relation entre ces concentrations dans l'œuf et la réussite d'incubation

de la ponte. En effet, l'exposition des stades embryonnaires sensibles à des contaminants peut être la cause d'une réduction du succès de reproduction. L'analyse de ces données est actuellement en cours et je vais vous présenter ici les premiers résultats. L'hypothèse de départ est que le transfert maternel de contaminants peut altérer le succès reproductif, en altérant la croissance des embryons et provoquer une réussite d'incubation plus faible pour les nids présentant des concentrations élevées de polluants dans les œufs.

La réussite d'incubation des nids a été obtenu en fouillant les nids et en divisant les œufs en différente catégories : œufs éclos, œufs avortés correspondant aux œufs pour lesquels il y a eu arrêt du développement embryonnaire à un moment de la période d'incubation, œufs prédatés par les courtilières, œufs pourris correspondant aux œufs pour lesquels aucun développement d'embryon n'a pu être observé et pour lesquels l'ensemble de l'œuf a pourri au long de la période d'incubation. La réussite d'incubation de chaque nid suivi a donc été calculée en divisant le nombre d'œufs éclos par le nombre d'œufs total. La réussite d'incubation moyenne pour 30 nids a été de 43,6%, avec 37,9% d'œufs pourris, 11,7% d'œufs prédatés et 1,3% d'œufs avortés. Puis, j'ai recherché l'existence de corrélations entre les concentrations en polluants et le pourcentage d'œufs éclos.

Les premiers résultats des corrélations entre la réussite d'incubation et les ETM ou les OCs n'ont pas montré de relations significatives entre la réussite d'incubation des nids et les concentrations en contaminants environnementaux. Les concentrations mesurées dans les œufs ne sont peut être pas suffisamment élevées pour provoquer une mortalité accrue des embryons. En revanche, aucune donnée ne permet de dire si ces concentrations peuvent provoquer d'autres effets délétères comme des malformations. Les données de la littérature portant sur les contaminants et la réussite d'incubation n'ont jamais mis non plus en évidence une relation dose-effet claire mais seulement des observations de faible réussite d'incubation ou de développement anormal dans des environnements contaminés (Bishop et al., 1991; Bishop et al., 1998; de Solla et al., 2003; Rauschenberger et al., 2007). La suite de l'analyse de ces données permettra peut être de mieux comprendre les relations potentielles entre réussite d'incubation et polluants environnementaux et les risques encourus. Des études expérimentales et/ou des études de terrain portant sur un nombre plus important de nids (ici n=30) restent nécessaires pour continuer de rechercher les effets des contaminants sur le développement des embryons chez les reptiles.

Perspectives

D'une manière générale, il existe encore trop peu de données de monitoring ou expérimentales chez les tortues marines pour évaluer correctement le risque écotoxicologique. L'incertitude liée à l'extrapolation à d'autres vertébrés (oiseaux, amphibiens, poissons) est trop grande pour produire l'évaluation des risques chez ces espèces avec une marge d'erreur raisonnable.

Il y a donc actuellement un réel besoin de suivi et d'études expérimentales pour évaluer les risques et les effets au niveau de la femelle comme au niveau des œufs, ainsi qu'un besoin de nouvelles approches pour améliorer l'évaluation du risque chez les reptiles. Cependant, le statut des espèces de tortues marines, les nombreuses menaces qui pèsent sur elles, ainsi que leur taille (pour des expérimentations en laboratoire par exemple) ne rendent pas les études facilement réalisables. De toute façon, l'étude des effets des contaminants sur ces espèces doit nécessairement utiliser des méthodes non-létales.

L'utilisation d'espèces proches (ex : tortues aquatiques) ou de méthodes non létales permettant d'évaluer directement les dommages (biomarqueurs) est donc nécessaire pour étudier les effets des contaminants.

1.1. Biomonitoring utilisant les organes

Il conviendrait dans un premier temps de regarder si les concentrations trouvées dans le sang de la femelle sont corrélées à celle des organes cibles des différents contaminants. En effet, les contaminants vont entrer dans l'organisme via les voies digestives, passer dans le sang qui va les transporter jusqu'aux organes où ils vont s'accumuler. Alors que les contaminants lipophiles vont se concentrer préférentiellement dans le foie, les ETM vont s'accumuler dans les reins (Sakai et al., 2000; Anan et al., 2001; McClellan-Green et al., 2006). De faibles concentrations sanguines peuvent donc signifier une faible exposition récente mais ne renseignent pas sur la bioaccumulation sur le long terme des polluants qui se concentrent dans les organes. Il est donc important d'avoir des données également dans les organes même si ce type d'échantillonnage implique de travailler soit sur des échouages (ce qui implique un biais potentiel vers des animaux en mauvaises conditions) soit sur des individus morts par prise accidentelle dans les bateaux de pêche si l'on se focalise sur les tortues marines.

J'envisage donc pour la suite de travailler sur des espèces non protégées, facilement manipulables et en conditions expérimentales (ex : tortues aquatiques) pour avoir accès aux différentes matrices nécessaires pour établir ces corrélations (sang et organes cibles).

1.2. Evaluation des risques par utilisation de biomarqueurs

Les effets biologiques des polluants peuvent être assimilés à des indicateurs biologiques de pollution, ou biomarqueurs, dans le règne animal. La caractérisation de ces marqueurs peut permettre la mise en évidence précoce d'effets de pollutions avant l'altération de la structure des organismes, et surtout avant que les populations et les communautés, voire l'écosystème, ne soient perturbés. Le besoin de détecter et d'évaluer l'impact des contaminants, particulièrement pour de faibles concentrations de mixtures, a conduit à l'étude et au développement de ces biomarqueurs pour évaluer les réponses biologiques des organismes exposés aux contaminants anthropogéniques.

Les biomarqueurs peuvent être utilisés à différents niveaux d'organisation de l'organisme (moléculaire, biochimique -enzymes, hormones stéroïdiennes ou protéines-, cellulaire -dégradation de l'ADN-, ou directement au niveau de l'individu -malformation, perturbation de la reproduction ou du comportement-) (Mitchelmore et al., 2006). Ils peuvent être utilisés dans les populations naturelles provenant de milieux contaminés ou dans des organismes exposés expérimentalement à des polluants ce qui renseigne sur la magnitude de la réponse de l'organisme en conditions contrôlées.

J'aimerais par la suite pouvoir développer certains biomarqueurs pour enfin avoir accès aux effets des contaminants. Les biomarqueurs qu'il me plairait d'utiliser dans le cadre d'une expérimentation en milieu contrôlé, seraient les essais comètes sur les stades adultes afin de déterminer les effets génotoxiques des contaminants, et des tests de comportement (vitesse de déplacement) sur les stades juvéniles.

1.3. Transfert de contaminants du nid vers les œufs et évaluation des risques associés pour le développement embryonnaire

Les reptiles pondent généralement leurs œufs dans un substrat pour lequel il existe un transfert potentiel du substrat du nid vers l'œuf de contaminants dissouts qui traverseraient la coquille. Ce phénomène est d'autant plus inquiétant chez les espèces qui ont des coquilles très perméables à l'eau et potentiellement aux contaminants dissouts (Brasfield et al., 2004). Les études portant sur ce type de transfert restent rares et pourtant nécessaires compte tenu des effets observés des contaminants sur le succès de reproduction.

La plage de Yalimapo, par sa proximité avec les rizières et sa situation à l'embouchure des fleuves Mana et Maroni, est potentiellement largement exposée à une contamination environnementale par des pesticides, issus du traitement des rizières, et par des ETM (ex : Hg, Pb), issus des souls sols guyanais naturellement riches en ETM ou des activités d'orpaillage dans les rejets sont transportés jusqu'à l'océan par le réseau hydrographique très dense de la Guyane. De plus, les températures élevées dans cette région équatoriale favorisent l'évaporation de certaines substances qui peuvent être plus bio disponibles pour certains organismes et le nombre de pores des coquilles augmente suite aux échanges d'eau et de gaz entre les œufs et l'environnement du nid, facilitant le transfert de contaminants (Hewavisenthi and Parmenter, 2001; Canas and Anderson, 2002; Marco et al., 2004). La perméabilité des coquilles, selon sa structure et son degré de calcification, ainsi que l'importance de la pollution du substrat du nid sont donc à considérer dans l'étude des facteurs impliqués dans la réussite d'incubation chez la luth, particulièrement en Guyane à cause de son importance en tant que site de ponte majeur des luths et de sa potentielle pollution environnementale marquée. Il se pourrait que ce transfert représente un risque écotoxicologique beaucoup plus important pour la réussite d'incubation à Yalimapo que le transfert maternel. J'ai prélevé des échantillons de sable au niveau des nids dont je connais la réussite d'incubation, mais je n'ai pas encore pu analyser pour l'instant ces échantillons. L'analyse de ce sable et la relation des concentrations au niveau des nids avec les réussites d'incubation observés permettraient d'évaluer le risque de ce type de transfert.

1.4. Tortues marines : transporteurs biologiques de nutriments ou vecteurs de contaminants ?

Les tortues marines sont confrontées à une ségrégation spatio-temporelle des sites et des épisodes d'alimentation et de reproduction : alors qu'elles se nourrissent exclusivement en mer, elles dépendent de la terre ferme pour se reproduire. Elles sont alors considérées comme des transporteurs biologiques, dans la mesure où elles apportent des nutriments et de l'énergie des zones d'alimentation (en plein océan) vers les sites de ponte (plages en zone tropicale et intertropicale). Les agrégations de ponte peuvent être très denses et fournissent finalement une quantité importante de nutriments à ces écosystèmes côtiers, souvent pauvres (Bouchard and Bjorndal, 2000). Cependant, du fait du transfert maternel, les nutriments déposés sur les plages vont être associés à différents contaminants. A Yalimapo, le nombre d'œufs déposés par km de plage est estimé à 100 000 par saison de ponte, dont près de 60% n'aboutissent pas à la formation de juvénile. Ainsi, les 60 000 œufs qui vont rester dans le sable peuvent

constituer une source de pollution pour les œufs pondus par la suite. Le rôle des tortues dans le transport des contaminants de l'océan vers le substrat de la plage de ponte est également à évaluer. La quantification et l'impact de cette pollution reste à déterminer et à sommer avec la pollution du substrat provenant des activités agricoles existant aux abords de la plage de Yalimapo (rizières).

Les études conduites pendant cette thèse ont confirmé que la réussite d'incubation était faible à Yalimapo et ont donné des pistes supplémentaires pour la compréhension des facteurs impliqués. Les facteurs naturels comme la prédation ou l'immersion et l'érosion des nids expliquent une part importante de l'échec du développement des œufs. Les facteurs écotoxicologiques intrinsèques semblent affecter également le développement des œufs de luths, mais les données commencent simplement à être analysées et il est trop tôt pour pouvoir conclure.

J'ai particulièrement aimé la partie sur l'écotoxicologie même si la mise en place des analyses a été difficile (il a fallu trouver un autre laboratoire spécialisé pour pouvoir réaliser les analyses). De plus, mes compétences en écotoxicologie étaient très limitées au début de ma thèse donc il m'a fallu beaucoup apprendre pour pouvoir analyser et interpréter mes données. Je regrette de n'avoir pas pu m'intéresser aux facteurs écotoxicologiques extrinsèques (pollution du substrat du nid) qui me semblent également très important à étudier par la suite et pourrait jouer selon moi un rôle majeur à Yalimapo. D'autres contaminants devront également être pris en compte, comme les hydrocarbures aromatiques polycycliques (HAPs), issus du dégazage des bateaux. J'ai d'ailleurs pu observer plusieurs fois pendant la campagne d'échantillonnage en 2006 des petites galettes de pétrole sur la plage.

Je voudrais maintenant poursuivre mes recherches par un post doctorat consacré à l'écotoxicologie chez les reptiles. Je souhaite particulièrement approfondir les conséquences des transferts maternel et environnemental sur le développement des embryons et la fitness des juvéniles issus de femelles plus ou moins contaminées. Le passage à l'expérimentation me parait une phase indispensable pour améliorer les connaissances dans le domaine.

L'importance de la contamination environnementale dans le déclin des populations est faiblement comprise, en partie à cause du manque d'information sur les effets des contaminants sur les individus ; il est donc nécessaire d'augmenter les connaissances sur les contaminants et leurs impacts sur la croissance, le stockage d'énergie, la reproduction ou la viabilité des embryons et des juvéniles produits (Gibbons et al., 2000; Hopkins et al., 2006; Selcer, 2006).

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Résumé

La tortue luth, *Dermochelys coriacea*, présente une réussite d'incubation relativement faible en comparaison des autres espèces de tortues marines. Ce faible taux a été attribué à une mortalité embryonnaire accrue plutôt qu'à une infertilité, mais les causes spécifiques de cette mortalité restent peu connues. Les espèces longévives, comme la luth, sont plutôt vulnérables à une mortalité adulte élevée, qui peut entraîner d'importants déclins des effectifs. Cependant, sur le long terme, un faible recrutement des juvéniles peut influencer défavorablement la dynamique d'une population. A Yalimapo, en Guyane Française, la réussite d'incubation est encore plus faible que sur les autres sites de ponte de l'espèce ce qui pose réellement le problème du recrutement des juvéniles et du renouvellement des effectifs de cette population. Comprendre les causes de ce faible succès de reproduction est donc primordial pour assurer la conservation de cette espèce.

Au cours de cette thèse, je me suis intéressée à l'effet de certains facteurs écologiques (prédation, place du nid sur la plage) et écotoxicologiques (niveaux de contamination dans le sang et les œufs en éléments traces métalliques et composés organochlorés) sur la réussite d'incubation des nids. Il est apparu en premier lieu que le choix du site de ponte par la femelle avait un impact direct sur la réussite d'incubation, en jouant sur la densité de prédation et les risques d'inondation des nids. Dans un second temps, la présence de contaminants dans les œufs a confirmé l'existence d'un transfert maternel de la femelle vers ses œufs qui expose les embryons à une source de pollution dès les premiers stades de développement, souvent les plus sensibles. Les relations doses-effets restent à évaluer pour déterminer le risque encouru par les tortues face au problème de la pollution environnementale. De plus, l'utilisation des isotopes stables a permis de détecter que les femelles différant dans le nombre d'années séparant deux saisons de reproduction, présentaient également des différences dans leurs sites d'alimentation (situation géographique, qualité environnementale du site) soulevant le problème de la contamination des adultes.

Cette thèse a permis de confirmer l'importance des facteurs écologiques dans la réussite d'incubation et de souligner l'existence des facteurs écotoxicologiques qui, jusqu'à ce jour, n'avaient pas été envisagés chez la tortue luth.

Abstract

Leatherback turtles, *Dermochelys coriacea*, have relatively low hatching success in comparison to other marine turtle species. This low hatching rate is largely a result of high embryonic mortality rather than infertility, but the specific causes remain unknown. Leatherbacks are vulnerable to excessive adult mortality (resulting in population decline) because they are long-lived species. However, low hatching success and corresponding low juvenile recruitment could also result in long term declines of leatherbacks. On the Yalimapo beach, in French Guiana, hatching success is lower for this species than on other nesting sites, emphasising the problem of recruitment for the population. Understanding the causes of low hatching success is therefore an important conservation step towards preventing extinction in this population.

During my thesis, I investigated the role of ecological (predation and nest site location) and ecotoxicological factors (blood and egg contamination by trace elements and organochlorine compounds) on the hatching success of leatherback nests. Firstly, nest location was shown to have an important effect on predation and inundation rate that decreased hatching success. Secondly, a maternal transfer of contaminants from females to their eggs was confirmed, raising the issue of the deleterious effects of environmental contaminants on embryos development, a developmental stage very sensitive to contaminants. Dose-effect relationships between contaminants and hatching success need to be assessed to establish the risk of environmental pollution for leatherback reproduction. Moreover, the use of stable isotope analysis for females differing in the number of years between two reproductive seasons revealed that they used different feeding areas. These feeding grounds differed in their geographical location, but also in the quality of the available prey in terms of their level of contamination by environmental pollutants, highlighting the issue of adult contamination.

This thesis confirmed the importance of ecological factors for hatching rate and highlighted the existence of ecotoxicological factors, which have not yet been studied for the leatherback turtle.