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TESIS DOCTORAL

Anidación y conservación de la tortuga laúd, *Dermochelys coriacea*, en la playa de Kingere, Gabón, Africa Central.

Nesting and conservation of the leatherback turtle, *Dermochelys coriacea,* at the beach of Kingere, Gabon, Central Africa.

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Introducción

Generalidades sobre la especie

La tortuga laúd, *Dermochelys coriacea*, es la única especie de la familia Dermochelyidae dentro del orden Testudines. Se cree que divergió de las otras especies de tortugas marinas durante el periodo Cretácico o Jurásico hace 100-150 millones de años (Zangerl, 1980) y presenta unas características anatómicas y fisiológicas únicas. Es la tortuga de mayor tamaño que existe en la actualidad, con pesos de hasta 600kg (Eckert *et al.* 2009). El mayor especimen encontrado, un macho varado en la costa de Gales, tenía un peso de 916kg y una envergadura de las aletas delanteras de 240cm (Eckert and Luginbhul, 1988). No tiene un caparazón queratinizado como la mayoría de las otras especies sino que posee una piel gruesa que recubre un mosaico de osículos dérmicos conectados por cartílago (Wyneken, 2003). Este caparazón tiene siete quillas longitudinales y forma ahusada, favoreciendo la natación (Wyneken, 2003). A pesar de ser un reptil, se considera que es parcialmente endotérmica ya que es capaz de mantener su temperatura corporal por encima del agua circundante (Frair *et al.* 1972). Esto parece ser posible gracias a un conjunto de adaptaciones morfológicas y fisiológicas que se han agrupado bajo el término de “gigantotermia”, similar a los extintos dinosaurios (Paladino *et al.* 1990). Entre estas adaptaciones se pueden citar su gran tamaño corporal, una gruesa capa de grasa aislante y un control del reflujo de sangre en las aletas delanteras (Greer *et al.* 1973). Esto supone que *D. coriacea* no está limitada por barreras climáticas y tiene un mayor rango de distribución en el mar. Se caracteriza por realizar largas migraciones de miles de kilómetros a través del océano entre las áreas de anidación y alimentación (Pritchard, 1976; Benson *et al.* 2007). También tiene inmersiones largas y profundas de hasta más de 1000 metros de profundidad durante las cuales se cree que busca alimento (Mendilaharsu *et al.*, 2008; Houghton *et al.* 2008). De hecho, esta especie está especializada en el consumo de medusas y especies asociadas (Davenport & Balazs, 1991, Houghton *et al.* 2006). Tiene un crecimiento más rápido que las especies con caparazón queratinizado y se cree que alcanza antes la madurez sexual, a una edad estimada de 9 años (Zug and Parham, 1996). Como especie longeva, también se ha estimado que puede pasar un mínimo de 20 años reproduciéndose (ver referencias en Reina *et al.* 2002).

Los hábitos son completamente marinos excepto para una fase de la reproducción (la puesta de huevos) en que necesita salir a tierra firme. A pesar de su amplia distribución en el mar, las áreas de reproducción se concentran en latitudes tropicales y subtropicales, entre los paralelos 40º N y 35 º S (Lutz and Musick, 1996). Es la tortuga marina de hábitos más pelágicos y habita los océanos Índico, Pacífico y Atlántico (Marco *et al*. 2009). La presencia en las costas ibéricas es común, tanto en el Cantábrico (Peñas-Patiño, 1989; Camiñas, 1997) como en el Mediterráneo (García y Chamorro, 1984).

La reproducción consiste en la cópula en alta mar y la salida de las hembras a las playas para depositar centenares de huevos en la arena. Los machos nunca salen a tierra firme y después del apareamiento regresan a las zonas de alimentación, situadas a miles de kilómetros (Ackerman, 1997). Las hembras salen a las playas y ponen varios nidos durante una misma temporada de anidación (cinco de media reportados para Gabón; Fretey & Billes, 2000). El proceso de anidación comprende varias fases desde la salida de la hembra del mar hasta su regreso. Asciende por la playa y forma “la cama”, un espacio que despeja con las aletas delanteras y traseras. Después, comienza a excavar el nido con las aletas traseras y sigue la puesta de los huevos. Recubre los huevos de arena con las aletas traseras y procede a camuflar todo el área. Cada fase dura entre 15 y 20 minutos (Marco *et al*. 2009). Las hembras depositan dos tipos de huevos: unos grandes y esféricos provistos de una yema (huevos normales o verdaderos) y otros más pequeños de formas irregulares. Los huevos verdaderos o viables son aquellos que han sido potencialmente fertilizados por el esperma del macho y que pueden originar un neonato. Los huevos “falsos” carecen de una yema (y por tanto de un núcleo) y no son más que sacos de albúmina recubiertos por una cáscara (Marco *et al*. 2009). Por sus siglas en inglés y acuñando la terminología de Bell *et al. (*2003) nos referiremos a ellos como SAG (Shelled Albumin Globules). Los huevos eclosionarán unos dos meses más tarde y las crías se dirigen instintivamente hacia el mar donde comienza la fase más desconocida de la vida de las tortugas marinas.

La mortalidad de huevos y crías es naturalmente alta; de ahí la razón por la que las hembras ponen tantos huevos (Manugsson *et al*. 1990). Sin embargo, una vez adultas tienen pocos depredadores naturales: tiburones y orcas en el mar y jaguares en las playas (Heitaus *et al*. 2009). *D. coriacea* esta incluida en la Lista Roja de especies amenazadas de la Unión Internacional para la Conservación de la Naturaleza con la categoría de “En Peligro Crítico” (Sarti, 2009). También está incluida en el Libro Rojo de los Anfibios y Reptiles de España con la categoría “En Peligro Crítico” (Camiñas, 2004). Los principales sitios de anidación se encuentran en la región Atlántica se encuentran en Gabon-Congo (Fretey *et al.* 2007), Guayana Francesa-Surinam (Girondot *et al.* 2007), Trinidad (Livingstone, 2006) y Caribe Centroamericano (Tröeng *et al*., 2004; Patiño-Martínez *et al.* 2008) y se cree que estas poblaciones están estables o incluso en auge. Sin embargo, las poblaciones Indo-Pacíficas están seriamente en declive y aparentemente al borde de la extinción como demuestran las tendencias en playas de Costa Rica (Reina *et al.* 2002; Spotila *et al*., 1996), Méjico (Sarti *et al.* 2007) o Malaysia (Chan and Liew, 1996). En general, las mayores amenazas antropogénicas sobre la especie son la captura accidental de individuos adultos por pesquerías industriales, la recolecta insostenible de huevos en las playas y la caza furtiva de hembras (Chan and Liew, 1996; Spotila *et al*., 2000; Tröeng *et al*., 2004; Carranza *et al*., 2006).

Conservación de Tortugas Marinas en la costa Atlántica de África

Se ha señalado la presencia de 6 especies de tortugas marinas en la costa Atlántica Africana (Fretey, 2001): tortuga verde (*Chelonia mydas*), carey (*Eretmochelys imbricata*), olivácea (Lepidochelys olivacea), tortuga de Kempii (*Lepidochelys kempii)* y la laúd (*Dermochelys coriacea*). La séptima especie (*Natator depressus*) está confinada a la región australiana. Existen importantes áreas de alimentación crecimiento y anidación repartidas desde Mauritania hasta Angola (Fretey, 2001). Parece haber un epicentro de anidación de tortuga laúd en el ecuador, en las costas de Gabón y Congo que se dispersa hacia el norte y hacia el sur (Fretey *et al.* 2007). Se ha confirmado anidación de laúd en Guinea Bissau, Sierra Leona, Liberia, Costa de Marfil, Ghana y Benin pero hay pocos datos existentes y en la mayoría de los casos se trata probablemente de eventos esporádicos (Fretey, 2001). Hacia el sur, parece haber un importante área de anidación en Angola (Carr & Carr, 1991). También hay anidación regular en las islas de Sao Tomé y Principe y Bioko (Tomas *et al.* 1999; Tomás, 2004; Rader *et al*. 2006). En general el estudio y conservación de tortugas marinas en África se encuentra a un nivel muy preliminar, comparado con otras regiones como América (Fretey *et al*. 2007) y la mayoría de los proyectos existentes llevan apenas funcionando una década. Por lo tanto, existen grandes lagunas en el conocimiento de muchos aspectos de la biología de estos reptiles.

Antecedentes de la tortuga laúd en Gabón

La importancia de las costas gabonesas como sitio de anidación para la tortuga laúd fue dada a conocer a la comunidad científica por Jacques Fretey en 1984, a raíz de las observaciones de una maestra (Nicole Girardin) que observó rastros de laúd en Pongara durante una excursión escolar (Fretey, 1984; Fretey y Girardin, 1989). Las primeras actividades de seguimiento fueron llevadas a cabo en las playas de Mayumba, al sur del país, donde se estimó una población anidante de 5.800 hembras (Billes, 2000), confirmándose así la importancia de este sitio a escala global. Se consideró inicialmente que era la segunda región más importante del mundo para la reproducción, después de la Guayana Francesa-Surinam (Fretey *et al.* 2007). En la actualidad, se cree incluso que pueda superar a la colonia Guyano-Surinamesa con una estima de entre 36.000 y 100.000 nidos puestos por temporada, estimados a partir de censos aéreos de toda la costa (Witt *et al.*, 2009). Considerando que cada hembra deposita una media de 5 nidos por temporada, esto podría corresponder a una colonia reproductora de entre 5.000 y 20.000 hembras (Witt *et al.* 2009).

Existen tres zonas con un proyecto mas o menos establecido para la conservación e investigación de tortugas marinas en Gabón: Mayumba (al sur, frontera con el Congo), Gamba y Pongara (al norte), a cargo de organizaciones no gubernamentales (ONGs) e instituciones nacionales e internacionale, entre las cuales se pueden citar: *Aventures Sans Frontières* (ASF), *Gabon Environnement*, *World Wildlife Fund* (WWF) y *Wildlife Conservation Society* (WCS). Además existen dos iniciativas que tratan de coordinar los diferentes proyectos a nivel regional: PROTOMAC (Protección de Tortugas Marinas de África Central), con fondos europeos y "Partenariat pour les Tortues Marines du Gabon" financiado por el Departamento de Pesca y Vida Silvestre de Estados Unidos.

El éxito de incubación de los nidos es uno de los principales parámetros que se evalúa en las playas de anidación que que es una medida del éxito reproductivo y la salud de la población anidante. Para la tortuga laúd, suele ser más bajo que en las otras especies, aparentemente debido a una alta mortalidad embrionaria en (Girondot *et al*. 1990; Bell *et al*. 2003; Whitmore and Dutton, 1985). Este parámetro ha sido extensamente evaluado en las playas del Atlántico Oriental con valores medios reportados de 38% en la Guayana Francesa (Caut *et al*. 2006, Torres *et al*. 2002), de 10 a 56% in Surinam (Hilterman *et al.* 2007), 41% en Costa Rica (Chacón *et al.* 2007), 67% en la isla de St Croix (Boulon *et al*. 1996), 67% en Florida (Stewart and Johnson 2006), de 44 a 75% in Colombia (Patiño *et al.* 2008) y 52.5% en Trinidad (Livingstone, 2006). Como se puede observar, hay valores altamente variables entre las diferentes regiones e incluso dentro de una misma región, como es el caso de las playas en Surinam. También ocurre que se utilizan diferentes metodologías para estimarlo. Mientras que lo realmente representativo es tomar una muestra de nidos repartidos al azar en la playa y marcados en el momento de la oviposición, muchos estimas se basan en una muestra de sólo nidos exitosos de manera que se puede estar sobrevalorando el valor (Manugsson *et al*. 1990; Torres *et al*. 2002). La amplia información existente en el continente americano contrasta con la falta de datos en el lado Africano. Tomás (2004) estimó un éxito medio de emergencia del 57% para 12 nidos en la isla de Bioko, Guinea Ecuatorial. En Gabón, a pesar de tener más de 800 km de playas de anidación, sólo existían tres estudios hasta la fecha con valores estimados del 70% para 43 nidos en Punta Denis (Bourgeois, 2007), 56% para 35 nidos en Gamba (Livingstone, 2007) y 67% para 49 nidos en Mayumba. Sin embargo, estos tres estudios se basan, como explicamos anteriormente en una muestra de nidos encontrados en la playa que presentaban señales de haber eclosionado.

Determinación sexual en tortugas marinas y estimación de la razón de sexos

La razón de sexos es otro parámetro clave a la hora de evaluar el éxito reproductivo y la continuidad a largo plazo de una determinada población. Todas las especies de tortugas marinas tienen un mecanismo de determinación sexual regido por la temperatura a la que se incuban los embriones. Aunque este mecanismo no está del todo comprendido, en general se ha comprobado que existe una temperatura pivotal a la que se producen 50% de ambos sexos y que temperaturas por encima o por debajo de ésta producen mayor cantidad de hembras y machos respectivamente (Mrosovsky and Pieau, 1991; Shine 1999; Davenport, 1997). Otro parámetro importante, es el rango de temperaturas a las cuales se producen crías de ambos sexos, y las temperaturas extremas a las cuales se producen un 100% de un sólo sexo. Para la tortuga laúd, la temperatura pivotal se ha estimado en 29.5º C y el rango de temperaturas para producción mixta de sexos está entre 29 y 30º C (Rimblot-Baly *et al*. 1987). Estos datos se obtuvieron con huevos de la Guayan Francesa bajo condiciones de incubación artificial en laboratorio lo que permitió elaborar una curva de razón de sexos para la especie. Aunque se cree que estos rasgos son bastante constantes entre poblaciones, no existe un estudio detallado sobre la población en Gabón.

Normalmente se asume que la proporción es 50% hembras-50% machos (Fisher, 1930); sin embargo, para tortugas marinas se han encontrado numerosas poblaciones donde la producción de crías está sesgada hacia las hembras (Mrosovsky and Provancha, 1991; Marcovaldi *et al*. 1997; Godfrey *et al*. 1997; Broderick *et al*. 2000; Godley *et al*. 2001). Para la tortuga laúd, se han encontrado playas sesgadas hacia las hembras en Malasia (Chan, 1995) y en la costa Pacífica de Costa Rica (Binckley *et al*. 1998) mientras que en Surinam parece que la producción de crías está bastante equilibrada (Mrosovsky *et al*. 1984, Godfrey *et al*. 1996).

Las crías de tortugas marinas no tienen dimorfismo sexual y por tanto es imposible diferenciar externamente el sexo, a diferencia de los adultos en los que los machos poseen una cola más larga (Marco *et al*. 2009). Así, la única manera de determinar el sexo de una cría o embrión es mediante cortes histológicos de las gónadas, lo cual implica el sacrificio del individuo (Rimblot-Baly *et al*. 1987). Considerando que muchas poblaciones están amenazadas, esta práctica resulta invasiva y poco ética, de manera que a lo largo de los años se han desarrollado métodos indirectos para estimar la razón de sexos producida en un nido. Se estima que la determinación sexual, cuando las gónadas se diferencian en ovarios o testículos, ocurre durante el segundo tercio de la incubación (Rimblot *et al.* 1987) y se considera que la temperatura prevaleciente durante este periodo crítico es un buen indicador de la razón de sexos en un determinado nido (Kaska *et al.* 1998). Dado que medir la temperatura de nidos individuales es una práctica limitada, la mayoría de las estimas a gran escala se basan en la temperatura de la arena a la profundidad media de los nidos.

Tortugas marinas y cambio climático

Existe una preocupación general por el efecto que pueda tener el cambio climático en las poblaciones de tortugas marinas, como lo demuestran el creciente número de estudios dedicados a este tema (Hawkes *et al*. 2009). Según los últimos registros meteorológicos, la temperatura global del aire y océanos están aumentando a un ritmo acelerado (IPCC, 2007). Este calentamiento global podrá acarrear otros fenómenos como la subida del nivel del mar, episodios climáticos extremos o cambios en las propiedades químicas del agua de mar (Leatherman *et al*. 2000; MacLean *et al*. 2001; Zhang *et al*. 2004). En general la temperatura es un parámetro extremadamente importante en la biología de las tortugas marinas, no sólo en el mar determinando su distribución (Davenport, 1997; McMahon and Hays, 2006) sino también en las zonas de reproducción, donde la temperatura de la arena influye en la incubación de los huevos y la determinación sexual de las crías (Mrosovsky and Pieau, 1991). El rango óptimo para el desarrollo embrionario tiene una amplitud de unos 10ºC, entre los 25-27ºC y 33-35ºC (Ackerman, 1997). Valores fuera de este límite pueden ser causa de muerte embrionaria o afectar a las crías y esta es la causa de la repartición actual de las playas de anidación. Por todo esto, las tortugas marinas son consideradas como un indicador biológico ideal para evaluar el impacto del calentamiento global (Janzen, 1994; Hawkes *et al*. 2009). Todas las especies de tortugas marinas actuales estaban presentes en la Tierra durante la Era Cuaternaria, que se caracterizó por tener grandes fluctuaciones climáticas, y por lo tanto, ya han estado expuestas a condiciones extremas sin haberse extinguido (Davenport, 1989). El problema con la situación actual es el carácter acelerado del cambio climático y el hecho de que algunas poblaciones están seriamente amenazadas (Davenport, 1989).

Uno de los principales impactos que se está evaluando es el aumento de la temperatura de la arena en las playas de anidación ya que esto puede llevar a una proporción de sexos sesgada hacia las hembras y una alta mortalidad de nidos por estrés térmico (Broderick, *et al*. 2001; Godley *et al*. 2001; Hays *et al*. 2005; Hawkes *et al*. 2007). La pérdida o alteración del hábitat de anidación es otro de los efectos negativos que se predice (Hawkes *et al*. 2009; Fuentes *et al*.

2009b). A pesar de ello, también se contemplan aspectos positivos, como el hecho de que aumentará la disponibilidad tanto espacial como temporal de nicho ecológico para la reproducción y alimentación (Hawkes *et al*. 2009), favoreciendo la expansión de las poblaciones. Davenport (1989) incluso sugiere que, de no ser amenazadas, un calentamiento global favorecería a estos reptiles, considerando que son supervivientes de la Era Terciaria. La combinación de estos potenciales efectos positivos y negativos, junto con la incertidumbre sobre la capacidad de evolución que puedan tener las tortugas marinas frente a un ritmo acelerado de cambio hace que sea difícil dar pronósticos sobre su supervivencia.

Planteamiento de la actividad investigadora

La tortuga laúd, *Dermochelys coriacea,* está incluida en los Libros Rojos Nacional y Mundial bajo la categoría de « *En Peligro Crítico* ». El océano Atlántico representa un área clave para su supervivencia con la colonia anidante más importante a nivel mundial en las costas de Gabón en África Central. Sin embargo, la importancia de esta región contrasta con la falta de conocimiento sobre la biología reproductiva de la especie en la costa Africana Atlántica en general. Debido a su amplia dispersión entre playas de anidación y zonas de alimentación en el mar, la perpetuación de esta especie depende de un esfuerzo interregional que combine programas de intervención tanto en las playas como en el mar.

Para animales marinos y migratorios como la tortuga laúd, los estudios en playas de anidación son una de las herramientas más asequibles para evaluar el estado de salud general de la población. La perpetuación de un sitio de puesta depende del éxito en la producción de crías que volverán a reproducirse a la misma zona. El éxito de incubación de los nidos y la razón de sexos producida en una determinada playa de anidación son una medida del éxito reproductivo y proveen una indicación sobre la idoneidad del sitio como sistema de incubación.

Por todo ello, era necesario realizar una primera incursión en el campo de la biología de la anidación de esta especie en Gabón. Se seleccionó la playa de Kingere por las siguientes razones: 1) la importancia del sitio, en términos de densidad de anidación , 2) la existencia de infraestructuras básicas para establecer un campamento de investigación 3) la ausencia de estudios previos en esta zona, convirtiéndolo en un estudio pionero.

Los resultados aportados pretenden contribuir significativamente a los esfuerzos de conservación y gestión de las poblaciones de *D. coriacea* en el Atlántico. Este trabajo constituye un diagnóstico general del estado de conservación de la especie en una zona de anidación hasta ahora desconocida para la comunidad científica, y a la vez, deja sentadas unas bases de continuidad para futuras actividades de manejo e investigación en Gabón.

Objetivo General:

Estudiar la anidación de la tortuga laúd, *Dermochelys coriacea*, en la playa de Kingere y evaluar su éxito reproductivo en términos de producción de crías para completar los conocimientos que se tienen de la especie en Gabón y en el resto del Atlántico así como dar recomendaciones para su conservación.

Objetivos Específicos:

1. Desarrollar una metodología adaptada al sitio y a la especie que pueda ser estandarizada y utilizada en otros proyectos de Gabón.
2. Describir los parámetros biológicos de las hembras, los huevos y los neonatos.
3. Estudiar la ecología del nido.
4. Determinar el éxito de supervivencia e incubación de los nidos, en condiciones naturales.
5. Identificar cuáles son las principales amenazas para las supervivencia de hembras, huevos y crías en las playas de Pongara.
6. Describir las condiciones térmicas de las playas y los nidos
7. Estimar la razón de sexos producida
8. Dar recomendaciones para futuras líneas de investigación.

Área de estudio: Parque Nacional de Pongara

La República Gabonesa, con una superficie de 267.667 km^2, pertenece a la región de África Central, bordeando el Golfo de Guinea. Limita al Norte con Camerún y Guinea Ecuatorial, al sur y al este con el Congo y se abre al océano Atlántico por el oeste. Se encuentra a caballo en la línea del ecuador entre las latitudes 2º 30´ N y 3º 55´ S. El 85% del país está recubierto por bosque ecuatorial, formando parte de lo que se podría considerar la "Amazonia" de África. En agosto del 2002, el difunto Presidente de la República Omar Bongo Ondimba anunció la creación de una nueva red de Parques Nacionales extendiéndose aproximadamente sobre unos 3 millones de hectáreas, lo que supone un 10% del territorio nacional. Hay cuatro Parques Nacionales en la costa: Akanda, Pongara, Loango and Mayumba, de los cuales sólo el último es marino. Otras regiones costeras están incluidas dentro de diferentes categorías de protección: las reservas de Wonga-Wongé, Sette Cama y Ouangu y el Complejo de Áreas Protegidas de Gamba. Todo ello constituye una red de áreas protegidas que abarca 354 de costa (~58%) (Witt *et al.* 2009).

El Parque Nacional de Pongara, con una superficie de 89000 ha. y 25 kilómetros de costa oceánica, está separado de la capital de Gabón, Libreville por el estuario de Komo (Vande weghe, 2005). Su gestión queda a cargo de la Agencia Nacional de Parques Nacionales (ANPN). Punta Denis es el único poblado dentro del Parque que cuenta con unos 200 habitantes en permanencia. Por su proximidad de Libreville es el lugar de veraneo de los diplomáticos del país. Existen pequeñas aldeas de cazadores-recolectores distribuidas por el interior del Parque que no suman más de 100 personas. Las actividades de subsistencia son la pesca artesanal, la caza furtiva, la recolección de plantas medicinales y recientemente el turismo. Los habitantes locales son los Mpongwé, un sub-grupo de los Myéné y poseen territorios ancestrales dentro del Parque donde yacen las tumbas de sus antepasados. También hay un gran porcentaje de habitantes de los países vecinos y de África del oeste que se dedican a la pesca y a cuidar las casas de veraneo. Existe un gran problema de contaminación lumínica de Libreville y el propio poblado de Punta Denis (Bourgeois *et al.* 2008).

Al sur de Punta Denis, el PN Pongara cuenta con 20km de playas arenosas y prácticamente vírgenes. La vegetación litoral es principalmente bosque esclerófilo de tipo matorral o arbustivo. Se desarrolla frente al océano y suele constituir una estrecha banda de unas decenas de metros con árboles que no sobrepasan los 10, 15 metros. La mayoría de estos árboles tienen hojas coriáceas y perennes adaptadas a la brisa marina. Las lagunas costeras se encuentran bordeadas de manglares dominados por Rhizophora y Avicennia en la parte anterior al comienzo del bosque húmedo. En la parte marina hay vegetación acuática, arbustos y plantas rastreras. Las sabanas son formaciones naturales que consisten en grandes explanadas ocupadas por herbáceas y tienen la misma morfología que las lagunas costeras. Cabe destacar la presencia de grandes mamíferos en la zona costera como elefantes de bosque (*Loxodonta africana cyclotis*), búfalos (*Syncerus caffer nanus*) e hipopótamos (*Hippopotamus amphibius*) que suelen ir al mar a desparasitarse.

Logística

Este trabajo se ha realizado dentro del marco logístico ofrecido por la ONG *Gabon Environnement*. El alojamiento consiste en un campamento situado a pie de playa accesible únicamente a pie o por vía marítima. Existe un camino para vehículos por el bosque pero suele estar impracticable en época de lluvias. El punto más cercano para comprar comida es la Punta Denis a 13 km pero sólo hay algunos productos básicos y muy caros de manera que el mercado del mes se hace en Libreville. la capital, situada del otro lado del estuario. Todo el transporte se hace por el mar. No hay electricidad ni generador. Los aparatos eléctricos necesarios para la investigación tienen que llevarse a cargar al campamento base situado a 6 km. La iluminación por la noche se consigue con lámparas de petróleo y linternas. El avituallamiento en agua, petróleo y gas para cocinar se efectúa vía mar no siempre con la frecuencia deseable por lo que muchas veces hay que tomar agua para beber de un arroyo cercano y cocinar con fuego de leña. Toda actividad que precise de agua dulce (higiene personal y tareas domésticas) se hace en arroyos que se encuentran a proximidad del campamento.

Vista aérea de la zona de la playa de Kingere y la laguna costera.

Zona ensanchada de la playa que corresponde a una antigua desembocadura de la laguna.

Zona de escorrentía de la laguna, altamente inestable y con talud de erosión.

Protocolo del trabajo de campo

Se llevaron a cabo un total de 4 campañas de investigación durante los años 2005 a 2009. Cada campaña tuvo una duración de 5 a 6 meses, durante los meses de anidación, de noviembre a abril. La parte fundamental de este estudio se ha llevado a cabo en la playa de Kingere dentro del Parque Nacional Pongara (0º 18´N, 9º 18´E). Durante la campaña 2008-2009, se recogieron datos adicionales en las playas de Punta Denis (PN Pongara), Gamba y Bame (PN Mayumba).

Las actividades básicas en playas de anidación consisten en realizar recuentos diarios de rastros y salidas nocturnas para la identificación de hembras. Estas quedaron a cargo del equipo de guardas de la ONG *Gabon Environnement* y se llevaron a cabo de acuerdo a los protocolos internacionales (Eckert *et al.* 1999). Durante las salidas nocturnas se hace un reconocimiento completo de las hembras anidantes, con toma de datos biométricos (ancho y largo curvo del caparazón) y se las identifica mediante un doble marcaje externo e interno. El marcaje externo consiste en anillas metálicas de tipo Monel aplicadas en las aletas traseras y el interno es un microchip (PITs por sus siglas en inglés: Passive Intehrated Transponder) que se inserta en el músculo en la zona del hombro mediante un inyector. Asimismo, cada mañana se realizan censos de rastros para contabilizar el número total de salidas de la noche anterior.

Estudio de éxito reproductivo

El trabajo básico ha consistido en realizar exhumaciones de nidos naturales previamente señalados para determinar el éxito de incubación. Para ello hay que salir a buscar hembras que se encuentren anidando a la noche y marcar los nidos de manera que se puedan vigilar durante todo el periodo de incubación hasta el nacimiento de los neonatos. El método de señalamiento está adaptado específicamente a la alta densidad de hembras y a la gran profundidad de los nidos de laúd (hasta más de un metro).

Para cada nido seleccionado se tomó la siguiente información en el momento de la puesta: fecha y hora de la puesta, identificación de la hembra (anillas y microchip), posición GPS del nido, profundidad del fondo del nido, distancia desde la superficie de la arena hasta el huevo más superficial, tamaño de la puesta, biometría de la hembra, *Selección y señalamiento de los nidos*

Cada nido se ha nombrado con un código único numerado por orden cronológico y con las siglas Dc (iniciales de la especie *D. coriacea*). Los nidos se marcan de forma provisional a la noche y permanentemente a la mañana siguiente. El marcaje provisional se hace con una cuerda de nylon atada a una estaca que se deja plantada en la playa.

Etiqueta de nido: Se ha utilizado una placa de madera de unos 10x15 cm con el código y plastificada para identificar cada nido. Esta etiqueta se coloca horizontalmente cuando la tortuga está tapando los huevos y queda enterrada en la arena a unos 20 cm por encima del nido. Se ha comprobado que no obstaculiza la emergencia de los neonatos y resulta extremadamente útil a la hora de excavar el nido ya que asegura la identidad del mismo (recordar la alta densidad de nidos en esta playa).

Estacas: La señalización permanente se hizo con cuatro estacas de aproximadamente 150 cm de alto de manera que el nido quedara en le vértice de una V imaginaria. Dos estacas (P1 y P2) se colocan el la parte alta de la playa y las otras dos (V1 y V2) se colocan en la vegetación. V1 y P1 están alineadas con el nido formando una línea perpendicular al mar. V2 y P2 están alineadas con el nido formando un cierto ángulo con respecto a P1 y V1. Las distancias se miden con una cinta métrica enrollable desde el extremo superior de la estaca hasta la superficie del nido en la arena. También se tomaron las distancias del nido a la vegetación (entendiendo por vegetación la zona en la que la cobertura vegetal sobre la arena es mas del 50%) y a la última línea de marea alta.

Este sistema de señalamiento permanente permite dejar al nido en condiciones absolutamente naturales y encontrar su emplazamiento exacto gracias a las cuatro medidas en cualquier momento de la incubación; ya sea para colocar la rejilla antes de la emergencia de los neonatos o para excavar el nido. Las estacas han de colocarse lo mas atrás posible debido a la alta frecuentación de hembras en la playa que pueden derribarlas a su paso. Aún así, es necesario revisar las estacas todos los días para hacer las reparaciones oportunas.

Dimensiones verticales del nido

La profundidad máxima del fondo del nido (desde la superficie de la arena hasta el fondo de la cámara de incubación) se midió introduciendo un metro de albañil durante las últimas fases de la excavación y justo antes del comienzo de la caída de los huevos. La medida se lee en el punto de intersección con la proyección caudal de la tortuga que se ha tomado como el nivel de la superficie. Suele ser conveniente tomar la medida a ambos lados del caparazón y hacer la media. En cuanto la tortuga empieza a mover la pata trasera con la que está tapando el nido (síntoma de que va a empezar a tapar los huevos) se prepara el metro para medir la distancia desde el ultimo huevo caído hasta la superficie de la arena. Para esta medida se toma el mismo punto de referencia en la proyección caudal.

Recuento de huevos

Los huevos se contaron a medida que estos caen de la cloaca de la hembra introduciendo la mano con un guante bajo la cloaca. En la otra mano se sujeta un contador automático. Los huevos verdaderos se van contando con el contador automático mientras que los SAG se cuentan mentalmente.

Biometría de la hembra

La tortuga se midió siempre cuando está tapando el nido tras el desove. Se tomaron el ancho y largo curvo del caparazón con un metro de costurero flexible. Antes de proceder a la medición siempre se quitó la arena. El largo corresponde a la curva mínima del caparazón tomada a un lado de la quilla central desde la hendidura nucal hasta el extremo más distal de la proyección caudal.

Recogida de los neonatos

Para facilitar la recogida de los neonatos que emergen de cada nido se decidió colocar algún dispositivo que permitiera retenerlos a su salida de la arena. De esta manera se pueden contar exactamente el nº de neonatos emergidos y se conoce el tiempo exacto de emergencia. La contención se colocó al día 50 de la incubación. Para esto se tiene que encontrar el lugar exacto del nido gracias a las medidas de las 4 estacas. Se excava hasta encontrar la etiqueta y los huevos. De paso, se aprovecha para observar el aspecto externo de los huevos y posibles síntomas de depredación subterránea. En la primera tanda de nidos la contención consistía en un cilindro de malla metálica forrado con tela mosquitera de 50 cm de alto y enterrada 10 cm. En la segunda tanda se eliminó la malla metálica ya que esta terminaba roñada y suponía más bien un peligro para los neonatos que otra cosa. Se utilizó la tela mosquitera colocada con 4 estacas formando un corral de unos 60 cm de diámetro.

Todos los neonatos se examinaron para detectar posibles aberraciones. De cada nido, se tomaron medidas biométricas de 10 seleccionados al azar. El peso se tomó con una balanza Pocket-350 de la marca GRAM. El largo y ancho recto del caparazón se miden con un calibre. Es importante quitar la arena pegada con ayuda de una brocha antes de pesarlos.

Biometría de los huevos y la puesta

La biometría de los huevos se tomó en 53 nidos. De cada nido se seleccionaron al azar 10 huevos que fueron pesados con una balanza de bolsillo Pocket-350 de la marca GRAM y medidos con un calibre manual (3 diámetros). Se tomó el peso total de la puesta con una pesola de 0-25kg. Los nidos utilizados para estudios biométricos se transplantaron a corrales de incubación donde pudieron ser vigilados hasta la emergencia de los neonatos. Se midieron 10 neonatos de cada nido seleccionados al azar.

Exhumaciones de los nidos

Los nidos se excavaron al día siguiente a la emergencia masiva de los neonatos o, en su defecto, 5 días después de la fecha prevista de las emergencias. Las primeras capas de arena se quitaron con una pala para hacer una trinchera alrededor de la zona del nido. Cuando se encuentra la etiqueta del nido se prosigue a mano hasta delimitar completamente la masa de huevos y arena. A continuación, se procede a retirar huevo por huevo todo el contenido del nido.

El contenido del nido se clasificó según lo propuesto por Miller, 2000:

- **Cáscaras enteras** (que tengan por lo menos el 50%): Son las cáscaras abiertas dejadas por los neonatos tras su eclosión y emergencia. Son de color blanco y están limpias en el interior (a diferencia de los huevos depredados donde se observan restos de materia orgánica).
- **SAG**: depredados y no depredados.
- **Crías atrapadas dentro del nido:** Neonatos muertos y/o vivos en la cámara de incubación y/o cuello del nido.
- **Huevos “pipped”:** huevos en los que la cría logró romper la cáscara pero, por causas desconocidas” quedó muerta en su interior.
- **Huevos no eclosionados** : Se clasificaron a su vez en depredaos y no depredados. Cada huevo se abrió para examinar su contenido:
    - **Sin desarrollo aparente:** no hay un embrión visible.
    - **Desarrollo temprano**: se observa un embrión macroscópico no pigmentado (de color blanquecino de 1 a 3 cm que todavía no ha adquirido la pigmentación en el cuerpo salvo en los ojos).
    - **Desarrollo tardío**: se observa un embrión que ya ha adquirido la pigmentación característica por todo el cuerpo.
    - **Indeterminado**: no se puede distinguir presencia o ausencia de embrión, frecuentemente debido a que el huevo se encuentra en descomposición, afectado por microorganismos.

El éxito de eclosión y emergencia se calculó en base al nº de cáscaras presentes en cada nido y a lo propuesto por (Miller, 2000):

$$\text{ÉXITO ECLOSIÓN}= \frac{\#\text{cáscaras}}{\text{Huevos totales}} \text{ X } 100$$

huevos totales =
pipped
+ cáscaras
+ huevos no eclosionados

$$\text{ÉXITO EMERGENCIA}= \frac{\#\text{cáscaras} -}{\text{Huevos totales}} \text{ X } 100$$

Estudio sobre biología térmica

La temperatura de la arena y los nidos se midió usando registradores automáticos de temperatura de la marca y modelo TidbiT ONSET Optic Stoaway que se programaron para una toma de datos sincronizada a intervalos de 30 minutos. Tienen una precisión de 0.3ºC y se calibraron mediante intercomparación antes y después del periodo de medición. La intercomparación se realizó por un periodo de entre 24 y 72 horas en el que los registradores estuvieron en el mismo espacio físico y sometidos a variaciones de temperatura. Se calculó la desviación de cada registrador respecto de la media total de todos los registradores. Desviaciones de ± 0,3 ºC fueron consideradas como normales dentro del rango de medida. Todo registrador presentando desviaciones mayores de este valor fue eliminado a efectos de análisis de datos y resultados.

La temperatura de la arena se midió a la profundidad media de los nidos de laúd, 70cm, durante cuatro temporadas de anidación en la playa de Kingere y durante la temporada 2008/2009 en las playas de Pointe Denis, Gamba y Mayumba. Los registradores se enterraron en zonas características de la playa donde permanecieron durante toda la temporada de anidación. Para medir la temperatura dentro los nidos, estos se colocaron durante la oviposición cuando la hembra ya había puesto unos 40 huevos de manera que quedaran en el centro de la puesta y se recuperaron al hacer la exhumación del nido.

Los registradores de temperatura utilizados y su colocación dentro de una nidada.

Análisis de datos

Los análisis estadísticos convencionales se realizaron con el programa SPSS, versión 16. Los datos de posición GPS de los nidos y mapas de la zona de estudio se trataron con Arc GIS (Arc Map, version 9.3).

Se asignó a cada nido una fecha juliana, considerando como primer y último día de la temporada de anidación el 1 de noviembre (día 1) y 31 de marzo (día 150) respectivamente. Para determinar los factores que intuyeron en el éxito de incubación se llevó a cabo un Modelo Lineal Generalizado usando el programa GenStat v12.1 (GenStat 2009). En el modelo, no se incluyeron ni los nidos perdidos por erosión ni las pseudo réplicas (nidos de la misma hembra).

Se consiguieron datos climatológicos de la Agencia de Meteorología ASECNA en Libreville para toda la temporada de estudio, en forma de temperatura del aire (lectura cada 3h) y lluvia (mm totales por cada 24 horas). Libreville se encuentra a 18km de la zona de estudio, del otro lado del Estuario Komo. La temperatura media del aire y la arena a la profundidad del nido se correlacionaron significativamente ($n=16$, Pearson $r=0.556$, $P<0.05$, $R^2 = 0.3096$) para un periodo total de 8 meses en que se tuvieron ambos datos disponibles. Así, se utilizó la temperatura del aire para predecir la de la arena en aquellos momentos en que los registradores no estuvieron midiendo, mediante la ecuación de la regresión: T arena= 0.9958 (T aire) + 1.77. Se encontró que la lluvia no tuvo efecto significativo por lo que no se incluyó en la regresión.

Para la estimación de la razón de sexos, los datos de temperatura de la arena fueron agrupados primero por medias diarias y posteriormente por periodos de 15 días para toda la temporada. Así, se llamaron 1N al periodo comprendido entre el 1 y el 15 de noviembre y 15N al comprendido entre el 16 y el 30, para todos los meses de la anidación. Para cada periodo de 15 días, se estimó la razón neta de sexos producida en base a la temperatura media prevaleciente. Dicha razón se multiplicó por la proporción de nidos incubando en ese momento para obtener una estima total de la temporada. Los pares de valores “temperatura-razón de sexos neta” se obtuvieron a partir de las dos curvas de determinación sexual disponibles para tortuga laúd correspondientes a las poblaciones de la Guayana Francesa y Pacífico Centroamericano. Asimismo, se utilizaron tres escenarios térmicos: la temperatura de la playa de Kingere (llamada temperatura 0 o índice, TK) y ±0.3ºC que fue la variación observada entre playas.

Resumen de las principales aportaciones

Capítulo 2: Ecología de la anidación

En este capítulo, se aportan datos descriptivos sobre la ecología de la anidación de *D. coriacea* en la playa de Kingere (7km) en el Parque Nacional Pongara durante tres años consecutivos (2005/2008). La temporada de anidación transcurre de noviembre a marzo, con un pico de intensidad entre diciembre y enero. La fenología es similar a la del resto de Gabón y África Oriental y también al Pacífico Americano pero opuesta a la del Atlántico Occidental. Durante la temporada 2006/2007 hubo un total de 4.783 nidos, mientras que en la temporada 2007/2008 la actividad de anidación se redujo en un 65%, con sólo 1.559 nidos. Este tipo de fluctuaciones interanuales son comunes en otros sitios de puesta para la tortuga laúd y pueden deberse a las condiciones ambientales de las zonas de alimentación. La actividad observada durante las dos primeras temporadas se puede calificar como de "anidación masiva" para esta especie, llegándose a ver hembras saliendo del agua de forma casi ininterrumpida durante toda la noche. Se sugiere que las hembras llegan en grupos a la playa y salen en forma de abanico.

La biometría de hembras, huevos y neonatos fueron similares a los valores reportados en otros sitios de Gabón pero difieren de otras regiones y parecen ocupar un valor intermedio entre las poblaciones anidantes del Pacífico y las del Atlántico Occidental. Las hembras anidantes tienen una longitud curva del caparazón (LCC) media de 150.5cm y un ancho curvo medio del caparazón (ACC) de 107.1cm. Se registró una gran frecuencia de individuos con longitudes comprendidas entre los 130 y 140cm (13%) y una hembra con un tamaño mínimo de 123cm que puso huevos normalmente. Esto podría indicar que se trata de individuos jóvenes pero también puede deberse a condiciones relacionadas con su alimentación. Por otro lado, algunas hembras fueron observadas con cicatrices de marcas previas en las aletas delanteras, que podrían corresponder a programas de marcaje efectuados en Guayana Francesa o Tongaland en Sudáfrica a finales de los años 70´. Esta observación podría constituir una evidencia de que un mismo individuo es capaz de anidar en ambos lados del Atlántico o incluso cambiar de océano migrando a través del Cabo de Buena Esperanza. Además, constituiría una evidencia de la longevidad del periodo reproductor de estas hembras, que llevarían más de 30 años anidando. Los traumas observados en las hembras anidantes son similares a los descritos en otros sitios de puesta y reflejan su interacción con artes de pesca (anzuelos, redes y filamentos) y con depredadores como tiburones. Ciertas hembras presentaban una sustancia no identificada en la zona nucal y caparazón que podría reflejar algún tipo de contaminación marina o residuos de petróleo.

Una nidada típica consta de 78.2 huevos viables y 30.4 SAG y tiene un peso medio de 7kg. Los huevos sin yema representan un 28% del total de la nidada en términos de nº de huevos y un 8% en términos de masa. Se detectó una correlación negativa entre el nº de huevos viables y los sin yema. El tamaño de la hembra (LCC) se correlacionó positivamente con el nº de huevos viables y el peso total de la nidada. Sin embargo, no se encontró relación con el tamaño de los huevos. Esta relación es similar a la encontrada en otros estudios y sugiere una estrategia reproductiva en la laúd que apuesta por maximizar el nº de huevos antes que el tamaño. Las hembras excavan el nido a una profundidad media de 71.3cm. El último huevo depositado queda a una distancia media de la superficie de 48.2cm. Por tanto, la altura media de la cámara de incubación es de 23.2cm. Durante la incubación, el 76% de los nidos termina a una profundidad mayor debido al aporte de arena por las mareas y los huevos se compactan de media 3cm. Tamaño de la hembra (LCC) y profundidad del nido están positivamente correlacionados de manera que hembras más grandes excavarían nidos más profundos.

No existe saqueo de huevos por humanos. Las principales causas de mortalidad de los nidos tiene un origen natural: inundación por mareas, depredación o pérdida por erosión de la playa. Los principales depredadores identificados son: cangrejos, insectos, mangostas, pequeños felinos y el lagarto *Varanus ornatus*. Tampoco se observó mortalidad de hembras por humanos (a diferencia de otros lugares en Gabón donde sigue existiendo). Sin embargo, se detectó una fuente de mortalidad preocupante causada por troncos de dimensiones gigantescas que aparecen varados en las playas procedentes de la industria maderera. Las hembras quedan atrapadas entre los troncos y mueren deshidratadas o heridas sin poder regresar al mar.

Capítulo 3: Éxito de incubación de los nidos

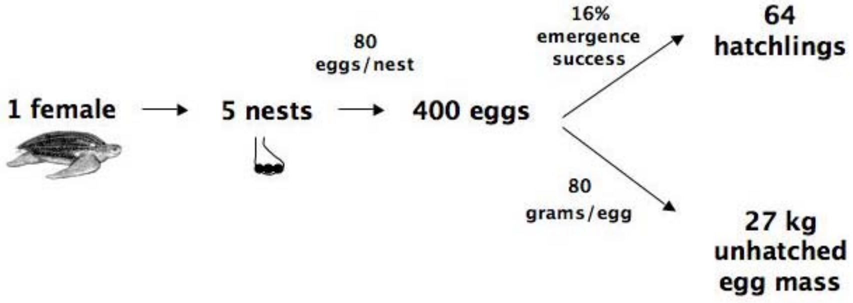
En este capítulo se aportan los resultados del primer estudio detallado sobre el éxito de incubación de nidos de tortuga laúd en la playa de Kingere en condiciones naturales y se compara con otros lugares de puesta en Gabón y la región Atlántica. Se desarrolló una metodología de trabajo adaptada a la especie y a las condiciones de alta densidad de nidos. Además se proveen recomendaciones sobre la metodología y colecta de datos sobre este parámetro, con vistas a la estandarización de protocolos. Se marcaron un total de 170 nidos durante las tres temporadas de estudio. La supervivencia de nidos fue de tan sólo el 36% de los nidos El éxito medio de eclosión y emergencia de los nidos fue del 16.7 y 15.8 % para el periodo completo, con medias anuales de 21%, 17% y 10% para las tres temporadas de estudio. Considerando sólo los nidos exitosos (n=61), la media de emergencia fue del 43.4% para el periodo completo, con medias anuales de 44%, 44% y 41%.

Para determinar los factores que afectaron al éxito de incubación, se llevó a cabo un Modelo Lineal Generalizado donde se incluyeron como variables independientes: año, fecha de puesta, LCC de la hembra, distancia del nido a la vegetación, profundidad media del nido, número de huevos viables, número de SAG y zona de la playa. Se consideró el que un nido sea exitoso o no como variable respuesta (dependiente). Los factores que resultaron significativos en el modelo fueron el sector de la playa, la fecha de puesta, la profundidad del nido y el número de huevos viables del nido. El factor que más influyó en la supervivencia de nidos fue la posición del nido en la playa. Se detectaron unos sectores mucho mas productivos que otros. En concreto, existe una formación arenosa que corresponde a una antigua salida de laguna costera al mar donde el porcentaje de supervivencia y emergencia de nidos es hasta más del doble que en el resto de formación típica de playa. Se atribuye esto a que la presión por depredación de insectos en este sector es mucho menor que en le resto de la playa y probablemente se deba a la ausencia de vegetación cercana. Los nidos puestos al principio de la temporada tuvieron un éxito menor que los puestos hacia la mitad de la temporada. Esto puede ser debido a un mayor régimen de precipitaciones durante los primeros meses de la anidación que afectó negativamente a la incubación de los nidos tempranos. Nidos más profundos tuvieron más probabilidad de eclosionar y esto se atribuye a una mayor estabilidad de las condiciones físico-químicas, como humedad, temperatura e intercambio gaseoso, durante el desarrollo embrionario. Finalmente, el número de huevos viables afectó negativamente a la supervivencia de los nidos, posiblemente debido a un efecto relacionado con la disposición espacial de los huevos dentro del nido. Nidadas de menor tamaño tienen una mayor cantidad de huevos en la periferia del nido que

resultan favorecidos en términos de temperatura y respiración metabólica, mientras que los huevos en el centro, pueden resultar desfavorecidos en este aspecto.
Los valores medios de éxito de incubación de nidos encontrados en este estudio son extremadamente bajos comparados con otras playas en Gabón. A nivel de la región Atlántica, se han encontrado valores igualmente bajos en la Guayan Francesa y Surinam y otros sitios del Caribe; sin embargo, existen otras regiones donde los éxitos superan el 70%.

De este estudio, se concluye que, en condiciones de alta mortalidad de nidos, como es el caso de Kingere, los valores medios de éxito de incubación (tanto eclosión como emergencia) difieren significativamente según se incluyen o no los nidos con éxito 0 en la media total. En el caso de Kingere, la diferencia es de más del 30%. El valor real y representativo es el que incluye a todos los nidos estudiados mientras que el otro es una sobreestimación ya que incluye sólo a los nidos eclosionados. Sin embargo, muchos estudios se basan en analizar únicamente nidos eclosionados y esto resulta un problema de cara a la comparación. Por otro lado, se recomienda especificar claramente en todos los estudios de incubación de nidos si se hace referencia al parámetro eclosión o emergencia ya que puede haber diferencias significativas entre ambos. En este estudio, la diferencia media entre ambos fue del 2.2% correspondiente a crías que quedan retenidas dentro del nido sin emerger a la superficie. El porcentaje de supervivencia de nidos es un parámetro igualmente informativo que debería de estimarse en todos los estudios de productividad.

La siguiente figura ilustra de manera esquemática la repercusión que tiene el bajo éxito de emergencia de los nidos en el balance final de la actividad reproductora de una hembra en Kingere, suponiendo que una hembra pone de media 5 nidos por temporada (Fretey and Billes, 2000) y con los parámetros reproductores que hemos estimado durante este estudio:



Capítulo 4: Depredación e Historia Natural de los Nidos

Los resultados de las exhumaciones de 163 nidos revelaron una mayoría de huevos no eclosionados (82.5%) y tan sólo un 16.7% de cáscaras vacías dejadas por crías vivas emergidas. Se encontró que el 59% de los nidos tenía más de la mitad de los huevos viables depredados y el 94% tenía al menos un huevo depredado. La tasa de depredación (% de huevos verdaderos depredados por nido) varió entre el 0 y el 100% y no implica necesariamente que el nido no produzca crías. Cada nido tuvo una media de 8.7 ± 8.8 SAG y 36.6 ± 26.4 huevos viables depredados. La mayoría de los huevos depredados estaba en estado avanzado de descomposición por lo que no fue posible discernir en qué momento de la incubación se produjo la muerte embrionaria y/o depredación. Los huevos no depredados carecían en la mayoría de los casos de un embrión visible sugiriendo que o bien no estaba fertilizados o bien hubo una muerte temprana del embrión, antes de que llegara a ser macroscópico.

Los depredadores principales sobre los nidos marcados fueron insectos y cangrejos (*Ocypodes cursor*). El tipo de depredador se identificó según la forma de los agujeros en la cáscara. Los cangrejos abren el huevo dejando una raja en forma de media luna característica. El resto de los huevos depredados que presentan otros tipos de agujeros claramente diferentes de los de cangrejo se catalogaron como depredados por insecto. Un 49% de los nidos depredados se atribuyeron a los insectos (n=79), un 18% a cangrejos (n=28) y en el 33% se observaron evidencias de los dos tipos (n=51).

La presencia de hormigas, identificadas como *Dorylus spininodis*, se observó en el 56% de los nidos afectados por la depredación, sugiriendo que se trata de uno de los principales actores. Esta es además la primera cita de esta especie en Gabón. El hallazgo más impresionante fue la observación de centenares de cadáveres de hormigas dentro de los huevos depredados. Otras veces se observaron nidos completamente infestados. Otros grupos de invertebrados asociados al nido fueron identificados pero ninguno parece ser potencialmente un depredador de huevos sino más bien oportunistas. Se trata fundamentalmente de insectos (hormigas, termitas y coleópteros), arácnidos (Acaridae), y anélidos (Lumbricidae). Se detectó una alta tasa de infección por microorganismos (hongos y/o bacterias) de diversas morfologías y colores, de los cuales se pudo identificar al hongo patógeno *Fusarium Oxysporum* creciendo sobre las cáscaras de huevos y SAG.

Se encontró una inusual interacción entre termitas y hormigas dentro del nido que podría corresponder a una relación de depredador presa. Las termitas fueron observadas en el 11% de los nidos, algunas veces muertas dentro de los huevos depredados y otras habiendo construido galerías sobre el molde de arena y huevos. Es muy posible que haya una colonización progresiva del nido con algunas especies pioneras como termitas y hormigas que podrían haberse especializado en explotar nidos y otras oportunistas que llegarían más tarde como los acáridos, frecuentemente asociados a huevos ya en estado de humus o cáscaras vacías.

Capítulo 5: Razón de sexos y predicciones frente al calentamiento global

La temperatura de la arena a la profundidad media de los nidos de *D. coriacea* en Kingere sigue un patrón estacional con valores más bajos durante los primeros meses de la anidación y aumentando hacia el final de la temporada. El rango térmico observado fue de 28 a 30ºC. Un patrón similar fue también encontrado en las otras playas donde se midió la temperatura: Gamba y Mayumba. En general, las variaciones entre las tres playas se mantuvieron dentro de un rango de ±0.3ºC sugiriendo unas condiciones térmicas homogéneas a lo largo de la costa.

Para estimar la razón de sexos, se utilizaron las curvas de determinación sexual disponibles para la especie, que corresponden a poblaciones de la Guayana Francesa y Pacífico centroamericano, ya que no existe un estudio detallado de la población gabonesa. Digitalizando las curvas se obtuvieron pares de valores de correspondencia temperatura-razón de sexos producidas. Así, se obtuvieron razones de sexos medias para periodos de 15 días a lo largo de la temporada. La proporción total para toda la temporada se calculó como la suma de todos los periodos de 15 días. Se usaron ambas curvas para evaluar posibles diferencias en el resultado. Asimismo, se añadió un factor de corrección de 0.6ºC a la temperatura de la arena, correspondiente a la media de calentamiento metabólico durante el periodo crítico para la determinación sexual. El calentamiento metabólico fue medido en 15 nidos donde se tenía un registrador de temperatura en el centro de la puesta y otro colocado a la misma profundidad pero a una distancia de un metro, fuera del nido. La temperatura dentro del nido aumenta a lo largo de la incubación, alcanzando valores máximos hacia el final del periodo. La media del calentamiento metabólico fue de 0.9ºC, alcanzando valores máximos de hasta 3.5ºC durante el final de la incubación. Durante el primer tercio, es casi inexistente mientras que durante el periodo crítico para la determinación sexual (alrededor del segundo tercio de la incubación), los huevos estuvieron de media 0.6ºC mas calientes que la arena, oscilando entre valores de 0.4 y 1.1ºC. Se comprobó, por tanto, que este factor tiene un efecto considerable a la hora de calcular la razón de sexos por medio de la temperatura de la arena.

La proporción media de sexos producida durante las cuatro temporadas de estudio fue estimada en un 60% (SD=21) de machos, con valores anuales de 60%, 37%, 54% y 87%. De acuerdo con la temperatura de la arena, la razón de sexos también sigue un patrón estacional, con tendencia a producir machos durante los primeros meses de la temporada y más hembras hacia el final. Las proporciones de sexos se estimaron también considerando tres escenarios diferentes en la temperatura de la arena: playa índice (Kingere), playa índice +0.3ºC y playa índice -0.3ºC (rango de variación observado entre playas). Los resultados muestran cómo con un escenario de

+0.3ºC ó -0.3ºC las proporciones quedan sesgadas hacia hembras y machos respectivamente. No se encontraron diferencias mayores en las estimas utilizando la curva Atlántica o la Pacífica. De hecho, la temperatura pivotal es semejante para ambas, difiriendo sólo en el rango de temperaturas que producen ambos sexos.

Para predecir la razón de sexos en el futuro, se estimaron las temperaturas resultantes en la arena con un hipotético aumento de 1ºC y 4ºC en la temperatura del aire, pronosticado por el IPCC para los años 2029 y 2099 respectivamente. Asimismo, se añadió el factor de corrección de 0.6ºC para calentamiento metabólico. Los resultados muestran cómo para el 2029 ya se alcanzaría un escenario de 100% producción de hembras. Para el 2099, la arena podría llegar a tener valores medios por encima de los límites tolerados para el desarrollo embrionario.

Discusión general

A pesar de estar su cercanía a la capital de Gabón, Libreville, la playa de Kingere permanece todavía virgen y prácticamente inalterada. Sin embargo, la principal amenaza la constituyen el desarrollo costero y el incremento de la actividad turística. Hay varios proyectos emergentes de construcción de centros turísticos y el número de personas que acuden para la observación de tortugas marinas va en aumento. Los promotores turísticos debería de recibir la información adecuada sobre la importancia del sitio para la anidación de tortugas marinas y evitar construir infraestructura cerca la playa. Asimismo, debería de mantenerse unas consignas estrictas acerca de las actividades de observación de tortugas marinas, como el uso de luces en la playa durante la noche o el número máximo de personas permitidas por grupo. Al norte de Kingere, Punta Denis se caracteriza por un turismo de tipo “sol y playa”, con actividades como los quadraciclos y motos acuáticas que son nefastas para el medio ambiente en general y la anidación de tortugas marinas en particular. Además, por su cercanía a Libreville, estas actividades son cada vez más populares y difíciles de controlar. Ante esta situación, sería recomendable que la playa de Kingere permanezca como un “santuario” para la anidación de la tortuga laúd, con acceso restringido, mientras que Punta Denis podría jugar un papel de centro educativo de cara a los visitantes.

La segunda amenaza más importante la constituyen los troncos varados en la playa procedentes de la industria maderera. En este estudio, se ha comprobado la alta incidencia de troncos enterrados en Kingere que puede igualar o incluso superar a los que están en superficie. Este problema genera un grave impacto sobre las hembras y crías, la incubación de los huevos y el hábitat costero en general. Tendiendo en cuenta que la presencia de troncos parece ser generalizada en toda la costa de Gabón (Laurance *et al*. 2008) y probablemente en los países vecinos, el numero de muertes de hembras anidantes podría alcanzar cifras alarmantes a escala regional y comparables a las causadas por otras amenazas como la caza furtiva o las pesquerías (Verhage *et al*. 2006, Carranza *et al*. 2006). Por el momento, no parece haber una solución viable para este problema y la única actuación eficaz es la de la vigilancia de las playas para llevar a cabo operaciones de rescate de las hembras atrapadas.

Si se considera el principio de la impronta, por la que las tortugas marinas regresan a anidar a la misma playa donde nacieron (Lohman *et al*. 1997), la baja supervivencia de los nidos en Kingere resulta paradójica comparada con la alta actividad de anidación. Sin embargo, dada la ausencia de registros históricos sobre esta playa y Gabón en general, no es posible saber si la anidación masiva y/o baja productividad son fenómenos recientes o no. Se cree que la tortuga laúd exhibe menor fidelidad al sitio de puesta, probablemente debido en parte a la naturaleza inestable de las playas en las que prefiere anidar, que suelen estar localizadas cerca de grandes estuarios, son altamente dinámicas y aparecen y desparecen por el efecto de la erosión y las corrientes marinas. Por ejemplo, se ha observado que una misma hembra puede anidar durante una misma temporada en Colombia y en Costa Rica y los intercambios entre playas de la Guayana Francesa y el país vecino Surinam parecen ser bastante frecuentes. De hecho, parece que la teoría de la impronta, no se cumple tan estrictamente en la tortuga laúd (Dutton *et al*. 1999).

Por lo tanto, no sería de extrañar que la playa de Kingere, y tal vez la costa de Gabón, sea un lugar recientemente colonizado por una población neófita o desplazada. Se cree que las hembras jóvenes podrían seguir a las experimentadas durante las migraciones y así llegar a una determinada playa de puesta. En la playa de Kingere, hemos observado una alta frecuencia de hembras con tamaños reducidos que podrían indicar que se trata de individuos jóvenes. Por otro lado, se han observado hembras con unas cicatrices en las aletas delanteras que podrían corresponder a programas de marcaje en otras playas de anidación, tan distantes como la Guayana Francesa del otro lado del Atlántico o Tongaland en Sudáfrica Occidental. En definitiva, la población anidante de Kingere y zonas aledañas podría corresponder a una mezcla de individuos jóvenes y experimentados procedentes de otras zonas de anidación que han colonizado recientemente este lugar.

En condiciones naturales, las tortugas marinas están adaptadas a soportar altas tasas de mortalidad sobre huevos y crías, de ahí su estrategia reproductiva de poner múltiples nidadas repartidas esporádicamente por la playa y durante la temporada de puesta (Manugsson *et al*. 1990). De hecho, las crías y los huevos son los estadíos de menor valor reproductivo comparados con adultos y juveniles (Manugsson *et al*. 1990). Por tanto, la baja productividad encontrada en Kingere no debería ser en principio ser un hecho alarmante. Sin embargo un bajo éxito de incubación de nidos prolongado en las playas combinado con tasas de mortalidad de adultos en el mar inusualmente altas puede ser una causa de declive poblacional (Heppel, 1997). Así, se cree que la causa por la que las poblaciones Indo-Pacíficas están al borde de la extinción fue el saqueo insostenible de huevos en playas de anidación combinado con la alta mortalidad de hembras en el mar por pesquerías. En el caso de la laúd, se estima que la captura accidental por pesquerías en el océano Atlántico es alarmante (Carranza *et al*. 2006) y por tanto, el bajo reclutamiento prolongado en Kingere podría tener consecuencias nefastas para la supervivencia de la especie a largo plazo.

Aún así, el hecho de que en otras playas de Gabón e incluso dentro de la propia playa de Kingere parece haber mejores condiciones para la incubación de los nidos es un hecho alentador, ya que el reclutamiento de nuevos individuos podría compensarse a una escala regional. Es más, dada la extensión de la costa de Gabón y Congo, con centenares de kilómetros aptos para la anidación, y la alta densidad de nidos, esta región tiene un rol extremadamente importante en el mantenimiento de las poblaciones Atlánticas. Bajo estas circunstancias, se recomienda mantener el estudio sobre éxito de incubación de nidos a largo plazo y extenderlo a otras playas.

Las altas tasas de depredación encontradas en Kingere parecen ser debidas mayormente a insectos y en particular, la hormiga *Dorylus spininodis* parece ser un depredador principal. A pesar de ser la primera vez que se cita la especie en Gabón (Ikaran *et al*. 2006), se trata de una especie pan-africana, nativa y no introducida por lo que se puede considerar una amenaza natural. Es además difícil de controlar ya que actúa de manera subterránea. Por este factor y la alta densidad de nidos, la posibilidad de llevar a cabo programas de reubicación de nidos a viveros o corrales de incubación para tratar de mitigar esta amenaza no es viable en Kingere. Además, el escaso personal disponible a cargo de la ONG y el Parque Nacional resulta insuficiente para abarcar el área de estudio y los requerimientos básicos de mantenimiento de dicha practica. A efectos de gestión de esta playa, resulta mucho más imprescindible mantener las actividades básicas de censos de rastros e identificación de hembras para concentrar los esfuerzos en el salvamento de los individuos atrapados entre los troncos.

Las principales fuentes de mortalidad sobre los huevos son de origen natural y parecen ser una consecuencia de las características de la arena y el efecto combinado de los ecosistemas bosque y mar sobre la playa. Por un lado, inundación y erosión afectan a un gran porcentaje de nidos ya que la franja de arena es muy estrecha, de apenas 20 metros de ancho. Por otro lado no existe prácticamente transición entre el suelo del bosque y la arena por lo que esta ultima recibe grandes aportes de material vegetal e invertebrados que posiblemente tengan un efecto negativo sobre la incubación de los huevos. Igualmente, la enorme cantidad de huevos que quedan sin eclosionar enterrados en la arena tras una temporada de anidación, que se ha estimado entre 1 y 3.5 toneladas por kilómetro, debe de suponer un aporte considerable de materia orgánica y microorganismos que pueden afectar al desarrollo embrionario. (Frente a estas dos presiones ambientales, mar y vegetación, a tortuga laúd parece haber desarrollado un patrón de comportamiento en el que pone sus nidos preferentemente en la zona media de la playa, como sugieren Kamel and Mrosovsky.) En el caso de Kingere, parece haber un factor añadido de origen antropogénico que influye potencialmente en la incubación de los nidos. Los troncos enterrados se descomponen lentamente y contribuyen a incrementar la cantidad de materia orgánica en la arena. Además, esta fuente de madera en descomposición constituye un foco de atracción para numerosos invertebrados procedentes del bosque, como por ejemplo las termitas que son potencialmente dañinos para los huevos.

Aunque la playa de Kingere no parece ser un sistema idóneo para la incubación de los nidos en términos de éxito de eclosión, sí que parece serlo en términos de razón de sexos. Las temperaturas de la arena parecen fluctuar naturalmente alrededor de la temperatura pivotal, a la cual se producen 50% de machos y hembras y ser favorables para una producción de sexos equilibrada o incluso sesgada hacia los machos. Sin embargo, nuestras estimas pronostican que en menos de dos décadas esta situación podría ser revertida con un sesgo del 100% hacia las hembras, una situación totalmente insostenible para la supervivencia de la especie. En efecto, *D. coriacea* tiene un patrón de determinación sexual por temperatura muy estricto. El rango de temperaturas a las cuales se producen ambos sexos es extremadamente estrecho para esta especie, con apenas un grado de amplitud. Esto implica que pequeñas fluctuaciones en la temperatura de incubación conllevan grandes sesgos en la proporción de sexos producida, como demuestran los resultados estimados con el rango de ± 0.3ºC. Así, se ha sugerido que especies como esta son altamente vulnerables a variaciones ambientales. Según las últimas predicciones del Panel Intergubernamental sobre Cambio Climático para la región Centro Africana, la temperatura del aire subirá una media de 1ºC y 4ºC para los años 2029 y 2099 respectivamente y esto produciría temperaturas de la arena totalmente desfavorables para el desarrollo embrionario y la determinación sexual.

De todas las especulaciones sobre los mecanismos de adaptación de las tortugas marinas al cambio climático, posibles cambios en la fenología de la anidación y desplazamientos hacia otras playas de puesta parecen ser los más asequibles para la laúd en esta región. Las temperaturas de la arena en Gabón parecen ser más bajas durante la estación seca, previa al periodo actual de anidación y por tanto, podría empezar a anidar antes. Por otro lado, hay grandes extensiones aptas para la anidación tanto al norte como al sur del epicentro Gabón-Congo y también podría desplazar sus sitios de puesta hacia latitudes donde las temperaturas sean más favorables. En definitiva, la disponibilidad de nicho ecológico espacial y temporal ofrecen un panorama esperanzador ante un inminente aumento en la temperatura de la arena. Esto se ve reforzado por el hecho de que la tortuga laúd parece tener un comportamiento de anidación mucho más flexible que las otras especies de tortugas marinas en términos de fidelidad al sitio de puesta. Sin embargo, otros efectos adversos del cambio climático, como la subida del nivel del mar o la destrucción de hábitats costeros podrían agravar al calentamiento global y tener consecuencias irreversibles para la especie.

Asimismo, gracias a este estudio, quedan abiertos numerosos campos de investigación relevantes para avanzar en el conocimiento y la conservación de la tortuga laúd en la playa de Kingere en particular y en Gabón y la región Atlántico en general. Hay varios factores que pueden estar afectando al éxito reproductivo de la especie pero no se tuvieron en cuenta en este estudio. Por ejemplo, sería necesario cuantificar el impacto e incidencia de los troncos enterrados sobre la incubación de los nidos. También se recomienda realizar un estudio sobre ecotoxicología para estudiar la posible presencia de contaminantes tóxicos en la playa o los huevos que podrían estar afectando al éxito de incubación y a la salud de la población anidante en general. En la Guayana Francesa, se han encontrado agroquímicos procedentes de las plantaciones cercanas a la playa y de la profilaxis para la malaria (Guirlet, 2005) y se sugiere que podría haber residuos de mercurio arrastrados por los ríos y procedentes de las minas de oro en el interior del país (Fossette *et al*. 2008). En Gabón, no existe prácticamente agricultura intensiva pero una de las mayores riquezas del país son los minerales, existiendo multitud de minas repartidas por el territorio. A esto se unen las plataformas petrolíferas, tanto en el interior como en el mar. Por otro lado, un estudio profundizado sobre la interacción entre nidos e insectos como las hormigas y termitas para determinar el mecanismo de la depredación y entender por que este fenómeno parece ser tan intenso en Kingere. Se recomienda igualmente realizar un inventario de los microorganismos encontrados en los nidos para evaluar su potencial impacto sobre el desarrollo embrionario y la posible identificación de patógenos humanos o causantes de plagas (Marco *et al*. 2006; Abella *et al*. 2008). A nivel más general, convendría cuantificar y estudiar las otras especies de tortugas marinas que anidan en Pongara (verde, olivácea y posiblemente carey) para determinar si se encuentran las mimas tasas de depredación y éxito de incubación de sus nidos. Esto podría ayudar a aclarar si lo observado para laúd es específico de esta especie o si deriva de las condiciones del medio de incubación.

Por último, cabe resaltar la continuidad de esta investigación, a través de la metodología y recolección de datos sobre los éxitos de incubación de nidos que se ha extendido a otros proyectos en la costa de Gabón (Ver Apéndice I). Esta contribución tiene doble significado ya que por un lado se han apoyado las iniciativas de estandarización de protocolos y por otro implica la disponibilidad de datos a largo plazo.

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Chapter 2: Nesting Ecology of *Dermochelys coriacea* at the beach of Kingere.

Summary

In this chapter, we provide descriptive information on the nesting ecology of *D. coriacea* at the beach of Kingere (7km) in Pongara National Park, from data and observations collected during three consecutive nesting seasons (2005/2008). Data on track counts was available for two of the years, 2006/2007 and 2007/2008. Nesting activity during 2006/2007 was massive, with almost uninterrupted emergences of females from the water during the peak period. There was a decrease of ~ 65% in the number of nests between the two years. The phenology was similar to that described for the rest of Gabon, spanning from november to march but there was a delayed peak of the nesting activity during season 2006/2007 that occurred between january and february. The biometry of females, eggs and hatchlings was similar to reported values at other sites in Gabon, but differed from those at other geographic regions and seem to occupy an intermediate position between the Western Atlantic and Pacific regions. A considerable proportion (13%) of the females had a curved carapace length below 145cm, the assumed size for sexual maturity, suggesting that this nesting population might be partly composed of young individuals. On the other hand, several females where seen with scars on the front limbs that most likely corresponded to lost tags applied previously either in French Guyana-Surinam or in South Africa. Poaching of egg was inexistent, the main sources of egg mortality were natural and originated from the conditions of the beach: erosion, flooding or predation. The viability of egg relocation programs was tested at different sites and predation by insects appeared as a major constraint for the establishment of hatcheries. The high incidence of lost timber on the beach caused the death of a considerable number of females. During the month of november 2006/2007, we counted 15 dead females at Kingere as the local NGO teams where not on duty yet. Once the regular monitoring activities started, ~ 30 females were rescued during the season. Apart from the logs, increasing coastal development, visitors and leisure activities such as quads or jet skies coming from Pointe Denis, the tourist resort in Pongara, were the main identified threats to the beach of Kingere. Despite this menaces, the site is still relatively wild and virgin. While Pointe Denis should play a role as an environmental education centre inside the National Park, we recommend that the beach of Kingere is kept as a sanctuary for the nesting of the leatherback turtle.

Introduction

Sea Turtles in the Eastern Atlantic Region

The presence of six out of the seven existing species of sea turtles have been reported on the Atlantic coast of Africa: olive ridley (*Lepidochelys olivacea*), leatherback turtle (*Dermochelys coriacea*), green turtle (*Chelonia mydas*), hawksbill (*Eretmochelys imbricata*), loggerhead (*Caretta caretta*), and the Kemp´s Ridley (*L. Kempii*) (Fretey, 2001). From Morocco to South Africa, important habitats have been identified for these species including feeding grounds, developmental habitats and nesting beaches (Fretey, 2001; Formia, 2002). The main nesting sites for green turtles are found in The Bijagos islands, Guinea Bissau (Catry *et al.*, 2002), Sao Tomé and Principe (Dontaine & Neves, 1999) and Bioko, Equatorial Guinea (Tomás *et al.*, 1999). Nesting of olive ridley is quite important all over the coast, from Guinea to Angola (Fretey, 2001). There is scattered nesting of hawksbills in the Bijagos Archipel, Bioko and Sao Tomé and Principe (Fretey, pers. comm.). As for leatherbacks, there seems to be an epicentre of nesting right at the Equator in Gabon-Congo from which the activity is dispersed northwards (Mauritania) and southwards (Angola) (Fretey *et al.* 2007). There is confirmed evidence of nesting northwards in Guinea Bissau, Sierra Leone, Liberia, Ivory Coast, Ghana, Benin but in most of the cases quantitative data is lacking and it is probably only scattered (Fretey, 2001). In the southern hemisphere, an important nesting site was reported in Angola (Carr & Carr, 1991). Regular nesting also happens in the islands of Sao Tome, Principe and Bioko (Tomas *et al.* 1999; Rader *et al*. 2006).

The leatherback turtle: generalities about the species

The leatherback turtle is the sole living species of the Dermochelyidae family, of the order Testudines*. Dermochelys coriacea* diverged from the other species during the Cretaceous or Jurassic Period, 100-150 million years ago (Zangerl, 1980) and is unique in many anatomical and behavioural features. It is the largest turtle in the world and one of the largest living reptiles. Although hatchlings weigh no more than 50g, an adult gravid female can weigh up to 600kg (see review on biometry by Eckert *et al.* 2009). The biggest specimen ever found, an adult male stranded in the coast of Wales, UK, weighed 916kg, had a curved carapace length of 256cm and a foreflipper span of 240cm (Eckert and Luginbhul, 1988). Unlike most turtles, the leatherback shell has no keratinized scales but a thick leathery skin covering a mosaic of thin bony plates connected by cartilage (Wyneken, 2003). The streamlined carapace is run by seven longitudinal keels (Wyneken, 2003) favouring hydrodynamics when swimming. Despite being reptiles, leatherbacks are partially endothermic, being able to maintain its body temperature well above the surrounding water (Frair *et al.* 1972). A combination of strategies (termed “gigantothermy”, Paladino *et al.* 1990) such as a large body size, effective fatty insulation and control of blood flow allow them to inhabit cold waters and dive to great depths. They also have a mechanism of counter-current flows in the flippers (Greer *et al.* 1973). Therefore, this species is not limited by climatic barriers and has a greater distribution range at sea. Sea turtles typically migrate between foraging and nesting grounds and the leatherbacks have the most extreme records with distances of more than 5000 km across the ocean (Pritchard, 1976; Benson *et al.* 2007). They also exhibit record dives of more than 1000m (Mendilaharsu *et al*. 2008; Houghton *et al.* 2008). Leatherbacks are specialized medusivores eating a wide range of jellyfish and associated animals (see review on feeding in Eckert *et al.* 2009; Davenport & Balazs, 1991, Houghton *et al.* 2006). They grow faster and reach sexual maturity at an earlier age, 9 years on average, than other sea turtle species (Zug and Parham, 1996). As long-lived animals, they are thought to spend at least 20 years as breeding adults (see references in Reina *et al.* 2002).

The leatherback turtle is included in the Red List of the International Union for the Conservation of Nature as “critically endangered” (Sarti, 2009). Major nesting sites around the world are in Gabon-Congo (Fretey *et al.* 2007), French-Guyana-Surinam (Girondot *et al.* 2007; Hilterman and Goverse, 2007), Trinidad (Livingstone 2006), St. Croix (Dutton *et al.* 2005) and Caribbean Central America (Tröeng *et al*., 2004, Patiño-Martínez *et al.* 2008). These nesting populations seem to be stable or even increasing. However, over the last decades, there have been drastic declines in most of the major Pacific nesting rookeries, as in Costa Rica (Reina *et al.* 2002; Spotila *et al*., 1996), Mexico (Sarti *et al.* 2007) and Malaysia (Chan and Liew, 1996). In general, the major threats to the species are unsustainable poaching of eggs and killing of females on nesting beaches and incidental capture by industrial fisheries at sea (Chan and Liew, 1996; Spotila *et al*. 2000; Tröeng *et al*. 2004; Carranza *et al*. 2006).

Background for Dermochelys coriacea in Gabon

Loveridge and Williams (1957) and Duguy (1983) reported the presence of the leatherback turtle in Gabon but without referring specifically to nesting activity. In the early 80´s, the observation of substantial numbers of tracks on the beaches south of Libreville (Fretey and Girardin, 1988; Fretey, 1984) triggered the international sea turtle community concern. The first monitoring activities on the beaches of Mayumba, south Gabon-Congo border, revealed 29.000 nests corresponding to an estimated rookery of 5800 females (Billes, 2000) confirming its importance at a global scale. It was initially considered as the second major nesting sites for leatherbacks in the world after French Guyana-Surinam (Fretey *et al.* 2007). Current monitoring activities, combining aerial and ground surveys estimate that 5,000 to 20,000 females nest annually in Gabon leaving between 36,000 and 100,000 nests per season, suggesting that Gabon hosts the largest leatherback nesting aggregation in the world (Witt *et al.* 2009).

Sea Turtle Conservation in Gabon

The country of Gabon is situated in the heart of Central Africa, divided by the equator line and bordered by the Guinea Gulf in the Atlantic Ocean. It has 885km of coastline of which the vast majority, are long continuous stretches of sandy beaches almost uninterrupted except by a few mangrove or rocky areas (Fretey, 2001). Concern about conservation and biodiversity in Gabon took a major step in 2002, when 10% of the national surface was declared protected areas into 13 National Parks, of which four; Akanda, Pongara, Loango and Mayumba, are on the coast. There are other coastal protected areas, under various regimes of protection such as Wonga-Wongé, Sette Cama and Ouangu Reserves and The Gamba Complex of Protected Areas. This network of coastal protected areas sums up 354 km (58% of the coastline) and encompasses 79% of the annual leatherback nests laid in Gabon (Witt *et al.* 2009). Main nesting sites identified during the course of aerial surveys are Mayumba, Gamba and Pongara (Fretey and Girardin 1989; Witt *et al.* 2009) where several conservation projects have been established by local and/or international NGOs. Sea turtle research and conservation is relatively new and emergent in Africa (Fretey *et al.* 2007) and most of these projects in Gabon have been running for less than a decade.

Pongara National Park

The major components of conservation interest at Pongara National Park, with 89000ha and 25 km of coastal line, are vast extensions of mangroves and nesting sea turtles (Vande weghe, 2005). Its location near the capital, Libreville, just across the Komo Estuary, makes it a very popular place with regular week-end visitors and tourists. North of Pongara, at Pointe Denis, NGO *Aventures Sans Frontières* has been doing research and conservation of sea turtles for over 10 years. This 5 km beach is the one receiving the majority of visitors coming to the National Park and suffers the bigger human impact because of its immediate proximity from Libreville (Bourgeois *et al.* 2008). Light pollution from Libreville and the village of Pointe Denis is a major threat to nesting females and hatchlings that get disorientated and lost in the surrounding savannah (Bourgeois *et al.* 2008). Another major environmental problem, both to sea turtles and the nesting habitat, is the highly abundant lost timber on the beaches originated from forestry activities (Laurance *et al.* 2008).

It is surprising that only a few kilometers south of the touristy Pointe Denis, and despite its proximity from the capital, there are stretches of uninterrupted wild and unexplored beaches. Recent aerial surveys showed that the beach of Kingere receives a great proportion of leatherback turtle nests every season (Witt *et al.* 2009) but so far, no specific research on the reproductive biology of the species had been undertaken at this site. This area could receive more than 150 females per night during the peak nesting season. There is almost no human presence or settlements apart from a few hotels and residential houses. The beach is almost entirely bordered by forest vegetation and the presence of large mammals such as elephants, buffaloes, antelopes and hippos is relatively common.

Methods

Study site and period

The fieldwork was carried out during three entire consecutive nesting seasons, from november to march in 2005/2006, 2006/2007 and 2007/2008. A research camp was set up at the beach of Kingere (0º 18´N, 9º 18´E) by local NGO, *Gabon Environnement* who provided basic logistic conditions. The typical landscape is a 10m wide sandy beach bordered by savannas, forest vegetation or lagoons. Lagoons´ water level is subject to rainfall and they can burst at specific places when the get too full. Beaches are subject to periodical erosion/accretion episodes by the sea resulting in total or partial loss of the sand and revealing many buried logs.

Terms and Definitions

The following are based on Eckert *et al*. 1999; Miller, 2000; Bell *et al.* 2003 and Eckert *et al*. 2009.

- **Viable eggs:** normal eggs that contain a yolk and can produce hatchlings.
- **SAG:** commonly known as "yolkless eggs", these are in fact "shelled albumin globules" or SAGs, that contain no yolk and will therefore not have a developing embryo.
- **Clutch:** although in strict terms of the sense, clutch should refer only to the viable eggs, we will use this to refer to all the viable eggs and SAG laid by the female.
- **Nest:** a track left by the female that presumably corresponds to a buried clutch and its immediate environment.
- **False crawl:** a track left by the female that returns to the water without having completed the process of nesting.
- **Track:** nests + false crawls.

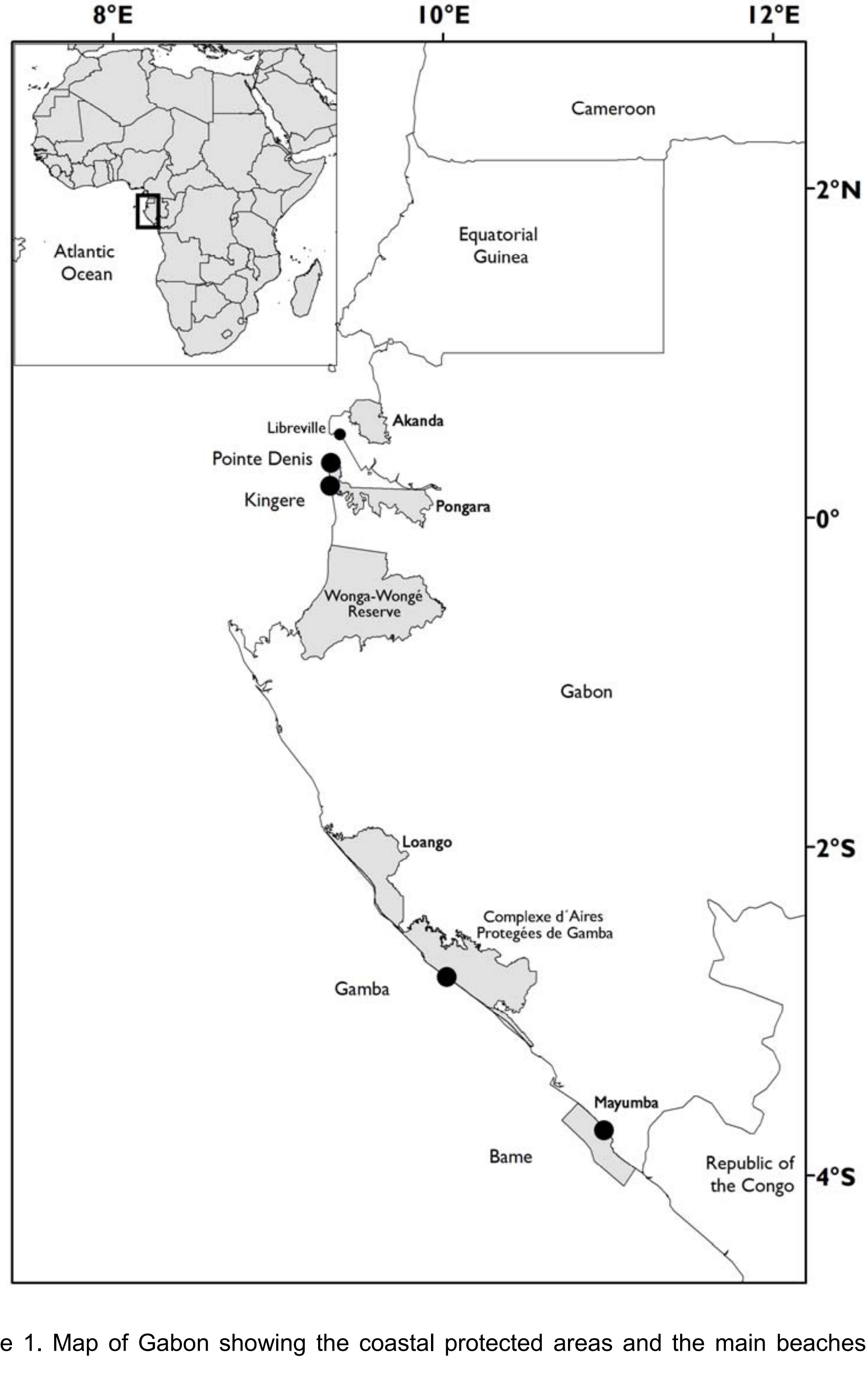

Figure 1. Map of Gabon showing the coastal protected areas and the main beaches were sea turtle conservation projects are established.

Basic monitoring activities during the nesting season were carried out by field staff of *Gabon Environnement*, according to the standard international protocols (Eckert *et al.* 2000). These consisted of nightly tagging surveys of females and early morning track counts.

Female identification

During night patrols, females were tagged with Passive Integrated Transponder (PIT) in the shoulder area and with Monel metal tags in both rear flippers for identification. The carapace of the females was measured with a flexible tape measure (curved carapace length, CCL and curved carapace width, CCW; ±0.1cm) and they were examined for possible injuries, epibionts or other abnormalities. Samples of epibionts were collected in eppendorf tubes and preserved in alcohol for identification. They were sent to the Smithsonian National Zoological Park, Washington, DC.

Track counts

Tracks were counted early in the morning over a 10km beach section. Based on the shape of the crawl; a distinction was made between real nests and false crawls (the female emerges and goes back to water without laying eggs). Data on track counts were available for two of the study seasons through the Gabon Sea Turtle Partnership, which holds the global database of all projects in Gabon. However, these datasets are incomplete and there were also difficulties in the interpretation of the data. We did a simple linear interpolation to complete the missing data and considered as 1 the number of nests laid on the first (1st of november) and last day of the nesting season (31st March).

Reproductive parameters

This specific study was carried out in conjunction with the basic monitoring activities of *Gabon Environnement* staff but involved taking additional data on the main reproductive parameters using in situ nests (n=170) and clutches that were relocated to hatcheries (n=53). The main methodology was based on Miller (2000). Whenever the emergence of a nest was observed, ten hatchlings were selected randomly for biometry. They were weighed using a GRAM scale, Pocket-350 (±0.1g) and their carapace measured with a caliper (straight carapace length and width, ±0.1mm). Over the course of the study, 53 nests were relocated to hatcheries (n=13 during 05/06, n=20 during 06/07, n= 20 during 07/08). These were nests laid close to seawater and the eggs were collected before being inundated. A plastic bag was placed underneath the female cloacae to collect the totality of the clutch. Artificial nests were dug by hand at mean depth for the species (70cm in this study) and the eggs were reburied no longer than 2 hours after collection. Ten eggs selected randomly from these 53 clutches were weighed using the same scale and three different diameters were measured with the calliper. The whole clutch was weighed in the same plastic bag where it was collected. For some of the clutches, SAG were separated and weighed apart. Additionally, a random sample of *in situ* nests was marked at oviposition and followed to term in order to evaluate incubation success (chapter 3). Clutch size was determined by direct counting during oviposition and again when nests were excavated. We counted SAG and viable eggs as they were laid, by lying in the sand behind the female and placing a gloved hand underneath the cloacae. The free hand was holding an automatic counter that was used for viable eggs. SAG could be differentiated by their smaller weight and size and were counted by memory. For all *in situ* nests, we measured the depth to the bottom of the nest before the female started to lay the eggs (maximum depth, A in fig. 2) and the distance from the last laid egg to surface before she started to cover the nest (minimum depth, B in fig. 2). These were taken using a rigid tape measure both at the beginning (oviposition) and end of incubation (during excavations).

Figure 2. Schematic figure of the nests and the vertical measurements that were taken at the beginning and end of the incubation period: A) maximum depth (depth of the bottom of the nest), B) minimum depth (distance from the top egg to sand surface) and C) estimated height of the egg chamber.

Results

Phenology and track counts

The bulk of the nesting activity at Kingere happened between november and march, although there is sporadical nesting in october and april. A total of 5,119 and 1,723 tracks were counted during seasons 2006/2007 and 2007/2008. False crawls accounted for 6 and 10% of the total tracks in both seasons. We estimated a rough density of 470 and 150 nests laid per beach kilometer during 2006/2007 and 2007/2008 respectively with a drop of nearly 65 % in the total number of nests between the two years. The peak of activity occurred later in 2006/2007 (between january and february) compared to 2007/2008 (between december and january) (Figure 3).

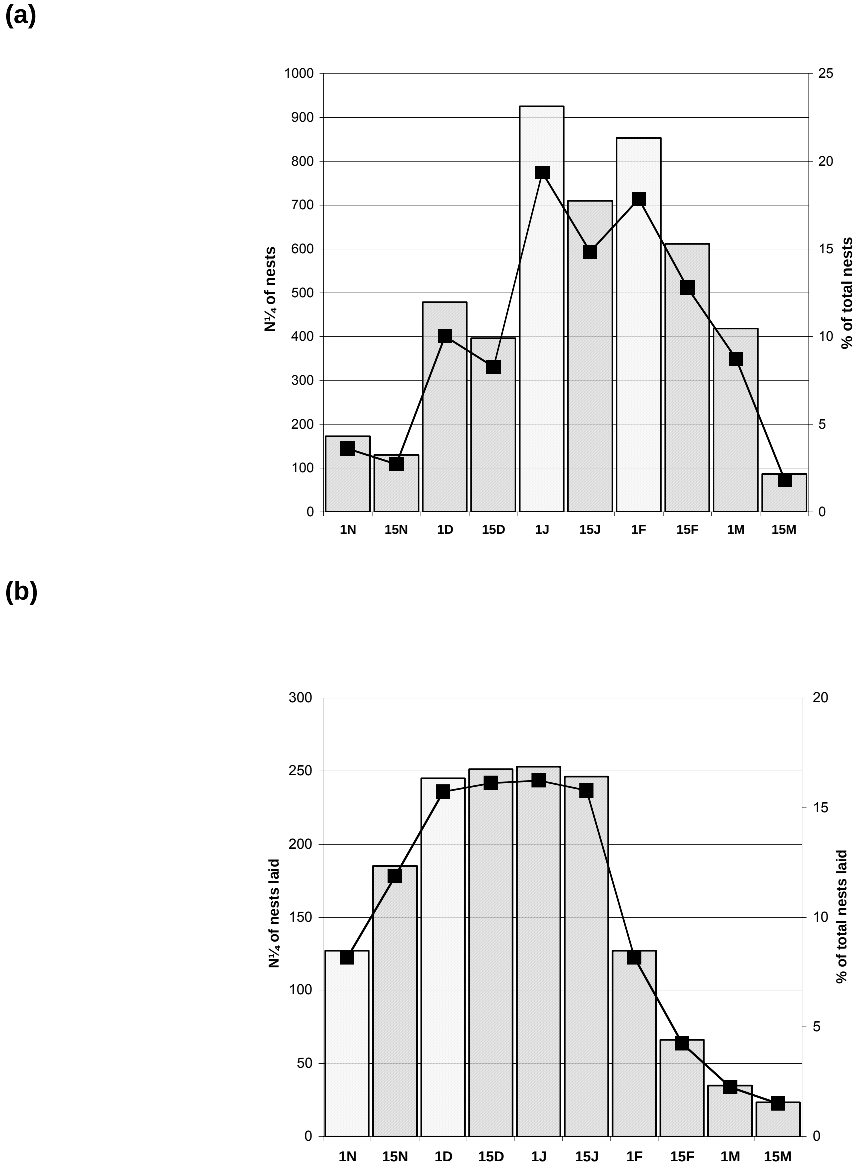

Figure 3. Leatherback nesting activity at Kingere during (a) season 2006/2007 and (b) 2008/2009 expressed as total number of nests laid (bars, left axis) and percentage of total nests laid during the season (line, right axis). Data are given on a 15 day window basis from the 1st of november (1N=1-15 november) to the 31st of march (15M= 16-31 march).

External condition of females and hatchlings

The most common injuries observed on nesting females were amputations, scars or bites on flippers and carapace, but there were other observations, as listed below:

- total or partial amputation of front and/or rear flippers (mainly from bites),
- bites or partial amputations of the carapace (Plate 1b),
- Scars produced by entanglement with gillnets or fishing hooks (especially frequent in the axilla area),
- Scars or fresh wounds produced by the logs on the beach or collision with boats,
- Fishing gear: hooks, lines or nets entangled over the flippers (Plate 1d),
- Malformations on the cloacae and carapace (Plate 1b),
- Self-injuries: Adult leatherbacks have the ridges and margins of the carapace adorned with rows of knobs that can be quite sharp. Several females were observed with fresh, bleeding wounds caused by the friction of the carapace knobs during the nesting movements. These were especially frequent in the soft fleshy areas like the neck or back limbs (Plate 1a),
- Characteristic neat circular holes of 1-2 cm diameter in the flippers that could be due to the loss of tags applied before (Plate 1e),
- Fresh, bleeding wounds on the top of the neck area. We presume that these were produced during copulation by the beak of the male,
- A greasy unknown substance was frequently observed on the carapace and neck area of the females (Plate 1c) with a rotten fish-like smell.

Some hatchlings (usually found during excavations, trapped in the nest) presented deformities in the carapace. Observations of adult females with similar deformities in the carapace suggest that these hatchlings can survive and reach sexual maturity.

Small barnacles (*Platylepas coriacea*) were often seen on the skin and carapace, specially axila, neck and back flippers area (the “fleshy” areas) and they seem to camouflage well among the white spots. Pedunculated barnacles (*Stomatolepas elegans*) were sometimes seen on the metal tags. Quite frequently, nesting females would come ashore with remoras or suckerfishes (*Remora remora*) still stuck on the carapace that eventually fall in the sand and die before the turtle returns to the sea.

PLATE 1: CONDITION OF NESTING FEMALES

(a) Self injuries at the rear limbs

(b) Malformation in the cloacae and partial amputation of the carapace

29/12/2006

(c) Unknown substance in the neck area

28/12/2006

(d) Necrosis from fishing hook in the axila

(e) Circular hole from lost tag in the front limb

(f) *Remora remora* on the carapace

Observations about nesting behaviour

Females invariably nested at night and, as a general observation, rarely came out of the water before 11pm. During massive nesting events, the timing of emergence did not seem to be related to tide cycles or moonphases. Females could emerge from the water even at extremely low tides, having to crawl up more than twice the normal width of the beach. Sometimes during the peak of the season and full moon nights, females would be found finishing their nests at daylight. During high-density seasons, like 2006/2007 an almost continuous succession of females could be seen coming out from the water and it seemed that this occurred in a synchronous way as if they were travelling in groups from offshore. This was especially noticeable during the peak months of nesting activity as the first females started to emerge from the water exactly at the same time, spaced by 50 meters in parallel. We hypothesize that groups of 10-15 females travel together from offshore and spread in a fan shape when emerging to the beach (Figure 4).

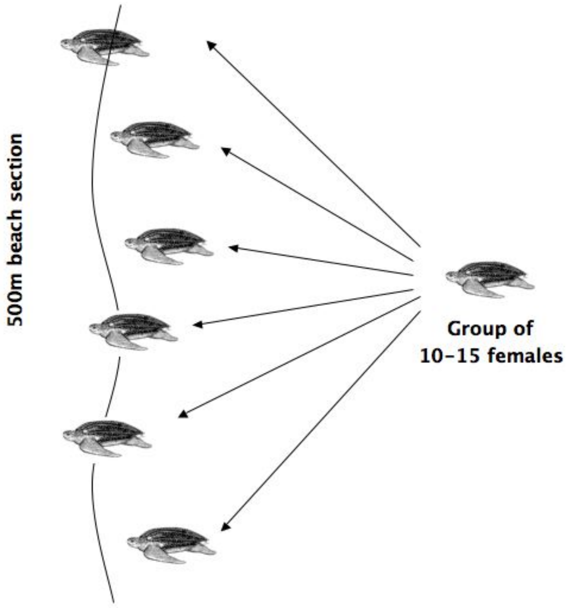

Figure 4. Schematic representation of the hypothesized fan-shaped synchronous emergence of female leatherbacks from the water during mass nesting periods.

Leatherback females dig the nest with alternating movements of the rear flippers. The resulting structure has the shape of a boot or an elephant foot (Billes and Fretey, 2000) with a wide incubation chamber at the bottom. At the time of oviposition, average maximum and minimum depths were 71.3 and 48.2 cm (Table 1). Natural beach dynamics can lead to considerable fluctuations in the depth of the egg chamber during incubation. On average, 76% of the clutches ended up being deeper because of sand build up by tides. Increase in depth ranged from 1 to 44cm. Differences in vertical measurements were found to be statistically significant between the beginning and end of the incubation (paired samples *t* tests) both for maximum (df=116, t=-6.501, p<0.01) and minimum depth (df=84, t=-5.770, p<0.01).

Table 1. Vertical nest dimensions measured at the beginning and end of the incubation

	Beggining of incubation		***End of incubation***	
Nest dimensions	**Mean ± SD**	**Range**	**Mean ± SD**	**Range**
Maximum depth (n=117)	71.3 ± 4.0	53-82	78.0 ± 12.8	42-117
Minimum depth (n=85)	48.2 ± 6.7	30-63	57.1 ± 14.1	26-98
Midpoint depth (n=80)	59.8 ± 4.5	46-71	67.8 ± 13.5	34-103
Height of the incubation chamber (n=80)	23.2 ± 6.3	9-40	20.9 ± 6.9	2-39

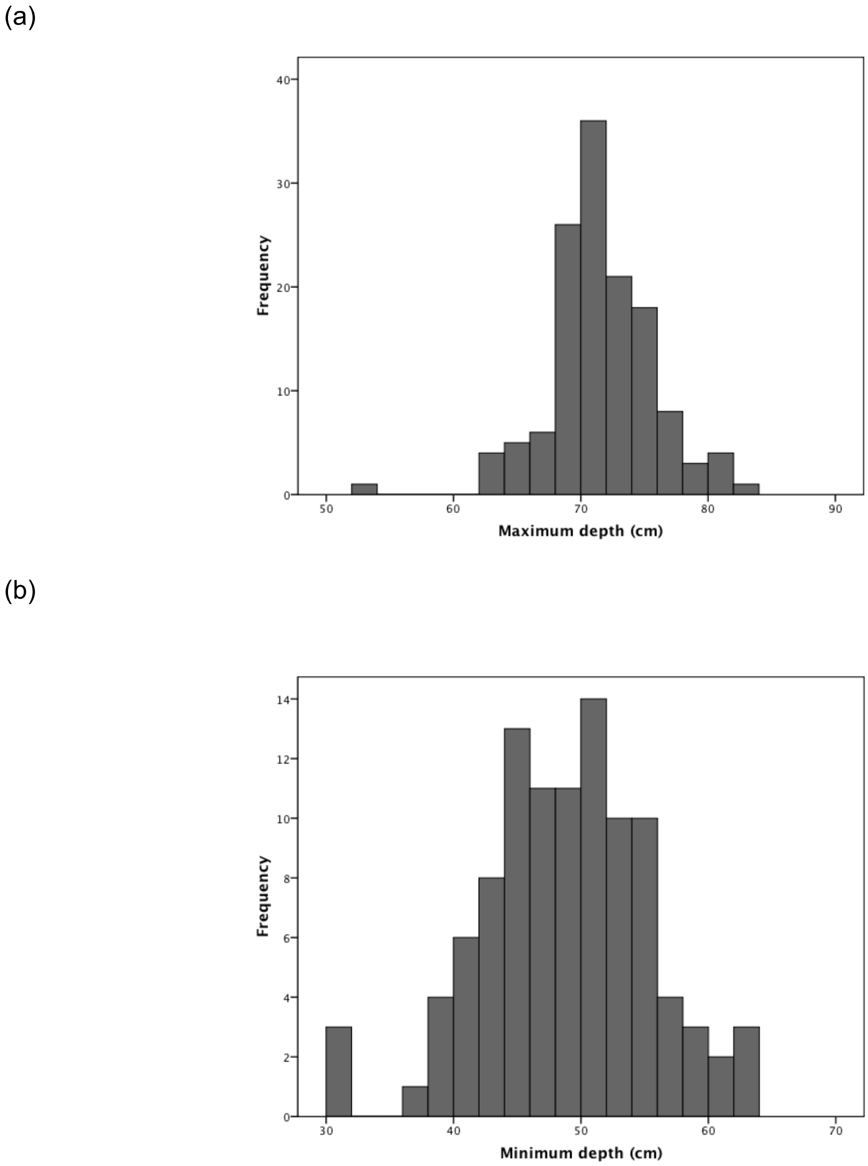

Figure 5. Frequency distribution of (a) the maximum (n=117) and (b) the minimum (n=85) depth of *in situ* nests measured at the time of oviposition.

Basic reproductive parameters of females, clutches and hatchlings

Females had a mean curved carapace length (CCL) of 150.5 cm (95% confidence intervals for mean 149.7-151.7) and a mean curved carapace width of 107.1cm (Table 2). Size distribution shows a peak of abundance between 150 and 154 cm CCL (Figure 6). The majority of the nesting females (77%) had a CCL between 145 and 160 cm. CCL and CCW were strongly correlated (Pearson, n=163, r=0.704, p=0.000, r^2 =0.496).

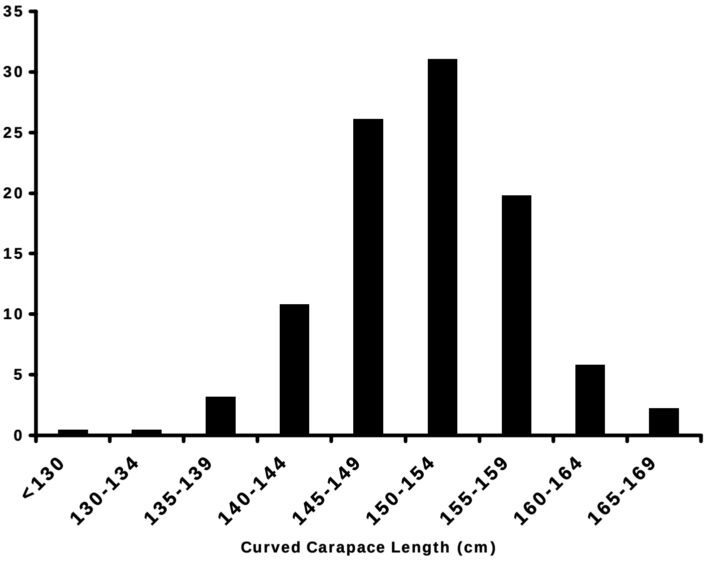

Figure 6. Distribution of sizes of female leatherbacks (n=223) measured during the course of the study.

During season 2007/2008 we found a female measuring only 123cm of CCL. She was slightly hunchbacked but had a normal caudal projection of the carapace, so there was no error in the measurement. Although she completed the process of nesting normally (dug a nest of 62cm deep) she laid fewer eggs than normal (34 SAG and 37 viable eggs). Despite the low size; suggesting that it was a young individual, this turtle had a scar of entanglement with a fishing net on the front flipper.

Hatchlings weighed 42.87g on average with 58.90 mm and 41.67 mm of straight carapace length and width respectively (Table 2). Viable eggs are spherical, white and have a soft membrane at the time of laying. Mean viable egg weight was 80.2 g and mean egg diameter was 51.8 mm. SAGs were very variable in size and shape, with weights between 1 and 78 grams (Table 2).

A typical leatherback clutch consisted on average of 78.2 viable eggs and 30.4 SAG (Table 2). SAGs are normally laid at the very end of oviposition, after all viable eggs. However, we observed that sometimes the female laid groups of SAG scattered throughout oviposition or even at the beginning. This happened in 20 out of 84 observed oviposition events (24%). Mean clutch weight (including SAG) was 6.96kg, ranging from 2.9 to 10.3 kg, whereas SAGs alone weighed 0.56kg on average (Table 2). SAG represent 28% of the clutch in terms of number of eggs and approximately 8% in terms of clutch weight. The number of SAG and eggs were negatively correlated ($n=170$, $r=0.335$, $p=0.000$, $r^2=0.112$) (Figure 7a).

The size of the females (CCL) was positively correlated to both clutch weight ($n=47$, $r=0.396$, $p=0.006$, $r^2=0.157$) and number of viable eggs ($n=172$, $r=0.417$, $p=0.000$, $r^2=0.174$) (Figure 7 b & c). There was no correlation between egg size and female biometry. There was a weak but significant correlation between the depth of the nest and female CCL ($n=139$, $r=0.439$, $p<0.01$, $r^2=0.19$) (Figure 7d).

Table 2. Main reproductive parameters (female, egg and hatchling biometry)

	n	mean	SD	Max	Min
Female size					
CCL (cm)	223	150.55	6.63	169	123
CCW (cm)	223	107.08	4.70	119	93
Hatchling size					
weight (g)	157	42.87	3.41	50.9	33.1
SCL (mm)	157	58.97	2.44	63.6	52.0
SCW (mm)	157	41.66	1.84	48.7	35.7
Egg biometry					
weight (g)	530	80.22	5.71	93.6	68.3
diameter (mm)	530	51.75	1.87	56.1	46.4
Clutches					
Nº SAG	171	30.4	15.0	77	0
Nº eggs	171	78.2	17.7	26	123
Total weight (kg)	47	6.96	1.65	2.9	10.3
Total SAG weight (g)	20	556	332.3	0	1300
Nests dimensions					

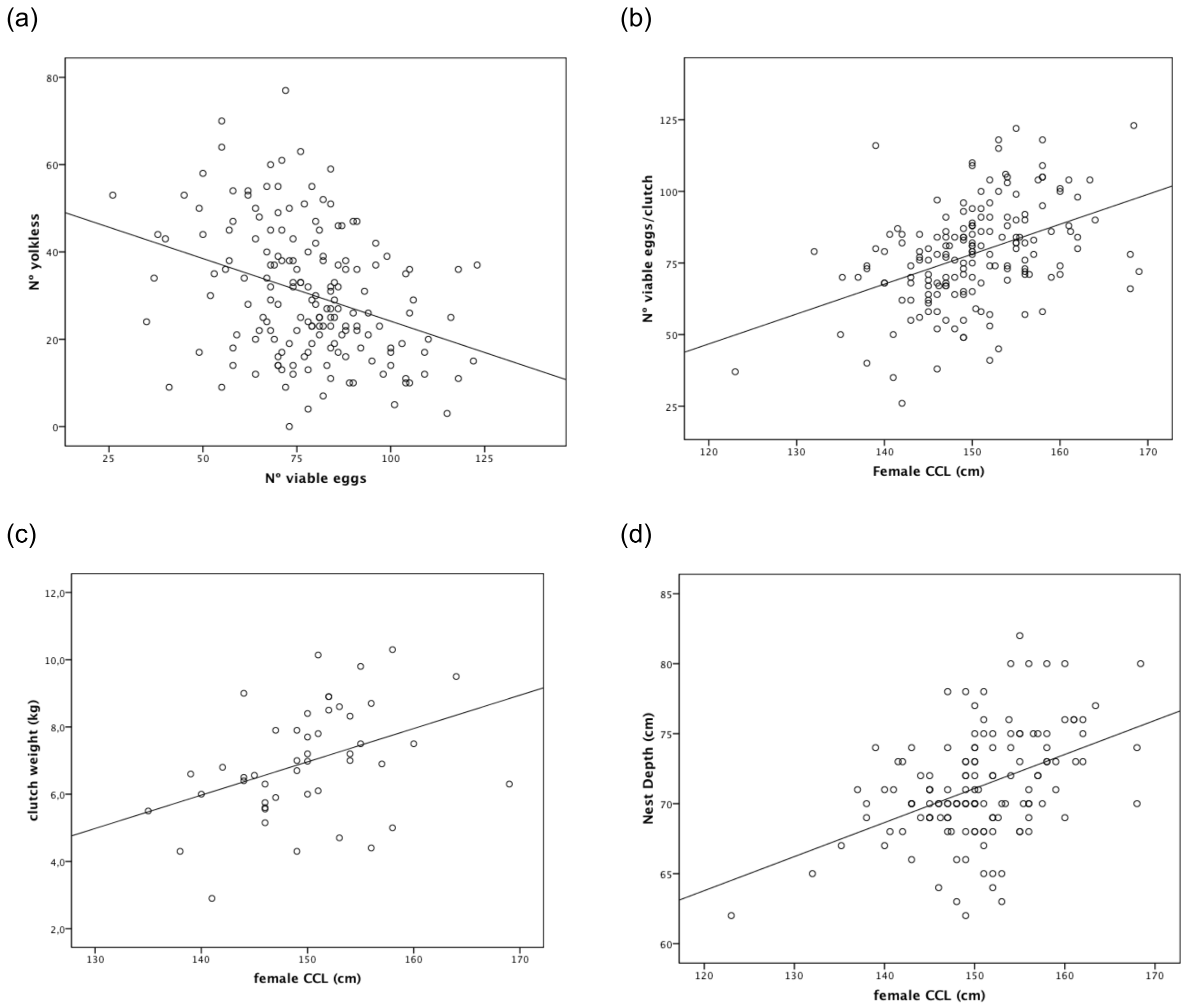

Figure 7. Scatter plots for the significant correlations found between basic reproductive parameters: (a) the number of SAG and viable eggs in a clutch (b) the size of the female (CCL) and the number of eggs laid per clutch, (c) the size of the female (CCL) and the total mass of the clutch and (d) the size of the female (CCL) and the maximum depth of the nest.

Threats to the species

The main identified threats upon nests were predation, flooding by tides and erosion. Also, eggs could be unburied and destroyed by subsequent nesting females when they dig new nests. Main observed predators on eggs were crabs (*Ocypodes cursor*), lizards, mongooses and small felines (Table 3). The degree of flooding by the sea could range from occasional during spring tides to other nests that were completely submerged for long periods of time. Beach erosion would result in total or partial destruction of a great proportion of nests that are washed over by the sea. The resulting vertical flood cliffs, sometimes up to two meters high constitute a barrier for nesting females as well.

The major threat to females was the high incidence of stranded logs on the beach that blocked their way either before or after nesting. As leatherbacks cannot crawl backwards, they sometimes got trapped in between the logs and die because of the shock or dehydration (Plate 2). During season 2006/2007 we counted 15 dead females that had been caught among these lethal traps in a 7km beach section, only during the month of november. Females can also get injured by the oxidised metal rings on the logs (Plate 2) or by the logs themselves and such scars or fresh injuries were commonly observed. On the other hand, females can find buried logs while digging the nest, which can in turn produce injuries in the rear flippers.

Another observed minor threat was that some females could accidentally enter the coastal lagoon and get stranded once the tide goes out. Over the course of the study, 4 dead females were found stranded on the beach with head and front flippers amputated by fishermen but this were isolated incidents more likely to be accidental and not intentional catch.

Stranded logs, plastic and other marine debris were the major obstacles for hatchlings on their way to the sea. Main observed predators upon hatchlings were crabs and small felines (*Civettictis civetta*). Snakes (*Naja* sp.) were observed several times around the hatchery when hatchlings had emerged but there is no direct evidence that it predated upon them.

There is no coastal infrastructure at Kingere apart from two hotels and a private property, where ultra bright projectors remained switched on all night. Leisure activities such as quads, jet skies and boats were relatively common, especially during the weekends.

Table 3. Main identified predators on leatherback eggs (E) and hatchlings (H) at the beach of Kingere.

Predator	Stage	Evidence	Other reports in Gabon
Ants			
Dorylus sp.	E	Found dead inside predated eggs durimng excavations. Nests infested	First report (see chapter 4)
Crabs			
Ocypodes cursor	E/H	Direct observation	Mayumba, Billes (2000) Gamba (Livinstone, 2007)
Reptiles			
Varanus ornatus	E	Direct observation	Mayumba, Billes (2000)
Naja sp.	H?	Frequent around the hatchery whenever emergences occurred	?
Mammals			
Civettictis civetta	H	Dead hatchlings and tracks in the sand	Mayumba (Billes, 2000)
Mongooses (unknown sp.)	E	Tracks in the sand	

Conservation measures: hatcheries and female rescue operations

Over the course of the study, we tested the viability of egg relocation programs and the possibility to establish a hatchery at three different sites on the beach (Table 4). The first location was adjacent to the beach but inside the forest canopy. As beaches are very narrow at Kingere, the idea was to avoid the danger of flooding by tides. The second location was in a typical sector of the beach and the third location was at an ancient lagoonmouth (see map and description of sectors in Figure 1, chapter 3). All attempts of relocation failed because of predation except at the lagoonmoouth, were mean hatching success was 72% (Table 4).

Table 4. Summary of the nests that were relocated and the different sites tested for hatcheries at Kingere during the study period

Site	Season	Nº of nests	Hatched nests	Mean hatching success
Inside forest canopy	2005/2006	13	0	0
Regular beach	2006/2007	20	5	9%
Regular beach	2007/2008	11	0	0
Lagoonmouth	2007/2008	9	8	72%

During the big season, 2006/2007, we did rescue operations and released around 30 females back to the sea that that were trapped among stranded logs and would have died otherwise. These operations were sometimes quite complicated and required several people because of the weight of the females and depending on the position in which it was trapped (Plate 3). We developed a technique in which harnesses (usually our clothes or bed sheets) were passed around the armpits to pull the female while others would push at the back (Plate 3). On one occasion, a female was found on its back and flipped to normal position (Plate 2c). Rescue operations were also made trying to direct females out of the coastal lagoons, before low tide would have left them stranded and unable to get out.

Table 5 (next page). Comparison between basic parameters of females, hatchlings, eggs and clutches at Kingere (mean ± SD) and at other main nesting sites in the Atlantic and Pacific region. Sample sizes for each site and parameter appear in brackets in the shaded area. For female CCL, CCW and clutch weight range of reported values appears in brackets

	Gabon			**Western Atlantic Region (WAR)**			**Pacific (P)**
	Pongara-Kingere	**Gamba**	**Mayumba**	**French Guyana**	**Surinam**	**Costa Rica**	**Costa Rica**
DATA SOURCE	This study	Verhage *et al.*. 2006	Billes. 2000	Fretey. 1980 Girondot & Fretey 1996	Hilterman and Goverse 2007	Chacón *et al.* 2007 Chacón 1997	Wallace *et al.* 2004
Females	(223)	(819)	(902)	(1328)	(1840)	(2751)	(146)
CCL (cm)	150.5 ± 6.6 (123-169)	150.4 ± 7.6 (130-172)	150.9 ± 7.3 (130-179)	154.6 ± 8.9 (127-252)	154.1 ± 4.9 (128-184)	153.2 ± 7.4 (135-198)	145.2 ± 6.4 (125-162)
CCW (cm)	107.1 ± 4.7 (93-119)	108.3 ± 6.6 (80-144)	108.4 ± 4.5 (86-124)	87.3 ± 6.21 (67-109)	113.2 ± 5.0 (97-135)	112.0 ± 5.5	104.4 ± 4.3 (93-116)
Hatchlings	(157)		(289)			(2237)	
Weight (g)	42.9 ± 3.4	-	44.2 ± 3.3	-	44.7 ± 3.5	44.4 ± 6.1	40.1 ± 2.7
SCL (mm)	59.0 ± 2.4	-	60.3 ± 2.5	-	59.5 ± 2.0	59.6 ± 4.26	56.9 ± 2.1
SCW (mm)	41.6 ±1.8	-	42.9 ± 1.6	-	-	-	38.8 ± 1.8
Eggs	(530)		(308)	(27 clutches)		(3250)	(334 clutches)
Weight (g)	80.2 ± 5.7	-	80.1 ± 3.72	87.9 ± 8.2			80.9 ± 7.0
Diameter (mm)	51.7 ± 1.9	-	51.9 ± 1.5	50.4 ± 4.5		53.2 ± 0.9	
Clutches	(171)	(95)	(40)	(27)		(5260)	(34)
Nº SAG	30.4 ± 15.0	31.5 ± 14.6	31.3 ± 14.9	30.2±15.1	0-223	32.1 ± 14.2	-
Nº eggs	78.2 ± 17.7	65.9 ± 17.2	77.8 ± 20.4	84.3 ± 15.6	23-147	81.2 ± 17.9	61.8 ± 16.3
Total viable egg weight (kg)	6.96 ± 1.65 (2.9 - 10.3)			8.1 ± 1.8 (3.8 - 11.6)			5.0 ± 1.4 (1.5 - 9.2)
Total SAG weight (kg)	0.55 ± 0.33 (0 - 1.3)						0.95 ± 0.39 (0 - 1.9)

PLATE 2: IMPACT OF STRANDED LOGS ON FEMALES

PLATE 3: RESCUE OPERATIONS

Discussion

Phenology and nesting activity

The phenology for *D. coriacea* at Kingere followed a similar pattern to that described at other nesting sites in Gabon such as Mayumba (Billes 2003) or Gamba (Verhage *et al.* 2006), ranging from november to march with a peak of activity around december and january. It is also similar to other nesting sites in the Eastern Atlantic Region like Congo (Parnell *et al.* 2007) or Bioko (Tomas *et al.* 1999) and to American beaches in the Eastern Pacific like in Costa Rica or Mexico (Eckert *et al.* 2009). On the other side of the Atlantic, as in French Guyana, Surinam, Trinidad or Central America, the nesting season occurs roughly during the other half of the year, from march to august (see review by Eckert *et al.* 2009).

However, the distribution and intensity of the nesting activity showed considerable variation between the two seasons of which data are available. First, there was a decrease of nearly 65% in the number of nests laid from one year to another and second, the peak of activity was delayed during the high density year (2006/2007). Although data was not available for the 2005/2006 nesting season, it was also a mass-nesting year. This seems to correspond to a pattern of high-density years followed by low-density years. Similar fluctuations were also observed in Gamba, further south on the coast of Gabon with a decrease of 77% in the number of nests laid over 3 seasons (Verhage and Magaya 2006) and Bioko (Rader *et al*. 2006) but since these databases are not longer than a few years it is not possible to jump to any conclusion. In French Guyana, reconstructed databases over 34 years of beach monitoring show several up and down events (Girondot *et al.* 2002). Thus, high inter annual variability in nest numbers seems to be common at leatherback nesting but the exact causes for this phenomenon remain uncertain. Long term monitoring in Gabon will help understanding these nesting patterns and try to elucidate whether they correspond to population trends or behavioural/physiological aspects of reproductive females.

Reproductive parameters

The biometry of females, eggs and hatchlings found at Kingere is similar to the reported values found at other sites in Gabon where information is available (Table 5). Comparing at a global scale, average CCL of the females is smaller than on the Western Atlantic Region (WAR) but bigger that in the Pacific (P) (Table 5). Therefore, the size of nesting leatherbacks in Kingere and Gabon appears to have an intermediate position between the two regions. The size of the clutches, eggs and hatchlings is also similar to the other sites in Gabon but there are differences with reported values in the other regions (Table 5). For example, the number of viable eggs laid per clutch, the size of the eggs and thereby the total weight of the clutches also appear to be at an intermediate position between the WA and P regions (Table 5). Such differences between nesting populations could be a reflection of the migration and feeding behaviour at sea. It appears that leatherbacks nesting in the WAR migrate to feeding grounds in the North Atlantic whereas those nesting in Gabon remain either in the Central Atlantic or Eastern South Atlantic (Marco *et al*. 2009). Differential productivity of the two feeding grounds might be having an influence on the reproductive parameters

Curved carapace length is the most commonly collected size measurement of sea turtles and a threshold value of 145cm is normally used for adult leatherback classification (Stewart *et al.* 2007). However, reproductively fit females smaller than 145 are often observed at many nesting sites around the world (Stewart *et al*. 2007) as it is the case in our study site, with 13% of the females measuring less than 145cm. This relatively high proportion of females with small carapace sizes could be the consequence of at- sea environmental factors that determine their growth patterns, as we mentioned before; but it could also reflect the fact that this is a young nesting population, composed partly by neophytes or new nesters. The aspect of the carapace of many individuals with the knobs on the keels sharp and almost not eroded seemed to confirm this. In fact, the smallest leatherback found during the course of this study, with only 123 cm of CCL, is also among the smallest females ever found on nesting beaches around the world (Stewart, 20007).

Condition and Behaviour of nesting females

The external condition of females on nesting beaches gives us additional information about their ecology and behaviour. For example, the observed injuries give evidence of interaction with predators (bites probably by sharks or *Orcinus orca*), fisheries (hooks and nets) or boats. These traumas are similar to the ones described at other nesting sites such as French Guyana (Fretey 1981), Surinam (Hilterman *et al.* 2007) or Costa Rica (Chacón *et al.* 1996). Self injuries on the fleshy areas of neck and rear flippers were also described by Fretey (1986) in French Guyana but there are not many other citations about this. Such injuries only happen while the turtle is on land and due to the nesting movements. The observation about bleeding wounds in the neck that could correspond to a mating scar due to the beak of the male has, as far as we know, never been reported on nesting beaches before.

We found a correlation between the size of the female and the number but not the size of viable eggs similarly to other studies (Wallace 2004; Quiñones *et al*., in press) and this seems to corroborate the hypothesis that the reproductive strategy of the leatherback turtle is maximizing the number of eggs rather than their mass, possibly to compensate the low success of nests (Wallace *et al*. 2004). Also, bigger females seemed to dig deeper nests, which can favour better conditions for incubation (see chapter 3). Abella *et al*. (in press) found that laying deeper nests will also favour the fitness of the hatchlings, enhanced synchrony at emergence and an equilibrated proportion of sexes for the loggerhead turtle.

One of the most interesting observations was the presence of circular holes in the front limbs of several females that, as suggested by Fretey and Girardin (1989), almost certainly corresponded to ancient tags applied before. According to the standard protocols, tags are currently applied on both rear limbs (Eckert *et al*. 2000). However, when the first tagging programs started in the 70s, they were applied on one of the front limbs (Fretey, pers. comm). The extremely high tag loss was the reason to eventually change to the current protocol, which is the one that has been used in Gabon since the beginning. The question is: if these females were not tagged in Gabon, where do they come from? Tagging programs in the front limbs happened in considerable numbers at two other geographic areas: French Guyana and Tongaland in South Africa at the end of the 70s (Fretey, pers. comm). Therefore, the females observed nesting in Gabon during the period of this study 2005 to 2008 may have previously nested in either one of these two sites almost 30 years ago. This hypothesis bears important assumptions about the longevity and nest site fidelity of leatherback turtles. First, the fact that these females might have been reproductively active for at least 30 years, second, that some individuals may be able to migrate form the Indian to the Atlantic Ocean, crossing the Cape of Good Hope and finally, that a same female during its lifetime might be able to nest in such distant places. There is evidence, from non lost tags, that individual nesting females can move between beaches in Colombia, Honduras, Costa Rica and even Florida within and between seasons (Tröeng *et al*., 2004). Also, satellite telemetry studies are showing how leatherbacks are capable of travelling over extensive geographic areas during their lifetime, migrating thousand of kilometers from nesting to foraging grounds (Marco *et al*. 2009) However, the possibility that some individuals might use nesting areas on both sides of the Atlantic Ocean has not been proven to date, although it has been considered many times (Fretey and Billes, 2000; Fretey, 1992, Formia *et al.* 2003).

Threats

The high incidence of stranded logs is probably the major environmental that was detected at Kingere. Harvested logs from inland timber operations are transported via rivers on barges or cabled together into floating rafts to open sea and then the major ports (see references in Laurance *et al*. 2008). During transport, some of these logs, that can measure up to 15 m in length and 120cm diameter, might be accidentally or intentionally (because being defective) lost and end up on the stranded on the coast. We found that these logs can cause the death of the females and disorientation of hatchlings towards the sea. Also, they constitute impassable barriers that can make the female return to the sea or nest in a flooded area closer to the water. Females often encountered logs while digging the egg chamber that made them abandon the nest and even cause injuries in the flippers. Hence, the visible stranded logs on the beaches could only be the tip of the iceberg with an equal or even higher amount of logs below sand at some points (see chapter 4). Laurance *et al* (2008) quantified the occurrence of logs all over the coast of Gabon and confirmed the magnitude of this problem not only as a threat to sea turtles but also as an economic waste. The incidence of logs seems to be particularly high at Pongara blocking up to 30% of the beach at some critical points.

The main sources of mortality of eggs and hatchlings had a natural origin as a consequence of the conditions of the beach (predation, flooding or erosion). There is currently no human poaching of eggs or killing of females at Kingere. At Pointe Denis in the 1980´s all nests were systematically poached and sometimes females killed for local consumption or sale at Libreville markets (Fretey & Girardin, 1989; Fretey, 2001) but since conservation initiatives started, these practices have been abandoned completely.

Oil exploitation is one of the major economic activities in Gabon with platforms for extraction present offshore and inland. In Mayumba, several females were observed with petrol residues in the carapace and one female was found dead covered in petrol (Billes 2003). Also, the sand of the beaches in Gamba and Mayumba receives frequently oil residues from the offshore platforms (Billes 2003, Verhage *et al.* 2006). There are no petrol platforms off the coast of Pongara but marine debris covered in oil appeared on the beaches several times. The greasy substance observed with relative frequency on the neck and carapace of females (Plate 1c) could be an indication of interaction with petrol at sea during the internesting period.

Our study area was virtually free of human settlements at the time we did this research. Unlike at Pointe Denis (Bourgeois *et al.* 2008), light pollution from Libreville was not a problem in Kingere because the coast is no more oriented towards the capital. However, coastal development is increasing at quite accelerated rates in Gabon and there are some emergent touristy projects in this study area. There is also an increasing number of visitors to Kingere coming from Pointe Denis. Although not permitted by the Park authorities, day trips on quads are becoming more and more popular and use the beach even at night. The same happens with jet skies and boats (as for sports fishing) but this relies outside the Park regulations because it is only terrestrial.

Recommendations for management and conservation

1. By patrolling the beaches regularly, the staff from local NGOs, can save substantial numbers of females every season. Female rescue operations alone are well worth the cost of running a conservation project because it implies saving adult individuals that have a higher reproductive value than eggs.

2. The problem of logs should be considered from two perspectives; the first one involving the elimination of the existing logs on the beaches and the second one involving the eradication of the source of the problem.

3. Although considerable amounts of nests are lost due to erosion and flooding, the practice of egg relocation to large scale hatcheries as a way to improve hatchling productivity is not recommended at Kingere because the beach is too narrow and the problem of underground predation appears as a major constraint with a difficult solution. A small hatchery (10-20 nests) with educational purposes for visitors could be kept at the lagoon mouth, the only place were nests successfully hatched.

4. Considering the increasing number of visitors and popularity of sea turtles in this area it is very important that strict guidelines about marine turtle ecotourism be applied from the beginning such as the maximum number of persons allowed per group and the code of conduct towards nesting females on the beach (for example, the use of lights).

5. The fact that research and conservation is recently new in Gabon is an advantage in the sense that coordinated efforts to standardise methodologies and data collection can be done from the beginning. There are currently two initiatives working on this: PROTOMAC (Programme des Tortues Marines de l´Afrique Centrale) and the Sea Turtle Partnership.

6. Other species of sea turtles were observed to nest at Kingere: *Chelonia mydas* and *Lepidochelys olivacea*. It would be recommended to extend monitoring activities outside the leatherback nesting season to evaluate their abundance.

6. We recommend keeping the beach of Kingere as a sanctuary for the nesting of sea turtles where leisure activities and visitors are regulated, especially during the nesting season

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Chapter 3: Incubation success of the clutches in natural conditions

Summary

The general objective of this study was to estimate hatching and emergence success of the nests in natural conditions at the beach of Kingere and look at what factors might be affecting the survival of nests. We had to develop a field methodology that allowed to track individual nests under the particular conditions of high nest density at this site. The study area (7km) was divided into 5 different sectors according to the coastal landscape to look for possible spatial variation in productivity. A total of 170 nests were marked at oviposition and followed to term for excavation during three consecutive nesting seasons (2005/2008). We found that only 36% of the nests produced live hatchlings while the rest failed. Overall hatching and emergence success for the whole study period and considering all tracked nests (n=170) was 16.7% and 15.8% respectively. Mean difference between hatching and emergence success was 2.18%. Considering only the successful nests (n=61), hatching and emergence success were 46.4% and 44.2% respectively. Overall values found in this study are at the lowest range of reported values for the species within Gabon and the Atlantic region. A generalized linear model was done to assess what factors influenced whether a nest successfully completed incubation or not. The major determinant parameter of nest survival was the beach sector. Minor determinants were the date of laying, nest depth and clutch size. Our results show that the hatchability of nests showed considerable within beach variation; with some sectors being much more productive than others and that this seemed to be related to differential clutch predation pressure. Deeper and smaller clutches had better chances to survive probably related to more stable incubation conditions in the former and the spatial distribution of the eggs in the latter. Nests laid later during the marking period hatched better than those marked earlier and this might be related to a negative effect of rainfall on the early-laid nests. Our results show that, under conditions of low nest survival, as it is the case at Kingere, values of incubation success (both hatching and emergence) differ significantly (over 30% in this study) depending on whether failed nests are included or not in the mean. It is recommended that all studies specify clearly which methodology was used (i.e. random sample of nests selected at oviposition or nests observed after hatching only) with views to easier comparison between sites.

Introduction

Sea turtles are migratory reptiles that evolved from terrestrial ancestors developing an almost 100% marine life excepting for one stage, which is the laying of eggs on sandy beaches. Their reproduction strategy involves laying multiple clutches within one nesting season (Miller, 1997) and assumes high mortality during the first stages of their life (Manugsson *et al*. 1990). Success in reproduction involves several behavioural and physiological aspects that range from mating and courtship at sea to the event of nesting on the beaches (Pough *et al* 2003). The nests are laid scattered on the beach and, as no parental care occurs, the fate of the eggs and future hatchlings depends on the successful incubation of the nests, which, in turn is influenced by the nest microenvironment and the conditions of the nesting beach (Miller 1997). There is a wide range of abiotic parameters affecting the incubation of eggs that range from the physical and chemical properties of the sand, such as temperature, gases and humidity (Ackerman, 1997), to environmental factors like rain (Houghton *et al*.), sea flooding (Whitmore and Dutton, 1985 or beach erosion (Patiño-Martinez *et al*. 2008).

Definition and methods to estimate incubation success of sea turtle clutches

Incubation success can be considered as an indication of the general health of the nesting population and the suitability of a particular nesting beach as an incubation system for the eggs. The term refers to a combination of two consecutive processes: hatching and emergence (Miller, 1999). Hatching occurs in the incubation chamber when the fully developed hatchling breaks the shell and comes out of the egg. Then all hatchlings emerge to surface through the sand column by "social facilitation" helping each other and this can take several days to happen. Incubation success is measured and reported in literature in two different ways (Torres 2002, Manugsson *et al.* 1990). In the first one a random sample of nests are marked as they are laid by the female and followed to term. The resulting value is an average considering all selected nests whether they hatch or not. In the second way, only nests that are observed to hatch on the beach are sampled, automatically excluding zero or low success nests. The former method is the best measure and mostly recommended (Manugsson *et al.* 1990) because when using the latter method there is a bias towards successful nests and the value can be overestimated. Values using both methods can be substantially different, especially when the nest survival is low and there are many failed nests (zero hatching). Because field conditions are usually challenging and difficult at marine turtle nesting sites, and the first method requires more work, there is a tendency to use the second method. What is more, there literature is usually confusing, as most of the times, the method that was used is not clearly specified.

Overview on incubation success of leatherback clutches

Incubation success of the leatherback turtle has been extensively studied at the major nesting sites in the Western Atlantic region. Mean reported values are 38% in French Guyana (Caut *et al*. 2006, Torres *et al*. 2002), 10 to 56% in Surinam (Hilterman *et al.* 2007), 41% in Costa Rica (Chacón *et al.* 2007), 67% in St Croix (Boulon *et al*. 1996), 67% in Florida (Stewart and Johnson 2006), 44 to 75% in Colombia (Patiño *et al.* 2008) and 52.5% in Trinidad (Livingstone, 2006). However, on the other side of the Atlantic, in the African continent, there are almost no studies or data available because sea turtle conservation initiatives are relatively new and emergent and logistic conditions are quite challenging at most sites (Fretey *et al*. 2007). Therefore, there are still many unexplored areas where this parameter needs to be determined so as to complete the general picture of the species in the Atlantic. Tomás (2004) monitored 12 nests in the island of Bioko, Equatorial Guinea that had a mean success of 57%. In Gabon, three studies have assessed this parameter with relatively small sample sizes, despite having more than 800km of important nesting grounds. Bourgeois (2007) found a mean hatching success of 70% in 43 nests at Pointe Denis, Livingstone (2007) reports 56% for 35 nests at Gamba and Billes (2000) reports 67% for 49 nests at Mayumba.

In this study, we estimate hatching and emergence success of nests in natural conditions at the beach of Kingere and look at what factors might be influencing their survival. We also compare the results at a regional scale within Gabon and at a global scale in the Atlantic region. On the other hand, a side-objective was to set up a methodology for fieldwork and data collection that suited the conditions of the beach and eventually could be used at other sites in Gabon in a standardised way.

Methods

Study site

The research was carried out at Kingere beach in Pongara National Park (0º 18´N, 9º 18´E), which is one of the main protected areas along the coast of Gabon. This beach is located between Pointe Ekwata and Pointe Kingere (Figure 1). Data on nests and females were collected during three entire consecutive nesting seasons (2005/2008), from november to march. Additional data was collected during season 2008/2009 at Pointe Denis and Mayumba (see Appendix I), using the methodology described here.

Characterization of the beach

The study (7 km) was divided into 5 sectors according to geo-morphological aspects like the width of the sand bar and the presence of lagoons or type of vegetation at the back (Figure 1). Sector 1 (2km) is different from the others because it is an arc beach forming a bay instead of open beach. Also nesting activity was found to be lower than in the rest of the study area. The dominating landscape is that of sectors 2 (800m) and 5 (3.5km): open and straight beach with forest vegetation at the back. These are quite narrow stretches of sand (10-15 m wide) periodically flooded or eroded by the sea, highly unstable and dynamic. There is a coastal lagoon behind sectors 3 and 4 that opens and closes seasonally according to the rain. Sector 3 is only 100m long but it is a very particular area because it corresponds to an ancient opening area of the lagoon, which has been refilled with sand, forming a vast extension of 20-30m wide. Sector 4 (600m) corresponds to a narrow sand bar in between the lagoon and the sea, with low vegetation and highly unstable due to seepage of lagoon waters combined with erosion.

Basic Field protocol

The bulk of this study involved finding females at night and marking the nest at the moment of oviposition so that it could be monitored and excavated after hatching to evaluate its success. An average of 2-4 nests were selected each night randomly in november, december and january. We did not mark nests during the second half of the season (february and march) because they would have hatched outside or field campaigns. Nests were named by chronological order of laying with the initials Dc for *Dermochelys coriacea*. The female turtle had to be encountered at the very beginning of the nesting process so as to have enough time to record all data properly.

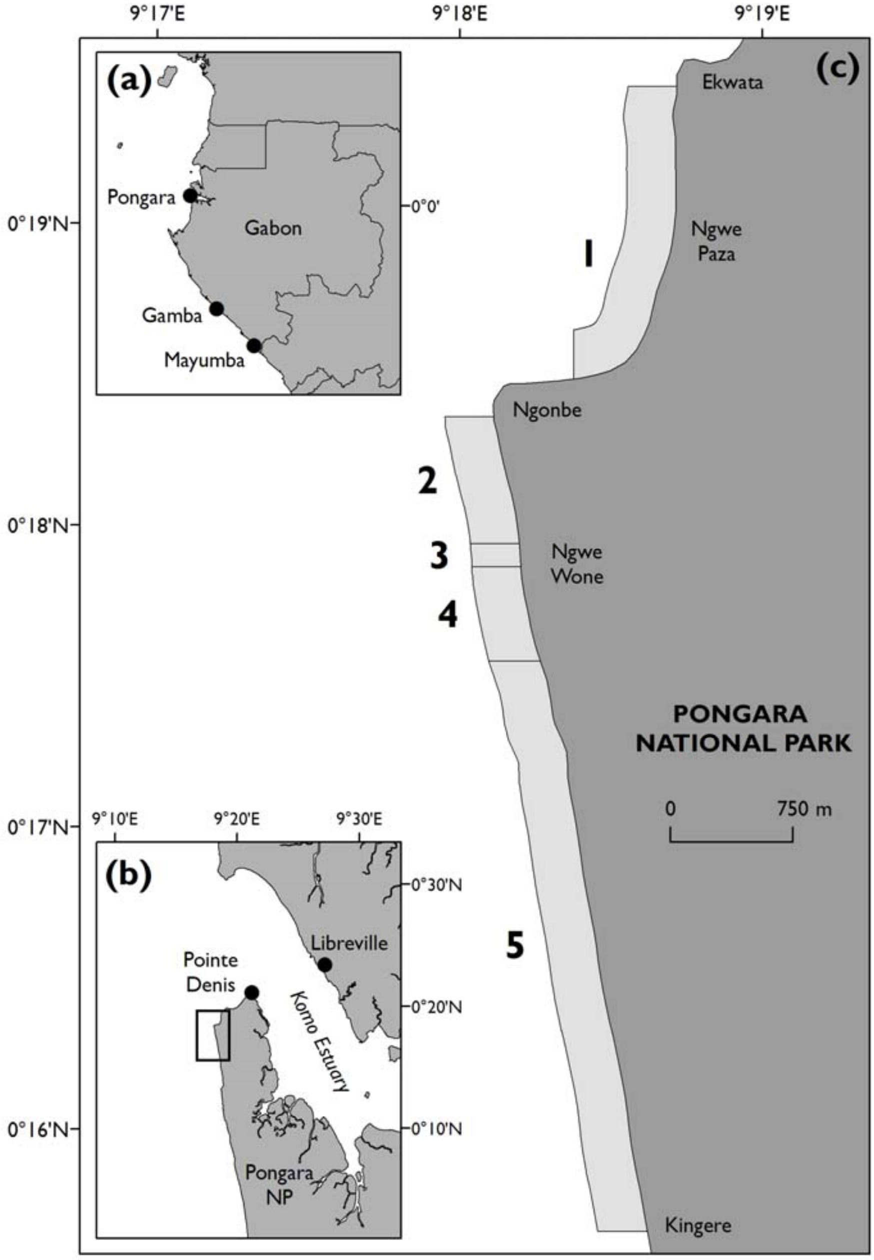

Figure 1. Map of the study area: (a) The Republic of Gabon and the major nesting sites: Pongara, Gamba and Mayumba, (b) Pongara National Park with the only human settlement at Pointe Denis separated from the capital of Gabon, Libreville, by the Komo Estuary and (c) the beach of Kingere with the 5 sectors that were identified and the vernacular names for the main topographic points.

For each pair of female/nest the following data were collected: date & time of laying, GPS coordinates, nest location along the beach (sector), nest location across the beach (distance to vegetation and to high tide line), nest vertical measurements (bottom depth, top egg depth both at laying and excavation time), clutch size (number of yolkless and viable eggs), and female biometry (curved carapace length and width, as CCL and CCW). Females were tagged and identified to detect possible pseudo replication in the sample of nests.

Nest tracking technique

Because of the high density of turtles and nests on the beach, the main challenge in this study was finding a technique of tracking the fate of individual nests in absolute natural conditions throughout incubation. Any artefact used to mark the nest *in situ* could have interfered with environmental factors such as predation and would have been destroyed by subsequent nesting females the following nights. Also, a precise way of confirming the identity of the nest was necessary because there could be several clutches side to side.

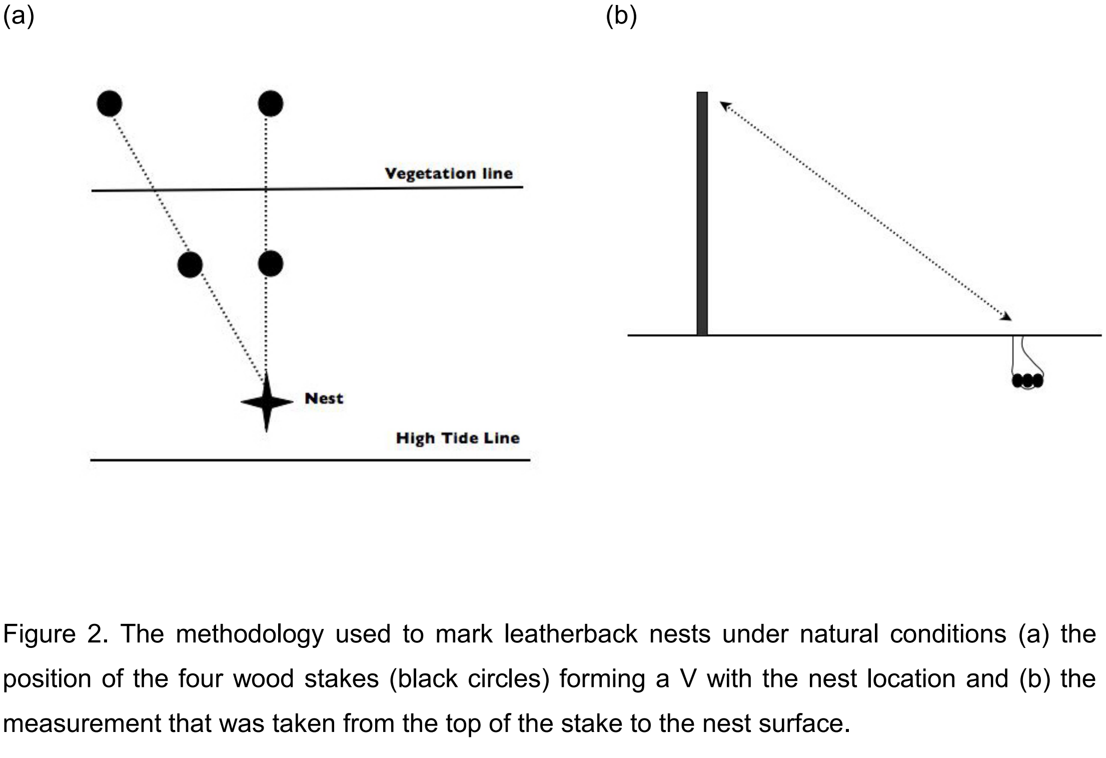

Figure 2. The methodology used to mark leatherback nests under natural conditions (a) the position of the four wood stakes (black circles) forming a V with the nest location and (b) the measurement that was taken from the top of the stake to the nest surface.

Nests were marked on a provisional way at night and then permanently the next morning. The permanent marking system consisted of 4 wooden stakes placed at the rear vegetation forming a V in which the position of the egg chamber was right at the intersection point (Figure 2a). For each nest, we took 4 measurements from the top of each stake to the sand surface (Figure 2b) that allowed finding the egg chamber for excavation after emergence. In addition, a wooden label of 5x5 cm with the nest code was placed 30-40 cm above the egg chamber, while the female was covering the eggs. This label remained buried in the sand throughout the incubation period and allowed to confirm the nest identity during excavation. Distance from the nest to vegetation line (defined as the forest tree line or the point at which vegetation covers more than 50% of the surface) and latest high tide line (that was recognized as a visible line of debris on the sand) were also measured.

Nest incubation success

A total of 170 nests were marked and followed to term during the 3 seasons of the study, (n=55 in season 2005/2006, n=60 in 2006/2007 and n=55 in 2007/2008). Direct emergence of hatchlings was rarely observed because very few nests hatched and also because no retaining artefact was placed on the sand surface. Therefore, the number of eggshells found during the excavation was considered as a reference for the number of hatchlings produced. The nests were excavated after emergence evidence was observed or at day 70 of incubation if emergence was not observed. The exact location of the nest was found using the four measurements of the wooden stakes and confirmed by the finding of the nest code label when excavating through the sand profile. Nest contents where classified into eggshells, unhatched eggs and pipped eggs (pipping is the process in which hatchlings pierce the egg shell). Also, trapped dead or live hatchlings could be found either during excavation or in the egg chamber. Hatching success (HS) was calculated as HS=[(#eggshells)/(total number of eggs)] x100 and emergence success (ES) as ES= [(#eggshells-trapped hatchlings)/(total number of eggs)] x100, where total number of eggs= eggshells + unhatched eggs+ pipped eggs. Egg predation rates were calculated in each clutch as the percentage of unhatched viable eggs having a hole in the membrane with respect to clutch size. Average values of incubation (hatching and emergence) were calculated considering all tracked nests (failed and successful) and considering only the successful nests. We used the terms “successful” and “failed” nest to refer to the nests that successfully hatched or not respectively. “Nest survival” refers to the proportion of successful nests.

Data analysis

We used Arc GIS (Arc Map, version 9.3) to map the study area, beach sectors and the position of the study nests. GPS positions of the total number of tracks and nests laid during season 2006/2007 (available from Gabon Sea Turtle Partnership) were plotted against projected map of the study area to quantify the relative track and nest density occurring in each sector. We refer to tracks as traces of crawling left on the sand by the females that may or may not correspond to a real nest. We calculated nest survival and mean emergence success rates in each sector using the total number of nests that were tracked during the three nesting seasons. Beach productivity was estimated as (nest density) x (emergence success).

A julian day was assigned to each nest considering day 1 as the 1st of november for each season. All data collected from the 3 seasons were pooled together and were analyzed using GenStat v12.1 (GenStat 2009). A Generalized Linear Model (GLM) approach with a binomial error distribution and a logit link function was used to assess whether a clutch successfully completed incubation or not. In the model, all potential explanatory terms were entered and dropped sequentially until only those terms that explained significant deviance remained (Crawley, 1993). Each dropped term was then put back into the minimal model to check that significant terms had not been wrongly excluded. Whether a nest successfully completed incubation or not was used as the response variable and explanatory variables included in the starting model were year, julian date, female curved carapace length, distance to the vegetation line, depth to the midpoint of the egg chamber, number of viable eggs and beach zone. We used the depth to the midpoint of the egg chamber (mean depth of the bottom and top of the egg chamber combined) and clutch size when measured during excavation because there were less missing values than at laying time. All variables were included as covariates in the analyses except beach zone, which was included as a categorical variable. All two-way interactions were tested, but results are only presented if found to explain significant variation. Three females were known to have laid two clutches but only a single randomly chosen clutch from each female was included in the analyses to avoid any potential pseudo replication issues. Eroded nests (n=7) were not included in the model but were included to give overall descriptive values of incubation success. All statistical tests were two-tailed and unless otherwise stated, means are quoted ± 1 SE.

Results

Nest survival and factors affecting

The main observed events during the regular surveys of the study nests were flooding by tides (22%) and crab holes in the sand (28%). A small proportion of the nests (4%) was completely eroded and washed away by the sea. Additionally, during excavations, we found that 94% of the nests had eggs predated by either insects or crabs. Therefore, it appeared that the main causes for failure were flooding, erosion and predation or sometimes even a combined effect of these.

The emergence of hatchlings usually happened at night and the exact date could be determined in 50% of the successful nests. This was because we either saw directly the hatchlings emerging, or because their tracks were seen in the sand the next morning. Also, there was usually a crater or depression in the sand at the place where they had emerged. Mean incubation time for observed nests was 64.6 days ranging from 57 to 72 days (n=30, SD=3.2). For the rest of the nests it was unknown.

Only 36% (n=61) of the study nests were successful (had at least one emerged hatchling) (Table 1). A few nests (n=4) had some eggs that hatched but none of the hatchlings successfully emerged to surface. The rest of the nests (58%) did not have any single egg hatched nor emergences at all.

Overall hatching and emergence success for the whole study period and considering all tracked nests (n=170) was 16.7% and 15.8% respectively (Table 2a). Mean difference between hatching and emergence success was 2.18% (n= 61, paired T-test, p=0.005; 95% Confidence Interval= 0.7-3.7) and this corresponded to hatchlings that were found dead or alive trapped inside the nest. Considering only the successful nests (n=61), hatching and emergence success were 46.4% and 44.2% respectively (Table 2b).

Table 1. Summary of the number of marked nests and their final fate during three nesting seasons at Kingere. In brakets, the relative percentage of the total.

	2005/2006	**2006/2007**	**2007/2008**	**Overall period**
Number of tracked nests	55	60	55	170
Successful	26 (47%)	22 (37%)	13 (24%)	61 (36%)
Some eggs hatched but no emergences	0	4 (6%)	0	4 (2%)
Failed	26 (47%)	31 (52%)	41 (75%)	98 (58%)
Eroded	3	3	1	7 (4%)

Table 2. Descriptive statistics of hatching and emergence success of the clutches during the three nesting seasons: (a) considering all tracked nests, including those that did not hatch (n=170) and (b) considering only the nests that hatched (n=61). CI: 95% Confidence Interval for mean.

(a)

ALL NESTS (n=170)		**Mean**	**Std. Dev**	**Std. Error**	**95% CI**		**Minimum**	**Maximum**
					Lower	**Upper**		
Hatching	Season 1	21,1	30,3	4,1	12,9	29,3	0	98
	Season 2	18,4	30,6	4,0	10,4	26,3	0	93
	Season 3	10,5	25,0	3,4	3,7	17,2	0	95
	Overall Period	16,7	29,0	2,2	12,3	21,1	0	98
Emergence	Season 1	21,0	30,2	4,1	12,9	29,2	0	98
	Season 2	16,8	28,5	3,7	9,5	24,2	0	87
	Season 3	9,7	23,6	3,2	3,3	16,0	0	95
	Overall Period	15,9	27,8	2,1	11,7	20,1	0	98

(b)

ONLY SUCCESSFUL NESTS (n=61)		**Mean**	**Std. Dev**	**Std. Error**	**95% CI**		**Minimum**	**Maximum**
Hatching	Season 1	44,6	29,8	5,8	32,6	56,7	3	98
	Season 2	47,7	32,5	6,8	33,6	61,7	3	93
	Season 3	44,2	34,6	9,6	23,3	65,1	4	95
	Overall Period	45,7	31,4	4,0	37,7	53,6	3	98
Emergence	Season 1	44,4	29,7	5,8	32,4	56,4	3	98
	Season 2	43,9	30,6	6,4	30,6	57,2	2	87
	Season 3	40,8	33,5	9,3	20,6	61,1	4	95
	Overall Period	43,5	30,4	3,9	35,8	51,2	2	98

Factors affecting incubation of the clutches

The generalized linear model was addressing the question of what determines whether a clutch hatches or not and we included the following factors: season, date of laying, distance to vegetation, beach sector, number of viable and yolkless eggs in the clutch and depth of the egg chamber. The final model explained 18% of the total deviance. Beach sector was found to be the most important factor in determining whether a nest hatches or not (Table 3). This parameter accounted for 16.2% of the total deviance with the lagoon mouth (sector 3) being the zone with the highest proportion of nests that hatched (Figure 3a). The other significant determinant factors in the model were clutch size (the number of viable eggs), the date of laying (julian day) and the midpoint depth of the egg chamber (Table 3). According to this, nests with a higher number of viable eggs had less chances to survive (Figure 3b) , whereas nest laid deeper hatched better (Figure 3d). Mean clutch size (as number of viable eggs) found at excavation was 71.0, ranging from 10 to 120 (n=160, SD= 21.8). Average midpoint depth of the egg chamber at excavation was 69.4cm and ranged from 34 to 102cm (n=141, SD=12.95). Also, nests that were laid later during the marking period had more chances to survive (Figure 3c). Note that the marking period of the nests was during the months of November to January. We used the model's fitted values, as this way, for each significant interaction, all the other significant variables are taken into account and it gives a much more discernable pattern. Other variables that were initially included in the maximal model but dropped from the final model due to non significance were year, distance to vegetation and the number of yolkless eggs.

Table 3. The determinant factors in the Generalized Linear Model analysis that affected whether a leatherback nest hatched or not under natural conditions at the beach of Kingere, Pongara National Park.

Factor	**df**	**Deviance**	**P**
Beach Zone	4	16.2	0.003
No. viable eggs	1	5.7	0.017
Julian day	1	5.4	0.02
Egg chamber depth	1	4.4	0.036

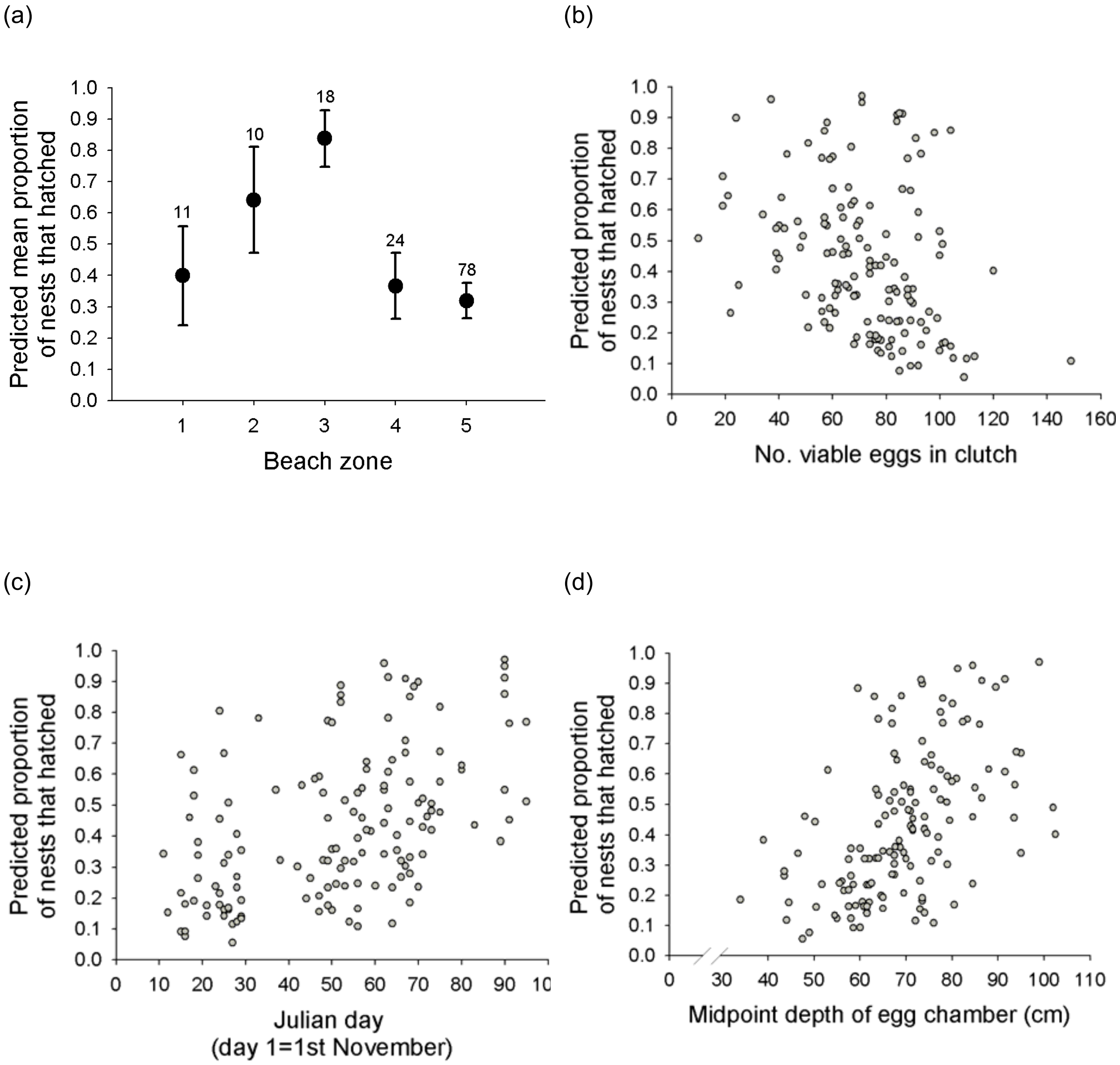

Figure 3. Relationship between the predicted proportion of leatherback nests that hatched at the beach of Kingere and the factors affecting: (a) beach zone, (b) number of viable eggs in a clutch c) date of laying (julian day) and (d) midpoint of the egg chamber. Data points show the model' s fitted values for the hatchability of nests and incorporate all the variables of the final model.

Productivity of the beach

Nesting activity varied considerably among sectors considering both tracks and nests per unit surface. The lagoon mouth (sector 3) had the highest densities, with 1.3 tracks/m and 1.2 nests/m, while the lowest density corresponded to the arc beach (sector 1) (Figures 4 a&b). Values of density ranged between 0.3 and 0.7 nests/m in the other sectors. The proportion of false crawls was higher in sector 4 (13%), probably because of the unstable nature of this zone due to seepage waters from the lagoon (Figure 4c) and lowest at the lagoonmouth (3%) suggesting that this area is favourable for nesting. Survival and emergence success of the clutches also varied greatly among sectors (Figures 4 d&e). The lagoonmouth (sector 3) had the highest percentage of successful nests (76%) and the highest rates of emergence success (47%). On the contrary, the proportion of eggs predated per clutch was lowest at the lagoon mouth (19%) while in the other sectors it ranged from 50 to 60% (Figure 4f).

To summarise, although the lagoonmouth is a relatively small sector of the beach (only 100m, see Figure 1), it seemed to constitute a particular area, different from the rest of the sectors with not only a higher nesting activity and emergence success of the clutches but also with lower predation rates upon eggs. This was therefore, by far the most productive sector in the beach in terms of hatchling production (Figure 5).

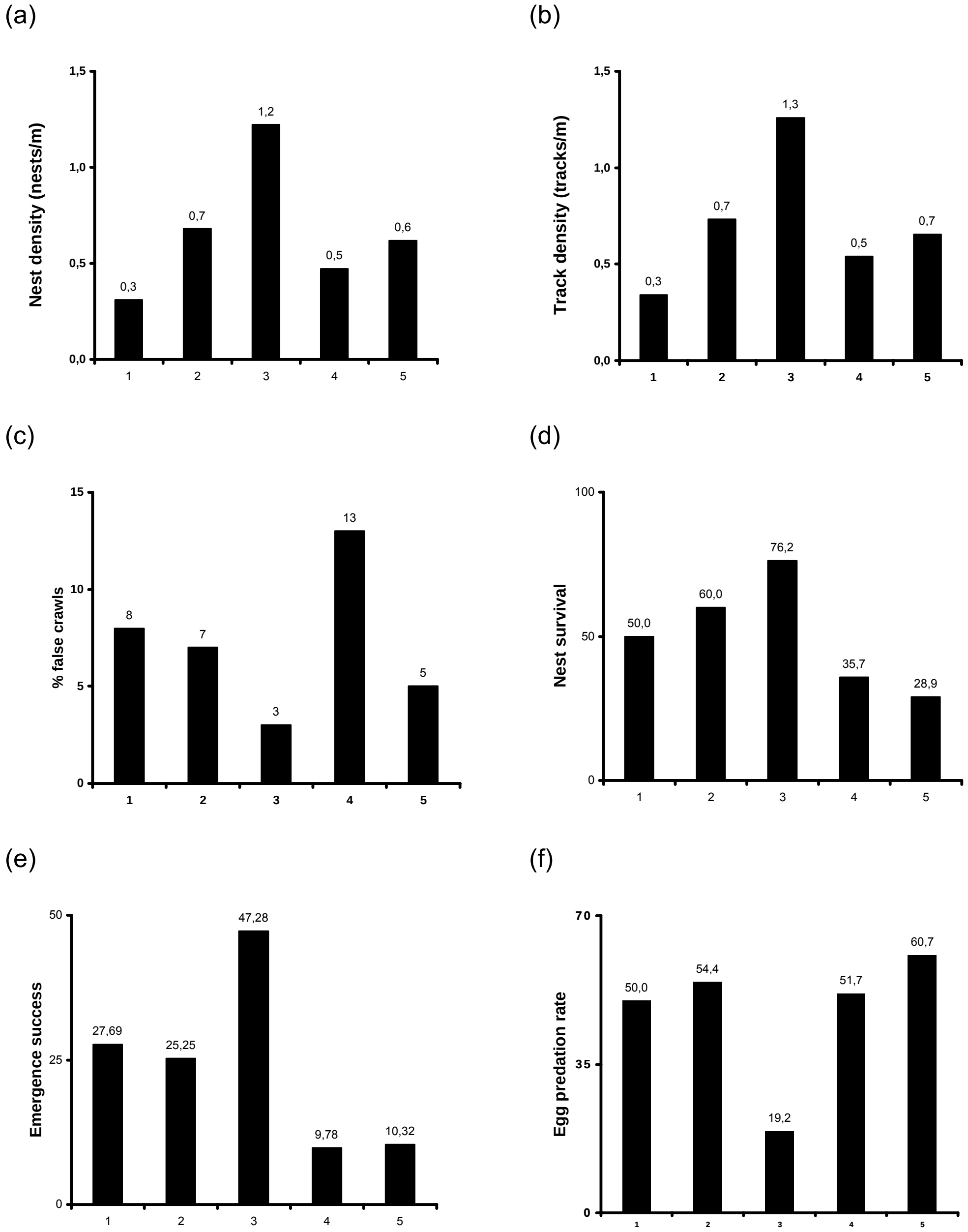

Figure 4. Characterization of the different sectors that were identified at the beach of Kingere according to (a) track density, (b) nest density (c) proportion of false crawls, (d) nest survival (proportion of nests that successfully hatched), (e) mean emergence success of the clutches and (f) egg predation rate (average number of viable eggs predated per clutch).

(g)

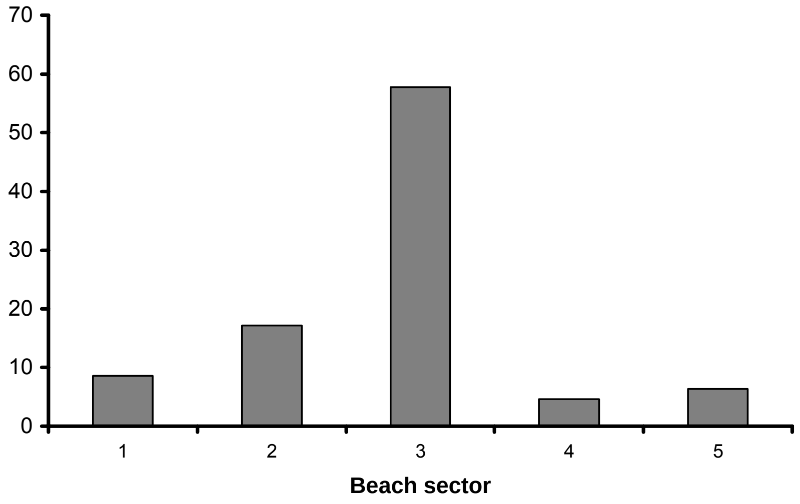

Fig. 5. Mean productivity, defined as (nest survival) x (mean emergence success) at the five sectors identified in Kingere.

Discussion

Low survival of nests at Kingere: causes and consequences

Leatherback turtles have among the highest reproductive output of any reptile, laying several large clutches of eggs per season and at relatively frequent intervals (Miller, 1997; Reina *et al*. 2002; Wallace *et al*. 2004). However, this species is also characterized by a low reproductive success, with high mortality rates at the egg stage (Bell *et al*. 2003, Wallace *et al*. 2004). There are several studies showing that embryonic mortality is particularly high at certain stages during early and late incubation (Whitmore and Dutton, 1983, Girondot *et al*. 1990, Bell *et al*. 2003) but the reasons for this are still not clear. Whitmore and Dutton (1985) found that inundation by the tides was one of the causes for the high embryonic mortality of nests in Surinam, but there seemed to be additional causes that were not clarified. In fact, poor nest site selection seems to be a typical trait in leatherbacks as they show a tendency to lay their nests near the sea (Mrosovsky, 1983). This fact is worsened as they usually nest in very dynamic and unstable beaches. It is estimated that, at some nesting sites, half of the reproductive potential is lost due to either flooding or erosion (Mrosovsky, 1983). On the other hand, nesting too near the vegetation can favour the presence of invading roots (Livingstone, 2007) or increase predation rates by insects (Caut *et al*. 2006). Kamel and Mrosovsky (2004) suggested that nest site selection in leatherbacks is compromised between the advantages and disadvantages of nesting too low or too high across the beach. Also, at nesting beaches with high density of females, the amount of eggs from past and present seasons might have a negative effect on nest incubation by favouring the presence of micro organisms (Clusella-Trullas and Paladino, 2007). For example, this was thought to be the reason for the low hatching success at some beaches in Surinam (Hilterman and Goverse, 2007).

Hatching and emergence rates found at Kingere are extremely low, with more than half of the nests that failed and an overall hatching success of only 16%. The reason for this might be a combination of the above-mentioned effects. First, the beach is very narrow and squeezed between the sea and dense forest. Many eggs get flooded by the tides periodically or lost by erosion. The proportion of flooded and eroded nests in this study is probably underestimated because of a bias towards marking nests above high tide line. On the other hand, we found high rates of predation exerted by insects that certainly come from the surrounding forest. Also, given the high density of nesting, the amount of unhatchedd eggs remaining in the sand might be favouring a thriving microbial activity that could infect freshly laid eggs. Finally, the sand might not be of good quality because of a high organic material content originated from vegetation debris and the surrounding forest.

All in all, such low rates of nest survival have important repercussions for the reproductive success of the species at this site as they cause a significant reduction in both the individual and collective annual production of hatchlings. If we consider that leatherbacks in Gabon lay an average of 5 clutches per season (Fretey and Billes, 2000) and that each clutch contains an average of 78 viable eggs (chapter 2), we can estimate the seasonal reproductive output of an individual female at 390 eggs. This is slightly lower but similar to the value of 450 eggs calculated for leatherbacks at Playa Grande in Costa Rica (Reina *et al.* 2002). However, the outcome in terms of hatchlings production between these two sites differs significantly. Using a mean emergence success of 16% at Kingere, only 62 hatchlings per female would be produced out of those 390 eggs, compared to 252 estimated in Playa Grande (Santidrián *et al*. 2009).

Within beach differential productivity

The beach sector was the major determinant of nest survival at Kingere. Although, only 100m long, the lagoon mouth was, by far, the most productive sector with the highest nest survival and emergence success. The main reason for this enhanced incubation conditions seemed to be that predation pressure was lowest at this site. This was partly confirmed by the different sites tested for hatcheries (see chapter 2) as all attempts of nest relocation failed because of underground predation except at the lagoon mouth. In fact, this area constitutes a wide extension of sand free of vegetation and relatively stable compared to the narrow stretches of beach that predominate otherwise. On the other hand, sector 5, which was 3.5 long and most representative of the study area, was the least productive, with mean emergence success rates of 10%. Surprisingly, the lagoon mouth proved to be also a high frequentation area, with a higher density of both tracks and nests per length. This suggests that females might prefer to nest in this sector, but whether they choose somehow to emerge from the water at this particular areas remains a mystery. Coastal lagoons are typically found along the coast of Pongara and similar sand formations are probably present at other places. Small sectors like this one may play a major role in compensating the overall productivity of the area at a large scale.

Depth, clutch size and date of laying as minor factors affecting nest survival

We found that deeper and smaller clutches have better chances to survive. It can be hypothesized that deeper sand provides a more stable environment in terms of temperature, gas exchange or humidity, which are of crucial importance for egg incubation (Ackerman, 1997). For example, at Ascension Island, Mortimer (1990) found a positive relationship between the depth of green turtle nests and hatching success and suggested that it could be due to more suitable moisture conditions in deeper nests. It is also possible that temperatures are cooler and more stable. For other species that lay shallower nests, like the loggerhead turtle, heat stress can be a major reason of embryonic or hatchling death (Matsuzawa *et al*. 2002). On the other hand, an excessive depth of the clutches due to tidal sand accretion during incubation can cause the eggs to collapse and have fatal consequences for the survival of the nest, as it was reported in Mayumba (Billes, 2003) and Colombia (Patiño-Martínez pers. comm). Although we found that 76% of the nests ended up being deeper at the end of incubation (chapter 2), this did not seem to have an adverse effect at Kingere.

The finding that smaller leatherback clutches hatch better is not new and it was even been applied with management purposes on some nesting beaches in Malaysia. Hall (1990) found that hatching success in Culebra Island, Puerto Rico was best at 52-56 eggs. Balasingam (1967) found similar results in Malaysia (46-60 eggs). This is well below the averaged size of leatherback clutches (70-80 eggs). Conservation programs in Malaysia used to relocate divided clutches of 50 eggs to hatcheries to improve hatchling production (Chan, 1989). The practice of splitting clutches was found to increase emergence success by 10% and reduce mortality during the last stages of incubation (Mortimer *et al.* 1993). The reason for this seems to lie on the spatial distribution of eggs within a clutch. Smaller clutches would have more eggs in the periphery and less eggs in the center that are more vulnerable to experience higher or even lethal temperatures, detrimental gas exchange or dehydrate (Balasingam, 1967; see references in Hall, 1990).

Although we did not include specifically environmental parameters such as rain and temperature in the model, the fact that the date of laying was significant in the model suggests that hatching success is subject to a certain seasonality, possibly driven by climatic condition. Rain and air temperature vary along the nesting season with more humid and cooler conditions during the months of november and december. The peak of air and sand temperatures is achieved around the month of february, when rain is lowest (see chapter 5). Our study nests were laid in november, december and january and were incubating from november to march. According to the model, a given nest had better chances to survive as it was laid later during the marking period. It is possible that early-laid nests received more rainfall during the first phases of incubation and that this had a negative outcome in their success. In Pacific Costa Rica, Santidrián *et al*. (2009) found an inverse tendency, with nests laid later during the season having decreased emergence success and this was attributed to heat stress during the dry season. In our particular case, it appears that the detrimental effects of rain or humidity might have prevailed over a temperature-related effect at Kingere. In fact, our results about sand temperatures show that heat stress does not seem to be a problem on the beaches of Gabon (see chapter 5).

Comparison within the Atlantic region

The coast of Gabon spreads over more than 800 km of which the majority is available for nesting. Therefore, it would be important to determine whether this condition of low reproductive success is general or particular to Kingere. There is not much information apart from three studies in Pointe Denis, Gamba and Mayumba (Figure 1) and the supplementary data that were collected during the course of this research (Table 4). Survival rates of nests also seem to be quite low at Pointe Denis due to extreme beach erosion episodes (see Appendix I). In fact, this beach is situated next to the Komo Estuary and is practically disappearing because of the strong marine currents. Bourgeois (2007) reported a mean hatching success of 70% but this corresponded to a sample of successful nests that were selected after hatching and therefore this value is certainly overestimated (see discussion about methodology). However, success rates twice as high as in Pongara were found at the beach of Bame in Mayumba (Appendix I). In Gamba, Livingstone (2007) estimated a nest survival of 54%, which is also higher than the 36% found at Kingere during this study. It appears therefore, that hatching success is variable among sites within Gabon and this is probably due to the particular conditions of each beach. Also, predation rates seem to be lower in Gamba and Mayumba (see Apendix I and chapter 4). At a global scale, there is also a wide range of reported values for hatching success between regions (see table 4). There are reported values over 50% of hatching success at some beaches in North America (as in Florida) or the Caribbean region (as in Trinidad or the U.S. Virgin Islands). However, at some other beaches in South America (as in French Guyana and Surinam), hatching success can be as low as 10%, similar to our values at Kingere. Therefore, while some particular sector at Kingere seem to have success rates comparable to beaches such as in Florida or Trinidad, the overall general picture is at the lowest range of reported values for the Atlantic region (Table 4) and more similar to the other major nesting site in the Atlantic at French Guyana and Surinam.

Methodological recommendations

When studying a new population or nesting site, it is important to use standard methods so that results are comparable at a global scale. Here, we make the point about two aspects of how incubation success data is collected and presented. First, our results shows that values of incubation success differ greatly depending on whether we consider nests with zero hatching or not with differences of more than 30% between the two methodologies. There seems to be a tendency to estimate incubation success from only successful nests (see table 4) and these values could be overestimated, especially when nest survival is low. This was found to be a major constraint when doing our comparison. To be truly representative, real accurate values of incubation success on a particular beach should be obtained from a random sample from pre-marked nests. Also, the “nest survival” parameter is as important as overall mean incubation success and we recommend that both values be given when assessing the productivity of a given beach. Second, whether hatching or emergence success is the described parameter should be clearly specified. In our study, mean difference between hatching and emergence lies within the normal reported range of 1-2% (Miller 1999) but differences of up to 7% are reported at other nesting beaches (Wallace *et al*. 2004). While in terms of hatchling production, emergence success seems more relevant, most sea turtle studies use hatching success as a measure. Also, sometimes it is not really known which of the two processes is being considered. We recommend that every study specify clearly this point, with views to method standardisation and easier comparison.

The model explained only 18% of the deviance and this means that there are certainly other parameters that were not measured but were having a major effect on the hatchability of nests at Kingere. The contribution of this study is important in the sense that it brings to light an extremely low productivity at an important beach where no data was available; yet there are many open fields for research to be carried on. For example, it is not known to what extent maternal reproductive health or chemical contaminants could be affecting egg incubation and hatching success (see references in Bell *et al.* 2003). In French Guyana, toxic contaminants from the surrounding agricultural crops and malaria profilaxis where found in the sand (Guirlet, 2005) and it is even suggested that mercury from inland gold mines could also en up on the beaches (Fossette *et al*. 2008). Although extensive agriculture is almost inexistent in Gabon, one of the main economical resources is mineral and oil exploitation, with countless mines and petrol platforms both inland and offshore. Therefore studies on the potential ecotoxicology of the beaches in Gabon would be recommended. Finally, evaluating incubation success of the nests of the other species of sea turtles nesting at Kingere, such as *C. mydas* or *L. olivacea* could help to elucidate whether the low success observed for *D. coriacea* is due to inherent aspects related to the biology of this species or a consequence of the incubation medium.

Table 4. Hatching success of leatherback nests at main nesting sites in the Atlantic.

ATLANTIC Nesting regions	**Beach**	**Season (number of seasons)**	**Sample size**	**Nest Survival**	**Hatching success (Mean ± S.D.)**		**Data source**
					Successful nests	**All nests**	
CENTRAL AMERICA AND CARIBBEAN							
Colombia-Panama	Armila	2006	29	-	69.4 ± 3.3		Patiño-Martínez *et al.* 2008
	Acandi-Chilingos		86	-	75.7 ± 1.9		
	Playona		65	32%	43.8 ± 21.5		
Costa Rica	Gandoca	1990-2004 (n=15)	818	-		41.0 ± 25.78	Chacon *et al.* 2007
	Tortuguero	1990 – 1991 (n=2)	206	51.5%	63.3		Leslie *et al.*, 1996
		1998 – 2005 (n= 8)	256	37.4 ± 19.9	49.0 ± 16.3	23.2 ± 9.6	Troeng *et al.* 2007 CCC reports (1998-2007)
St. Croix USVI	Sandy Point	1982-1995 (n=13)	-	-	67.1		Boulon *et al.*, 1996
		1996-2006 (n=11)	2060	-		57.4 ± 7.9	Garner *et al.* 2006
Trinidad	5 beaches North Coast	2002- 2004 (n=3)	53	73%		52.5	Livingstone, 2006
			746	-	73		
NORTH AMERICA							
Florida	Juno beach	2001-2003	208	-		67 ± 24.7	Stewart & Johnson, 2006 Stewart, pers. com.
SOUTH AMERICA							
French Guyana	Awala-Yalimapo	2002	86	86 %		38.2 ± 2.4	Caut *et al.*, 2006
		2001	41	-	-	38.9 ± 38.9	Torres, 2002
			53	-	38.0 ± 2.75		
Surinam	Babunsanti	2001 – 2004 (n=4)	380	48–79%	21.6 ± 17.7 to 34.9 ± 22.1	10.6±16.4 to 25.8±24.4	Hilterman *et al.* 2007
	Matapica	2001-2002 (n=2)	151	86–90%	58.3 ± 25.4 to 63.7±24.2	52.7±29.7 to 56.0±30.8	
	Krofajasapi	1981-1982 (n=2)	46	-		52.4 ± 4.0	Whitmore & Dutton, 1985
EASTERN ATLANTIC REGION							
Equatorial Guinea	Moraca, South Bioko	1996-1998 (n=2)	12	100%		57.85±25.06	Tomas, 2004
Gabon	Pongara-Pointe Denis	2008-2009	30	30%	57.3±30.6	17.3 ± 31.1	Appendix I
		2006-2007	43	-	70.9 ± 24.6		Bourgeois, 2007
	Pongara Kingere	2005-2008 (n=3)	170	30%	46.3±31.1	16.7±28.9	This study
	Gamba	2006-2007	35	56%	67.0	-	Livingstone, 2007
	Mayumba	1999-2000	49	-	67.3 ± 18.6	-	Billes, 2000
		2008-2009	25	56%	71.0±24.6	42.2 ± 40.8	Appendix I

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Chapter 4: Natural History of Nests with a focus on underground predation

Summary

The results of 163 nest excavations at Kingere revealed a majority of unhatched eggs (82.5%) and only 16.7% of eggshells corresponding to emerged hatchlings. We found that, 59% of the excavated nests had more than half of the viable eggs predated and 94% had at least one egg predated. Egg and SAG predation rates within a clutch ranged from 0-100%. Typically, a clutch had 8.7 ± 8.8 SAG and 36.6 ± 26.4 viable eggs predated corresponding to an average proportion of 39% and 52% respectively. The great majority of the predated eggs were highly decomposed and it was not possible determine at which stage of incubation embryonic death and/or predation occurred. The majority of non predated eggs had no visible embryo within, suggesting that they were either not fertilized or that the embryo died before becoming macroscopic. In general, there was a high degree of bacterial and fungal infection. The pathogen fungus *Fusarium oxysporum* was identified growing on the shells of SAG and viable eggs. The main predators were crabs (Ocypodidae family) and insects that affected 51 and 82% of the nests respectively. Crab and insect predation co-ocurred in 33% of the affected nests. Ants, identified as *Dorylus spininodis,* were found in 56% of the excavated nests suggesting that it is a major predator of eggs and it might be the first record of this species in Gabon. The most striking observation was that most of the times, large numbers (hundreds of ants) were found dead within predated eggs. In some occasions they were also infesting some of the nests. Other invertebrates were associated to the clutch environment and identified. None of them was likely to be an egg predator but most probably opportunistic feeders. We found an unusual ecological interaction within the leatherback clutches between termites and ants that is possibly related to a predator-prey interaction between the two and the high incidence of buried logs on the beach. Termites were found in 11% of the nests either dead inside predated eggs or even building what could be foraging galleries over the nests. We suggest that the conditions of the beach at Kingere, with an abrupt transition between the soil forest and the sand, might be favouring a thriving microbial and invertebrate activity in the sand profile that colonizes the nests.

Introduction

Overview on clutch predation

Sea turtles are typically long-lived animals and thus an adult individual produces considerable amounts of offspring during its lifetime. The stages that are normally differentiated during their life cycle are eggs, hatchlings, juveniles and adults. The reproductive strategy of sea turtles assumes high natural mortality rates at early life stages (Magnugsson *et al*. 2009). However, they have few predators once they reach the adult size. It is known that sharks or orcas can attack leatherbacks at sea (Heithaus *et al*. 2009). Also, at some nesting beaches they can be killed by jaguars (Tröeng *et al*. 2007) and there is one citation of an attack by crocodiles (Hirth *et al*. 1993). Little is known about the juvenile stage since there is not much information about at sea behaviour but they probably have higher mortality rates. However, the greatest mortality occurs at the hatchling and egg stage and these have been much more documented, due to the ease of taking observational data.

There is a wide documented range of predators of sea turtle eggs from wild to domestic animals like pigs or dogs. Wild predators include crabs (Tomás, 2004; Livingstone, 2007), insects (Maros *et al.* 2003), lizards (Tomás, 2004; Livingstone and Verhage, 2006) or mammals such as mongooses (Nellis and Small, 1983). Bacterial and fungal infection of eggs is also relatively common and may cause the death of the embryos (Wyneken *et al*. 1988).

As sea turtle clutches are buried in the sand, the presence of invertebrate fauna associated to the nest environment is relatively common but the nature of such interactions is not always easy to determine. Most of the times, it appears that invertebrates prey upon eggs causing considerable damage to the survival of the clutches. For example, in Florida, a native species of coleopteran larvae, the click beetle *Lanellater sallei*, was identified as a major predator of eggs of the loggerhead turtle, *Caretta caretta* with, 78% of the nests presenting pierced eggs with small circular holes and/or the presence of larvae in the incubation chamber (Donlan *et al.* 2004). There are other reports of coleopterans inside loggerhead nests and presumably feeding on eggs in Cyprus (McGowan, 2001) and in Turkey (Baran *et al.* 2001). In French Guyana, the last instar nymph of the mole cricket (*Scapteriscus didactylus*) appears to predate opportunistically on leatherback eggs (Maros *et al.* 2005) reaching a peak of adult abundance on the beaches at the peak time of turtle nesting activity. Dipterian larvae have been recorded in the nests of green and loggerhead turtles in the Mediterranean (McGowan *et al*. 2001) but did not appear to have any adverse effect on incubation success as only a low percentage of eggs were affected.

There are several reports of predaceous ants might feed on eggs as well as on emerging hatchlings: green turtles in Costa Rica, (Fowler, 1979), loggerheads in South Africa (Maxwell, 1998), hawksbills in Malaysia (Chan and Liew, 1999) or leatherbacks in Surinam (Whitmore and Dutton, 1985). Ants can also attack eggs and hatchlings of terrestrial and freshwater turtles (Burger, 1977; Allen at al. 2001). On the south Atlantic coast of North America there seems to be a major problem with red imported fire ants (*Solenopsis spp.*) that have a devastating effect on sea turtle eggs (Moulis, 1997; Allen *et al*. 2001; Parris *et al*. 2002; Allen *et al*. 2004).

So far, main reported predators on sea turtle eggs in Gabon were Ocypodidae crabs, the lizard *Varanus niloticus* and small felines like *Civettictis civetta* (Billes, 2000; Verhage *et al*. 2006; Livingstone, 2007). However, during the course of the study on incubation success at Kingere, we found ants in massive amounts inside leatherback nests. In this chapter, we focus on the results of the nest excavations and particularly on the process of predation that appeared to be one of the main causes of nest failure. We provide quantitative data on the mortality of eggs and nests and describe some of the main interactions between the clutches and the associated fauna found within.

Methods

This study was conducted in The Republic of Gabon, at Kingere beach in Pongara National Park (0º 18´N, 9º 18´E) during 3 nesting seasons (2005 to 2008). A total of 163 nests were excavated to evaluate incubation success. The procedure to mark and track the nest is explained in the previous chapter and the methodology explained here refers specifically to the excavation of nests and data collection on predation.

Nest excavations

Once the exact location of the nest was found on the beach, the first layers of sand were removed with a shovel taking care not to harm any other eggs or emerging hatchlings nearby. We then excavated around the presumed position of the nest until finding the nest tag, and subsequently scraped by hand to minimise impact. Then the rest was done by hand. The objective was to leave the mould of eggs and sand as if doing “sand archaeology”. We then proceeded to remove the contents of the nest, again taking care not to harm the eggs. The eggs were classified in the sand surface first by morphology (hatched vs. unhatched, predated vs. non predated) (Plate 1 d and f) and secondly by their contents (presence or absence of an embryo). The following categories were differentiated:

- **SAG**
- **Eggshells**: empty eggs remaining after hatchlings emerge from the nest
- **Pipped**: eggs where the hatchling died within after piercing the membrane but has been unable to get out of the egg and died within. These were rarely found and the exact causes why it happens remain unknown.
- **Unhatched**: These were initially separated into predated (P) and non predated (NP) and then opened to examine the contents. A second classification was done according to the presence or not of a visible embryo:
    - **no visible embryo**
    - **early embryo**: a macroscopic embryo in early stages of development with no pigmentation (Plate 1a)
    - **late embryo**: macroscopic embryo that has acquired the pigmentation (Plate 1b).
    - **unknown**: unidentifiable content, usually decomposed by microorganisms fungus or bacteria.

Note that the classification into early and late embryo should be considered an estimate not corresponding in detail to developmental stages.

Quantitative and qualitative data on underground predation

The presence of a hole of any shape on the egg shell (clearly different from the pipped eggs) was considered as evidence of predation. For each nest, the following information was collected: presence of invertebrates, presence/absence of ants and/ or termites, how ants were found, type of predator and number of predated eggs. Egg and SAG predation rates for each nest were calculated as percentage of each presenting a hole in the membrane relative to total number. We used two criteria to assume that a nest was "affected by predation": 1) the nest had at least one viable egg predated or 2) the nest had more than 50% of the viable eggs predated. For each nest, a type of predator was assigned according to the holes observed in the membrane of the eggs: insects, crabs or mixed. Predator type and ant presence were quantified at a nest level, not at an egg level. Samples of invertebrates were collected and stored in eppendorf tubes with 96º alcohol. They were sent to the following organizations for identification: The Faculté Universitaire des Sciences Agronomiques, Belgium; Department of Enthomology of the National History Museum of London and Jardín Real Botánico, CSIC-Madrid.

PLATE 1


(a) early stage of embryo development


(b) late stage of embryo development


(c) two nest moulds side by side


(d) typical aspect of a clutch with termites


(e) Hatched nest


(f) predated nest

Results

Classification of nest contents

A total of 11613 viable eggs were examined corresponding to the 163 excavated nests during the course of three nesting seasons at the beach of Kingere.

Mean proportion of eggshells was 16.7% while the rest of the viable eggs (82.5%) were unhatched and a very small proportion (1.1%), pipped eggs (Figure 1a). Predated eggs accounted for 51.2% of the total. Inside the eggs that were not predated, a dead embryo in early or late stages of development was found in 6.3 and 16.3% of the cases respectively but most of the times, there was no visible embryo (58.6%) (Figure 1b). In most of the predated eggs (89.3%), it was not possible to determine the presence or absence of an embryo and the egg was classified as “unknown” (Figure 1c). Thus, the most abundant categories inside the excavated nests were predated eggs with unknown contents and non-predated eggs with no visible embryo (Figure 1a). SAG were not considered for this analysis.

The nature of the contents of predated eggs was highly variable. Sometimes the eggs were completely filled with sand or they were completely decomposed. In other occasions sand was mixed with egg remains that had a characteristic grey-blue metallic colour (Plate 2e). This grey colouration was attributed to either microbial infection or to the pigmentation of totally liquefied and decomposed embryo. There were also eggs in which the content was a mixture of these categories (Plate 2f), e.g. plain/grey sand or grey sand / egg remains or egg remains/dry contents reflecting a succession in the degradation and decomposition process of an egg. The most striking finding during excavations was the presence of thousand of dead ants inside predated eggs that seemed to be especially frequent in the eggs filed with sand.

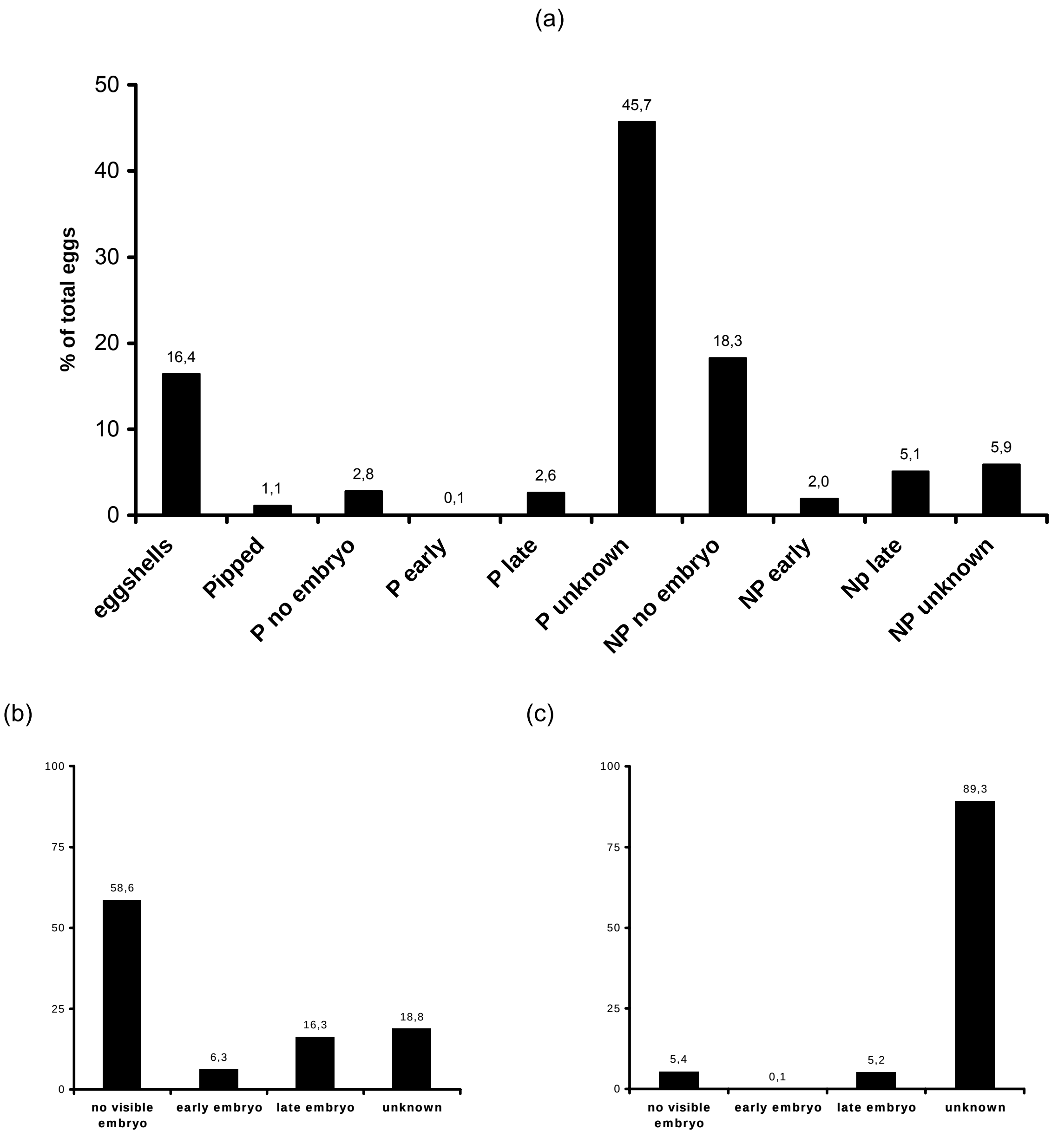

Figure 1. Results of the excavations (a) all viable eggs (P: predated, NP: non predated), (b) non predated eggs and (c) predated eggs. Y scale bar is average frequency for the totality of excavated nests.

Rates of predation to nests and eggs

Using the two criteria to consider a nest as predated, we found that 94% (n=154) of the nests had at least one egg predated and 59% (n=96) of the nests had more than half of the eggs predated. Although predation by the lizard *Varanus ornatus*, and mammals like mongooses was frequently observed on the beach, all study nests were affected by either crabs or insects. Insect and crab holes were easy to differentiate: crabs produce characteristic slit marks (Plate 2c) and disperse the eggs over the sand surface or at different depths above the incubation chamber. Also, there were usually holes on the sand surface from the burrowing galleries (Plate 2d). Insects produced different shapes of holes but did not disperse the eggs. Insect type holes ranged from small and circular (1mm to 1cm diameter) (Plate 2b) to irregular with serrated edges (1-3cm width) and a typical amoeba shape (Plate 2a). Numerar fotos y poner correspondiente cita. Insect and crab affected eggs were recognized in 49% and 18% of the nests respectively. Both types of predation were evident in 33% of the nests (Table 1). This means that insect affected eggs were found in 82% of the nests in total. Both SAG and viable eggs were attacked. Mean number of viable eggs and SAG predated in a nest was 36.6±26.4 and 8.7±8.8 respectively that corresponded to a mean proportion of 52% and 39% of the total eggs and SAG in a clutch (Table 1). These rates ranged from 0-100%. The fact that a nest was partially predated did not necessarily mean that none of the eggs hatched.

Table 1. Quantitative data on predation levels to eggs and clutches and on the occurrence of ants inside leatherback nests at Kingere. Number of nests appears in brackets.

UNDEGROUND PREDATION	
On nests	
% Nests with >1 egg predated	94% (154)
% Nest with >50% eggs predated	59% (96)
On SAG and eggs	
% SAG predated per clutch ± SD	39.0 ± 30.8
% viable eggs predated per clutch ± SD	51.9 ± 33.1
Predator	
Insects	49% (75)
Crab	18% (28)
Co-ocurrence crabs and insects	33% (51)
Ants inside the nest	
Presence	56% (91)
Absence	44% (72)
Presence of ants	
Dead inside predated eggs	88% (80)
Colonising	4% (4)
Alive solitary	30% (27)

Inventory of associated fauna in the nest

Most of the specimens were found at nest depth (average 70 cm) in the incubation chamber among the eggs or inside predated eggs. The following groups were recorded: insects (ants, termites, adult beetles and fly and beetle larvae), arachnids (acarids), crustaceans (crabs) and annelids (red worms) (Table 2). There was also a wide variety of micro organisms (fungi and bacteria) on the shell and inside the eggs. We only identified the pathogen fungus *Fusarium oxysporum* that was quite frequently growing on the eggshells and conferred an orange colouration. Ants, termites and acarids were found in large numbers/aggregations while the rest were solitary individuals.

The ant genera Dorylus and Hypoponera (Hymenoptera) were identified inside the nests. Found in most abundance was *Dorylus spininodis* (Emery, 1901), belonging to the Dorylinae subfamily commonly known as “Army ants” (Plate 3). Although it is a pan-african species this was the first record in Gabon. Termites (soldiers and workers) of the genera Coptotermes (Rhinotermtidiae), Nasutitermes and Amitermes (both Termitidae) were identified. Different morphologies and sizes of fly larvae were observed but not identified, some of them probably belonging to Cypselidae flies that are commonly found in the sand surface. Isolated individuals of adult beetles from the following families were identified: Staphylinidae, Carabidae and Histeridae (carrion feeders). Beetle larvae were identified as belonging to Tenebrioidae, Elateridae and Dynastinae (rhino beetle). Most of these feed on carrion, mold, fungus or rotten plant material and are probably opportunistic. Acarids were usually present in large numbers covering the inner membranes of predated eggs, specially those that were already at the humus stage or empty, and are also probably opportunistic feeders on humus or microorganisms. On occasion, crabs (Ocypodidae family) were found in the egg chamber although most frequently, only their galleries or the predated eggs dispersed around the nest were observed. It was quite surprising to find red worms, belonging to Lumbricidae family in between the eggs and they seemed to occur more often in flooded nests.

Ants were never seen on the sand surface during nest monitoring activities but were found during excavations inside nests that were presumed to be undisturbed during incubation. Direct presence of Dorylus ants was observed in 56% of the excavated nests (n=91) (Table 1). Most frequently, large numbers (hundreds of ants) were found dead within predated eggs (n=80 nests), in particular those that were filled with plain sand or grey sand (Plate 3b). Four nests were found completely infested by ants and their galleries through the sand. Otherwise, live solitary individuals were found in the incubation chamber.

Termites were found in 11% of the excavated nests (n=19). They were often associated with buried logs (50% of the findings) and high organic material contents (plant debris or other). More often isolated individuals were found, while others where dead individuals inside predated eggs. Identification of the termites revealed that sampled individuals were workers and soldiers of the genus Nasutitermes and on a few occasions Amitermes and Coptotermes. We observed galleries of dark brown colour built on top of the mould of eggs and sand in 6 nests conferring a characteristic oxidised aspect to the eggs. Ants were always found in the nests occupied by termites.

An interesting observation was that of a nest that was opened 11 days after oviposition to check fertility using the white spot technique. This nest had already several eggs predated with tiny circular holes and Dorylus ants were drowned in the yolk (Plate 3 a & b). Also, sand coagula were already present in the yolk. Other predated eggs were filled with sand and alive termites inside.

Table 2 Main groups of invertebrates that were found during excavations in the leatherback nest environment and their observed or hypothesized interactions with eggs.

Taxon	Behaviour	Presumed interaction with the eggs
INSECTA		
HYMENOPTERA (Ants) Dorylus sp. Hypopomera sp.	Dead in huge amounts inside predated eggs (≥1000). Alive isolated (1-10) in the egg chamber or actively infestating the nest with galleries (≥ 10.000).	Predation (on eggs or termites). Primary or secondary predator.
ISOPTERA (Termites) Nasutitermes sp Amitermes sp. Coptotermes sp.	Frequently associated to decomposing buried logs. Alive isolated or dead inside predated eggs. Build galleries above the nest mould (could be foraging galleries).	Commensalism with collateral damage: possible consumption of egg shells, however likely to foraging on plant material in sand profile Inquilinism
DIPTERA (Fly larvae)	Inside eggs or attacking embryos or hatchlings.	Predation ??
COLEOPTERA (Adult beetles) Staphylinidae Scaratinidae Histeridae	Isolated individuals in the egg chamber, not directly associated to eggs.	Commensalism (Opportunistic feeder on carrion, mold, fungus)
COLEPOTERA (beetle larvae) Tenebrionidae Dynastinae Elateridae	Alive and isolated in the incubation chamber. Sand dwelling.	Opportunistic feeder
ARACHNIDA		
Acarids	Alive in large numbers. Covering the inner side of the membrane of predated eggs.	Comensalism (opportunistic feeder)
CRUSTACEA		
Ocypodes cursor (ghost crab)	Build galleries around the nest and is sometimes found inside the egg chamber.	Predator of eggs and hatchlings
ANNELIDA		
LUMBRICIDAE (Red Worms)	Alive, isolated in between the eggs. Frequently associated to flooded nests.	Comensalism (opportunistic feeder)

PLATE 2: PREDATION HOLES AND EGG CONTENTS

(a) amoeba shape 1-3 cm width

(b) circular hole 0.1-1mm diameter

(c) crab-type slit marks

(d) crab holes from burrowing galleries

(e) unidentified content: sand, egg remains and dead ants

(f) unidentifed content: egg filled with sand and dead ants (red circle).

PLATE 3: *Dorylus spininodis*


(a) drowned ants


(b) dead ants in a predated egg filled with sand


(c)

Discussion

We found that 59% of the nests had more than half of the viable eggs predated at Kingere and that insect predation was evident in 81% of these nests. Although it would need to be confirmed, ants are apparently major responsible for this predation as they were found in more than half of the predated nests. Such rates do not seem to happen at other beaches in Gabon. During a study conducted in season 2008/2009, the presence of ants was recorded only in 3% of the nests at Pointe Denis and at 22% of the nests in Mayumba (Appendix I). Livingstone (2007) reports finding ants at Gamba in a 12.5% of hatched nests. Otherwise, the main predators at other beaches in Gabon seem to be crabs, lizards and small mammals.

The typical nest profile found in the majority of excavations was that of a failed nest, almost transformed into humus, with the majority of eggs predated by insects. We ignore the moment of incubation at which predation occurred and whether this is the primary cause of death of the embryo, or if this is a secondary process once the embryo has died for some other unknown reason. The most abundant categories were predated eggs with unknown contents. These contents probably corresponded to remains of eggs infected by fungi and bacteria or even to liquefied and decomposed embryos. It was therefore impossible to tell at which stage of development predation occurred. The second most abundant category were non predated eggs with no visible embryo. These could correspond to an egg that was not fertilized or where the embryo died before becoming visible. Bell *et al*. (2003) suggested that fertility was high in the leatherback nesting clutches at Playa Grande in Costa Rica. Our preliminary results show that there was a considerable difference between the clutches laid at the peak (over 90% fertility) and end (50%) of the season (Appendix III). The clutches that were analyzed in this study were laid during the early- middle season and further research into this would be recommended so as to determine the exact causes of embryonic death or infertility of the eggs.

Gabon is situated in the heart of Central Africa and belongs geographically to the Congo basin area, which can be considered as the equivalent of the American Amazonian region in the African continent in terms of plant biomass. 85% of the surface of Gabon is covered by dense equatorial rainforest, which borders almost entirely the 800km of Atlantic coastline. The typical landscape of the Gabonese coast is that of almost uninterrupted stretches of sandy beaches bordered by forest and occasional savannas and coastal lagoons. Most of the time, there is either a savannah or a transition strip of lowland vegetation of 50-100 meters separating the beach and the forest (as in Mayumba and Gamba and Pointe Denis). However, at Kingere, the forest reaches the beach abruptly resulting in almost no transition habitat in between. Hence, the influence of the forest on the beach is likely to be much stronger resulting in sand being very similar to forest soil. On the other hand, there is a specially high incidence of stranded logs at Kingere with some sectors being almost entirely covered and many of them ending up buried in the sand by beach dynamics (Laurance *et al.* 2008). The high organic material content (buried logs and forest contribution) may support a thriving invertebrate and micro-organism community that is attracted into the beach soil profile, and subsequently monopolises on the high energetic values of turtle nests. This would explain the low incubation success of nests and the high levels of predation that have been found in this study.

Dorylus spininodis in leatherback nests

The presence of ants in leatherback nests is reported in Surinam (Whitmore & Dutton, 1985), French Guyana (Maros *et al* 2005), and Bioko (Tomás 2004) but without specifying the species and corresponding apparently to sporadic observations in lower proportions. The magnitude of the occurrence of ants at Kingere seems to be only comparable to that of red-imported fire ants (Solenopsis sp.) in the coast of Florida in green and loggerhead turtle nests. However, while these are introduced species, *Dorylus spininodis* is a native pan-african ant that occurs naturally in equatorial rainforests. However, it appeared to be thr first citation of this species in Gabon (Ikaran *et al*. 2006). It belongs to the Dorylinae family, commonly known as “army ants” (or magnan in french), due to their foraging behaviour in mass raids. The activities of *Dorylus spininodis* are mainly subterranean; that is why, no signs of predation were recorded above sand during daily surveys of the nests. Two aspects of their feeding behaviour are interesting regarding their interaction with sea turtle nests: 1) they can exploit large bulky sources of food, such as termite nests, over several weeks or months and 2) their feeding regime is composed of foods rich in lipids (Berghoff, 2002). In fact, the only effective method to study these subterranean ants is by using oil-rich baits, such as palm oil (Berghoff, 2002). The trophic dynamics of army ants within tropical ecosystems is far from being negligible, due to their mass feeding habit and an ability to conduct highly organised raids (Berghoff, 2002), which confers with the predation patterns observed in this study.

Observed predation levels in our study don’t seem to be produced by isolated individuals but rather by some kind of social insect or highly abundant predator in a very organized way. We therefore suggest that *Dorylus spininodis* is a major source of predation on leatherback eggs, which may be determining the nesting success rates at Kingere. As opportunistic predators, they are likely to be able to efficiently detect and exploit leatherback nests, an abundant yet patchy lipid-rich nutrient source available seasonally with a certain regularity and abundance. Eggs would become filled with sand as a result of constant traffic in and out, while some individuals would die drowned in the egg. Considering that the average size of a colony can exceed 325,000 individuals, this small number of deaths is probably negligible compared to the benefits to the colony in terms of nutrient gain. The mechanism by which ants detect a nest remain unknown: it could be by chance, during foraging expeditions to the beach (density of nests), or because they are initially foraging on termites attracted to buried logs in the sand profile.

Termites in leatherback nests

Termites are particularly diverse and abundant in forest systems of equatorial Africa (Davies *et al*. 2003a), where they consume dead plant material at different stages of decomposition, from woody material to clay soil. Generally they have static colony centres, located for instance in constructed mounds, such as the arboreal mounds of Nasutitermes, or nesting in the same dead wood material that they consume, such as Coptotermes. Nasutitermes was the most commonly found termite in the turtle nests. It is likely to nest exclusively in the forest adjacent to the beach, with worker termites leaving the forest to forage for dead plant material in the beach profile, constructing protective galleries over the foraging columns. Due to the difficulty of direct observations of termites, very little is known about the foraging distances of termites from the colony centre. Foraging distances of up to 15 m have been recorded from *Globitermes sulphurous*, a wood feeding species in a Borneo forest (Ngee and Lee 2002). Our study indicates that worker Nasutitermes are foraging a minimum of 10-15m from a colony centre located within the forest habitat.

All three genera reported from the turtle nests are termed “wood-feeding termites”. Amitermes is completely subterranean, and consumes more decayed wood material (Donovan *et al*. 2001a). It is generally accepted that termites only consume plant material, although at various stages of decomposition. They are highly adapted to this feeding habit, with complex gut morphology and associated micro-flora to mineralise material with a high carbon/nitrogen ratio (Slaytor 2000). This therefore does not lend itself to consuming high protein food material such as the contents of turtle eggs. However in the tropical forest in Panama, groups of Nasutitermes have been observed amongst decomposing animal carcasses (Thorne & Kimsey, 1983), upon which they had constructed carton foraging galleries. Foraging termites have also been observed at the contact point between the soil surface and elephant bones with possible foraging tunnels through the bone material, from an in-land forest in Gabon (Scholtz, pers. comm.).

In this study we found a clear interaction between termites and turtle eggs but we cannot be conclusive about its nature. First, in several failed nests, foraging galleries similar to those described by Thorne & Kimley were found over the mould of sand and eggs. The nests where termites were found had a typical oxidized aspect (collateral damage). Secondly, dead termites were sometimes found inside predated eggs, in a similar way to ants, suggesting that they might also have get drowned when entering the egg. Finally, termites were found in nests as young as 11 days after oviposition, suggesting that there is a rapid contact between newly laid turtle nests and foraging termites. Considering all this, we suggest that termites might be occasional or accidental consumers of leatherback eggshells, however this would need to be confirmed by examination of the termite gut contents. Rather, the buried plant material seems to play a major role in attracting termites to the beach sand profile away from the colony centre in the adjacent forest. Again the termites may be accidentally passing the turtle nest environment, which are often in close proximity to pieces of buried logs and roots. Buried logs are found in particularly high abundance at Kingere, which originate from the forestry sector (Laurance *et al* 2008), as logs are lost into the sea during loading onto ships. As a consequence army ants may be predating on the foraging termites, providing a possible mechanism by which the army ants detect the turtle nests. Another possibility is that termites would use leatherback nests just as an architectural support for their galleries without feeding on it. As far as we know, this is the first record of interaction between termites and sea turtle eggs.

Ecological role of nests

Our results show how a leatherback nest provides an underground microenvironment (micro-ecosystem) hosting a variety of invertebrates with different ecological interactions among them and with the eggs themselves. Beetles, acarids and worms are probably attracted as opportunistic hosts to feed on mold, fungus, bacteria growing in the eggs. Termites, ants and buried logs are possibly inter-related in a predator-prey relationship (ants prey upon termites and even exploit their nests). There is probably a progressive colonisation (micro-succession) of the nest, with some species being pioneers and others opportunistic arriving later. The ultimate stage in the succession is probably the nest being transformed into humus. The fate of a leatherback nest thus is far from being merely hatchling production: unhatched eggs provide a substantial amount of nutrients that are either used by host invertebrates or could have a potential fertiliser role of the coastal area. Hannan *et al*. (2007) found that dune vegetation in sandy ecosystems assimilated nitrogen most likely coming from sea turtle eggs at some beaches in Florida. In fact, through nesting activity on the beaches, sea turtles are considered as nutrient and energy transporters between terrestrial and marine habitats (Bouchard and Bjorndal 2000, Hannan *et al*

2007). Considering that a leatherback clutch weighs on average 7kg and the nests densities at Kingere range between 150 to 470 nests per kilometer and season (see chapter 2), we can estimate that the mass of unhatched eggs remaining in the sand after one nesting season is between 1 and 3.5 tons per kilometer (Figure 2); a contribution that is certainly non negligible.

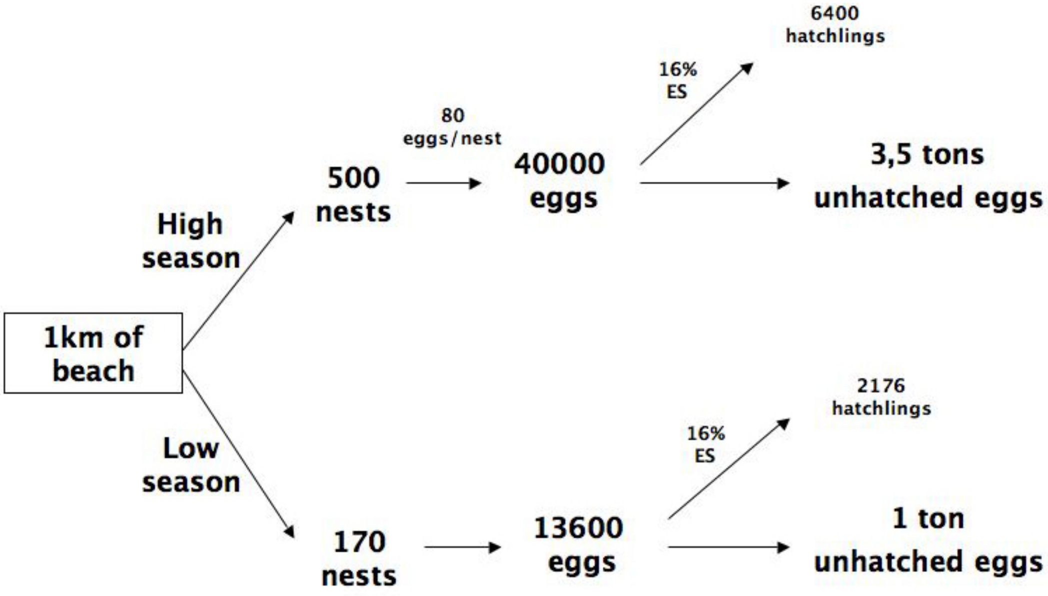

Figure 2. Estimated mass of unhatched eggs that remain in a 1km section of beach at Kingere after one nesting sesons, considering a high density and low density year.

Recommendations for future studies

The present study has to be considered as a first insight into the natural history of leatherback nests at Kingere and provides grounds for further enhanced research into the topic of predation. Several questions remain uncertain and need to be elucidated: Is predation the real cause of egg mortality? Are ants primary or secondary predators? Is the primary predator gone by the time of excavation? According to the different hole structure, predation by multiple insects was apparent within a single nest. Although some of the observed holes could be similar to those made by the mole cricket in French Guyana and described by Maros *et al*. (2005) we did not find a single specimen during nest excavations. For a better understanding of the mechanism of predation; we suggest to conduct further studies in which nests are opened at regular intervals throughout incubation so as to monitor predation in a chronological way, assign type of hole to predator and study the evolution of predated eggs**.** Also, a great proportion of the eggs was infected by fungi or bacteria and could be a source of embryo mortality (Wyneken *et al*. 1988; Marco *et al*. 2006). We only identified the pathogenic fungus *Fusarium oxysporum* that has also been described to infect loggerhead turtle eggs in Cabo Verde (Marco *et al*. 2006; Abella *et al*. 2008). This plant pathogen can affect both embryos and hatchlings and is also potentially harmful for humans. Further research into this topic with a detailed identification of micro organisms is recommended. Finally, in this study, we found that there is a high percentage of logs, equally or even higher to those visible on the beach, that end up being buried deep in sand and it would be recommended to evaluate quantitatively the potential impact of these on the incubation of eggs.

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Chapter 5: Estimating current (2005/2009) and future primary sex ratios of *Dermochelys coriacea* hatchlings from sand temperatures in Gabon.

Summary

In this study, we combine information on sand temperatures and thermal regimes of nests with meteorological data, nest counts and the sex ratio curves available for *D. coriacea* to estimate primary sex ratios produced in Gabon over four nesting seasons (2005 to 2009). Sand temperature at nest depth (70cm) was measured at the beach of Kingere during the whole study period and at two other beaches along the coast of Gabon, Gamba and Mayumba during season 2008/2009. Although the study beaches are several hundreds of kilometers apart, interbeach thermal variation was only ± 0.3ºC. Therefore, this allowed to make an estimation for the whole coast, using values recorded at Kingere over the 4 seasons. We used different scenarios to look at possible differences in the outcome of the estimations. First, as there is no precise study on TSD for leatherbacks in Gabon, paired data of sand temperatures-sex ratio were obtained from both the French Guyana and Pacific sex ratio curves. Also, we considered three thermal scenarios: index beach (Kingere), index beach +0.3ºC and index beach -0.3ºC (observed interbeach variation). There was a seasonal pattern in which both air and sand temperatures increase towards the nesting period, which resulted in a seasonal fluctuation in sex ratios. Mean estimated male proportion over the four year period was 60% ± 21% with annual values ranging from 37% to 87%. The nesting season for the leatherback turtle in Gabon starts during the rainiest months of the year (october and november) when, according to sand temperatures, there is almost 100% hatchling male production. Sand temperature increases towards the end of the season and more females are produced but rarely attaining 100% female proportions. This within seasonal pattern seems to be an important factor inducing variability in sex ratio and is similar to that found at the other major Atlantic nesting area for the species in French Guyana-Surinam. There were no major differences by using the French Guyana and Pacific curves; however, scenarios of ±0.3º lead to male or female skewed situations. Our results highlight the importance of Gabon as a nesting site in terms of male hatchling production in the Atlantic region. Having a very restricted pattern of temperature dependent sex determination, with a transitional range of temperatures of only 1ºC wide, the leatherback turtle is particularly vulnerable to global warming effects on sex ratio. We used air temperatures to predict sand temperatures and the resulting sex ratios in the future. A mean rise of 1ºC in air temperature, predicted by 2029 for the Central African region, would be enough to lead to a 100% female skewed scenario. The vulnerability of this species to climate change is discussed.

Introduction

Climate Change and sea turtles

There is an increasing concern about the effects that climate change will have upon sea turtle populations with more and more studies dedicated to this topic (Hawkes *et al*. 2009). According to the latest meteorological data, air temperatures and global mean ocean temperatures are increasing at accelerated rates (IPCC, 2007). These warming trends will carry other phenomena such as a rise in sea level, extreme weather events, changes in seawater chemistry or erosion of the beaches (Leatherman *et al*. 2000; MacLean *et al*. 2001; Zhang *et al*. 2004). Temperature is a major parameter in the life cycle of marine turtles, not only at sea in determining the limits for adult distribution (Davenport, 1997; McMahon & Hays, 2006) but also on nesting beaches where sand temperature influences embryonic development (Ackerman, 1997) and the sex of the hatchlings (Mrosovsky and Pieau, 1991). Furthermore, the optimal thermal range at which incubation of sea turtle eggs can occur is no more than 10 ºC wide, from 25-27ºC to 33-35ºC (Ackerman, 1997). Sand temperatures above and below this range can lead to embryonic death and this is one of the main reasons for the distribution of nesting areas around the world. Thereby, sea turtles are considered as an ideal indicator of the biological impact that global warming might carry in future (Janzen, 1994; Hawkes *et al*. 2009). All species of living sea turtles appear to have lived through the Quaternary Era, that was characterized by pronounced fluctuations in global temperature (Davenport, 1989), and therefore have already been exposed to extreme conditions without going extinct. Sea turtles can probably adapt to environmental changes behaviourally, through phenological or spatial shifts, or even physiologically. The problem with the current situation is the speed at which climate conditions are changing and the fact that sea turtle population numbers might not be as flourishing as in the past (Davenport, 1989).

Negative effects of global warming include the presumed female bias that increasing sand temperatures would produce on nesting beaches, as well as reducing survivorship of the clutches (Broderick, *et al*. 2001; Godley *et al*. 2001; Hays *et al*. 2005; Hawkes *et al*. 2007). Also, increasing sea level and extreme weather events would result in damage or alteration of nesting beaches (“coastal squeeze” in Hawkes *et al*. 2009; Fuentes *et al*. 2009b) and human activities such as shoreline protection and beach renourishment could worsen the situation. On the other hand, positive effects of climate change have also been suggested, like increasing thermally suitable nesting or feeding habitats (food abundance and availability), both geographically and temporally (for reviews see Hawkes *et al*. 2009), as it would allow the expansion of sea turtle populations. Davenport (1989) even suggested that, if species were not threatened, a warming tendency would even be favourable to sea turtles as they are survivors of a warmer Tertiary Era. The combination of these potential negative and positive effects together with the uncertainty about the ability that sea turtles will have to adapt to this accelerated environmental changes make it difficult to make assumptions about the future.

Sea turtles and Temperature Dependent Sex Determination

Sex determining mechanisms in vertebrates have been broadly classified into either genotypic (GSD) or environmental sex determination (ESD). GSD is universal in birds (female heterogamety), mammals (male heterogamety) and some reptiles, amphibians and fish (male and female heterogamety). ESD is common in reptiles, but also exist in amphibians and fish. Temperature dependent sex determination (TSD) is a particular case of ESD, that has been shown in three orders of reptiles (lizards, turtles and crocodiles), in which temperature is the decisive environmental factor (Hardy, 2002). Some authors consider TSD as a primitive character in reptiles (Rimblot-Baly *et al*. 1987) and the evolutionary advantages that it might bring to the species remain uncertain because it seems rather a constraint than a favourable character for a population (limit geographic expansion for example). All species of sea turtles typically exhibit a pattern in which higher incubation temperatures produce females and lower temperatures produce males (Mrosovsky, 1994; Shine 1999; Davenport, 1997). Although several aspects of the true mechanism of TSD remain unclear and the exact way in that it operates is not fully understood (Mrosovsky, 1994; Shine 1999), the are two key parameters that have been extensively described and seem to be universal: the pivotal temperature and the transitional range of temperatures (TRT) (for definitions see Mrosovsky and Pieau, 1991 or Hulin *et al*. 2009). The former is the constant incubation temperature at which both sexes are produced in equal proportions and the latter refers to the range of temperatures in which a variable mixed proportion of sexes is produced. Even having the same pivotal temperature, differences in TRT can have significant effects in the resulting global sex ratios (Chevalier *et al*, 1999), as it was found for green and leatherback turtles in Surinam (Godfrey *et al*. 1997). Both parameters provide an insight into species evolutionary history: the pivotal temperature has been found to be a rather conservative trait in sea turtles, lying between 28 and 29ºC for all species (Ackerman, 1997; Davenport, 1997) and TRT possibly reflects the level of plasticity of a given species to environmental changes and even the genetic diversity (Chevalier *et al*., 1998; Hulin *et al*., 2009).

Estimating sex ratios for sea turtles

Sex ratio is an important parameter to consider when evaluating the reproductive success and long-term sustainability of a given population and demographically viable proportions should be expected (Fisher, 1930). However, many studies in different rookeries of the world have found female biased scenarios of hatchling production as shown for green turtles (Broderick *et al*. 2000), loggerheads (Godley *et al*. 2001; Marcovaldi *et al*. 1997; Mrosovsky & Provancha, 1991) and hawksbills (Godfrey *et al*. 1997). As for leatherbacks, female biased situations has also been found in Malaysia (Chan, 1995) and on the Pacific coast of Costa Rica (Binckley *et al*. 1998) whereas in Surinam, fairly equilibrated proportions seem to be produced (Mrosovsky *et al*. 1984; Godfrey *et al*., 1996).

Unlike adults, with males having a longer tail, sea turtle hatchlings have no sexual dimorphism and the only direct method of estimating the proportion of sexes in a clutch is by histological dissection of the gonads (Rimblot-Baly *et al*. 1987), which involves the death of the embryos or hatchlings. Apart from the inherent difficulties from a technical point of view, this method might also be considered as invasive and inappropriate because of the endangered status of many sea turtle populations and, therefore, other indirect methods of estimating primary sex ratios at nesting beaches have been tested over the years. The critical moment for sex determination (gonadal differentiation into either ovaries or testis) occurs during a particular period, which normally corresponds to the middle third of incubation (Rimblot *et al* 1987). The basic assumption for all indirect methods is that the temperature prevailing during this critical period is a good indicator of the resulting sex ratio of a given clutch (Kaska *et al* 1998). As monitoring nest temperatures in a given beach will always be limited in terms of sample size and coverage, the majority of large scale estimations have been done using sand temperature measurements at nest depth.

However, this parameter can fluctuate both in space and time and several factors should be considered when trying to estimate primary sex ratios by this method. There can be intra or inter beach thermal variations (Broderick, 2001, Hays *et al*., 2001) as well as seasonal changes (Mrosovsky *et al*., 1984, Godfrey *et al*., 1996). Environmental factors such as the depth of the nest (Kaska *et al* 1998), rain (Houghton *et al*,) or tides (Kaska *et al* 1998, Broderick *et al* 2001) can produce significant fluctuations in temperatures. Also, during incubation, turtle eggs generate some level of metabolic heat (Broderick, 2000; Godley, 2001; Godfrey, 1997; Mrosovsky & Yntema, 1980; Maxwell, 1988) that, if overlapping with the thermo sensitive period (Desvages, 1993), can influence the resultant sex ratio of the clutch (Maxwell *et al*., 1988; Godfrey 1997, Broderick 2000). To improve accuracy when estimating sex ratios by means of sand temperature alone, it is convenient to add a correction factor for metabolic heating (Godfrey 1997, Broderick 2000).

The pattern of TSD for the leatherback turtle

Rimblot *et al*. (1985) first demonstrated the pattern of TSD for leatherbacks by using eggs from French Guyana nesting beaches incubated in laboratory conditions and gonadal dissection. They initially found that incubation of eggs at and below 28.5 ºC produced 100% phenotypic males at hatching, whereas at 29.75ºC or above temperatures all individuals were females. In a consecutive study (Rimblot-Bay *et al*. 1987), they showed that the temperature producing both sexes was 29.5 ºC and it has since then been considered as the pivotal temperature for *Dermochelys coriacea*. They also found that the thermosensitive period started between day 20 and 30 of incubation and lasted for 10 to 15 days (Rimblot-Baly *et al*. 1987). Another sex ratio curve was obtained from eggs taken at Playa Grande, Pacific Costa Rica (Binckley *et al*. 1998). Laboratory incubation and histology of gonads showed a similar pivotal temperature of 29.4ºC, with 100% males below 29ºC and 100% females above 30ºC. Although the results looked similar for both populations, further statistical analysis performed in another study showed that the French Guyana population had a narrower TRT (Chevalier *et al*. 1999) and the authors suggested that this might be related to differences in genetic diversity between the two populations.

Gabon leatherback nesting population

Recent estimates combining aerial and ground survey data suggest that Gabon hosts the world's largest nesting population of leatherback turtles with an average of 36185 to 126480 nests deposited every year (Witt *et al*. 2009) and a total of 15730 to 41373 breeding females Hence, the contribution of Gabonese beaches to Atlantic stocks is far from being negligible. Furthermore, as *Dermochelys coriacea* is an endangered species (IUCN, Sarti, 2009) and Pacific populations are on the verge of extinction (Spotila *et al*. 2000), the Atlantic Ocean remains as a critical area for the conservation of this species. The objectives of this study were (a) to describe the thermal conditions of the beaches in Gabon and evaluate their suitability with regards to the nesting of *Dermochelys coriacea*, (b) to describe the thermal regimes of leatherback nests and particularly the metabolic heating of eggs during the critical period for sex determination, (c) to estimate current primary sex ratios in Gabon and (d) to predict future scenarios due to global warming and discuss the repercussion for the conservation of this species.

Methods

Study site

This study was conducted on the beaches of The Republic of Gabon situated on the Atlantic coast of Central Africa during four nesting seasons (2005 to 2009). There is a coastal protected network that covers 354 of the 600 km of nesting grounds available with four National Parks (Akanda, Pongara, Loango and Mayumba) and two Nature reserves (Wonga-Wongé and The Gamba Protected Areas Complex). The bulk of the fieldwork took place at Kingere beach in Pongara National Park (0º 18´N, 9º 18´E) and additional data on sand temperatures were gathered during season 2008/2009 at Pointe Denis, Gamba and Mayumba National Park.

Data loggers

Information on sand at nest depth and nest temperatures in the centre of clutches was gathered using TidbiT ONSET Optic Stoaway data loggers that recorded synchronously at 30 minutes interval. These data loggers have a default precision of 0.3ºC and were cross-calibrated before and after the season. Cross-calibration was done for a period of time (24 to 72 hours) when all loggers were recording data synchronously in the same physical place and exposed to a wide range of ambient temperatures. Deviation of each logger with respect to the mean value of all loggers at a given time was calculated for the whole cross-calibration period. Deviations of ± 0.3ºC were considered inside the measure range of the logger. Any logger showing a bigger deviation than the default 0.3 was not used for analysis.

Meteorological Data

Meteorological data in the form of air temperature (AT) (readings every 3 hours) and rainfall (total mm per 24 hours) from january 2005 to june 2009 were available from ASECNA meteorological station situated at Libreville airport (18km north of the study site). A daily mean temperature was calculated which was then correlated with daily mean sand temperatures for the periods available. As 2009 data year is incomplete, note that for the period january-april, n=5 years and for the rest of the months n=4 years.

Sand and intranest temperatures

Data on sand temperaure (ST) and thermal regimes of nests were collected during 4 consecutive seasons (2005 to 2009) at Kingere beach in Pongara and during one season (2008-2009) at Pointe Denis, Kingere, Gamba and Mayumba. Sand temperature (ST) was recorded at mean depth for leatherback nests (70 cm). Control sites were located in representative areas of the beach and the loggers generally remained buried in sand from the 1st of november to the 31st of march. To measure intranest temperatures (n=25 nests), the logger was placed in the center of the clutch, at the time of oviposition, after the female had laid 40 viable eggs. To measure differential heating of the eggs during incubation, specific data loggers, other than control sites, were used. These were buried one meter away from the nest parallel to the sea, at midpoint depth of the egg chamber. Metabolic heat was defined as the difference between nest temperature and specific control temperature at any point during incubation, minus the minimum observed difference between the two values. For data manipulation purposes, the first hours of incubation were considered as an equilibrating period and we framed the incubation period from midnight of the day after laying to 6 am on the night of the massive emergence of hatchlings. Hourly values were averaged successively to day and month mean values for all loggers. With views to sex ratio estimations, most of the data on ST are expressed as bimonthly values. We defined 15 day windows for the season, from november to march as follows: 1N, 15N, 1D, 15D, 1J, 15J, 1F, 15F, 1M 15M; where 1N comprises day 1 to day 15 of the month and 15N from day 16 to day 30 or 31.

Nest counts

We defined the span of a nesting season from the 1st of november (julian day 1) to the 31st of march (julian day 151) and used ground nest counts to calculate % of nesting throughout the season. Mean seasonality was calculated using nest counts for 2006-2007 and 2007-2008 seasons. Data was obtained from “Partenariat des Tortues Marines du Gabon”. We calculated seasonality for Gabon as the mean seasonality in 4 sites (Pointe Denis, Kingere, Gamba and Mayumba) and during the two nesting seasons. When datasets were incomplete (ie project monitoring started later than day 1 or ended before day 151 or missing days), a simple linear interpolation was done (as in Godley *et al*. 2001). For all day gaps an interpolated average number was given and, when missing, number of tracks at day 1 and 151 were supposed to be 1. Only real nests were used to calculate mean % of nesting as these data was calculated with the purpose of estimating sex ratios (and thus non nesting emergence would not count)

Sex ratio curves

Due to the absence of a specific study on TSD for *Dermochelys coriacea* in Gabon, we had to use the sex ratio curve from other nesting regions. We digitised the curves from Chevalier *et al*., (1998) so as to have a set of paired values: sand temperature-sex ratio. Pivotal temperature for the Atlantic is 29,5 ºC (53% males) and 29,4 ºC for the Pacific (50% males). Transitional Range of Temperatures (TRT) is 29 to 30ºC and 28,5 to 30,4 ºC for the Atlantic and Pacific population respectively. Upper and lower limits are temperatures producing 1% and 99% males.

Estimation of primary sex ratios

Sand temperature was averaged into 15 day bins for the whole nesting period, with an added correction factor of 0.6 ºC for metabolic heat (mean difference between nest and control sites during the 2/3 of the incubation period in the centre of the clutch). We used 3 different thermal scenarios: +0.3, 0.0 and -0.3ºC, which is the range of fluctuation observed in sand temperature among the different study sites. Temperature zero corresponds to Kingere in Pongara, which was considered as the index beach. Nest counts (as % of the total) were also averaged into 15 day bins. As the critical period for sex determination occurs during the second third of incubation we had to consider what proportion of nests were incubating at this particular moment in a given window. Therefore, we adjusted the proportion of nests to a lag time of two 15 day bin (30 days) between the date of laying and the critical period. This means that if 100% of the nests are laid between 1st november to the 31st march, the critical periods for sex determination will be happening between 1st of december to 31st april and that the latter was the period considered for seasonal sex ratio estimation. For each 15 day bin a mean neat sex ratio was obtained from the paired sex ratio-temperature dataset and then multiplied by mean proportion of nests incubating at the critical period (delayed 30 days from nesting day). Overall primary sex ratio for each season was then calculated as the sum of all average ratio produced every 15 day period.

Sand Temperature Prediction

We used air temperature as a predictor of sand temperature to fill in gaps at periods in which loggers had not been recording and also to make forecast predictions with views to global warming effects. Mean daily temperature of sand at a depth of 70 cm was correlated with mean daily values of air temperature at Libreville airport meteorological station. Rain was found to have no major additional effect on the variability of sand temperature and was not considered in the regression. Of the different periods in which both air and sand temperatures were available, we chose the one that gave the best regression equation (a total of 8 months in 2008 and 2009 and by grouping into 15 day bins). The equation used is ST= 0.9958 (AT) + 1.77 (n=16, Pearson r=0.556, $P<0.05$, $R^2 = 0.3096$).

Prediction of sex ratios

Future sex ratios were estimated by adding 1ºC and 4ºC (mean predicted rise according to IPCC) to monthly values of mean air temperature over the four study seasons, similarly to Abella et al. (2007). Using the regression equation Sand T = 0,9958* (AT) + 1,77, we then were able to predict monthly sand temperatures and the resulting neat sex ratios (adding the correction factor for metabolic heat). This was done using the Atlantic sex ratio curve and only one temperature scenario (index beach).

Results

Seasonal profile of climate and sand temperatures in Gabon

Average air temperature during the study period was 26.5ºC (n= 4, range of annual means= 26.4 - 26.6). Mean year thermal amplitude was 2.9ºC ± 0.4 (n=4) with the lowest temperatures during the months of july and august (range of minimum values= 24.5 - 25.2), and highest values during february (range of maximum values= 27.7 - 28.0) (Figure 1a). Average rainfall was 217 mm (n=4, range of annual means=183 - 276 mm). There is virtually no rain at all in june, july and august (the so called "big" dry season). The rainy period occurs from september to may with a drier period in january and february (the "small" dry season). Apart from a pronounced peak of rain in may 2007 (746 mm), the seasonal pattern of rain and temperature was fairly consistent within years (Figure 1b).

The temperature of the sand at nest depth followed a seasonal pattern similar to that of air (Figure 1c). To show the complete year round profile, values outside the nesting season, when loggers where not recording, were estimated from air temperature by using the regression equation ST= 0.9958 (AT) + 1.77 (n=16, Pearson r=0.556, $P<0.05$, $R^2 = 0.3096$). Mean thermal amplitude year round was 2.3ºC ± 0.4 (n=4) with minimum average values ranging from 26.9 to 27.6 and maximum from 29.4 to 31.1. Estimated sand temperatures during the big dry season (june, july and august) could be as low as the tolerated limit for sea turtle incubation of eggs (25-27ºC).

At the beach of Kingere in Pongara, sand temperatures at nest depth over the four seasons varied significantly (Figure 2) (paired T-tests, $p<0.01$). Overall mean sand temperature was 29.1ºC ± 0.6 (n=4) with annual means ranging from 28.3 in 2008/2009 to 29.5 in 2007/2008 (supplementary data). In general, temperature remained well below 29.5 ºC (pivotal temperature) for most of the nesting season (Figure 2) except during the months of february and march.

At other sites south of Pongara (Gamba and Mayumba), the same pattern of increasing sand temperatures over the nesting period was found (Figure 3). During 2008-2009 season, mean sand temperature was 28.3ºC ± 0.5 at Kingere, 28.7ºC ± 0.8 at Gamba and 28.0ºC ± 0.7 at Mayumba. Sand temperatures remained below 29.5 ºC for most of the time except at Gamba during the month of march. Interbeach thermal variation remained within a range of ± 0.3ºC. On average, Mayumba had - 0.3ºC and Gamba + 0.3ºC with respect to Kingere which was taken as the reference or index beach (values are the mean of deviations from each site to average temperature).

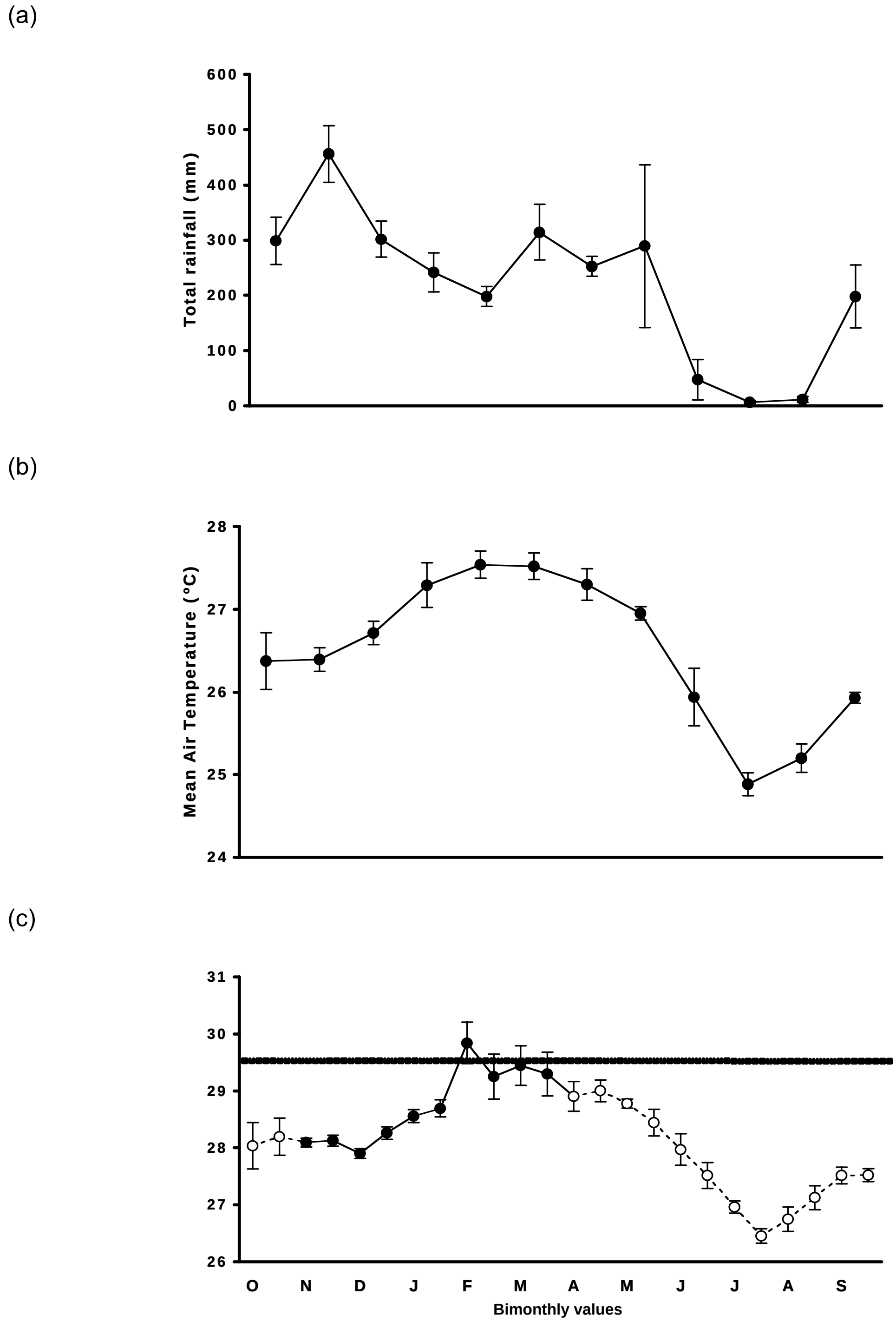

Figure 1. Seasonal pattern of climate in Gabon during the calendar year: (a) rainfall (mm), (b) mean air temperature (ºC) and (c) mean sand temperature ºC. Open circles denote real values from data loggers buried in the sand whereas closed circles refer to predicted values from air temperature. Bars show standard error of mean.

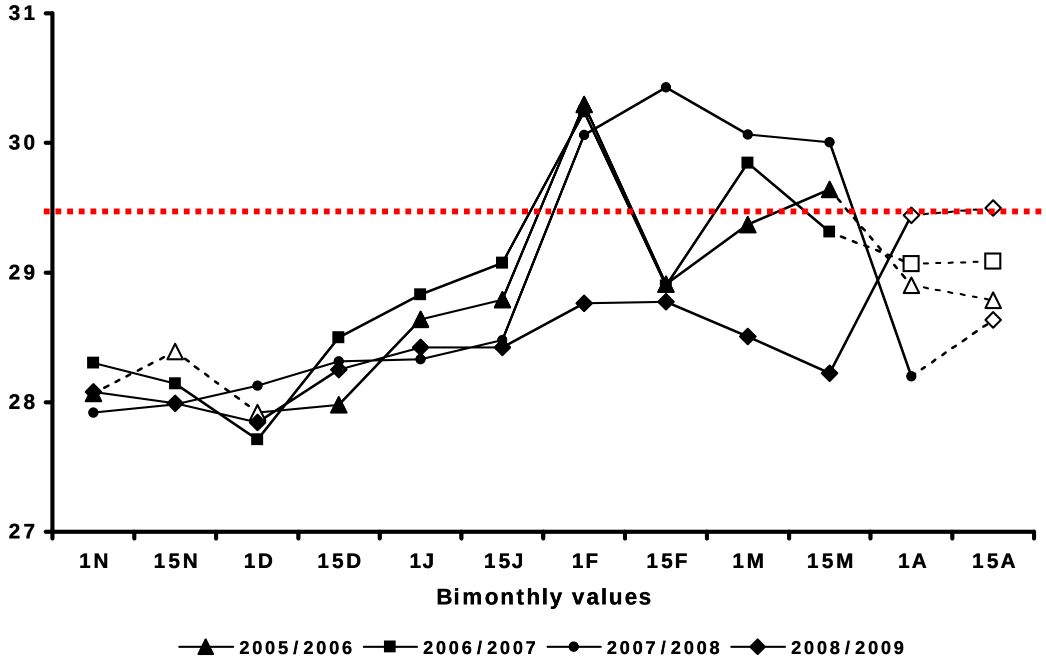

Figure 2. Sand temperature at Kingere over the four study seasons. Open signs and dotted lines correspond to predicted values for the month of april because a certain proportion of late-laid nests are still incubating at this time. The red dotted line shows the pivotal temperature for the leatherback turtle (29.5ºC)

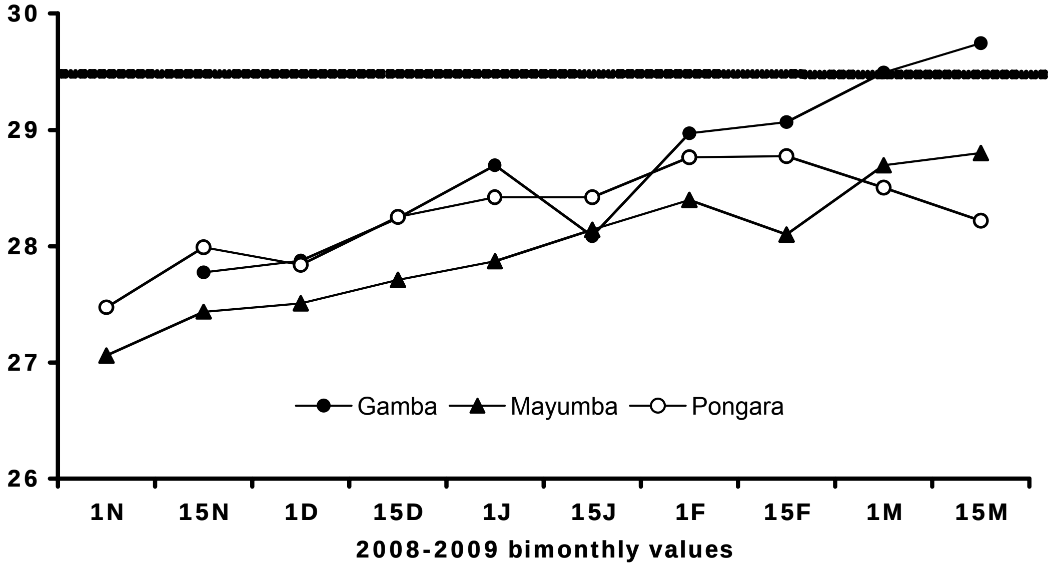

Figure 3. Sand temperatures at three beaches in Gabon during season 2007/2009: Gamba, Mayumba and Kingere in Pongara. Dotted line shows the pivotal temperature for the leatherback turtle (29.5ºC).

Thermal regimes of nests

Mean temperature of 25 nests at Pongara ranged from 29.0 to 31.2ºC for the whole incubation period and from 28.9 to 30.3ºC during the middle third. All these nests were laid during the second half of december or the month of january, which means that the critical period for sex determination occurred during the warmest months of the season. All nests experienced some diel fluctuation that ranged from 0.1 to 0.6ºC for the whole period and from 0.2 to 0.5ºC during the middle third of incubation. The date of laying was correlated to the mean temperature during the middle third of the incubation period (n= 25, r=0.756, p< 0.01, r^2= 0.571) suggesting that sex ratio is influenced by seasonality.

Levels of metabolic heat were measured in 15 of the nests. Differential heating of the eggs is gradual and starts to be noticeable during the middle third of incubation, reaching a peak at the end (Figure 4). Defining the onset of differential heating as 0.5ºC, it started on average at day 32 of incubation, which corresponds to 52% of the incubation period (Figure 6). Mean value for metabolic heating for the entire incubation period was 0.91ºC (n=15, SD= 0.27, range= 0.0-3.5). During the first third of incubation, differential heating of the eggs is almost inexistent with a mean value of 0.2 ºC (n= 15, SD= 0.1, range= 0.0-0.7). Levels of heating are highest during the final third of incubation, with maximum values of 3.5ºC in some nests (mean=1.7 ºC, SD= 0.4). During the thermosensitive period for sexual determination (middle third), metabolic heating values ranged from 0 to 1.9ºC with a mean of 0.6ºC (SD=0.2).

There was a pronounced peak followed by a sudden drop in all the metabolic heating profiles of the nests that seemed to correspond to hatching and emergence respectively. The time lag between hatching and emergence was estimated according to this for 13 nests and was 3 days on average (n=13, range=2-5 days) (Figure 5).

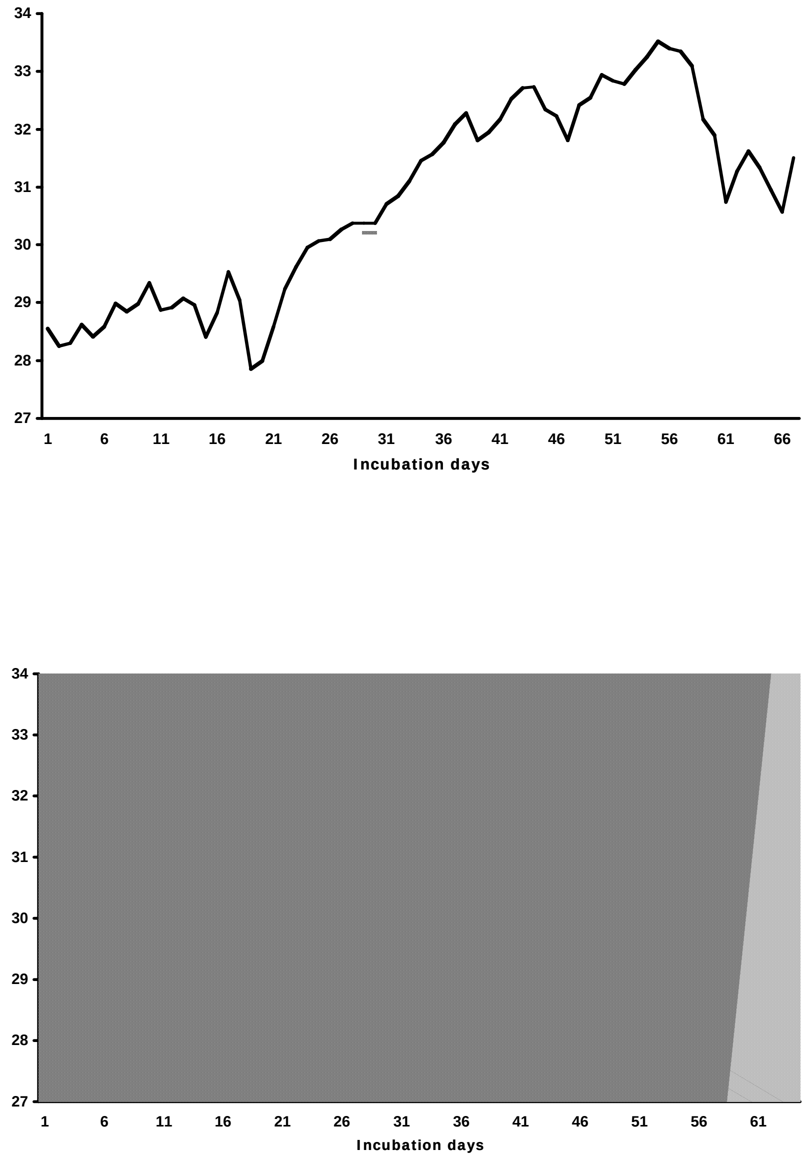

Figure 4. Thermal regimes of two leatherback nests (dark line) and the temperature of the surrounding sand (light lines) during the incubation period.

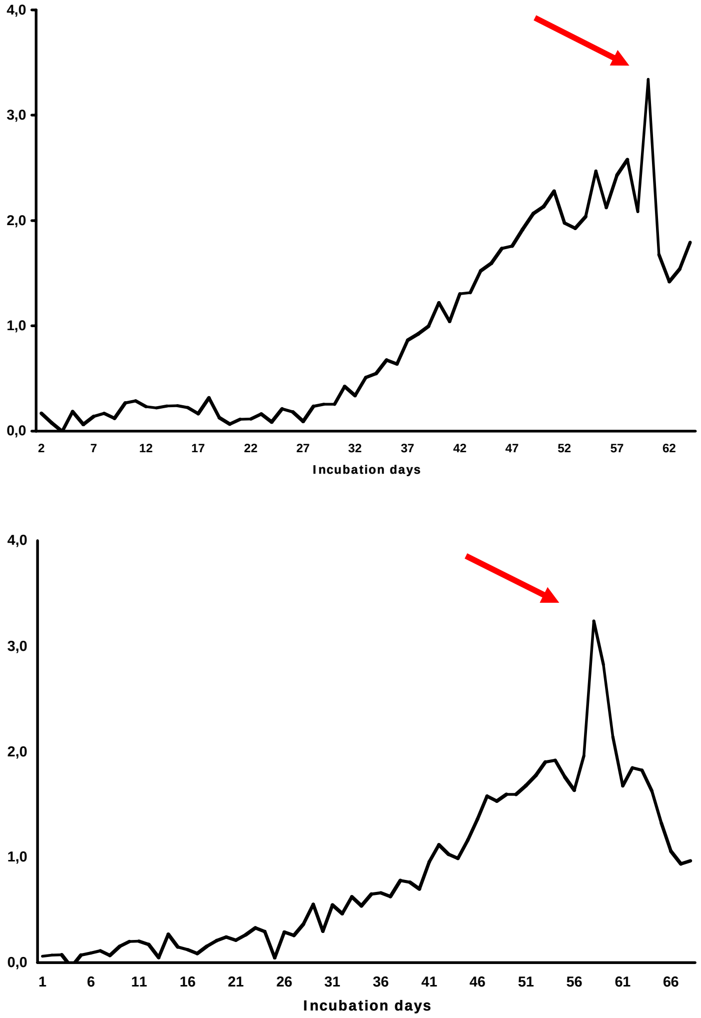

Figure 5. The level of differential heating between the clutch and the surrounding sand (in ºC) in two of the study nests during the incubation period. Arrows indicate the peak of heating that is achieved at the moment of hatching of the eggs.

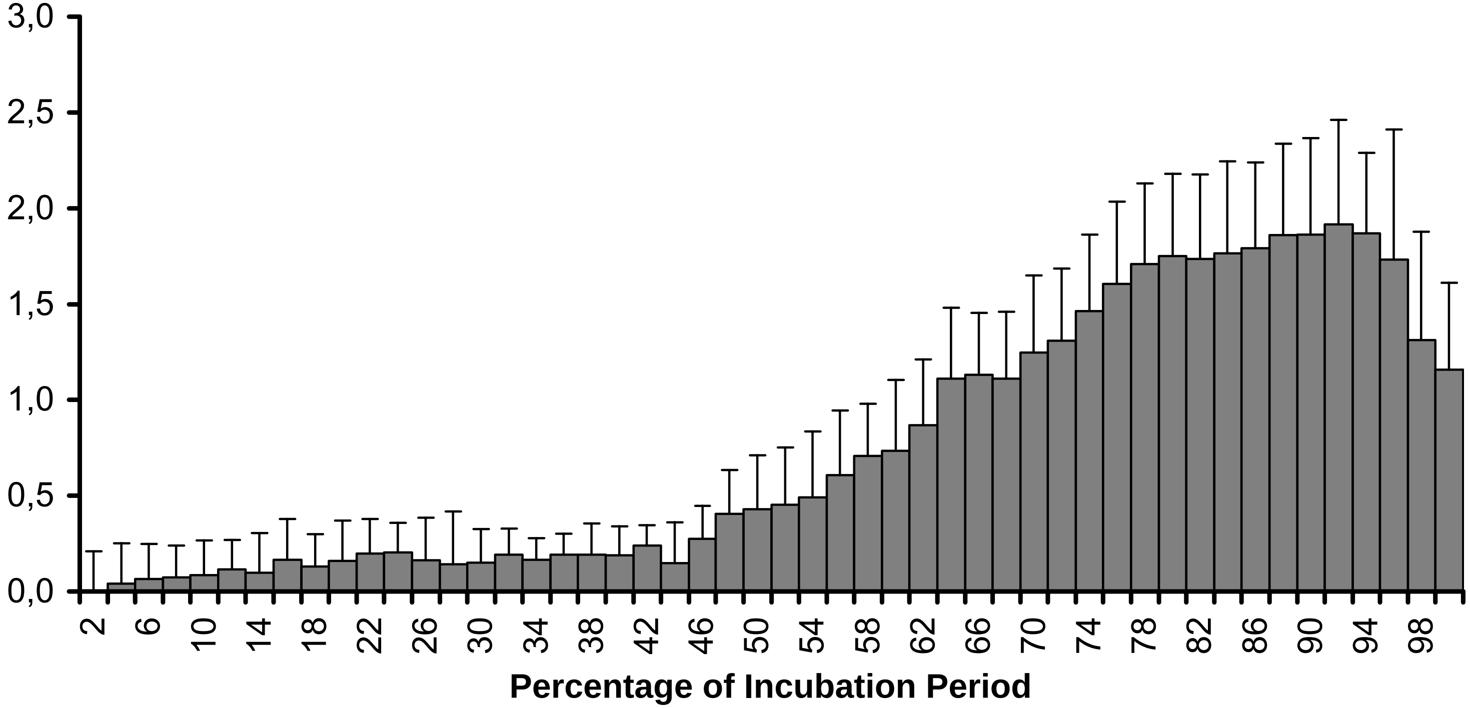

Figure 6. Mean metabolic heating (nest – control temperature) in each of two percentiles of incubation of clutches (n=15). Error bars denote standard deviation of mean.

Estimation of current sex ratio

Mean seasonality for Gabon (Figure 8c) was obtained by grouping nest counts at four different beaches along the coast (Pointe Denis, Kingere, Gamba and Bame) during two nesting seasons, 2006/2007 and 2007/2008 (Figure 8 a&b). The months with the highest nesting activity are december and january. At index beach temperature (Kingere) and over the 4 seasons, we estimated that 60% of males would be produced (Table 1). If sand temperatures where only 0.3ºC higher, the proportion of males would be reduced to 35%; whereas if 0.3ºC lower the proportion would rise to 70% (Table 1). Note that for all estimations, a correction factor for metabolic heat of 0.6ºC was added to sand temperature. If this value had not been added then our estimations would have been highly male biased. Values using the Pacific curve did not differ much than when using the Atlantic curve (Figure 7). There was a pronounced within seasonality in mean sex ratios with predominant male production during the first half of the season and more females being produced as the season progresses (Figure 9).

Table 1. Seasonal and overall primary sex ratios (as percentage of males) produced on leatherback nesting beaches in Gabon during four nesting seasons estimated using sand temperatures at nest depth with and added correction factor for metabolic heat. Estimations were done using the French Guyana and Pacific sex ratio curve and under three different thermal scenarios of ±0.3ºC (considering Kingere as the Index beach with temperature 0.0).

	Atlantic curve			**Pacific curve**		
	+0.3	**0.0**	**-0.3**	**+0.3**	**0.0**	**-0.3**
2005/2006	0,26	0,60	0,68	0,27	0,58	0,63
2006/2007	0,14	0,37	0,63	0,16	0,32	0,52
2007/2008	0,48	0,54	0,54	0,38	0,50	0,54
2008/2009	0,51	0,87	0,95	0,51	0,75	0,90
Overall Period	**0,35±0.18**	**0,60±0,21**	**0,70±0,18**	**0,33±0,15**	**0,54±0,18**	**0,65±0,18**

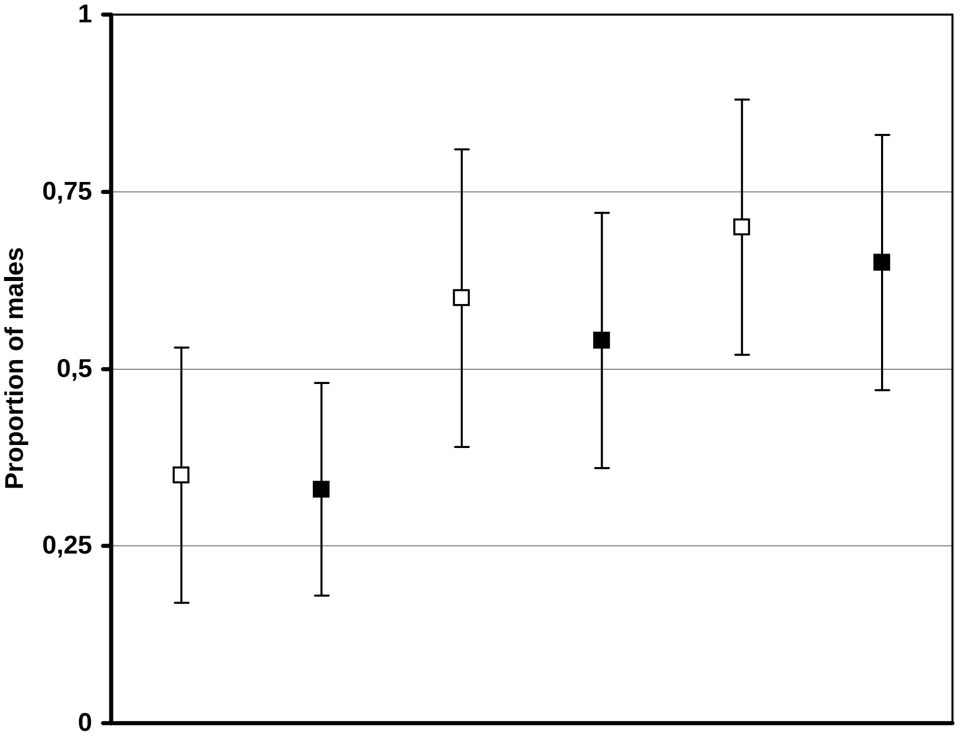

Figure 7. Primary sex ratios for the leatherback turtle in Gabon over a four year period (2005/2009) estimated from sand temperatures and under different scenarios: considering index beach temperature (Kingere in Pongara National Park) and ±0.3ºC, which is the observed interbeach variation. Open circles and closed circles refer to values estimated using the Atlantic and Pacific sex ratio curve respectively. Bars denote standard deviation (n=4 seasons).

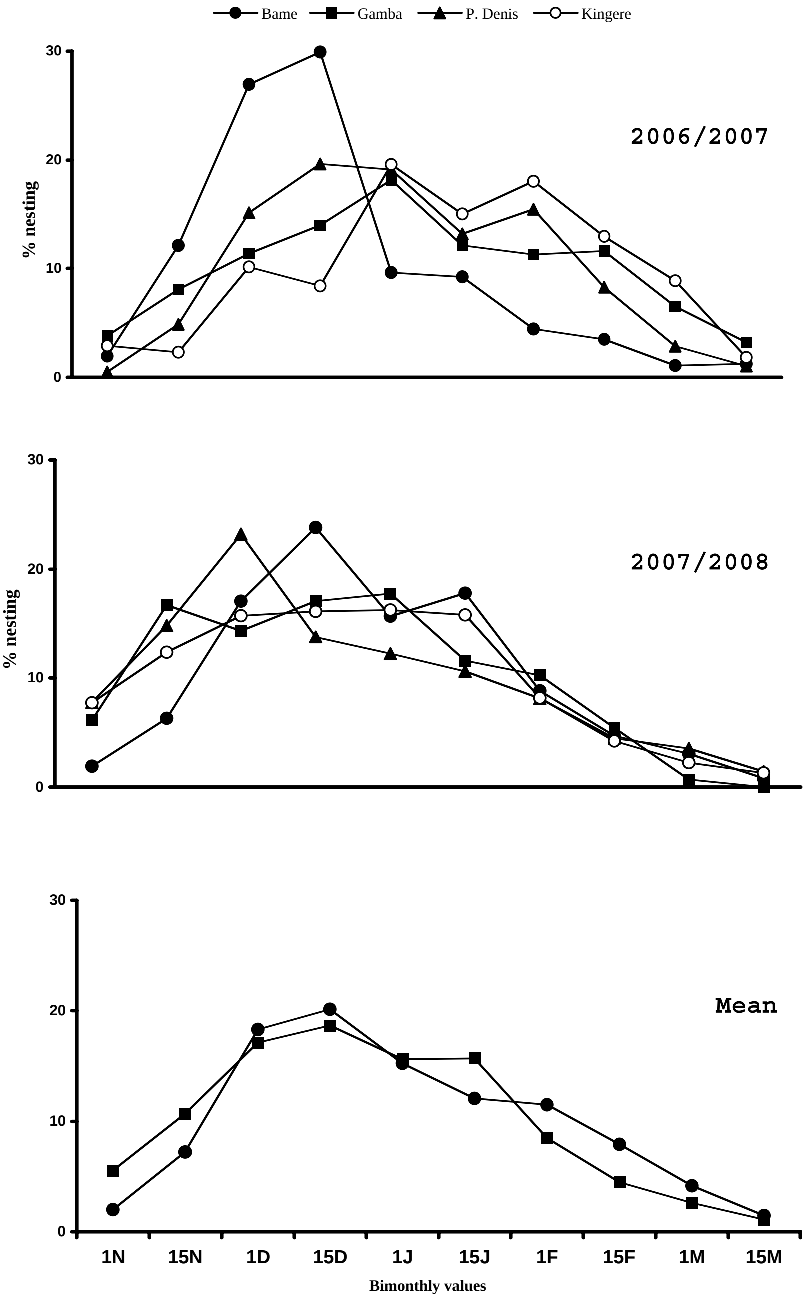

Figure 8. Seasonal distribution of nesting activity in Gabon at the main nesting sites expressed as proportion of the season´s total: (a) proportion of nests in 2006-2007 season, (b) proportion of nests in 2007-2008. Closed circles: Bame, squares: Gamba, triangles: Pointe Denis and open circles: Kingere. (c) mean of the 4 sites for the two seasons.

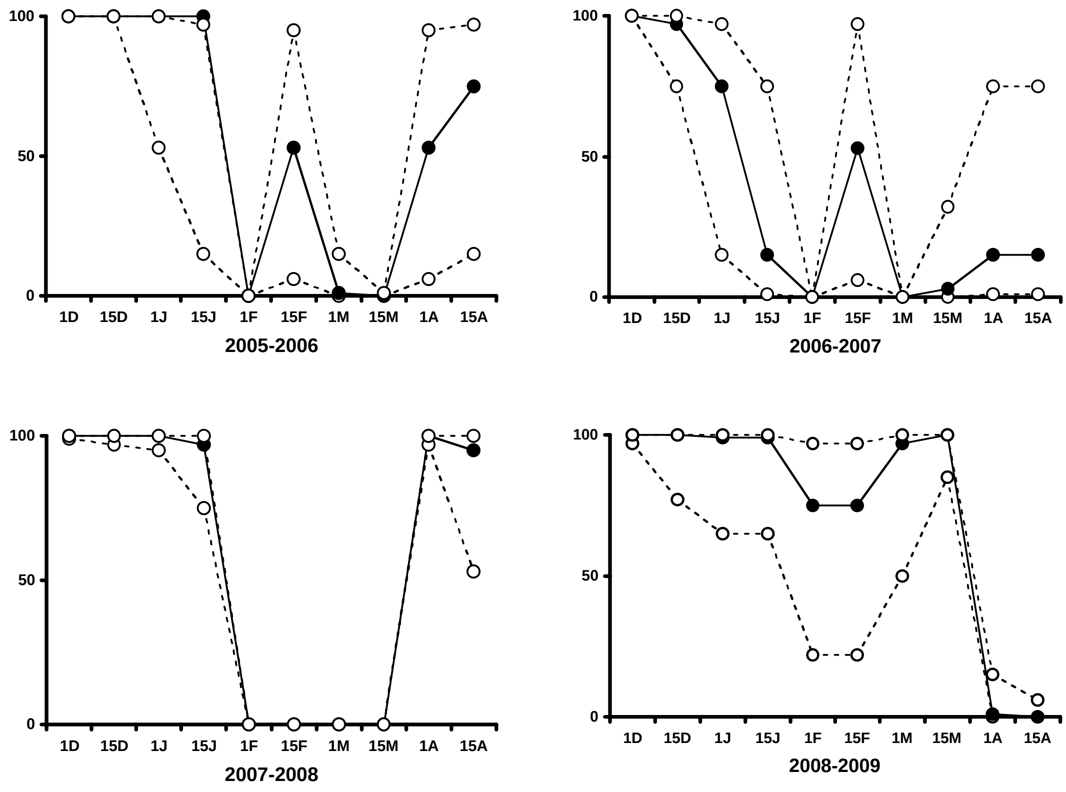

Figure 9. Seasonality in sex ratio (as % of males) in Gabon estimated from sand temperatures at nest depth during 4 consecutive nesting seasons (2005/2009) and considering the index beach temperature (solid line) and a thermal range of ± 0.3ºC (dotted line). Values are given on a bimonthly basis from the 1st of december to the 31st of april as these are the months that encompass the greatest proportion of nests incubating during the thermosensitive period.

Predicted sand temperatures and sex ratios

A 100% female scenario would occur when sand temperatures rise above 30ºC (upper limit of the TRT). According to the latest Intergovernmental Panel on Climate Change Report (IPCC, 2007), mean global warming in the Central African region will be 1ºC by 2020-2029 and 4ºC by 2099. A rise of 1ºC in air temperature would be enough to produce a totally female bias scenario on the beaches of Gabon with mean incubation temperatures (sand + correction factor) above 30ºC during most of the season. By 2099, sand temperatures would reach values above 33ºC, which in turn would lead to incubation at dangerous limits for the species.

Table 2. Current and predicted scenario of primary sex ratios in Gabon for *Dermochelys coriacea*. Air Temperature (AT), Sand Temperature (ST), Incubation Temperature (IT): sand temperature with an added correction factor for metabolic heat during the middle third of incubation) and resulting neat sex ratios (as % of males) according to the sex ratio curve for the species in the Atlantic Region.

	Current scenario				**2020-2029**				**2099**		
	AT	**ST**	**IT**	**Sex Ratio**	**AT +1ºC**	**ST**	**IT**	**Sex Ratio**	**AT +4ºC**	**ST**	**IT**
Nov	26,4	28,1	28,7	1,00	27,4	29,1	29,7	0,15	30,4	32,0	32,6
Dic	26,7	28,1	28,7	1,00	27,7	29,4	30,0	0,01	30,7	32,3	32,9
Jan	27,3	28,6	29,2	0,95	28,3	30,0	30,6	0,00	31,3	32,9	33,5
Feb	27,5	29,5	30,1	0,00	28,5	30,2	30,8	0,00	31,5	33,1	33,7
Mar	27,5	29,4	30,0	0,01	28,5	30,2	30,8	0,00	31,5	33,1	33,7
Apr	27,3	29,0	29,6	0,32	28,3	30,0	30,6	0	31,3	32,9	33,5

Discussion

Thermal conditions of the beaches

In Gabon, there are two rainy periods alternated with two dry seasons. It appears that leatherback turtles have chosen the most thermally suitable period of the year to nest both in terms of egg incubation and sex ratio. Nesting does not occur during the big dry season (june, july and august) when sand temperatures drop to levels that would be even dangerous for egg incubation. Significant thermal differences among beaches could have been expected, especially if they are hundreds of kilometers apart, like between Pongara, Gamba and Mayumba. However, we found little inter site variation suggesting rather homogenous thermal conditions. In fact, the general landscape along the coast of Gabon consists of white sand beaches bordered by forests and occasional savannahs or lagoons. This panorama is very different to that found at other sea turtle nesting sites, such as Ascension Island, where there can be differences of more than 4ºC between black and white sand beaches in a much smaller geographic range (Hays *et al*. 1995, Hays *et al.* 2001).

Current sex ratios

Our results suggest that the thermal scenario in Gabon is adequate for a balanced sex ratio production with sand temperatures fluctuating naturally around the pivotal during the nesting season. Balanced sex ratios were also found in Surinam, with 53.9% and 69.4% female production estimated in 1982 (Mrosovsky *et al*. 1984) and in 1993 (Godfrey *et al*. 1996) from sand temperatures. This general picture in the Atlantic region is encouraging as highly female skewed scenarios have been found in other regions. At Rantau Abang, in Malaysia sand temperatures on the beach exceeded the lower limits of 100% female production all season long (Chan and Liew 1995) and on the Pacific coast of Costa Rica estimated sex ratio was 0%, 6.5% and 25.7% male during 3 consecutive seasons (Binckley *et al*. 1988). However, we also found that this equilibrium in Gabon can easily be reversed with temperature variations of as little as 0.3ºC leading to female or male skewed scenarios. This is due to the narrow range of TRT described for the leatherback turtle and the fact that sand temperatures are close and fluctuating around the pivotal.

Sand temperatures and sex ratio in Gabon have a within seasonal pattern related to the climatic conditions with predominant male production in earlier nests laid during wet and cooler months and females being produced towards the final drier months. This same pattern seems to happen at other major leatherback nesting sites like French Guyana (Rimblot-Baly *et al.* 1987) or Surinam (Mrosovsky *et al*.1984; Godfrey *et al*. 1996) as well as for other sea turtles species like loggerheads in South Africa (Maxwell *et al*. 1988) and Cape Verde (Abella *et al*. 2007). In other sites like Rantau Abang in Malaysia however, no seasonal trends were observed in sand temperatures (Chan and Liew, 1995). Primary sex ratios can also vary among years, with some seasons, like 2008/2009 strongly male biased due to lower sand temperatures.

Predicted sex ratios

The effects of global warming will not be evenly distributed, with equatorial regions presumably being less affected than middle latitudes or the poles. Hence, it is likely that equatorial breeding areas, like Gabon-Congo beaches will not experience the same changes as others sites like in North America. Current trends for the Central African region predict a mean increase in AT of 1ºC for early 21st century (2020-2029) (IPCC, 2007). According to our estimations, this would be enough to lead to a 100% female scenario on the beaches of Gabon.

A situation of 99% female production is unsustainable and would drive any population to extinction unless some adaptation mechanisms occur. Therefore, the plasticity of a given species to environmental changes will play a major role for its survival. It is thought that sea turtles have experienced behavioural or physiological changes through their natural history that have allowed them to cope with a changing environment in the past (Davenport, 1989) but whether this will occur sufficiently quickly to meet current climate change rates remains unclear. Presumably, in the event of warming sand temperatures, sea turtles would be able to modify their nest locations either at a small scale (depth or position of the nest on the beach) or at a large scale (latitudinal shift of nesting beaches) so as to favour male production by lowering incubation temperatures. Also, they could modify the timing of the nesting season so that it occurs in a more thermally suitable period (phenological shift).

Vulnerability of the leatherback turtles nesting in Gabon to global warming

The way the leatherback turtle would be affected by global warming is marked by aspects inherent to its biology that seem to counterbalance the possible outcome. This species exhibits a very restricted pattern of TSD, with a narrow TRT (only 1ºC wide compared to other species like the loggerhead that have 4ºC wide) meaning that the range of temperatures at which both sexes are produced is very restricted and the majority of nests are unisex (Hulin *et al*. 2009). Small changes in temperature during the thermo sensitive period lead to substantial differences in the resultant sex ratios and this is clearly seen in our results, where differences of ± 0.3ºC in sand temperature resulted in male or female skewed scenarios. Thus, it has been suggested that species with a narrow TRT, like the leatherback turtle, would be more vulnerable to sudden environmental changes (Mrosovsky *et al*. 1984; Hulin *et al*. 2009). Also, considering that the pivotal temperature seems to be a rather conservative trait among sea turtles (Davenport, 1997; Mrosovsky *et al*. 2009), it is unlikely that such a physiological adaptation would occur in time to solve the problem.

Therefore, if the possibility of microevolution is discarded, behavioural adaptations would be necessary so as to maintain balanced sex ratios, in the event of accelerated global warming conditions. In this sense, leatherbacks seem to be in an advantageous position because of several behavioural features. For example, they could change their annual phenology by starting to nest earlier in the year, when sand temperatures are lower. This would not seem unlikely in Gabon, as there are reports of scattered nesting all year round. Also, they could shift their nesting beaches from equatorial to higher or lower latitudes. This again, seems a reasonable option as there are plenty of suitable nesting grounds along the Atlantic coast of Africa, both northwards and southwards of the Gabon-Congo epicentre and leatherback nesting is reported from Angola to Mauritania (Fretey *et al*., 2007). What is more, *Dermochelys coriacea* has less beach fidelity and wider dispersal among nesting sites and it seems that the natal homing behaviour (by which adults return to nest to the beach where they were born) is not as strict as in other species of sea turtles Dutton *et al*., 1999). Finally, other aspects, such as the great depth at which they lay their clutches or the tendency to place nests in the cooler wash over zone seem to suggest that this species might be the best able to cope with warming sand temperatures (Kamel and Mrosovsky, 2004). There are some indications that such behavioural modifications are starting to occur. For example, leatherback nesting has been recorded in North Carolina at the upper northerly limit ever registered (Rabon *et al*. 2003). Expansion at sea is probably occurring as well, as the summer position of the 15ºC isotherm, which is considered to be limiting for Dc distribution is moving northwards at a rate of 330km over the last 17 years (Hays *et al.* 2006).

Recommendations for future studies

The aim of this study was to give a rough estimation of primary sex ratios using the simplest possible approach. The finding of little inter beach thermal variation allowed us to simplify estimations by considering the whole coast as a single unit both in terms of seasonality and sand temperatures. Also, our control sites were located in the “open sand zone” of representative nesting sectors at mean nest depth (70cm). Therefore there are many particular details that were out of the scope of these estimations. For example, we did not take into account the fact that nests are laid in a scattered random way on the beach or at different depths. The depth at which the clutch is laid can affect incubation temperature and the resulting sex ratio (Abella *et al*., in press). Therefore, it might be possible that bigger differences than our observed ±0.3ºC range exist within or among beaches leading to a considerable spatial variation in sex ratio. Further long term monitoring of sand temperature at different beaches would be necessary to confirm this.

The general pattern of metabolic heating found in this study is similar to the one described for leatherback turtles in Surinam (Godfrey *et al*. 1997), for green turtles at Ascension Island (Broderick *et al*. 2001) or loggerheads in South Africa (Maxwell *et al*. 1988), increasing through incubation with a peak towards the end followed by a gradual decline. Our mean value for the middle third of incubation found (0.6ºC) is slightly lower but similar to that estimated in Surinam (0.8ºC) (Godfrey *et al.* 1997). However, mean values provided here should be considered maximal, as levels of heating are probably lower in the periphery and bottom of the nest (Kaska *et al*. 1998; Maxwell *et al*. 1998). If the correction factor for metabolic heating would not have been added, or if using a lower value, our results would have been highly male biased.

Given the absence of data on TSD for leatherbacks in Gabon, we were forced to use the sex ratio curves from other geographic regions. In terms of geography, the French Guyana curve should have been chosen first, but we used both the Pacific and Atlantic curves for possible differences in the outcome. The differences were small and considering that these are only estimations, further similar studies could use only the Atlantic curve. However, a precise study on TSD for Gabonese leatherback populations and beaches would be recommended.

A recent study shows how the prediction of ST from AT could be potentially improved by including high-resolution sea surface temperature (SST) as a covariate (Fuentes *et al*. 2009a). In this study, the regression between AT and ST explained only around 30% of the variance in sand temperature. Adding the rain as a covariate did not improve the regression either; probably because it has no major effect at a depth of 70cm. It would be recommended to use SST to augment accuracy in the prediction of sand temperatures both for the current period and for future estimations. However, given the fact that sex determination in *Dermochelys coriacea* is so sensitive to small changes in temperature, we are confident about the predictive estimation that is provided in this study. Using SST could also be useful to estimate sex ratio produced in the past or for other species of sea turtles nesting in Gabon.

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Supplementary data

Table.3 Mean sand temperatures (ºC) recorded in control sites (a) at Kingere in Pongara over 4 nesting seasons (2005/2009) and (b) at four beaches over the coast of Gabon during 2008/2009 season. Values are mean of day means. n=number of loggers that were recording at each site or year. Readings refer to the number of data recorded by the logger during the period.

Kingere	**Temperature ± SD**	**Range**	**n (readings)**	**period**
2005/2006 (n=3)	29.3 ± 0.8	27.6 - 31.1	4320	30 Dec - 31 March
2006/2007 (n=3)	28.9 ± 0.9	26.9 - 30.8	6576	10 Nov - 31 March
2007/2008 (n=5)	29.5 ± 1.0	27.3 - 31.0	4320	30 Dec - 31 March
2008/2009 (n=2)	28.3 ± 0.5	27.0 - 29.4	6576	10 Nov - 31 March
Mean Kingere	29.1 ± 0.6	27.7 - 30.2	4320	30 Dec - 31 March
2008/2009	Temperature ± SD	Range	n (readings)	period
Hatchery				
Pointe Denis (n=3)	29.7 ± 0.6	28.3 - 31.4	3408	8 Nov - 31 March
Kingere (n=2)	28.3 ± 0.5	27,0 - 29.4	3288	13 Nov - 31 March
Gamba (n=3)	28.7 ± 0.8	27.1 - 30.4	2952	27 Nov - 31 March
Mayumba (n=2)	28.0 ± 0.7	26.6 – 29.8	3600	1 Nov - 31 March
Mean Gabon	28.6 ± 0.6	27.0 – 29.8		

Table.4 Descriptive statistics of mean temperatures and metabolic heating in leatherback nests during the entire and separate thirds of the incubation period.

	All period	**First Third**	**Middle Third**	**Final Third**
Nest temperatures (ºC) (n=25)				
mean of means ± SD	30.33 ± 0.56	29.16 ± 0.68	30.33 ± 0.59	31.54 ± 0.94
range of means	29.0 - 31.2	27.9 - 30.3	28.9 - 31.1	29.5 - 32.9
extreme values	27.1-34.9	27.1-30.7	28.1-32.5	28.5-34.9
Metabolic heating (ºC) (n=15)				
mean of means ± SD	0.91 ± 0.27	0.18 ± 0.06	0.62 ± 0.24	1.66 ± 0.41
range of means	0.5-1.4	0.1-0.3	0.4-1.1	1-2.4
extreme values	0-3.5	0.0-0.7	0-1.9	0.2-3.5

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General Discussion and Conclusion

Despite its proximity from the capital of Gabon, Libreville, the beach of Kingere still remains almost unaltered, yet, coastal development and increasing tourism into Pongara National Park is probably one of the major potential threats. There are two emerging *eco-hotels* projects that are building infrastructure next to the beach. Also there is an increasing number of visitors that come to Kingere to watch nesting sea turtles. It is highly recommended that strict guidelines about the code of conduct, such as maximum number of persons per group and the use of lights, be implemented since the beginning. North of Kingere, Pointe Denis is characterised by a leisure type of tourism (beach and sun, quads, jet skies) that could not be precisely environmentally friendly and seems very difficult to control as being so near the capital. While Pointe Denis is the beach suffering the biggest impact of tourism and could play a role as an educational center, it would be necessary to keep Kingere as a sanctuary, with restricted access and more controlled human activities.

Another major environmental problem at Kingere is the high incidence of lost timber on the beach, as a threat to nesting females, hatchlings and the coastal habitat in general. What is more, in this study, we found that there seems to be an equal or even higher amount of logs that remain buried in the sand and potentially affecting incubation of the clutches. Considering that the presence of logs is generalised all along the coast of Gabon and probably the neighboring countries (Laurance *et al*. 2008), the numbers of dead adult females every season may reach alarming numbers at a regional scale and comparable to those caused by illegal hunting or fisheries bycatch (Verhage *et al*. 2006, Carranza *et al*. 2006). This problem seems to have a difficult solution, and the main conservation measure for the moment is to maintain female rescue operations through regular patrolling of the beach.

The beach of Kingere is paradoxically highest in terms of nesting numbers (Witt *et al.* 2009) and lowest in terms of hatchling productivity. Given the absence of historic information at the site, it is not possible to know whether this is a recent phenomena and it is difficult to make predictive assumptions about the long-term consequence of this low hatchling output. It appears that the leatherback turtle shows less site fidelity to the nesting beaches, partly due to their preference for highly unstable nesting beaches that are highly dynamic, usually located near big estuaries and prone to disappearing by the effect of beach erosion and marine currents. Therefore, it would not seem impossible that the beach of Kingere and possibly the whole coast of Gabon is a recently colonised area by a neophyte or shifted population. It has been suggested that neophyte females might follow experimented nesters during migrations and this way, arrive to a given beach. At Kingere, we have observed a high frequency of nesting females with a reduced size that might correspond to young individuals. On the other side, there was also a considerable amount of females with an evidence of a previous mark on the front limb that might correspond to a tagging program in Tongaland or French Guyana. To summarize, the nesting population at Kingere might be composed of a mixture of young and experienced individuals that have recently colonized this area. This is obviously just a hypothesis that might be elucidated by genetic analysis, as well as long-term monitoring and tagging programs.

Under natural circumstances, sea turtles are adapted to withstand high rates of mortality at the egg and hatchling stages and hence, their reproductive strategy of laying multiple clutches scattered on the beach and through the year (Manugsson *et al*. 1990). In fact, these are the stages that have the lowest reproductive value compared to juveniles and adults (Manugsson *et al*. 1990). Therefore, the low rates of productivity found at Kingere should not, in principle, be a cause for concern. However, if coupled with abnormal mortality rates of adults at sea, low recruitment over a long period of time can lead to a population decline (Heppel, 1997). For example unsustainable harvesting of egg on nesting beaches coupled to fisheries-related mortality might have been the causes for the severe declines at some Indo-Pacific rookeries (Chan and Liew, 1996; Sarti *et al*. 2007). Although leatherback populations in the Atlantic seem to be relatively stable, there is evidence that incidental captures by fishing activities are causing increased mortality rates in adults (Carranza *et al*. 2006).

On the other hand, despite the overall low nest success that seems to prevail at Pongara, it is encouraging to find out that there is also a patchy pattern in which some sectors are considerably more productive than others and could compensate somehow to increase overall rates. This within beach scale observation can possibly be inferred to a large scale considering the whole coast of Gabon as some beaches like Mayumba seem to have a much higher nest survival and incubation success. What is more, given the extension of nesting grounds and its importance in terms of nest numbers, Gabon must certainly play a major role in maintaining Atlantic population levels stable. Under such circumstances, it would be advisable that long-term data collection on incubation success be continued and extended to other sites so as to have a clearer picture at a regional scale.

The unusually high predation rates found at Kingere seem to be caused mainly by insects and, particularly, the ant *Dorylus spininodis* seems to be a major egg predator. Despite being the first report of this ant in Gabon (Ikaran *et al*. 2006), it is a native pan-african species and it should be considered as a natural source of mortality. It is also difficult to control as predation occurs in a subterranean way. This fact, together with the elevated nest density, makes unviable the practice of hatcheries as a way to augment hatchling production at Kingere. Furthermore, the human resources currently available at the site would be insufficient to cope with the maintenance requirements of a large-scale hatchery. As far as management is concerned, keeping the basic monitoring activities of night patrols and track counts are far more recommended because of the female rescue operations. As we explained in chapter 2, 15 dead females were counted on the beach trapped by the logs during the month of november 2006, because the NGO staff were not on duty yet and ~30 females were saved and released back to sea during the rest of the season.

The main identified sources of mortality on eggs have a natural origin and seem to be a consequence of the quality of the sand and a combined effect of the sea and forest ecosystems onto the beach. Flooding and erosion affect a great proportion of nests as the beach is relatively narrow, not more than 15-20m wide. The abrupt connection between the soil in the forest and the sand, is possibly favouring the presence of invertebrates and vegetation debris in the sand profile that might have a negative influence on the incubation of the clutches. Also, the amount of unhatched eggs remaining buried in the sand after one nesting season, that we have estimated at between 1 and 3.5 tons per kilometer, is certainly contributing to augment organic material and microorganisms that might affect negatively embryonic development. In fact, this situation seems to be the same described by Kamel and Mrosovsky (2004), with a compromise between the advantages and disadvantages of nesting too low or too high on the beach. However, there seems to be an additional human-induced factor at Kingere that has a potential negative effect on the incubation of eggs, which is that of stranded and buried logs. Each log constitutes a source of decaying organic material and probably an attraction point for invertebrates from the neighbouring forest, such as ants and termites.

The results in this study show alarming levels of predation by insects not apparent to the same frequency elsewhere in Gabon and we also described an unusual interaction between termites and ants within leatherback nests. As for the low hatching success, is not possible to determine whether the nest predation observed here is a recent or long-term phenomenon, because monitoring projects are not older than a decade. We hypothesize this is due to both natural and anthropogenic environmental conditions relatively unique to this beach system, i.e. an abrupt forest-beach interface and forestry originated drift wood. Predatory and colonising insect communities, especially the ants and termites are likely to be mostly derived from the neighbouring forest habitat. The higher frequency of foraging into the beach habitat may be facilitated by the abrupt connection between the beach and forest found at Kingere. Colonisation frequency may be further increased by the unusually high incidence of buried logs, attracting termites into the beach profile, followed by predatory ants.

Although seemingly not a good incubation system for eggs in terms of hatching success, Kingere appears to be favourable for sex ratio production. Sand temperatures fluctuate naturally around the pivotal temperature during the nesting season and current estimated sex ratios are near equilibrium or even male biased. In this study, we show that the importance of Gabon as a globally significant nesting site for the leatherback turtle is reinforced by the fact that it is a potentially male producing area. However, we also found that this situation could easily be reversed due to the effect of global warming on sand temperatures. Indeed a 100% female scenario is likely to be reached in less than two decades, according to the latest IPCC forecast that predicts for the Central African region a mean increase in air temperature of 1ºC and 4ºC for 2029 and 2099 respectively.

Of all the speculated mechanisms by which sea turtles might be able to cope with global warming (Hawkes *et al*. 2009; Hulin *et al*. 2009), behavioural adaptations such as phenological or spatial shifts seem to be the most feasible for the leatherback turtle in this particular region. Estimated sand temperatures in Gabon during the dry season, prior to the current nesting season are lower and therefore they could start nesting earlier during the year. Also, there are plenty of nesting grounds available both northwards or southwards of the Gabon-Congo epicentre (Fretey, 2001; Formia *et al*. 2003; Fretey *et al*. 2007) and a latitudinal shift to other nesting beaches would be possible. To sum up, there is both spatial and temporal niche available, which, together with the bigger behavioural plasticity *of D. coriacea*, seem to provide encouraging predictive views. However, other impacts of climate change such as projected sea-level rise are expected to cause detrimental effects on the nesting habitats (shoreline erosion, flooding of beaches, increasing egg mortality) (Fuentes *et al*., 2009b) to which these animals might not be able to respond.

With this study, we hope to have augmented the knowledge about the reproductive ecology of *D. coriacea,* and sea turtle biology in general, on the Eastern Atlantic Region. We also hope that the main findings highlighted will help in the conservation and management of the species. After the preliminary results gathered during this study, there are many interesting research fields that remain open. Several factors that were not considered in this study could be playing a role in the reproductive success. For example, a study on the potential presence of toxic contaminants in the sand would be recommended, considering that mineral and petrol exploitations are the major source of income of the country of Gabon. Also, quantitative data on the abundance and impact of buried logs on incubation success of the nests would be necessary.

Finally further research into the topic of underground predation would help to understand why it seems to be particularly intense at Kingere.

To sum up, this study is above all descriptive and provides grounds for future research topics as well as management activities. We have brought up to light two main sources of mortality upon *D. coriacea* in the Eastern Atlantic region. The first one, predation, concerns the egg stage and seems to have a natural origin, while the second one, the stranded logs, is human-induced and affecting adult females. We have also contributed to current efforts of data and methods standardisation by setting into motion a field protocol that is currently being used at the major nesting sites along the coast of Gabon and hopefully will provide a long term database that will help to fill in some gaps of knowledge about the species in this region..

APPENDIX I: Extending the nest study to other sites in Gabon

Here, we present additional data collected at the beaches of Pointe Denis and Mayumba during season 2008/2009 in the frame of The Gabon Sea Turtle Partnership. The objective was to gather information for inter-beach comparison and at the same time to cooperate in the current efforts to standardize methodologies and data collection. The three main sites for sea turtle conservation in Gabon (Pongara, Gamba and Mayumba) were involved with training programs for the field staff. However, due to logistical problems and the low density of females during this nesting season, no data was available from Kingere and Gamba.

Table 1. Mean values of nest survival and incubation success at Pointe Denis and Mayumba.

	Pointe Denis (n=30)		**Mayumba (n=25)**	
	Mean ± SD	Range	Mean ± SD	Range
ALL NESTS				
Hatching success	17.3 ± 31.1	0 - 83	43.0 ± 39.4	0 - 96
Emergence success	16.1 ± 29.4	0 - 83	40.4 ± 39.4	0 - 96
ONLY SUCCESSFUL NESTS				
Hatching success	57.3 ± 30.6	7 - 83	71.0 ± 24.6	12 - 96
Emergence success	53.8 ± 29.2	7 - 83	68 ± 25.9	2 - 96
Nest survival	30%		60%	

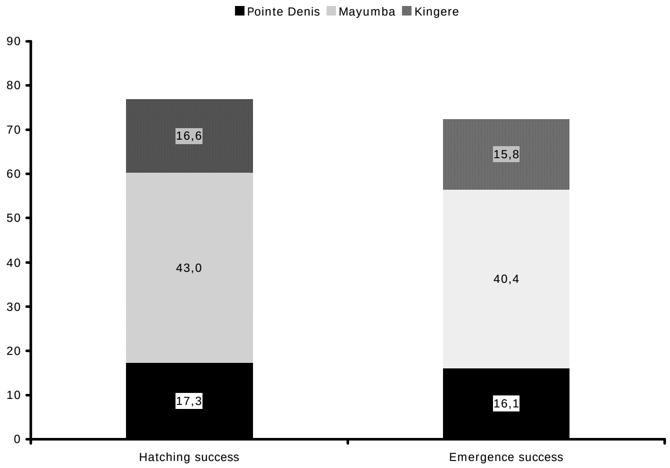

Figure 1. Comparison of Pointe Denis,Kingere and Mayumba in terms of hatching success.

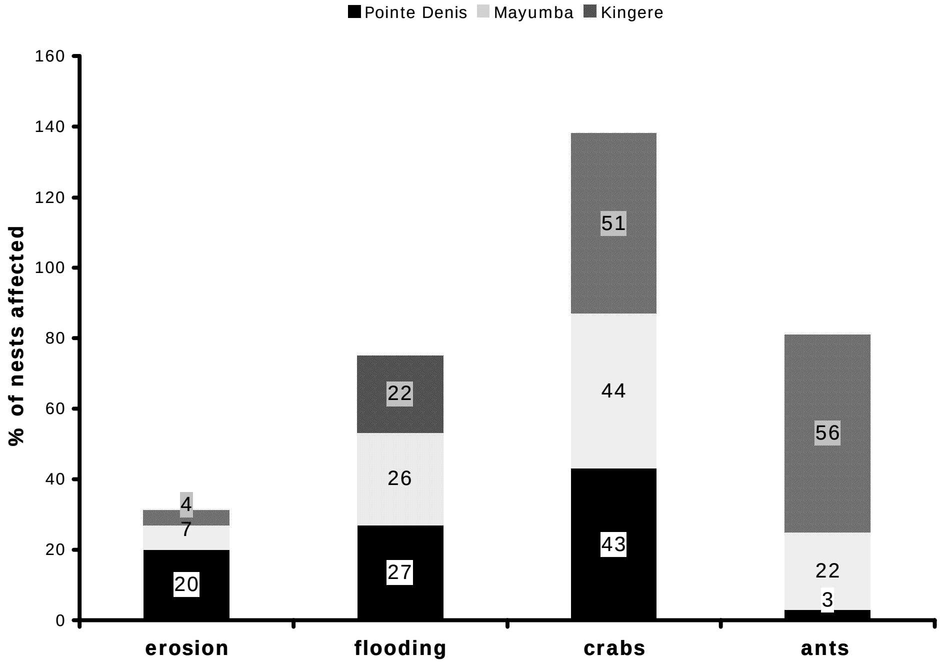

Figure 2. Comparison of main sources of egg mortality at the three sites. Bars express relative proportion of nests affected from data collected during three seasons (2005/2008) at Kingere (n=170 nests) and during one season (2008/2009) at Pointe Denis (n=30 nests) and Mayumba (n=25 nests).

Acknowledgements

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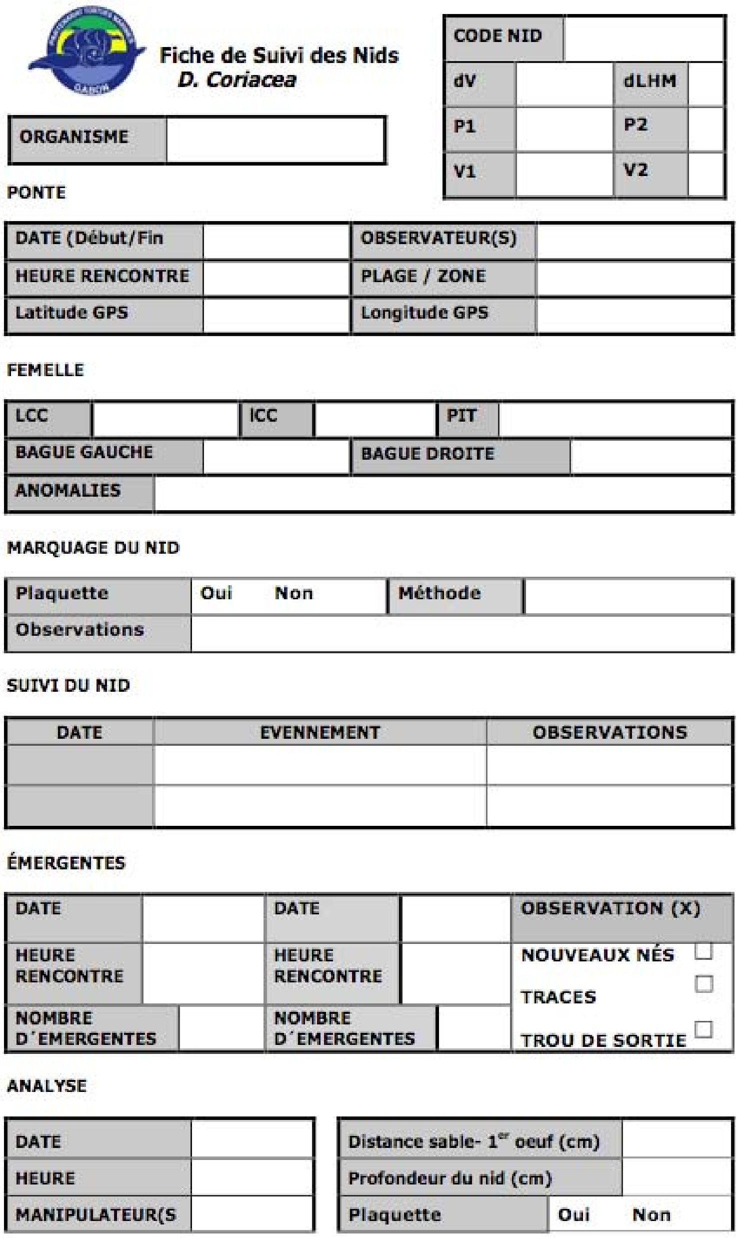
APPENDIX II: Datasheets



Fiche de Suivi des Nids
D. Coriacea

CODE NID			
dV		dLHM	
P1		P2	
V1		V2	

ORGANISME	

PONTE

DATE (Début/Fin		OBSERVATEUR(S)	
HEURE RENCONTRE		PLAGE / ZONE	
Latitude GPS		Longitude GPS	

FEMELLE

LCC		ICC		PIT	
BAGUE GAUCHE		BAGUE DROITE			
ANOMALIES					

MARQUAGE DU NID

Plaquette	Oui Non	Méthode	
Observations			

SUIVI DU NID

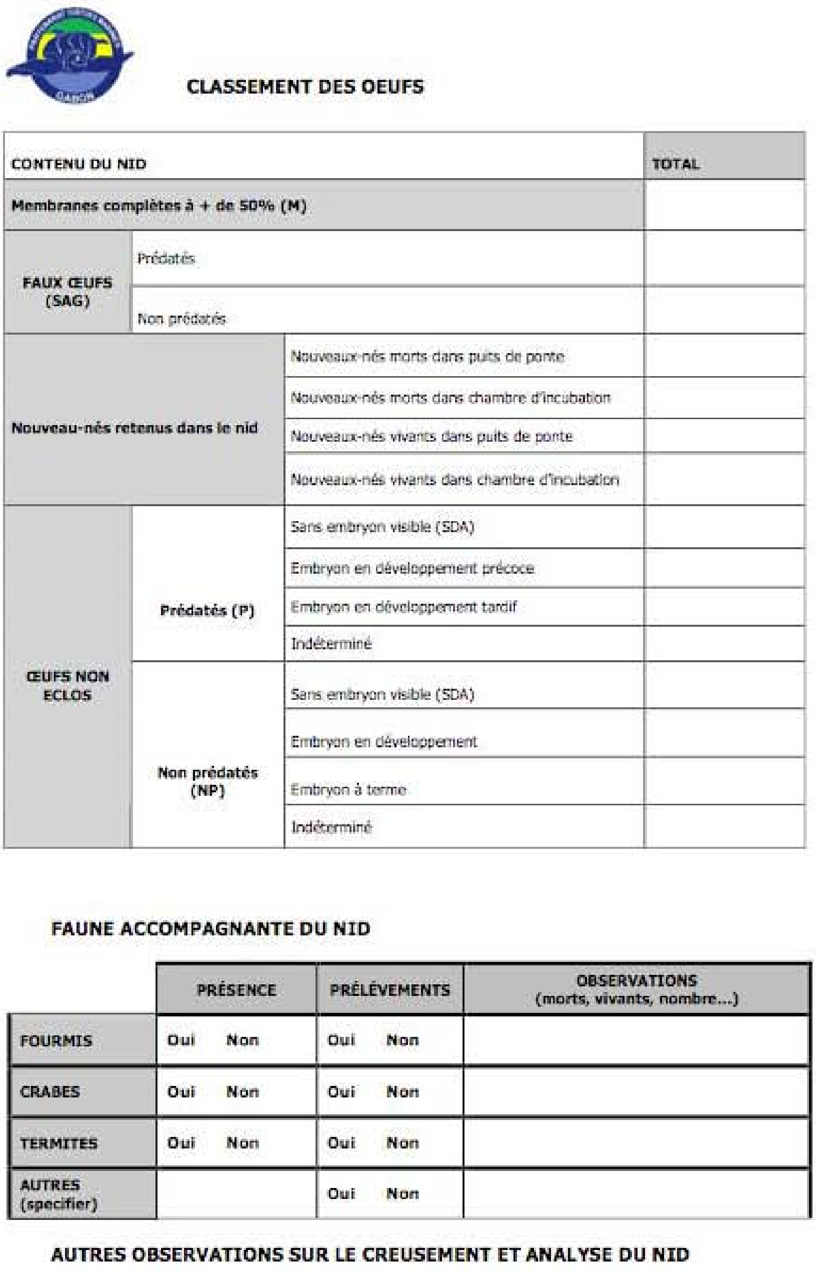
DATE	EVENNEMENT	OBSERVATIONS

ÉMERGENTES

DATE		DATE		OBSERVATION (X)
HEURE RENCONTRE		HEURE RENCONTRE		NOUVEAUX NÉS ☐ TRACES ☐ TROU DE SORTIE ☐
NOMBRE D´EMERGENTES		NOMBRE D´EMERGENTES		

ANALYSE

DATE		Distance sable- 1^{er} oeuf (cm)	
HEURE		Profondeur du nid (cm)	
MANIPULATEUR(S		Plaquette	Oui Non



CLASSEMENT DES OEUFS

CONTENU DU NID			TOTAL
Membranes complètes à + de 50% (M)			
FAUX ŒUFS (SAG)	Prédatés		
	Non prédatés		
Nouveau-nés retenus dans le nid		Nouveaux-nés morts dans puits de ponte	
		Nouveaux-nés morts dans chambre d'incubation	
		Nouveaux-nés vivants dans puits de ponte	
		Nouveaux-nés vivants dans chambre d'incubation	
ŒUFS NON ECLOS	**Prédatés (P)**	Sans embryon visible (SDA)	
		Embryon en développement précoce	
		Embryon en développement tardif	
		Indéterminé	
	Non prédatés (NP)	Sans embryon visible (SDA)	
		Embryon en développement	
		Embryon à terme	
		Indéterminé	

FAUNE ACCOMPAGNANTE DU NID

	PRÉSENCE	PRÉLÈVEMENTS	OBSERVATIONS (morts, vivants, nombre...)
FOURMIS	Oui Non	Oui Non	
CRABES	Oui Non	Oui Non	
TERMITES	Oui Non	Oui Non	
AUTRES (specifier)		Oui Non	

AUTRES OBSERVATIONS SUR LE CREUSEMENT ET ANALYSE DU NID

Appendix III. Observations about fertility and the white spot

Fertility (as the proportion of fertilized eggs) was quantified in 11 clutches (n=4 season 05/06, n=7 season 06/07) using the white spot technique (Chan, 1989; Abella *et al*. 2007). The clutches were collected as they were laid by the female and relocated in artificial nests dug by hand at specific places on the beach. The nests were opened at regular intervals during the first 10 days of incubation and the top eggs were carefully examined to check the appearance of the white spot. Eggs were hold against the light of a torch at night to minimise the possible thermal impacts. When the white spot was clearly visible, we checked the totality of the clutch and counted the number of fertilized eggs.

Main fertility was 53% for 4 nests that were laid at the end of season 2005/2006 and 97% for 7 nests of the peak season in 2006/2007 (Table 1). These were complemented with other observations on eroded clutches found on the beach allowing to make a “calendar” of white spot development. It first appeared between 4-5 days after the night of laying as a calcification area of 1-2cm diameter and expanded until day 20-21 when the whole egg gets calcified (Figure 1).

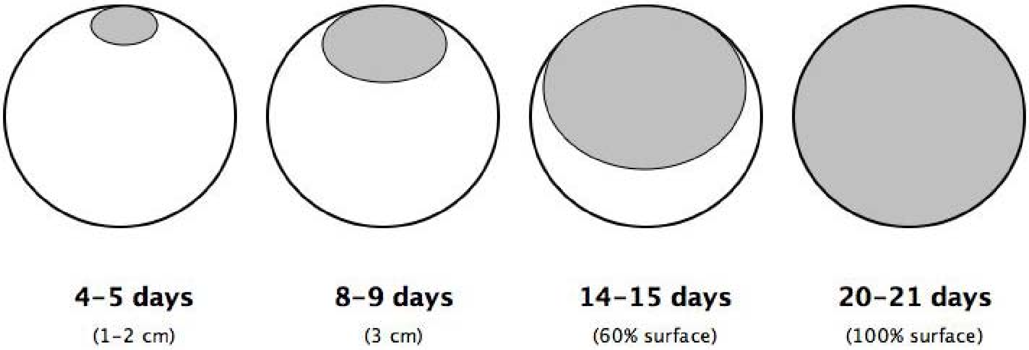


Figure 1. Suggested evolution of the white spot during the first third of the incubation period for the leatherback turtle eggs according to observations in 11 relocated clutches and other eroded nests found accidentally on the beach.

Table 1. Results of the fertility tests on 11 leatherback clutches at Kingere, using the white spot technique.

	Late season clutches (n=4)	**Peak season clutches (n=7)**
Fertility ± SD	53,3 ± 22,8	97,2 ± 2,0
range	26,0 – 78,1	94,3 – 100,0

Our observations about the white spot on leatherback eggs are similar to the ones described by Chan (1989) in Malaysia, appearing between 4-5 days after oviposition. This in fact, is thought to correspond to the moment when the vitelline membrane adheres to the inner shell surface and is taken as an evidence that the egg has been fertilized. In Cabo Verde, eggs of the loggerhead turtle showed the first signs of the white spot at 24 hours after laying and covered about 33% of the shell after 4 days (Abella *et al.* 2007). This technique has been used in Cabo Verde with loggerhead (Abella *et al*. 2007) and in Malaysia with leatherback clutches (Chan, 1989) and both studies concluded that it is an effective and non-invasive method to estimate the fertility of the clutches. Apart from being an indication of the reproductive success of a given population, identifying unfertilized eggs in a clutch can bear important management implications. Although unfertilized eggs do not seem to have a negative impact on the incubation success of the clutch (Abella *et al*. 2006), they could be a source of microbial activity as they decompose. Also, at places with high incidence of illegal poaching, as it is the case of Cabo Verde and Malaysia, unfertilized eggs could be removed from clutches that are relocated to hatcheries so as to be consumed by local populations.

At Kingere, we found that nests laid during the peak of the season had high fertility rates, over 90%, whereas those laid at the end of the season had lower but still acceptable fertility rates (50%). The proportion of unhatched eggs with no apparent signs of development found during nest excavations was ~20% (chapter 4), yet, the high degree of microbial infection did not allow a reliable indentification of most of the contents. Also, embryonic death in early microscopic stages could have ocurred (Bell *et al*. 2003). However, this preliminary test suggests that infertility of a certain porportion of the eggs should be considered as a potential cause for low hatching success and/or the high degree of bacterial and fungal infection of the clutches.

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APPENDIX IV. Estimation of pivotal incubation duration for sex ratio determination in the leatherback turtle

Background

This is an indirect method of estimating the proportion of sexes produced in a group of nests based on the relationship between incubation durations and temperatures during the critical moment for sex determination (middle third of incubation) (Marcovaldi *et al*. 1997). Long and short durations imply low and high temperatures and in consequence more males or females respectively with a "pivotal incubation time" in which 50% of each sex would be produced (similar to the pivotal temperature). This method has been proved to be quite accurate (Mrosovsky, 2009) and has been used to estimate primary sex ratios of green turtles (Broderick *et al*. 2000), loggerheads (Marcovaldi *et al*. 1999; Godley *et al*. 2001) and hawksbill turtles (Godfrey *et al* 1999) at different nesting sites where extensive datasets on incubation times of nests exist. It has the advantage of being simpler because it does not require any material but only field data and can be used when there are no economical means to acquire data loggers and has useful application in the field, for example to evaluate whether a hatchery is in a wrong location. There is only one estimation of pivotal durations for leatherbacks by Mrosovsky *et al*. (1984) in Surinam.

Pivotal incubation duration for the leatherback turtle in Gabon

We estimated pivotal incubation duration from a sample of 8 nests in which data loggers were recording intranest temperatures and with a known incubation duration. There was a strong correlation between the temperature during the middle third of incubation (IT middle third) and the incubation period (IP) of the nests (Pearson, r= -0.780, p=0.22, r^2=0.608, n=8). To calculate the pivotal and extreme incubation durations, we used the equation IP= -3.4326 * (IT middle third) + 165.45 and the French Guyana sex ratio curve by Rimblot *et al*, (1987) that predicts 100% males at and below 28.9ºC, 100% females at and above 30.1ºC and 50% of each sex at 29.5ºC. According to this, pivotal incubation duration for leatherbacks in Gabon is 64.2 days and mixed sexes are produced at durations in between 66.2 and 62.1 days. This means that average incubation durations longer than 66.2 days would produce 100% males and below 62.1 days 100% females.

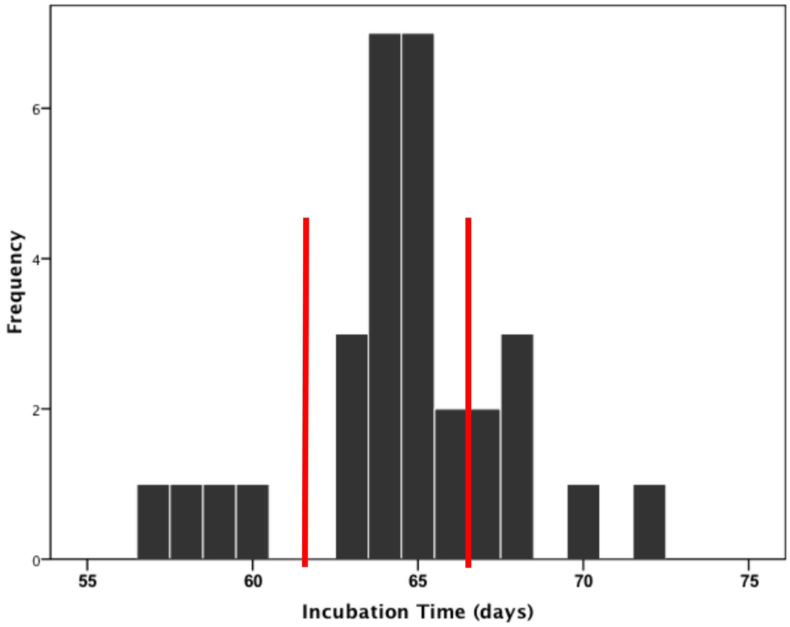

Figure 1. Frequency distribution of incubation durations of 30 leatherback *in situ* nests at the beach of Kingere. The line shows the range of durations at which mixed sexes would be produced, estimated in this study.

Table 1. Mean incubation durations data available for Gabon and the estimated sex ratio according to the pivotal duration obtained in this study.

Site		Mean Incubation time (range)	Data source	Sex Ratio
Kingere	In situ (n=30)	64.6 (57-72)	This study	Mixed
Gamba	In situ (n=12)	67.1	Livingstone (2007)	100% Male
	Hatchery (n=15)	63.1		Mixed, female skewed
Mayumba	In situ (n=9)	69.0	This study	100% males
	Hatchery	71	Billes, 2003	100% males

Data on incubation durations in Gabon are still quite scattered and it would still not be possible to make solid estimations using this method. Table 1 summarises the data available for the main beaches so far and the corresponding estimation and this seems to match sand temperature data showin a tendency towards male production in Gabon.

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