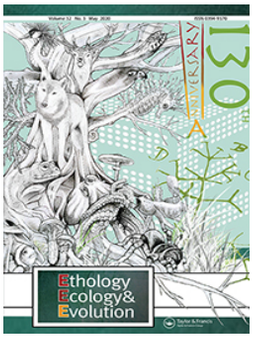

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Cryptic massive nest colonisation by ants and termites in the world's largest leatherback turtle rookery

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Egg mortality is one of the main factors affecting life history and conservation of oviparous species. A massive and cryptic colonisation of many leatherback turtle (*Dermochelys coriacea*) eggs is presented in the most important rookery for the species in Gabon. A total of 163 nests were exhumed at Kingere beach, revealing that only 16.7% of eggs produced hatchlings. In the 59% of the nests, more than half of the eggs were dead and attacked by invertebrates and 94% had at least one egg affected by invertebrates. The rate of eggs and SAGs (yolkless eggs) affected by invertebrates within a clutch ranged from 0% to 100%, with an average proportion of 39% and 52%, respectively. The most common invertebrates interacting with the eggs were ghost crabs and insects that affected 51% and 82% of the nests, respectively. Crab and insect co-occurred in 33% of the affected nests. Ants, identified as *Dorylus spininodis* (Emery 1901) were found in 56% of the excavated nests. However, it was not possible to determine if the ants predated alive eggs or scavenged dead eggs. Very often, hundreds of ants were found dead within dead eggs. Termites and other invertebrates were associated with the clutch environment and identified as opportunistic feeders. An unusual ecological interaction within the leatherback clutches between termites and ants was found in 11% of the nests. The abrupt transition between the soil forest and the beach might be favouring a thriving microbial and invertebrate activity in the sand profile that colonises the nests.

KEY WORDS: nests, leatherback turtle, ants, termites, survival, tropical beaches.

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INTRODUCTION

Sea turtles are typically long-lived animals where adults produce considerable amounts of offspring during its lifetime. The reproductive strategy of sea turtles assumes high natural mortality rates at early life stages (Magnuson et al. 1990). However, they have few predators and low natural mortality once they reach the adult size. It is known that sharks, orcas and large seals and sea lions can attack leatherbacks at sea (Heithaus et al. 2008). In addition, 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 no 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 (Leighton et al. 2011; Heithaus 2013). This ecological interaction can reduce hatchling recruitment and affect the recovery of threatened populations (Hamann et al. 2010; Leighton et al. 2011).

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; Marco et al. 2015), insects (Maros et al. 2005; Da Silva et al. 2016), lizards (Tomás 2004; Livingstone 2007) or mammals such as mongooses (Nellis & Small 1983). Bacterial and fungal infection of eggs is also relatively common and may cause the death of the embryos (Wyneken et al. 1988; Sarmiento-Ramírez et al. 2010, 2014).

As sea turtle clutches are buried in the sand, the presence of invertebrate fauna associated with 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 predate eggs causing considerable damage to the survival of the clutches. For example, in Florida, a native species of coleopteran larvae, the click beetle *Lanelater sallei* (Leconte 1853) 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 et al. 2001b) and in Turkey (Baran et al. 2001). In French Guyana, the last instar nymph of the mole cricket [*Scapteriscus didactylus* (Latreille 1804)] 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. Dipteran larvae have been recorded in the nests of green and loggerhead turtles in the Mediterranean (McGowan et al. 2001a) 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 predatory ants that might feed on eggs as well as on emerging hatchlings: green turtles in Costa Rica (Fowler 1979), loggerheads in South Africa (Maxwell et al. 1988), hawksbills in Malaysia (Chan & Liew 1999) or leatherbacks in Surinam (Whitmore & Dutton 1985). Ants can also attack eggs and hatchlings of terrestrial and freshwater turtles (Burger 1977; Allen et al. 2001). On the south Atlantic coast of North America, there seems to be a major problem with red imported fire ants (*Solenopsis invicta*) that have a devastating effect on sea turtle eggs (Moulis 1997; Allen et al. 2001, 2004; Parris et al. 2002; Wetterer et al. 2014). However, in many cases, it is not easy to be sure, whether the insects predated the eggs or scavenge dead eggs.

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 et al. 2000; Verhage et al. 2006; Livingstone 2007). However, other unknown factors could be killing turtle eggs, and insects could be at least just consuming the dead eggs. During the course of the study on incubation success at Kingere beach (Pongara National Park), we found ants in massive amounts inside leatherback nests. In this paper, we focus on the results of the nest excavations and particularly on the possibility of predation that could 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 (0°18′N, 9°18′E) during three nesting seasons (2005 to 2008). A total of 170 nests were marked and followed to term of which seven were eroded by the sea, leaving 163 for excavation.

Basic field protocol

Between November and January, we located nesting females at the point of oviposition so that nests could be monitored and excavated after hatching to evaluate success. On each night, 2–4 females were selected randomly. We recorded date and 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 prevent pseudo-replication in the sample of nests. Nests were marked in a provisional way at night with marks on the beach, and then permanently the next morning using wooden stakes. A wooden label (5 × 5 cm) with the nest code was placed 30–40 cm above the egg chamber, while the female was covering the eggs.

Nest incubation success

A total of 170 nests were marked and followed to term at Kingere during the three seasons of the study (n = 55 in season 2005/2006, n = 60 in 2006/2007 and n = 55 in 2007/2008). The emergence of hatchlings usually happened at night and the exact date could be determined in 50% of the successful nests either because we saw directly the hatchlings emerging or because their tracks were seen in the sand the next morning. The nests were excavated after emergence evidence was observed or at day 70 of incubation if emergence was not observed. Nest contents were classified into eggshells, unhatched eggs and pipped eggs (pipping is the process in which hatchlings pierce the eggshell). 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)] × 100 and emergence success (ES) as ES = [(#eggshells-trapped hatchlings)/(total number of eggs)] × 100, 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. 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.

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 scrapped by hand to minimise the 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) and secondly by their contents (presence or absence of an embryo). The following categories were differentiated: (1) SAG: stands for shelled albumin globules, commonly known as "yolkless eggs", contain no yolk and will therefore not have a developing embryo; (2) eggshells: empty eggs remaining after hatchlings emerge from the nest; (3) 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); (4) 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: a-no visible embryo; b-early embryo: a macroscopic embryo in early stages of development with no pigmentation; c-late embryo: macroscopic embryo that has acquired the pigmentation; d-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 eggshell (clearly different from the pipped eggs) was considered as evidence of an interaction with an invertebrate. For each nest, the following information was collected: presence of invertebrates, presence/absence of ants and/or termites, where and how ants were found, type of interaction and number of affected eggs. Egg and SAG rates of colonisation by invertebrates for each nest were calculated as the percentage of each presenting a hole in the membrane relative to the total number. We used two categories to classify nests "affected by invertebrates": (1) the nest had at least one viable egg affected or (2) the nest had more than 50% of the viable eggs affected. For each nest, a type of invertebrate was assigned according to the holes observed in the membrane of the eggs: insects, crabs or mixed. Invertebrate type and ants' 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 organisations for identification: Departement Entomologie Fonctionnelle et Evolutive, Gembloux Agro-Bio Tech, Liège, Belgium; Department of Entomology of the National History Museum of London and Real Jardín Botánico, CSIC, Madrid.

RESULTS

Classification of nest contents

A total of 11,613 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 (Fig. 2a). Eggs affected by invertebrates accounted for 51.2% of the total (Fig. 1a–b). Inside the eggs that were not affected by invertebrates, a dead embryo in early or late stages of development was found in 6.3%

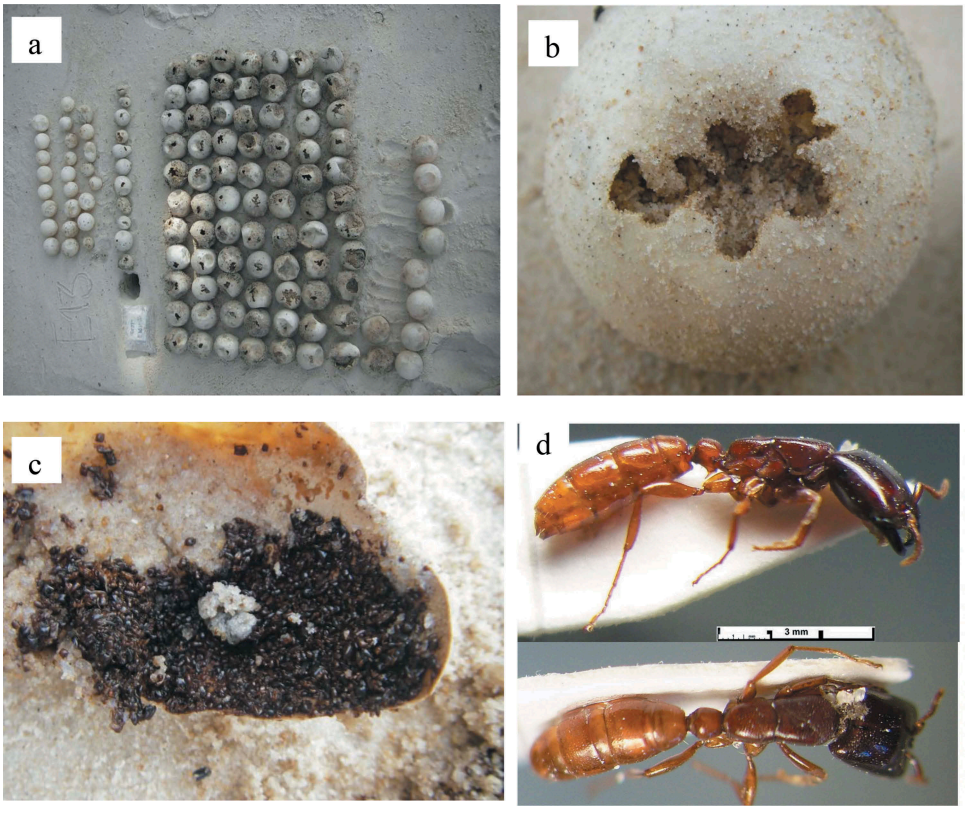

Fig. 1. — Leatherback turtle nest infested by army ants. (a) Leatherback clutch mass with most of the eggs predated. (b) Details of an egg colonised by ants. (c) Dead ants inside a dead leatherback turtle egg. (d) Army ants (*Dorylus spininodis*).

and 16.3% of the cases, respectively, but most of the times, there was no visible embryo (58.6%) (Fig. 2b). In most of the eggs affected by invertebrates (89.3%), it was not possible to determine the presence or absence of an embryo and the egg was classified as "unknown" (Fig. 2c). Thus, the most abundant categories inside the excavated nests were eggs affected by invertebrates with unknown contents and eggs unaffected by invertebrates with no visible embryo (Fig. 2a). SAG was not considered for this analysis.

The nature of the contents of eggs affected by invertebrates 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. 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, 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 thousands of dead ants inside eggs that seemed to be especially frequent in the eggs filled with sand (Fig. 1c).

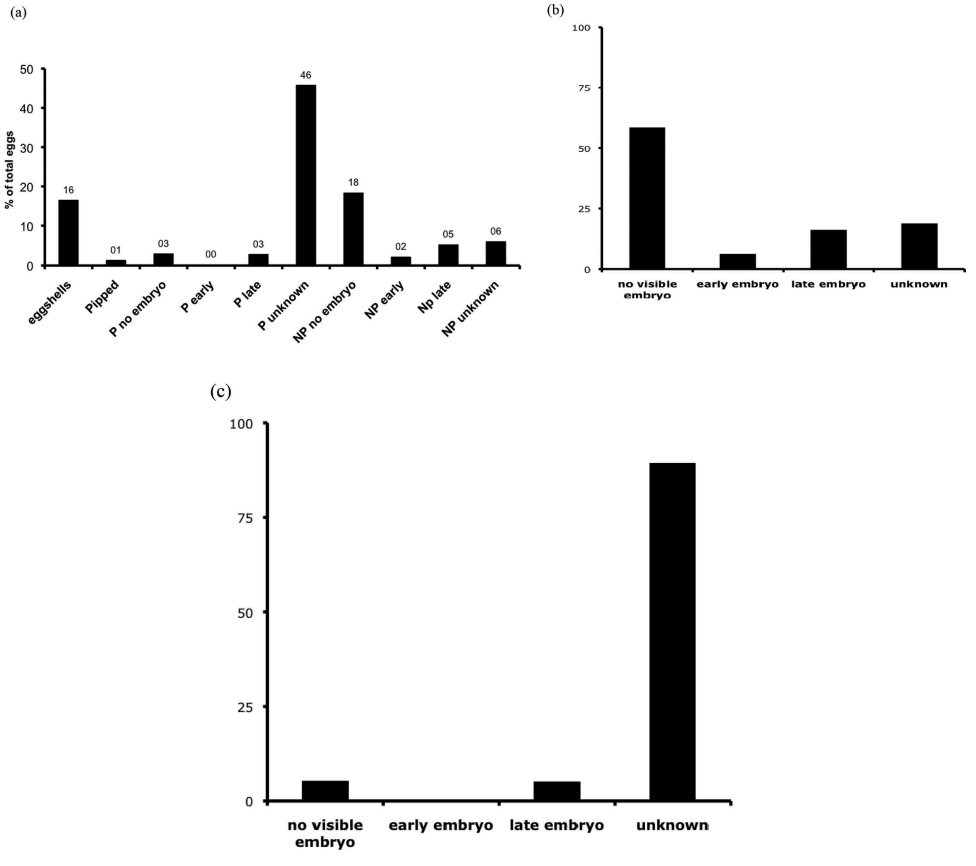

Fig. 2. — Results of the excavations: (a) all viable eggs (P: predated or scavenged, NP: non-predated nor scavenged), (b) eggs not colonised by invertebrates and (c) eggs colonised by invertebrates. Y scale bar is the average frequency for the totality of excavated nests.

Rates of invertebrate infestation to nests and eggs

Using the two criteria to consider a nest as invaded by invertebrates, we found that 94% (n = 154) of the nests had at least one egg affected and 59% (n = 96) of the nests had more than half of the eggs affected. Although predation by the lizard *Varanus ornatus* and mammals like mongooses was frequently observed on the beach, either crabs or insects affected all studied nests. Insect and crab holes were easy to differentiate: crabs produce characteristic slit marks and disperse the eggs over the sand surface or at different depths above the incubation chamber. Moreover, there were usually holes on the sand surface from the burrowing galleries. Insects produced different shapes of holes but did not disperse the eggs. Insect type holes ranged from small and circular (1 mm to 1 cm diameter) to irregular with serrated edges (1–3 cm width) and a typical amoeba shape. Insect and crab affected eggs were recognised in 49% and 18% of the nests, respectively. Both types of interactions 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 affected 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 affected by invertebrates did not necessarily mean that none of the eggs hatched.

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 microorganisms (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 mites 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" (Fig. 1d). Although it is a pan-African species this was the first record in Gabon. Termites (soldiers and workers) of the genera *Coptotermes* (Rhinotermitidae), *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 Tenebrionidae, Elateridae and Dynastinae (rhino beetle). Most of these feed on carrion, mould, fungus or rotten plant material and are probably opportunistic. Mites were usually present in large numbers covering the inner membranes of predated eggs, especially those that were already at the humus stage or empty, and are 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 dead eggs (n = 80 nests), in particular, those that were filled with plain sand or grey sand. 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

Table 1.

Quantitative data of levels of infestation by invertebrates of eggs and clutches and on the occurrence of ants inside leatherback nests at Kingere.

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

Number of nests appears in brackets.

a few occasions *Amitermes* and *Coptotermes*. We observed galleries of dark brown colour built on top of the mould of eggs and sand in six 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. In addition, sand coagula were already present in the yolk. Other predated eggs were filled with sand and alive termites inside.

DISCUSSION

We found that 59% of the nests had more than half of the viable eggs dead and colonised by invertebrates at Kingere and that colonisation was evident in 81% of these nests. Although it would need to be confirmed, ants are apparently major responsible for this infestation as they were found in more than half of the affected nests. Such rates do not seem to happen at other beaches in Gabon. During a study

Table 2.

Main groups of invertebrates found during excavations in the leatherback nest and their observed or hypothesised interactions with eggs.

Taxon	Behaviour	Presumed interaction with the eggs
INSECTA		
HYMENOPTERA (ants) *Dorylus* sp., *Hypoponera* sp.	Dead in huge amounts inside predated eggs (≥ 1000). Alive isolated (1–10) in the egg chamber or actively infesting the nest with galleries (≥ 10.000).	Predation or scavenging (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 mound (could be foraging galleries).	Commensalism with collateral damage: possible consumption of egg shells, however likely to foraging on plant material in sand profile. Inquilism.
DIPTERA (Fly larvae)	Inside eggs or attacking embryos or hatchlings.	Predation or scavenging.
COLEOPTERA (adults) Staphylinidae, Scaratinidae, Histeridae	Isolated individuals in the egg chamber, not directly associated to eggs.	Commensalism (opportunistic feeder on carrion, mold, fungus).
COLEOPTERA (larvae) Tenebrionidae, Dynastinae, Elateridae	Alive and isolated in the incubation chamber. Sand dwelling.	Opportunistic feeder.
ARACHNIDA		
Mites	Alive in large numbers. Covering the inner side of the membrane of predated eggs.	Commensalism (opportunistic feeder).
CRUSTACEA		
Ocypode sp. (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.	Commensalism (opportunistic feeder).

conducted in the 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. Livingstone (2007) reports finding ants at Gamba in 12.5% of the hatched nests. Otherwise, the main predators at other beaches in Gabon seem to be crabs, lizards and small mammals.

Turtle nests are usually threatened by a variety of vertebrate and invertebrate predators (Fowler 1979; Dodd 1988; Donlan et al. 2004). Carnivore mammals are considered as the most important predator of turtle nests (Ratnaswamy & Warren 1998), and the regulation of carnivore mammals to reduce nest predation is a common practice (Pennisi 2006; Barton & Roth 2007). In Florida beaches, predation by raccoons and ghost crabs together was of the 31% (Barton & Roth 2008). However, nest predation by invertebrates usually has lower intensity. On Key Biscayne, the average predation rate of loggerhead eggs by ghost crabs and beetles (*Lanelater sallei*) was, respectively, 1 and 5 eggs per nest (Donlan et al. 2004). Caldwell (1959) found predation by crabs on 61% of loggerhead nests in South Carolina though a much lower rate of egg predation per nest. Ghost crabs (*Ocypode cursor*) also caused significant predation of around 50% of nests on the Cape Verde loggerhead rookery (Marco et al. 2015). Hawksbill nest predation by ghost crabs (*Ocypode cordimana*) in the Cousine Island (Seychelles) in the absence of larger predators (Hitchins et al. 2004) was relatively low (approx. 17%). In the Galapagos Islands, 18.9% egg loss due to beetle predation was found early in the season (Allgöwer 1980). Mean predation of green turtle nests by the ghost crabs (*Ocypode ceratophtalmus* and *O. kuhlii*) in the Malaysian island of Mak Kepit was also very low (approx. 1.3%) (Ali & Ibrahim 2002). In Bijagos Archipelago (Guinea-Bissau), ghost crabs only predated the 1% of green turtle nests (Catry et al. 2002). In Turkey, insects from nine families and seven different orders have been detected, being the tenebrionid *Pimelia* sp. the most common one, which affected 36% of the clutches (Katılmış et al. 2006). In Cyprus, larvae of dipterans have been found in 15% of the clutches studied. These larvae belonged to 11 different species although the dominant species was the sarcophagid *Sarcotachina aegyptiaca* (McGowan et al. 2001b). If the invertebrates found on leatherback nests would cause the egg death, the predation on Gabon would be massive and exceptional, likely having a severe impact on the population dynamics. However, the invertebrates could have colonised the eggs when they were already dead. Turtle eggs are vulnerable to other threats such as flooding or an excess of humidity in the incubation chamber (Patino-Martinez et al. 2014) or the colonisation of pathogenic fungus (Sarmiento-Ramírez et al. 2010, 2014). The possibility of cryptic nest predation by ants and termites should be evaluated in Gabon and other sea turtle populations with unexpected low hatching success, discarding the possibility of necrophagy.

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 dead and colonised by insects. We ignore the percentage of eggs that were predated by the insects or the moment of incubation at which predation or scavenging occurred. The content of the infested eggs was usually liquefied and decomposed with the presence of fungi and bacteria and was therefore impossible to determine at which stage of development die the embryos. There were many eggs not invaded by invertebrates with no visible embryo. These could correspond to an egg that was not fertilised 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% fertility) of the season. The clutches that were analysed in this study were laid during the early-middle season and further research into this would be recommended 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. About 85% of the surface of Gabon is covered by dense equatorial rainforest, which borders almost entirely the 800 km 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 m 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 having more characteristics of forest soil than usual. On the other hand, there is an especially 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 could explain the low incubation success of nests and potentially high levels of predation that could suggest the results of this study. However, alternative causes of embryo death should be evaluated in the rockery in order to explain such high mortality. The possibility of invertebrates scavenging dead eggs should not be discarded in part or most of the eggs. Future studies should be conducted in order to clarify this uncertainty.

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. While these are introduced species, *Dorylus spininodis* is a native pan-African ant that occurs naturally in equatorial rainforests. However, it appeared to be the first citation of these 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 colonisation patterns observed in this study.

The colonisation levels found in our study do not seem to be produced by isolated individuals but rather by some kind of social insect or highly abundant

predator in a very organised way. We, therefore, suggest with caution that *Dorylus spininodis* could be a relevant 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 because 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 remains 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. 2003), where they consume dead plant material at different stages of decomposition, from woody material to clay soil. They usually 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 & Lee 2002). Our study indicates that worker *Nasutitermes* are foraging a minimum of 10–15 m from a colony centre located within the forest habitat.

All three genera reported from the turtle nests are termed "wood-feeding termites". *Amitermes* is fully subterranean and consumes more decayed wood material (Donovan et al. 2001). 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 inland forest in Gabon (O. Scholtz, personal observations).

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 and Kimsey (1983) were found over the mound of sand and eggs. The nests where termites were found had a typical oxidised aspect (collateral damage). Secondly, dead termites were sometimes found inside predated eggs, in a similar way to ants, suggesting that they might also have to

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 is 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. Consequently, 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, mites and worms are probably attracted as opportunistic hosts to feed on mould, 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 either are 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 & Bjorndal 2000; Hannan et al. 2007). Considering that a leatherback clutch weighs on average 7 kg and the nest densities at Kingere range between 150 and 470 nests per kilometre and season, 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 kilometre (Fig. 3), a contribution that is certainly non-negligible.

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 topics of predation or scavenging. Several questions remain uncertain and need to be elucidated: Is predation the main cause of egg mortality? Are ants primary or secondary predators? Is the primary predator gone by the time of excavation? How ants and other invertebrates find nests? Why so many ants die within the nests during

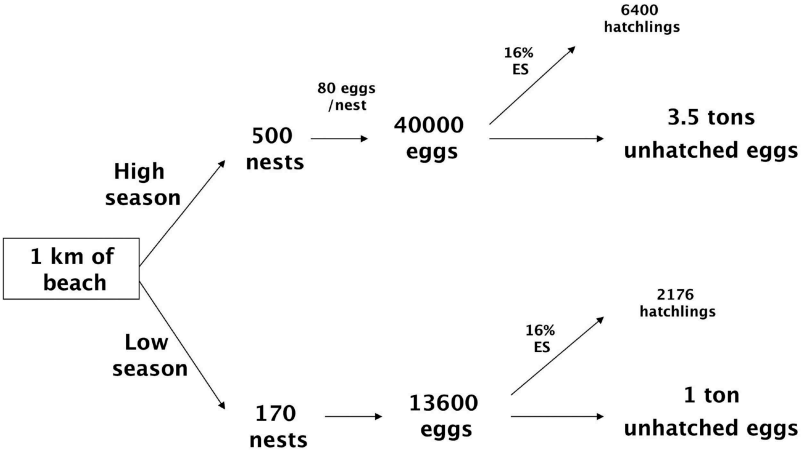

Fig. 3. — The estimated mass of unhatched eggs that remain in a 1 km section of beach at Kingere after one nesting seasons, considering a high density and low-density year.

their predation? Is the leatherback nest an unsuitable or risky site for ants? Are ants opportunistic scavengers of dead eggs? Can we determine or predict the ant predation risk in order to plan leatherback turtle nesting conservation? Is there any external evidence of massive underground ant predation of nests? May so many ants and termites attract to the nest to ant predators? Recent studies indicate that nest site choice by sea turtle females does not affect hatching success. This seems to be the case in the studied leatherback population regarding invertebrate colonisation. Is there a relationship between ant or termite abundance or ant selected microhabitats and nest site selection by leatherbacks?

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 infestation and the existence of predation by ants, we suggest to conduct further studies in which nests are opened at regular intervals throughout incubation to monitor the colonisation and potential predation in a chronological way, assign the type of hole to predator and study the evolution of predated eggs. In addition, 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 been described to infect loggerhead turtle eggs in Cabo Verde (Sarmiento-Ramírez et al. 2010, 2014). This plant pathogen can affect both embryos and hatchlings and is potentially harmful to humans. Further research into this topic with a detailed identification of microorganisms 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.

Recommendations for the management of endangered populations

The cryptic character of this potential predation on nests of an endangered species could make difficult the early detection of severe mortality that can negatively affect the recuperation of the population. The exhumation of nests to detect the level of predation could permit a late response to correct this natural impact. For the management of endangered populations it could be very useful the identification of external signals, interactions or correlations that can permit a quick assessment of this potential threat. This could permit the prioritisation of vigilance and habitat protection of nesting areas with higher hatching success. Moreover, it could permit the earlier relocation of threatened nests to safe beach locations or hatcheries.

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DISCLOSURE STATEMENT

No potential conflict of interest was reported by the authors.

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