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Fibropapillomatosis confirmed in *Chelonia mydas* in the Gulf of Guinea, West Africa

Angela Formia¹, Sharon Deem², Alexis Billes³, Solange Ngouesso⁴, Richard Parnell¹, Tim Collins¹, Guy-Philippe Sounguet⁵, Alain Gibudi³, Armando Villarubia¹, George H. Balazs⁶ & Terry R. Spraker⁷

¹Wildlife Conservation Society-Gabon, Libreville, Gabon (E-mail: aformia@seaturtle.org); ²Smithsonian National Zool. Park, Washington, DC, USA

³Protection Tortues Marines d'Afrique Centrale, Libreville, Gabon; ⁴Conseil National des Parcs Nationaux, Mayumba, Gabon

⁵Aventures Sans Frontières (ASF), Libreville, Gabon; ⁶NOAA-NMFS, Pacific Islands Fisheries Science Center, Honolulu, HI, USA

⁷Colorado Veterinary Diagnostic Laboratory, Colorado State University, CO, USA

Fibropapillomatosis has a global distribution and is the most important health problem affecting free-ranging sea turtles today (Herbst 1994; George 1997). While primarily a disease of immature and young adult green turtles, there is now evidence that fibropapillomatosis occurs in all seven of the extant sea turtle species (Aguirre *et al.* 2002). The underlying etiology is still not fully understood, although there is strong evidence of an underlying viral infection (Herbst *et al.* 1995; Quackenbush *et al.* 1998; Work *et al.* 2001; Work 2005). Tumors appear to manifest more often in stressful habitat conditions, such as regions with poor water quality, under eutrophication and in the presence of contaminants and toxins (Aguirre *et al.* 2002; Ehrhert *et al.* 1996; Adnyana *et al.* 1997). However, a number of documented cases of fibropapillomatosis have been reported in areas considered relatively uncontaminated and studies on the role of environmental contaminants are imperative (Herbst & Klein 1995).

External fibropapillomas sometimes regress if the turtle can maintain its body condition. Treatments include surgical tumor removal and secondary care for debilitation. Mortality rates have yet to be established, but data indicate that the outcome of the disease is highly variable and depends on a number of factors (Work *et al.* 2004; Work & Balazs 1999). When the tumors affect the eyes and mouth, preventing the turtle from foraging effectively, they can

lead to death from starvation. Additionally, internal tumors are particularly devastating (Work 2005).

The Gulf of Guinea has recently emerged as critical habitat for sea turtle populations and has been receiving increased attention from the research and conservation community (Fretey 2001; Formia *et al.* 2003). Corisco Bay is home to an important sea turtle foraging ground (Formia 2002). It is located in the Gulf of Guinea, on the border between Equatorial Guinea and Gabon, and comprises approximately 1500 km sq. of warm shallow waters on the west African continental shelf. The most common sea turtles in the Bay are adult and immature green turtles (*Chelonia mydas*). Hawksbills (*Eretmochelys imbricata*), leatherbacks (*Dermochelys coriacea*) and olive ridleys (*Lepidochelys olivacea*) also occur occasionally in the Bay and nest sporadically. The ecology of the area is still largely unknown, but both green turtles and hawksbills feed here throughout the year (Formia *et al.* unpublished data).

Genetic mixed stock analysis of the green turtles in Corisco Bay has shown that potentially contributing rookeries include Ascension Island, Bioko, São Tome, Principe, as well as Suriname, Comores and Mexico (Formia 2002). Local inhabitants have hunted green turtles for generations both for local and commercial consumption in the urban centers of Bata (Equatorial Guinea) and Libreville (Gabon). Research and conservation efforts are underway to halt

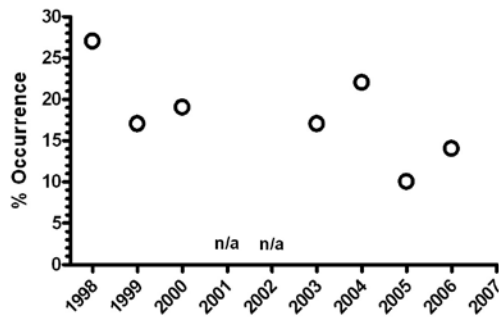


Figure 1. Annual prevalence of fibropapillomatosis in Corisco Bay green turtles (1998 n= 33; 1999 n=151; 2000 n=53; 2003 n=53; 2004 n=54; 2005 n=62; 2006 n=77).

turtle hunting in the Bay and decrease market demand for turtles in the cities.

Before transport and sale, captured turtles are often held in enclosures for several days where close observation by researchers can be carried out. Based on visual assessments, we have documented large abnormal masses of the skin on several individuals. These masses tend to be distributed around the eyes and mouth, neck, tail and the ventral surface of the anterior flippers. Based on the appearance and location of these growths (Balazs & Pooley 1991; Herbst 1994), we hypothesized that fibropapillomatosis was present in the population.

In April 2000, tissue biopsies were collected from an immature green turtle captured in Corisco Bay (near the islet of Mbanye, on the border between Equatorial Guinea and Gabon), West Africa. One skin biopsy was taken from healthy tissue, and seven biopsies from seven different tumors attached to the skin of the neck and inguinal regions. Biopsy sites were pre-treated with topical betadine, tissue samples collected using a 6 mm biopsy punch, and the site again cleaned with betadine at the end of the procedure. The turtle was then measured (67 cm curved carapace length and 62 cm curved carapace width), tagged on the anterior flippers (ECO-813 left,

ECO-815 right), and released.

All tumor biopsies were stored in 10% buffered formalin at room temperature, except a single biopsy from the abnormal tissue which was placed in 70% ethanol for parasite evaluation. A healthy skin sample was also collected for mitochondrial DNA analysis and was found to exhibit the common Atlantic haplotype CM-A8 (Formia 2002).

Histopathological analysis of the samples was carried out at the Colorado Veterinary Diagnostic Laboratory (USA). Microscopically, all masses were diagnosed as fibropapillomas based on proliferation of epidermal cells, dermal fibroblasts, or both (Jacobson *et al.* 1989). These tumors were similar to tumors from other regions of the world except there was increased blister formation and/or early microabscess formation within the epidermis. Additionally, there was a slightly increased infiltration of heterophils, both within the tumor and within the epidermis, and increased cellular vacuolation. None of the seven biopsies we evaluated had evidence of regeneration within the tumors. Intranuclear inclusion bodies, although not extremely distinct, were present in three cells of one of the biopsies observed. There were no parasite eggs detected in any of the biopsies.

Data collected over seven years (1998 – 2006) on the green turtle population of Corisco Bay reveal a 17% prevalence of probable fibropapillomatosis in captured turtles (82 out of 483 individuals) we examined. These 82 turtles had an average curved carapace length of 71.5 cm (n=82; SD 10.5 cm; range=49-97 cm), and six of the 82 were morphologically identified as males, the remainder were females or immatures. Sampling effort was not uniform during the seven years of data collection. However, we found no apparent trend in annual fibropapillomatosis prevalence (Figure 1).

In October 2006 we recorded a green turtle live stranded in Mayumba National Park, in southern Gabon, with multiple growths consistent with fibropapillomatosis (Figure 2). In addition, we noted the presence of leeches near one of the tumors (Figure 2). Interestingly, marine leeches have been found to carry high loads of fibropapilloma-associated turtle herpesvirus DNA, implicating them as a possible mechanical vector (Greenblatt *et al.* 2004). Histological evaluation of biopsy samples collected from this turtle is pending.

To the authors' knowledge, described here is the first histopathologically confirmed report of fibropapillomatosis of sea turtles in Central or West Africa. To date, the conditions contributing to the presence of fibropapillomatosis in Corisco Bay are unknown. There are no other cases reported from other sea turtle species in Gabon or Equatorial Guinea (hawksbills, leatherbacks and olive ridleys), or from green turtles elsewhere in the region, despite regular monitoring activities. No apparent fibropapillomatosis has been reported among the nesting females of Bioko Island (Equatorial Guinea; J. Tomas pers. comm.), or the adults and immatures found in continental Equatorial Guinea and in São Tome and Principe (AF, unpublished data).

However, field biologists and technicians in the region are receiving further training

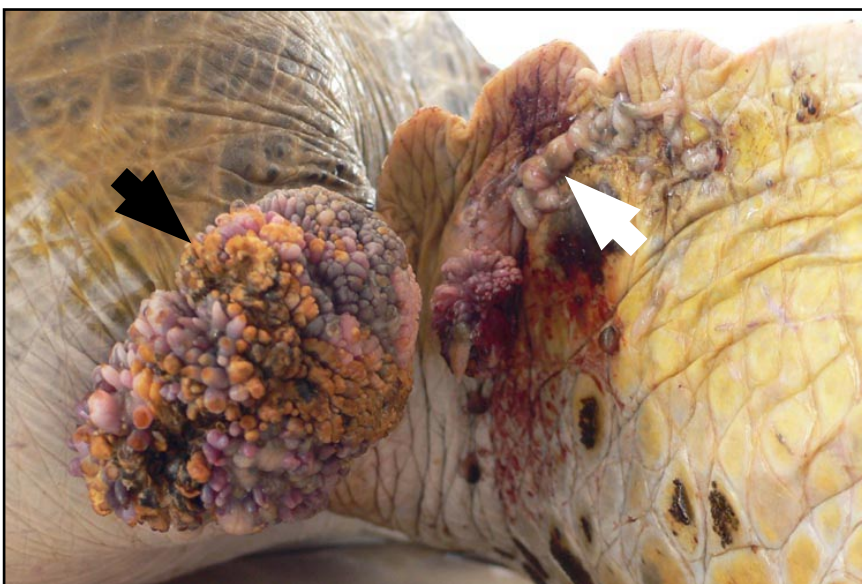


Figure 2. Right anterior flipper of a green turtle stranded in Mayumba National Park, southern Gabon. Note the fibropapilloma with highly rugose structure (black arrow) and presence of leeches (white arrow). Photo credit: Tim Collins.

in methods of health assessment based on visual examinations, including assessing the presence or absence of fibropapilloma-like growths, and documenting mortalities throughout the region. Training and data-collection will also be expanded to include necropsies and in-water prevalence studies. Future lines of investigation on this disease in the region should also include testing tumors for herpesvirus DNA. Overall, health monitoring of the sea turtles in the Gulf of Guinea will help us determine the true prevalence of fibropapillomatosis and other disease threats, in this region of such high conservation value.

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Measuring a male green turtle captured by Benga fishermen in Gabon (photo by Alejandro Fallabrino).

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