# Tumors in Sea Turtles

## THE INSIDIOUS MENACE OF FIBROPAPILLOMATOSIS

By Thierry M. Work and George H. Balazs

Credit: Jonathan Sleeman

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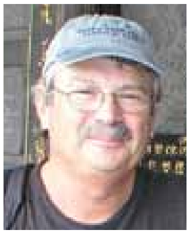

Credit: Thierry M. Work

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Early in July 2013, a colleague in New Caledonia reported the stranding of a green sea turtle on the far northwest of the island. The animal had washed up dead on a rocky beach with multiple large tumors on its neck and hind flippers. To all appearances, the turtle had fibropapillomatosis (FP), a tumor disease affecting marine turtles globally. This was the first known case of FP on the island—an alarming find, and another example of the creeping expansion of this disease in green turtles around the world.

Notably, this expansion garners little attention, for several reasons. Biologists have known about and observed FP in sea turtles since it was first described in the late-1930s in Florida green turtles (Smith and Coates 1938). We know that the disease is chronic, and that the degree of affliction is variable. Some turtles may have multiple massive tumors that affect their ability to swim or eat, leading to slow and lingering death, while others may have minimal tumors with little or no observable effect on the animal's lifespan. Because marine turtles are largely out of sight and difficult to access, FP remains something of an "orphan disease"—off on its own, little studied, even dismissed as old news.

That's unfortunate. All seven species of marine turtles are either threatened or endangered and face multiple threats from hazards such as trawler nets, egg poaching, and pollution. The expansion of FP tumors is just one more stressor, and it's one that scientists need to learn more about. The reasons for its emergence, the geographic pattern of infection, and the degree to which human and environmental stresses are exacerbating the disease all remain unclear. It's therefore essential that we devote more resources to studying and managing this illness, for the sake of sea turtles and, potentially, as a means to better understand coastal ecosystem health.

Working through the USGS National Wildlife Health Center and NOAA's Pacific Islands Fisheries Science Center, both in Hawaii, we have been studying FP in sea turtles for over 15 years. It's a challenging task, in part because of major gaps in knowledge about the animals' life histories, and because many of the tools and tests used to investigate disease in terrestrial animals are not suitable for sea turtles. Yet by teasing out information about FP disease, we're also learning more about sea turtles in general, marine ecosystem health, the epidemiology of chronic disease, and how viruses generate tumors in animals.

### Learning What's at Risk

To understand FP, it's helpful to understand the complex life history of sea turtles. The seven species—including the leatherback (*Dermochelys coriacea*), hawksbill (*Eretmochelys imbricata*), olive ridley (*Lepidochelys olivacea*), Kemp's ridley (*Lepidochelys kempii*), flatback (*Natator depressus*), loggerhead (*Caretta caretta*), and green (*Chelonia mydas*)—spend most of their lives in the tropics and subtropics.

To illustrate the complex life history of marine turtles, we'll focus on greens, which are plentiful in Hawaii. Of the seven sea turtle species, adult greens are the only herbivores, foraging on sea algae and sea grass; the remainder forage on various invertebrate prey including jellyfish, crustacea, or sponges. During the breeding season, resident adult green turtles migrate from their foraging pastures in the main Hawaiian islands to the principal nesting beaches at French Frigate Shoals, located about 800 kilometers north and west from Oahu. There, adult females breed, crawl up the beach, excavate a nest in the sand, lay and bury their eggs, then depart, having no further contact with the young.

Eggs hatch after about two months, and hatchlings strive to make their way from nest to sea, during which time they must cross a gauntlet of predatory crabs and other hazards. Turtles that survive to reach the sea eventually disappear into the pelagic zone, where they feed on small invertebrates and face additional hazards, such as predation. After four to nine years, surviving juveniles return to near-shore foraging pastures, where they transition from a carnivorous to herbivorous diet and grow to adulthood. Scientists estimate that less than 0.1 percent of hatchlings survive to become nesting adults (Frazer 1986).

To understand how all this complicates the study of FP, consider that it's analogous to a medical doctor wishing to understand a childhood disease but having fleeting access to babies who mysteriously disappear immediately after birth and magically show up later as four to nine year olds. Some answers to the illness may lie in that gap.

## An Oddly Selective Disease

The tumors of FP are essentially a form of cancer. Notably, the disease almost exclusively affects green turtles and, to a lesser extent, olive ridleys and loggerheads. The majority of papers on this topic—more than 80 percent—deal with green turtles. It is extremely rare in the remaining four species of sea turtles (Herbst et al. 1995), and we have no idea why. Turtles affected with FP typically get tumors on the skin of the flippers and neck, eyes, corners of the mouth, cloaca, and internal organs. Some tumors can exceed the size of a cantaloupe.

Interestingly, green turtles with FP will show different clinical signs depending on whether they live in Florida or Hawaii, the two areas where the illness has been most comprehensively studied. In Florida, for instance, liver tumors in turtles with FP are common (Herbst 1994), but such tumors are rare in Hawaii. Tumors in the mouth are relatively common in Hawaiian green turtles, but rare elsewhere (Aguirre et al. 2002). These oral tumors are particularly insidious because they impede closure of the trachea, and affected turtles therefore often aspirate foreign material, leading to infections and inflammation of the lungs. In such cases, the disease is invariably fatal.

Fibropapillomatosis debilitates sea turtles through a variety of other mechanisms. Skin tumors can become abraded, thereby opening portals to bacterial infections in affected animals. The sheer physical size of some tumors can lead to blindness (which interferes with swimming) or an inability to eat (leading to starvation). Severely affected animals can also become immunosuppressed. When sea turtles are severely ill or debilitated, they can't effectively swim or navigate and therefore often strand on shore. In Hawaii (Chaloupka et al. 2008) and Florida (Foley et al. 2005), FP is the leading cause of stranding in green turtles. Although we have no data on mortality rates, we know that turtles that are moderately to severely tumored become severely emaciated and immunosuppressed (Work et al. 2001) and thus have a low likelihood of survival.

Credit: George H. Balazs

A dead green turtle found stranded on Maui displays the grotesque tumors caused by fibropapillomatosis (FP), a viral disease affecting marine turtles around the globe. Large tumors can impact turtles' ability to see, swim, and eat, sometimes leading to their death. Veterinary disease specialist Thierry M. Work (below, left) and colleagues in Honolulu perform a necropsy on a green turtle with large FP tumors on vital internal organs, likely the cause of death.

Credit: Bob Rameyer

Despite its effect on individuals, there is no evidence that FP has significant demographic impacts. Growth rates in sea turtles with FP in Hawaii (Chaloupka and Balazs 2005) and Florida (Kubis et al. 2009) do not differ widely from the rates in unaffected animals. The same holds true for survivorship in Puerto Rico, where 53 to 83 percent of sea turtles survive to recapture, whether affected with FP or not (Patricio et al. 2011). In addition, about 84 percent of sea turtles in Florida have tumors that regress, (Hirama and Ehrhart 1999), meaning they either dramatically shrink in size or disappear completely.

Credit: Thierry M. Work

A green turtle in Hawaii glides through a cleaning station in Kauai, where fish graze on algae, barnacles, and other epibionts attached to the turtle's skin and shell. Some cleaner fish have tested positive for the virus associated with FP, leading some scientists to speculate that the fish may be involved in virus transmission.

Only 33 percent of turtles with FP in Hawaii experience tumor regression (Bennett et al. 1999), yet there are some very positive overall trends for green turtles in that region. For example, though the prevalence of FP in greens peaked to a rate of about 50 percent in the mid-1990s, prevalence has since declined to less than 20 percent (Chaloupka et al. 2009). Also in Hawaii, the numbers of nesting turtles have been increasing at approximately 5 percent annually since the 1970s (Balazs and Chaloupka 2004). The reasons behind all these trends remain a mystery.

## Speculations on the Cause

Clearly it's troubling to see charismatic sea creatures plagued by hideous tumors, which adds urgency to the question about what causes FP and to what extent people and climate may be spurring its geographic expansion. Early research suggested that FP might be associated with flatworms, or blood flukes, that live in the turtle's circulatory system. Indeed, 100 percent of turtles with FP are infected with these worms. Each day they lay thousands of eggs, which lodge in capillaries throughout internal organs. Because the eggs are foreign material, they prompt the host to mount a chronic inflammatory response that can be physiologically demanding (Aguirre et al. 1998). This parasite is difficult to study because we do not know its life cycle, although we suspect some sort of snail might be involved (Stacy et al. 2010). However, a high prevalence of turtles unaffected by FP are also infected with the blood flukes.



It is more likely that a virus is involved with this disease, as both epidemiology and physical observations point to an infectious cause of FP. Captive, tumor-free green turtles in Florida, for example, have developed FP after contact with seawater from a tank that housed a tumored turtle (Hoffman and Wells 1991). And microscopy has revealed the presence of herpesvirus particles in some tumors (Jacobson et al. 1991), an important discovery because herpes viruses are known to cause tumors in other animals, including humans. The AIDS-associated skin tumor in humans called Kaposi's sarcoma, for example, is closely associated with a herpes virus. After 50 years of people studying this disease, there is no evidence that the turtle herpes is transmissible to humans.

To confirm that FP was indeed transmissible among turtles, investigators took samples of tumor homogenates, filtered out all particles bigger than bacteria, and injected the material into tumor-free turtles, which soon developed tumors at the injection sites (Herbst et al. 1995). This showed that FP was caused by a "filterable agent" (smaller than a bacterium). Subsequent research using molecular tools revealed large amounts of herpes virus DNA in tumors but little to none in non-tumored tissues (Quackenbush et al. 1998). This particular herpes virus has been named chelonid fibropapilloma herpes virus (CFPHV) to reflect the virus type (herpes), the disease with which it is associated (FP), and the hosts affected (Cheloniidae) (Ene et al. 2005). Molecular studies suggest that CFPHV has existed in sea turtle populations for millions of years (Patricio et al. 2012).

Researchers have been exploring how CFPHV may be transmitted between turtles. Among the theories:

- Tumors are more numerous in the front of the animals, so perhaps turtles come into contact with infected material as they explore their environment (Work et al. 2004). We know that the virus can remain infectious in seawater (Curry et al. 2000).
- Virus DNA has been found in leeches that cling to tumors of some turtles, suggesting the leeches may act as a vector.
- Sea turtles commonly hang out in portions of the reef called "cleaning stations," where they allow cleaner fish to graze on algae and other epibionts that grow on their skin and carapace. Such cleaner fish have been observed biting tumors, and

CFPHV DNA has been detected in some species of cleaner fish (Lu et al. 2000), so perhaps cleaner fish transmit the disease. We aren't yet certain.

Equally perplexing: Prevalence of FP within a single area can be mysteriously erratic, with the distances between areas where resident turtles are affected or tumor free sometimes remarkably short. For example, on the island of Hawaii, FP is prevalent in turtles along the east coast but not the west coast (Work et al. 2001). Likewise, in Florida, turtles in the inner reefs of the Indian River Lagoon have a high prevalence of FP, whereas prevalence is low in the nearby Sabellid Reef less than one kilometer away (Holloway-Adkins and Ehrhart 2005). This characteristic complicates the question of transmission.

## Signs of Human Complicity

With the notable exception of Hawaii (Chaloupka et al. 2009), the prevalence of FP appears to be stable or increasing in most regions of the world, and human action may lie at the root of the expansion. For example, FP in Brazil is particularly prevalent in Espirito Santo Bay surrounded by heavy industry (Santos et al. 2010) or in areas where invasive algae are prominent in Hawaii (Van Houtan et al. 2010) suggesting that a dietary or other environmental co-factor may be involved. The disease is also more prevalent along coastlines with a broad continental shelf (Fick et al. 2000), but reasons for this are unclear.

To tease out the role of environment and virus in causation of FP, we must confirm whether herpesvirus is the causal agent of FP tumors, or whether tumored tissues somehow encourage herpesviral growth. To answer those questions and definitively establish causation between an infectious agent and a disease, we must be able to experimentally isolate and replicate the agent, observe it causing the disease, and re-isolate the agent from the diseased tissue. Unfortunately, it's been extremely hard to grow and purify CFPHV in the lab (Work et al. 2009) because the virus simply will not grow in cell culture. This has been a challenge with some human herpes viruses as well, such as the virus associated with human Kaposi's sarcoma. This inability to grow and manipulate the virus has prevented us from developing a blood test to detect FP.

If we had a blood test that could reliably detect antibodies to the FP herpesvirus infection in sea turtles, we could conceivably identify animal populations where the virus is circulating but where disease has not yet appeared. For instance, if turtles historically are testing negative but suddenly start becoming antibody positive to CFPHV and later develop tumors, this would be evidence of incursion of CFPHV into the environment and provide additional evidence of its role in causing tumors. Such forewarning would indicate that the virus is in the environment, alerting managers that tumor outbreaks may be on the way. Managers could then consider intervention, such as permanent removal of tumored animals and cleaning up coastal pollution.

Credit: Peter Bennett/www.turtles.org

**Tumor Regression** A green turtle photographed in Maui in 1993 (top) has FP tumors around its face and neck. The same turtle, photographed in 2004 (bottom), is tumor-free. Such tumor regression occurs in 33 percent of turtles with FP in Hawaii, but scientists don't yet know why.

As coastal ecosystems continue to degrade, it is likely that FP will continue plaguing sea turtles. The visibility of the disease in these charismatic animals invariably prompts public concern, one reason why researchers must strive to learn what is driving the illness. In the case of FP, we may ultimately come to find that prevention and cure lie in proper treatment of wastewater and agricultural and storm runoff. Discovering the cause of FP in sea turtles and addressing that cause may therefore lead to more responsible development and cleaner oceans for all marine life. ■

*This article has been reviewed by subject-matter experts.*