NOAA Technical Memorandum NMFS-PIFSC-54



August 2016 doi:10.7289/V5/TM-PIFSC-54

Proceedings of the 2015 International Summit on Fibropapillomatosis: Global Status, Trends, and Population Impacts



Stacy Hargrove, Thierry Work, Shandell Brunson, Allen M. Foley, and George Balazs

Pacific Islands Fisheries Science Center National Marine Fisheries Service National Oceanic and Atmospheric Administration U.S. Department of Commerce

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Hargrove, S., T. Work, S. Brunson, A. M. Foley, and G. Balazs.
2016. Proceedings of the 2015 international summit on fibropapillomatosis: global status, trends, and population impacts. U.S. Dep. Commer., NOAA Tech. Memo., NOAA-TM-NMFS-PIFSC-54, 87p. doi:10.7289/V5/TM-PIFSC-54

Cover Photos: (Left) Hawaiian green turtle with advanced fibropapillomatosis in 1993. (Right) Same turtle, tumor-free in 2004 (Chaloupka et al., 2009 Journal of Wildlife Diseases; photos by Peter Bennett and Ursula-Kueper Bennett used with permission).

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EXECUTIVE SUMMARY

The 2015 International Summit on Fibropapillomatosis (FP) was convened in Honolulu, Hawaii June 11-14, 2015. Scientists from around the world were invited to present results from sea turtle monitoring and research programs as they relate to the global status, trends, and population impacts of FP on green turtles. The participants engaged in discussions that resulted in the following conclusions:

- 1. Globally, FP has long been present in wild sea turtle populations—the earliest mention was in the late 1800s in the Florida Keys.
- 2. FP primarily affects medium-sized immature turtles in coastal foraging pastures.
- 3. Expression of FP differs across ocean basins and to some degree within basins. Turtles in the Southeast US, Caribbean, Brazil, and Australia rarely have oral tumors (inside the mouth cavity), whereas they are common and often severe in Hawaii. Internal tumors (on vital organs) occur in the Atlantic and Hawaii, but only rarely in Australia. Liver tumors are common in Florida but not in Hawaii.
- 4. Recovery from FP through natural processes, when the affliction is not severe, has been documented in wild populations globally.
- 5. FP causes reduced survivorship, but documented mortality rates in Australia and Hawaii are low. The mortality impact of FP is not currently exceeding population growth rates in some intensively monitored populations (e.g., Florida and Hawaii, USA and Southern Great Barrier Reef stock Queensland, Australia) as evidenced by increasing nesting trends despite the incidence of FP in immature foraging populations.
- 6. Pathogens, hosts, and potential disease and environmental cofactors have the capacity to change; while we are having success now, there needs to be continued monitoring to detect changes in the distribution, occurrence, and severity of the disease.
- 7. While we do not have clear evidence to provide the direct link, globally, the preponderance of sites with a high frequency of FP tumors are areas with some degree of degradation resulting from altered watersheds. Watershed management and responsible coastal development may be the best approach for reducing the spread and prevalence of the disease.
- 8. Future research efforts should employ a multi-factorial ecological approach (e.g., virology, parasitology, genetics, health, diet, habitat use, water quality, etc.) since there are likely several environmental cofactors involved in the expression of the disease, which is still thought to be caused by a herpesvirus.
- 9. Minimum FP data collection in new areas should include: individual identification (photo ID, PIT tags, etc.), standard measurements (length and weight), presence/absence of tumors, tumor severity, body condition, oral examination, method of capture, and effort.

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INTRODUCTION

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I am honored to have been asked by my Steering Committee colleagues to write this introduction to the Proceedings of the International Summit on Fibropapillomatosis, convened in Honolulu, Hawaii, June 11–15, 2015. In doing so, I have relied heavily upon decades of personal experience living and working in Hawaii. Depending upon my frame of mind, from year-toyear, and even month-to-month, I feel either fortunate, or unfortunate, to have spent the past 43 years of my professional life with Hawaiian green turtles witnessing and immersed within the tortuous up-and-down historical pathway of the grotesque, cruel, and often humanly heartbreaking disease known as fibropapillomatosis (FP). I am acutely aware that many others in Hawaii and globally, where afflicted turtles occur, deeply share these emotional and scientific concerns for the health of the seas and the survival of sea turtle populations. Indeed, to draw deeper upon the Hawaii human experience for comparative purposes, FP disease has outward similarities to the disfiguring and ultimately fatal disease of leprosy. Starting in 1888, Hawaiians and others found to have signs of leprosy were forcibly isolated and abandoned without hope, at the remote Kalaupapa Peninsula of Molokai. Eventually, in the 1930s, medical research resulted in a means to prevent progression of the disease (known as Hansen's disease) and eliminate its contagious nature. However, in the ensuing years, over 8,000 people, mostly Hawaiians, died at Kalaupapa, including Samaritans from overseas that risked infection to care for and comfort the afflicted, both physically and spiritually. The history of leprosy in Hawaii is highly recommended reading, as is a guided-tour to modern-day Kalaupapa National Park, for all those that grieve for the plight of sea turtles afflicted with FP disease.

There was a time when FP disease seemed to signify, to many of us, the biological extinction of the Hawaiian green turtle, just as leprosy and so many other adverse impacts seemed to foretell the fate of the indigenous Hawaiian people. Such prior worries, while fully justified, failed to come true and likely never will, given population resiliency, self-determination, and the helping hands of many that care. However, as eloquently stated herein in Brian Stacy's Keynote Address, we should make no mistake that FP remains an important disease in need of continuing attention, requiring an array of dedicated people, expertise, and resources. Although substantial progress has been made in understanding the disease, there are still many questions whose answers would facilitate effective conservation and management, especially at locations where new outbreaks of the disease may occur. Included amongst the most bewildering unknowns to me is the prominent manifestation of oral tumors in Hawaii that almost never occur in other populations worldwide.

The purpose for convening the 2015 International FP Summit was to "provide a forum to assess the status and trends of the disease globally and its demographic impact on sea turtles" as detailed in the Terms of Reference (Appendix A). To identify regions to be represented, the Steering Committee conducted a survey poll (Appendix B). From the 47 responses received, 6 broad regions of priority were selected and travel invitations extended to key scientists having longer-term data and insights for each region. Important cornerstones for the 2015 International FP Summit were two prior workshops held in Hawaii in 1997 and 1990, 19 and 26 years ago (Balazs and Pooley with multiple contributors; and Appendix C, 1997 Priorities for Research Summary). The workshop agenda (Appendix D), list of participants (Appendix E), map of the global distribution of FP (Appendix F), and an updated Bibliography of Fibropapillomatosis in Marine Turtles (Appendix G) are included in this report.

There can be no doubt that the story of FP disease will continue to unfold leading to improved understanding, resolution, and ultimately a cure, whether medical or ecological, or a combination of both. These Proceedings form a strong step forward in the challenging but necessary process.

ACKNOWLEDGEMENTS

The Steering Committee expresses its appreciation to the Joint Institute for Marine and Atmospheric Research with special thanks to staff members Devon Francke and Denise Parker for significantly contributing to the success of the FP Summit. The Western Pacific Regional Fishery Management Council kindly made possible the participation of quantitative specialist Milani Chaloupka, along with providing meeting facilities for the final session day of the FP Summit.

PRESENTATION ABSTRACTS

Keynote Address: Fibropapillomatosis in 2015: A Historical Review & Modern Perspective on Why It Remains an Important Disease

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Fibropapillomatosis (FP) is a neoplastic disease that affects the epidermis or outer layer of cells comprising the skin and the stromal cells or fibroblasts of the underlying dermis and other internal tissues. Its classical manifestation is multiple papillary or cauliflower-appearing tumors, especially on the proximal flippers, axilla, and inguinal areas that vary in size and appearance depending on the degree of pigmentation and stage of the disease. It is principally known as a cause of morbidity and mortality in green turtles, but has been reported in all sea turtle species. Neritic life phases, especially immature animals, are primarily affected. Available historical data indicate that the disease rose in prevalence most noticeably in the 1980s. It is now known to occur in sea turtle populations worldwide with regional and locality differences in prevalence.

Fibropapillomatosis first appeared in the scientific literature in the late 1930s. Smith and Coates described the disease in 1938 in a sea turtle at the New York aquarium that was originally captured in Key West. They also reported it in a small number of additional green turtles captured in the Key West turtle fishery. The same year, Lucké described FP in a turtle caught off Cape Sable, Florida. The first photo of an FP turtle from Hawaii dates from the late 1950s. Little to nothing is known about the status of the disease in Florida and Hawaii during the 1960s and 1970s, although green turtle populations had significantly declined under pressure from harvest. The first resurfacing of the disease in US waters was documented in the 1980s, when stranding monitoring programs and in-water studies began encountering increasing numbers of turtles with the disease. Against the backdrop of alarmingly low green turtle numbers, concerns escalated that the disease could be catastrophic to decimated populations. As attention turned to understanding the disease and its etiology, the earliest observations of herpesvirus-like particles in tumors was made in the late 1980s. Researchers made substantial progress studying the disease and its occurrence in various parts of world, revealing an association among FP, shallow inshore systems, and altered habitat. A seminal study by Herbst and others published in 1999, demonstrated tumor formation in captive-reared juvenile green turtles using cell free tumor homogenates treated in a manner to preserve the infectivity of enveloped DNA viruses, such as herpesviruses. As molecular tools became increasingly available, the associated herpesvirus was studied in various populations showing that it is actually a group of close viruses or variants, with regional distributions that diverged long before the disease emerged as a panzootic, suggesting that environmental or ecological factors are key to the global occurrence. Other potential etiologies were also investigated, including spirorchiid trematodes (blood flukes), individual health parameters (e.g., immune function), and contaminants, and were not shown to play a primary role in development of the disease. Positive news in terms of the outlook for FP in green turtle populations was reported the late 2000s when a decline in prevalence was reported

in some localities in Hawaii, suggesting that FP may eventually subside to some lower level of occurrence as an endemic disease.

Despite what can be a severely debilitating and lethal disease, with apparent prevalence exceeding 50% in some regions, the green turtle is on a positive course for recovery following mitigation of key direct anthropogenic threats in many areas where FP was originally observed. However, FP still remains an important disease for a number of reasons. There undoubtedly are negative effects at individual and population levels, although these costs are difficult to quantify. Although FP is not preventing success of conservation measures, the vision or objective of population recovery generally entails healthy turtles, not only numbers of animals.

Fibropapillomatosis is one of the most visible affronts to the notion that turtles within a given area are in good health. As charismatic species that draw considerable attention from the public, FP leaves a lasting impression upon people and quickly engenders concerns for the health of sea turtles and the environment. Providing humane treatment to sea turtles with FP, which is a common societal expectation, consumes substantial resources in terms of costs of responding to stranded turtles with FP and rehabilitation. In addition, the link with altered habitat warrants caution with regard to expectations of future disease trends. Coastal development, land use, and run-off are constant threats to marine ecosystems and undoubtedly will become an increasing concern as the human population grows. As green turtle numbers increase, recruitment into new areas with degraded habitat also may lead to expansion of the disease. Furthermore, the effects of climate change on FP, sea turtle physiology as it relates to manifestation of the disease, and habitat also are highly uncertain.

Management of FP with the objective of facilitating a decrease in the occurrence in sea turtle populations presents a number of formidable difficulties. Many of the tools applied to management of wildlife diseases in terrestrial taxa, such as vaccination and selective culling, are impractical to effectively implement in sea turtles, even if logistical and ethical issues were resolved. The reduction in apparent prevalence in some areas offers promise that the disease may subside or wax and wane as the result of natural factors, such as development of population-level immunity and natural selection. However, such processes are unlikely to provide reprieve to trustee agencies, resource managers, stranding and rehabilitation networks, and others who currently deal with FP in its current status.

Although the relationship between FP and environmental cofactors is not well understood, there are certainly other effects of degraded habitat that are readily apparent, such as algal blooms and mortality and diseases of other marine life, including seagrasses, corals, fish, and marine mammals. Perhaps synergy could be gained from an ecosystem-wide approach that mitigates habitat degradation and achieves a natural experiment of sorts in areas where turtles are affected by FP. Research should continue to explore associations between FP and environmental or ecological factors, but response to mitigation of impacts known to be ecologically harmful would be beneficial, at least as a parallel effort, and seems to be the principal management option emerging from research findings to date. Ultimately, getting definitive answers about a complicated, multifactorial disease may be on the same order of difficulty as effecting meaningful reversal of anthropogenic effects on marine habitat.

Marine Turtle Fibropapillomatosis Infection Etiology

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Fibropapillomatosis (FP) causes external and internal tumors in green turtles. External tumors are fibropapillomas with epidermal intranuclear inclusions occasionally visible that have herpesvirus-like morphology on electron microscopy. Internal tumors are fibromas, myxofibromas, or fibrosarcomas of low grade malignancy. Turtles with FP also have a high prevalence of infection with vascular trematodes comprising 4 species that cause chronic inflammation in multiple organs. Historically, in Hawaii, FP complicated by vascular flukes is the major cause of stranding, and ca. 25% turtles have internal tumors mainly in the lung, kidney, and musculoskeletal system. A scoring system was devised in Hawaii to assess severity of disease, and this, along with development of lab tests to assess immune response, revealed that FP causes immunosuppression in sea turtles but is not a prerequisite for development of disease. Animals in tumor score 2 or 3 category have decreased white cells, decreased cell mediated immunity, low plasma protein, and bacteremia. Parasites were thought to be associated with tumors because of presence of trematode eggs in various tissues. However, experiments in Florida using cell free filtrates revealed that FP is a transmissible filterable agent (virus). Subsequent molecular studies in Hawaii and Florida revealed close association with presence of herpesviral DNA (Chelonid herpesvirus 5-ChHV5) in tumors but not normal skin. Herpesviral DNA was also found in greater amounts in superficial tumors. Unfortunately, the virus cannot be cultured in the lab, and this has precluded development of laboratory tests to assess exposure. Molecular analyses in Florida suggest that there is geographic population structure in ChHV5 genotypes implicating that turtles get infected after recruitment to nearshore foraging pastures. A serological test was developed for Florida green turtles using bacculovirus expressed proteins (glycoprotein H) from ChHV5 and revealed that tumored and non-tumored turtles from FPendemic and FP-free areas have antibodies to ChHV5 emphasizing the fact that factors above and beyond virus infection may play a role in tumor development. No one knows for sure how the virus is transmitted. Molecular assays show that virus can be detected in leeches and cleaner fish, so these are possibilities. A recent study in Hawaii looked at shedding of virus in skin of tumors and showed that a minority of tumored turtles (superspreaders) are responsible for the majority of virus shedding and that smaller tumors shed more virus than larger ones.

Role of Environmental Pollution in Fibropapillomatosis of Marine Turtles

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The hypothesis that environmental pollutants may play a role in the etiology of fibropapillomatosis (FP) has spanned three decades. The rationale for this idea stems from three observations. Firstly, FP rates are correlated with degraded habitat quality, which has been seen in several locations across the world. Secondly, many environmental pollutants or marine natural products are carcinogenic and/or have the ability to promote tumors through mechanisms like immunosuppression. Finally, turtles with tumors, especially those at later, severe stages, show signs of suppressed immune systems. Thus, it is rational to suspect exogenous chemicals that are tumor promoting or immune suppressing could contribute to FP, whether they are manmade pollutants or natural products. Testing this hypothesis is challenging, where do you begin? There are over 35,000 man-made chemicals in high volume production in the U.S. and many more natural products. In the published scientific literature, only six studies have attempted to address this hypothesis. Three of them provided evidence that man-made organic pollutants, like polychlorinated biphenyls (PCBs) and pesticides, do not contribute to the disease onset. Heavy metals were investigated in one study, but the sample size was too small to investigate differences between tumored and non-tumored turtles. Natural products were examined in the remaining three studies. Okadaic acid is a known tumor promotor and is produced by the Prorocentrum dinoflagellate that grows on green turtle algal prey in Hawaii. The dinoflagellates were more prevalent in areas where FP was present compared to FP-free areas, and okadaic acid concentrations were higher in kidneys of turtles with severe FP compared to moderate FP. Lyngbyatoxin A is produced by Lyngbia majuscula, a cyanobacterium present in Hawaiian and Australian green turtle foraging habitats. Estimated daily exposure to lyngbyatoxin A was significantly higher for turtles from higher FP habitats. Finally, amino acid profiles were investigated in green turtle algal prey from a variety of sites across the Hawaiian Islands. In watersheds that have a higher nitrogen footprint from human land use, higher levels of arginine were found in the algae. Arginine stores four nitrogen atoms in each molecule and has tumor promoting activity in certain cases. A previous study showed that FP rates were higher in the watersheds with higher nitrogen footprint, but a direct link between arginine (or other amino acids) in the algae and FP has not been documented. While several man-made chemicals have been ruled out as contributors to FP, many more pollutant classes need to be investigated. Preliminary evidence has shown that biotoxins, like okadaic acid and lyngbyatoxin A, could play a role, but more studies are needed. Finally, nutrients and effects of eutrophication (blooms of harmful species) remain a strong co-factor without a clear understanding of the mechanisms behind FP tumor promotion.

Wildlife Epidemiological Investigations: Moving from Observation to Understanding Processes

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Epidemiology is the investigation of the patterns, causes, and effects of health and disease conditions on populations. Epidemiological investigations of animal populations have a long history, dating back at least to the Greek philosopher, Aristotle; however, investigations in wildlife populations are relatively new. The recent emphasis on wildlife stems from a growing recognition that the health of human and domestic animals is intricately linked to the health of wildlife, particularly as anthropogenic factors increase the interactions of these groups. Therefore, the main motivations for conducting wildlife epidemiological investigations are 1) detecting emerging or re-emerging diseases or changes in characteristics of infection, 2) investigating diseases that may affect the population dynamics of a species of interest, and 3) examining diseases that may affect human or domestic animal health. This is clearly a recapitulation of the "One-Health" paradigm. Given these motivations, the process of wildlife epidemiological investigations can generally be characterized in 3 stages: 1) the exploration stage, 2) the investigation stage, and 3) the action stage. The ultimate goal of wildlife epidemiological investigations is to obtain a "critical mass" of understanding to be able to move into the action stage and implement targeted and efficient disease management measures. However, a critical look at wildlife epidemiological investigations demonstrates that many investigations remain in the initial stages without moving to the action stage. This can be attributed to many causes outside of the investigator's sphere of influence including insufficient resources or longevity of resources, the complexity of the systems, situation resolution, and other sociological or political reasons. But wildlife epidemiological investigations may also be hampered by lack of adequate definition of the problem or hypotheses. Several tools or paradigms exist that can assist investigators in clear and productive hypotheses generation including utilizing multiple working hypotheses and employing mathematical models to guide the entire investigative process. When adopted, these tools can promote more efficient and productive wildlife epidemiological investigations leading to effective disease management strategies and interventions. Several wildlife disease systems provide opportunities to explore the epidemiological process as described and illustrate the use of these paradigms. These systems include Chronic Wasting Disease (CWD) in cervids, plague in prairie dogs, and respiratory disease in bighorn sheep. In conclusion, wildlife epidemiological investigations are an increasingly important component of wildlife management in aquatic and terrestrial systems. Given their significance, it is critical to understand the epidemiological process underpinning these investigations and employ all available tools and techniques that promote the acquisition of the essential understanding of disease ecology and lead to targeted and effective disease management.

Fibropapillomatosis in Marine Turtles of the Caribbean Region: the Case Study of Puerto Rico

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The incidence of fibropapillomatosis (FP) among sea turtles in the Caribbean appears to be under-estimated due to the lack of available information. The few reports available are from cases gathered on stranding and/or in-water studies of capture-mark-recaptures (CMR). There have been very few publications regarding this subject in the Caribbean. Williams et al., (1994) was the first peer reviewed publication to document over 120 cases of FP from different parts of the Caribbean (See figure 1). However, long term data of FP incidence are very rare, not only in the Caribbean but worldwide. After reviewing the existing published literature, unpublished reports, and interviewing specialists on different locations of the Caribbean, it was difficult to obtain prevalence information due to a variety of factors. For example, most of the few in-water studies in the Caribbean target hawksbill turtles on the reef, which are less susceptible to express FP tumors. Other studies have only recently started (< 3 yrs), and several projects are located at marine reserves or pristine areas where FP is not common. CMR information is also rare since not all the countries have the logistics to establish such a program, or the data are limited due to small sample sizes. With the restricted available information, we produced a map of FP occurrence in the Greater Caribbean (see Fig. 1). However, places where FP tumors are not reported cannot be confidently considered disease-free, due to the reasons mentioned above.

A few places were FP prevalence has been documented are Turks and Caicos, Bonaire, and Puerto Rico. In the case of Turks and Caicos, most of the information comes from the fisheries, as green turtle harvest is still permitted. Preliminary results indicate that 13.4% of green turtles captured showed external signs of FP (n = 239) (Stingel and Hart, in prep.). Since 2006, regular in-water surveys have been conducted at Lac Lagoon in Bonaire (Netherlands Antilles) (Stapleton et al., 2015). An increase of FP prevalence has been observed over the past 3 years, with a maximum of 22% last year (2014, n = 259). Turks and Caicos and Bonaire are currently in the process of analyzing these data.

Puerto Rico has the most long-term data on FP incidence in the Caribbean, with 24 years of information (Ortiz-Rivera et al., 2012, DNER, internal reports, Diez et al., 2010). FP tumors were officially reported in 1985 at several locations within the main coast of PR. A total of 840 cases of green turtles have been reported as strandings since 1985. From those, 268 (32%) had FP tumors (Ortiz-Rivera et al., 2008, DNER, internal reports). Efforts to study FP prevalence have concentrated in the Culebra Archipelago (located 17 km off the east coast of PR), where there are two high density foraging aggregations of juvenile green turtles with high recapture rates, and a CMR program has been ongoing for 18 years (1997 – 2014). Molecular studies and long distance tag recoveries indicate that these aggregations are mixed stocks from rookeries of the Wider Caribbean (Velez-Zuazo et al., 2010). From 2000 to the present, multifactorial studies have been conducted at two specific study sites within the Culebra Archipelago (i.e., Puerto Manglar and Tortuga Bay-Culebrita cay) to measure several aspects of FP among immature

green turtles. Captures ranged in size from 26.0 cm to 81.0 cm SCL (mean = 53.3 cm; SD = 11.7, n = 765), indicating a juvenile and subadult aggregation (Diez et al., 2010, Patrício et al., 2011). At Puerto Manglar, FP was first observed in 2000, and FP prevalence peaked in 2002 and 2003, with over 70% of the captured turtles expressing disease. At Tortuga Bay, FP was not detected until 2005, and the peak of prevalence was 33% in 2009 (Patrício et al., 2016). According to Patrício et al., (2011) juvenile turtles showed a higher survival probability and FP had no effect on survivorship. Trends in catch per unit effort and CMR indicate population growth at Manglar, the study site where FP prevalence is higher (Diez et al., 2010; Patrício et al., 2011). Additionally, estimated mean somatic growth at Manglar was higher than elsewhere in the world for wild green turtles and FP had no effect on growth rates (Patrício et al., 2014). Sonic tags attached to turtles aimed at understanding their home range and social behavior, demonstrated that these aggregations are not using the same resting places (Griffin et al., 2013), preventing contact, which could modulate FP transmission. Interestingly, some turtles from Puerto Manglar, where FP reached higher proportions, seem to aggregate after feeding (Griffin et al., 2013). These sites also show differences in physical and environmental features. The underwater vegetation at Puerto Manglar is dominated by macroalgae with few seagrasses, mostly of the species *Thalassia testudinum*, while Tortuga Bay has greater seagrass coverage, mainly Syringodium filiforme and Halodule wrightii, and coral reefs (Diez et al., 2010). Puerto Manglar is a narrower bay, slowing the circulation of currents, with a mangrove growing at the coast (*Rhizophora mangle*), and has high water turbidity. It is also located at the human populated mainland of the Culebra Archipelago, surrounded by minor development. Tortuga Bay is a natural reserve cay protected by USFW, with a wider opening basin and high energy currents. Its coast is surrounded by a white sandy beach and water transparency is high. Water quality levels were assessed at both sites. Enterococci, obtained with the DST (Enterolert) before and after a rain episode in April 2007, were detected at both Tortuga Bay and Manglar. DNA-markers identified the widespread human fecal contamination at Puerto Manglar, while at Tortuga Bay it only was detected next to a boat (Diez et al., 2010). Finally, nitrogen isotopic values (δ 15N) of macroalgae at Manglar suggested intermediate level of wastewater impact (Diez et al., 2010). The pathology of FP tumors at Puerto Manglar and Tortuga Bay was investigated by the School of Veterinary Medicine of the University of Georgia, from 2003 to 2009. Studies on blood chemistries and FP pathology analyses were published by Kang et al., (2008) and Page-Karjian et al., (2012, 2015). One of the most significant results of these studies was the presence of the virus in non-tumored turtles (Page-Karjian et al., 2012). Ongoing analyses on FP dynamics at Culebra's aggregations indicate that smaller turtles (< 40 cm SCL) do not exhibit FP tumors and middle sized turtles (~ 50-60 cm SCL) are the most affected (Patrício et al., in prep). Throughout 15 years of FP presence (2000 onwards), 59% of the turtles with FP were only mildly affected, 36% moderately, and only 6% had severe FP (Patrício et al., in prep). Additionally, a disease recovery rate of 31% was estimated after 1.5 - 4.0 years of tumor expression (Patrício et al., in prep).

In summary, green turtles with FP tumors are ubiquitous in the Greater Caribbean, but information on prevalence is scarce. Studies in Puerto Rico suggest that FP is not currently a major threat to green turtle populations and that higher disease prevalence was potentially associated with human contamination. Further monitoring is necessary to continue assessing the prevalence of this disease, since viruses have the capacity to change.

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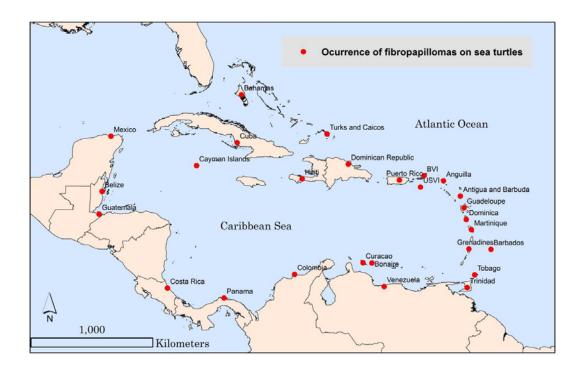


Figure 1.--Map of FP occurrence on green turtles in the Caribbean Region. Data source of Map: Williams et al., 1994; and personal communication: Stringel and Hart, Turks and Caicos; Leon, Dominican Rep; Moncada, Cuba; Horrocks, Barbados; Chacon, Costa Rica; Barrios and Vernet, Venezuela; Dummont-Dayot, Martinique; Chabrolle, Guadulope; Nava, Bonaire; Cazabon-Mannette, Tobago; Gumbs, Anguilla; Stewart, St. Kitts; Doyle, Grenadines; Muccio, Guatemala.

Characteristics of Green Turtle Fibropapillomatosis in the Northwest Atlantic as Indicated by Strandings

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Data from dead, sick, or injured (i.e., stranded) green turtles were collected during the period of 1980–2014, by the Sea Turtle Stranding and Salvage Network (STSSN), which comprises 18 U.S. states from Maine to Texas. Strandings included carcasses that were found washed ashore or floating and live turtles that were found debilitated by injury or disease. Although some stranded turtles were found floating far from shore, most were found beached along shorelines or floating close to shore. Observers used a standardized form to document data from each stranded turtle. Collected data included location, carapace length, and presence or absence of fibropapilloma-like tumors. STSSN observers ranged from professional sea turtle biologists to volunteers with no prior data-collection training. However, specific data collection and reporting methodology were a part of the STSSN protocol, as were periodic training workshops. All stranding reports were also reviewed, verified, and edited by coordinators of the STSSN.

During the first 2 years of work by the STSSN, FP was only documented in stranded green turtles from the very southern end of Florida (the Florida Keys). FP was documented in 6 of 15 green turtles found in the Florida Keys but in none of the green turtles found throughout the rest of Florida (N = 47). By 1985, FP was documented in stranded green turtles north to 29° N latitude (about to the middle of the Florida peninsula). FP was documented in 22 of 220 green turtles (10%) found south of 29° N, but in none of the 17 green turtles found in Florida north of this latitude. The northern limit of FP as indicated by stranded green turtles continued to be 29° N through 1999. At that time, FP had been documented in 742 of 3,212 green turtles (23%) found south of 29° N, but in none of the 244 green turtles found in Florida north of 29° N (or among the approximately 1,000 stranded green turtles found in the SE U.S. outside of Florida).

Beginning in 2000, FP was found in stranded green turtles in Florida, north of 29° N. By 2013, FP had been documented in 2,380 of 9,574 green turtles (25%) found south of 29° N and in 62 of 1,517 green turtles (4%) found in Florida north of 29° N. No FP was documented in the 176 green turtles found in the western half of the Florida Panhandle (west of Gulf County).

FP occurs from the northern portion of The Bahamas, but the time of first appearance there is not known. FP was documented in a stranded green turtle in Georgia (the US state to the north of Florida) for the first time in 2004. The frequency of FP among stranded green turtles in Georgia since that time has been 9%. Of thousands of immature green turtles captured in a long-term study in Bermuda, only one case of FP was ever documented (in 2007, Anne Meylan, pers. com.) FP was documented in stranded green turtles in Texas (three states west of Florida along the Gulf of Mexico) for the first time in 2010. The frequency of FP among green turtles found in Texas since then has been 3.1%. A single stranded green turtle with FP was documented in South Carolina (the state to the north of Georgia) late in 2014. No FP has been documented in the

hundreds of stranded green turtles found between Florida and Texas, and FP has not been documented in the hundreds of stranded green turtles found north of South Carolina.

From 1980 to 2014, the frequency of FP among stranded green turtles has generally risen from about 10% to 25% (with absolute values fluctuating between 15% and 30% since 1987). The increase in the frequency of FP among stranded green turtles has been concurrent with an increase in the annual number of stranded green turtles found in Florida (from around 100 in the mid-1980s to around 1000 in recent years).

The frequency of FP was highest among stranded green turtles found in areas of low energy, where water movement (i.e., flushing) tended to be the slowest. For example, the frequency of FP was higher among green turtles found along inshore areas (e.g., bays, lagoons, inlets, rivers, etc.) than among green turtles found along offshore areas (along beaches directly adjacent to the Atlantic Ocean or Gulf of Mexico, 33.7% vs. 15.5%, respectively). The frequency of FP was also higher among stranded green turtles found along the west coast of Florida (primarily characterized by a wide continental shelf and low-energy shorelines) than among stranded green turtles found along the anarrow continental shelf and high-energy shorelines, 47.7% vs. 15.3%, respectively).

The sizes and characteristics of FP tumors on stranded green turtles in Florida were typical of those found on green turtles in other parts of the world. Tumors were found on all external body surfaces and a variety of internal tumors were occasionally documented. Only three oral tumors were found among the 2,637 green turtles that were found with FP. Data on the frequency of internal tumors among stranded green turtles with external tumors are available but have yet to be evaluated. Standardized information on the sizes and numbers of tumors associated with FP began to be collected on some stranded green turtles in Florida beginning in 1999, with widespread collection of these data by 2005. However, these data still need to be entered and evaluated.

The frequency of FP among stranded green turtles varied with the size of the turtle. We did not document FP on any of the 405 stranded green turtles found in Florida that were less than 20 cm curved carapace length (CCL). The frequency of FP among stranded green turtles between 20 and 30 cm was 2.5%, and this frequency steadily rose and peaked in turtles between 40 and 50 cm (45.4%). The frequency of FP among stranded green turtles by 10-cm size class steadily decreased, and was less than 1% for adult turtles (> 100 cm CCL).

It is possible that the spatial associations of stranded green turtles with FP could misrepresent actual distributions because dead, sick, or injured sea turtles may strand far from where they were living. However, the spatial characteristics of FP occurrence indicated by the stranding data were confirmed by research projects that captured green turtles at foraging sites. In-water studies of green turtles in the southern half of Florida (south of 29° north latitude) also documented the occurrence of FP, while similar studies in the SE U.S. outside of Florida did not document green turtle FP. The finding that the frequency of FP among stranded green turtles was greater in inshore areas than in offshore areas was also corroborated by in-water studies. Finally, on a finer scale, no FP was documented among the 230 stranded green turtles found in Broward County (in southern Florida) through 1996. A research project conducted in that

county during the same time period also found no FP among green turtles captured there. It is also possible that green turtles with FP may be over-represented in the stranding data because green turtles with FP probably have a higher mortality rate than green turtles without FP. However, the frequency of FP among stranded green turtles was similar and often lower than that of green turtles captured on nearby foraging grounds.

Prevalence and Trends in Fibropapillomatosis in Green Turtles on Florida's Atlantic Coast

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INTRODUCTION

Our research group (at UCF) has been studying patterns and trends in green turtle biology (including green turtle fibropapillomatosis, GTFP) for nearly 40 years. The research is focused on that region of east-central Florida that Dr. Carr always referred to as the "Indian River Coast." Indeed, much of our work has involved juvenile *Chelonia* populations in the developmental habitats of the Indian River Lagoon system, which stretches for 250 km along Florida's eastern seaboard (Fig. 1). We have also studied juvenile green turtles in littoral developmental habitats, just off-shore in the Atlantic, and at a dredged-out submarine basin at Port Canaveral, Florida, for more than 20 years.

However, others have studied green turtles, including GTFP prevalence, in these lagoonal and littoral habitats of east Florida, as well. These include investigators with the In-water Research Group (J. Gorham and S. Weege) and with a NASA contractor (IMHA), namely Jane Provancha and her co-workers at the Kennedy Space Center, working mainly in the northern reach of the Indian River system, i.e., Mosquito Lagoon (Fig. 1). In the interest of a comprehensive treatment of the GTFP problem in this region, we invited those investigators to share their results and co-authorship of this report, and they agreed to do so. Altogether, we are dealing with seven data sets, with varying degrees of temporal and geographic variation. The data sets are, however, broadly divisible into two groups: first, those derived from green turtles inhabiting lagoonal habitats (shallow, brackish, water movement largely wind-driven), and secondly, littoral habitats are, of course, highly disparate ecologically and the differences between them apparently have serious consequences relative to GTFP epidemiology

EARLY ACCOUNTS

In a small, privately-published book entitled, "Saga of the Sea Turtle," Edison "Blackie" Cruz described life in the Florida Keys from about 1910 to 1960, with special reference to sea turtles. What he termed simply "warts," almost certainly were the verrucous excressences that we now know as fibropapillomatous tumors, or "FP." Cruz was familiar with them in the Keys as early as 1913, and he reported that older fishermen had seen these tumors (in the Keys) in the late 1800s.

Not until more than 25 years later did accounts of so-called "fibro-epithelial tumors" appear in the scientific literature, with the publication of papers by Smith and Coates (1938) and Lucke (1938). These two studies were apparently not related to one another in any way, although they

appeared coincidentally in the same year. The subject animals in those studies were from the Florida Keys and Dry Tortugas, so our understanding of the geographic distribution of the disease was restricted to that region early on.

THE MODERN ERA: ANNUAL PREVALENCE

Lagoonal Habitats Mosquito Lagoon

Our group (UCF) studied the green turtle population of the northern reach of the Indian River Lagoon system (Mosquito Lagoon) systematically from 1975 to 1981 (Fig. 1). Relatively small numbers of animals were captured in large-mesh tangle nets during that period, but we also examined more than 200 green turtles that were stunned by extremely low water temperatures during cold spells in January of 1977 and 1981. We never saw any evidence of the disease in any of those green turtles and we were completely unaware of the existence of what we now call "GTFP" through 1981.

Another severe cold spell occurred in January of 1985, and once again provided a sample of 145 Mosquito Lagoon green turtles for examination. Surprisingly, 29% of them presented GTFP! They were the first afflicted animals seen in the "Northern Region" of the Indian River and farther north along the SE US coast than any previous records. Clearly, fibropapillomatosis broke out in that population between 1981 and 1985.

To further confuse the situation, another cold spell occurred four years later (January 1989) and provided still another sample (this time 248) of Mosquito Lagoon green turtles for examination. Remarkably, only 2% (4/248) showed any evidence of the disease and none were severely afflicted. In other words, prevalence had gone from zero in the 1970s and early 1980s, to 29% in 1985, and back to near zero by 1989.

The geographic focus of our net sampling program shifted in 1982, to a location about 100 km south of Mosquito Lagoon, in the central reach of the Indian River, between Melbourne and Vero Beach, Florida (Fig. 1). We did, however, continue to assist with the evaluation of cold-stunning episodes in Mosquito Lagoon in 1985 and 1989.

About six years after the 1989 cold spell in which FP prevalence fell to 2%, Jane Provancha's NASA contract group resumed systematic assessment of the green turtle population in exactly the same location as our original work, Mosquito Lagoon (Fig. 1). They used tangle-net capture just as we had 14 years earlier. In their first year (1995), they found that FP prevalence stood right at 50%. Over the next 20 years, they documented an overall mean prevalence of 47.2% (SEM: 4.5%), with a range of 0.0% to 77.8%. There was no significant positive or negative trend by linear regression analysis (P = 0.5093).

Provancha's 20 years (1995–2015) of results, combined with ours from the period 14-20 years earlier (1975–1981), constitute the foundation of two patterns or tendencies that characterize GTFP epidemiology on the east Florida coast in the late 20th and early 21st centuries. First: a tendency for absence of the disease during the first five to ten years in populations where study

began in the late 1970s to early 1990s; followed by GTFP break-out, then by relatively level prevalence rates over periods ranging to three (3) decades. Second: relatively high prevalence rates in green turtle aggregations utilizing <u>lagoonal</u> developmental habitats in contrast to <u>littoral</u> ones.

Central Indian River Lagoon

In the central region of the Indian River system (CIRL), we were completely unaware of GTFP as the result of six (6) years of netting in the Northern Reach. We were thoroughly shocked when, immediately upon setting our nets in an area called "South Bay," near Sebastian, Florida (Figure 1), we encountered juvenile green turtles with heavy burdens of FP tumors. We know now that veterinarians are familiar with similar growths on horses, cattle, dogs, etc., but none of us had ever seen them.

A short while later, I (LME) received a note from George Balazs saying that he had heard that we were beginning to see tumors on Indian River turtles and that he was becoming quite concerned about a significant increase in FP on Hawaiian greens. Not long after that, he and I attended the Workshop on Marine Turtle Conservation & Biology in Waverly, Georgia. Both of us gave presentations on fibropapillomatosis in our respective *Chelonia* populations (Balazs, 1986). It can be argued that his note and those two papers constitute the beginning of attention to and concern for GTFP in the modern era.

We continue to set our nets and examine green turtles at Sebastian to the present day. The overall mean annual prevalence observed over the past 32 years is 48.9% (SEM: 1.98%), with a range of 26.9% to 71.6%. The data fit the "lagoonal model," with variable but relatively high rates, year over year, but a slope with no significant deviation from zero over the 32-year period (P = 0.5687).

Jennings Cove

Jenning's Cove is also in the Indian River Lagoon, about 50 km south of, and quite similar to, the Sebastian site (Fig. 1). Here, too, the green turtles exhibit high annual prevalence rates (mean: 67.5%, SEM: 5.6%), considerable variability year to year (range: 33.0% to 89.9%), and no significant trend over a nine (9) year period (P = .8206). As in other lagoonal foragers, prevalence is neither growing nor shrinking over time.

Lake Worth Lagoon

Lake Worth Lagoon is essentially an expansion of the Intra-Coastal Waterway and an extension of the Indian River Lagoon, about 140 km south of Sebastian (Fig. 1). Here again, over the course of nine (9) years, we documented a relatively high prevalence (mean: 48.7%, SEM: 7.2%), considerable variability year over year (range: 28.6% to 78.6%), but no significant positive or negative trend (P = .2871).

Littoral Habitats Trident Submarine Basin

Looking at green turtle populations occupying habitats much more oceanic in character, we start with the Trident Submarine Basin at Port Canaveral (Fig. 1). Geographically, it may appear to be more "lagoonal" in character but its entrance is so near the mouth of the port channel that it is thoroughly flushed with ocean water throughout the year, by swells driven by prevailing southeasterly winds. For the first eleven (11) years, during which we examined 1,107 animals, the prevalence of FP was zero. Beginning with one afflicted turtle in 2005, we observed a low and variable prevalence in this aggregation ever since. The mean for the last eight (8) years is only 4.2% (SEM: 0.95%; range: 0.0 to 17.5%), but it is probably significant that the rate has doubled in each of the past two years, to 17.5%.

Near-shore Worm-rock Reefs

Moving to the near-shore, worm-rock reefs, we saw that any expression of the disease was absent during the first eight (8) years (1989–1986) of sampling, involving 210 animals. Prevalence exceeded 20% in the first year in which we observed FP, then averaged 19% (SEM: 2.3%; range: 8.0 to 33.7%) over the next nine (9) years, at which time an enormous increase in turbidity, related to a nearby beach construction project, caused us to close down this study.

St. Lucie Power Plant

At the St. Lucie Power Plant (Fig. 1), three cooling water pipes ranging from 3.7 to 4.9 meters in diameter and extending 365 meters into the near-shore reef zone, serve as a sea turtle collecting and sampling device. Turtles swept into the cooling canal are captured in nets or by hand and, after examination, are released into the ocean. The GTFP prevalence rate for these reef dwellers is variable and quite small (mean: 6.84%; SEM: 1.3%; range: 2.3% to 12.7%), and there has been no tendency for increase or decrease in the past ten (10) years (P = 0.135). It is important to note here, that there are records of green turtles with tumors as early as 1977, at the power plant. While, as noted above, warty tumors on green turtles were known from the 1800s in the Florida Keys and Dry Tortugas, these power plant records from 1977 are the earliest we have been able to discover for the Florida Atlantic coast above the Keys-Tortugas region.

SIZE CLASS DISTRIBUTION

In lagoon-dwelling green turtles, FP prevalence is greatest (> 50% and as high as 70%) in individuals 30-50 cm (SCL), but in littoral habitats, the affliction rate is quite small (generally < b12%) and evenly distributed over all size classes.

SEASONAL VARIATION IN PREVALENCE

In the Indian River Lagoon, FP prevalence is significantly greater (P < 0.001) in the fall and significantly smaller (P < 0.001) in the summer. At the power plant, FP is most prevalent (P < 0.001) in the winter and least so (P < 0.001) in the summer.

TUMOR REGRESSION

Our treatment of regression has been hampered by a low recapture rate (12.5%) and the lack of consistency in our method of severity assessment. We use a three-category scale, as follows:

Category 1: mild affliction; fewer than 20 masses, relatively small (< 2 cm) tumors, none on the eyes

Category 2: moderate affliction; more and larger tumors, usually on soft skin and in inguinal and axillary regions, not more than an incipient tumor on the eye

Category 3: severe affliction; many larger, highly-vascularized, multi-colored neoplastic masses, usually some involvement of the eyes

Figure 2 provides an appreciation of the frequency of disease, population-wide, and of those severity categories described above. Our records contain 111 well-documented cases of tumor growth and regression. Fully 64% (71/111) exhibited partial or complete regression of tumors between initial capture and subsequent recapture. About 34% (38/111) either acquired tumors de novo or presented increased severity between first and subsequent captures. The fact that two of every three afflicted turtles exhibited partial or complete regression of tumors is impressive when one considers that just a few years ago there was a general consensus that FP was fatal to virtually all juvenile green turtles that acquired it.

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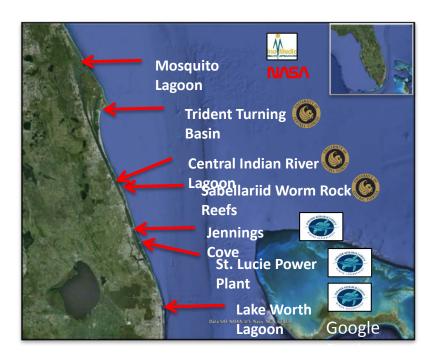


Figure 1.--GTFP Study Locations along the Florida Atlantic coast.

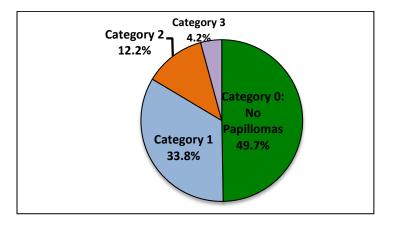


Figure 2.--Fibropapillomatosis Prevalence: First Time Central Indian River Lagoon Captures, October 2005 - February 2015; N = 1,277.

Fibropapillomatosis in Sea Turtles from South America – Brazil, Uruguay, and Argentina

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Since 1982, Projeto TAMAR-ICMBio, the Brazilian Sea Turtle Conservation and Research Program, has been studying and protecting the marine turtles that occur in Brazil. It has progressively established a national network of 21 field stations located across nine Brazilian states, covering 1100 km of the Brazilian mainland coast and oceanic islands. Most of these stations are operated year-round and are located in the main sea turtle nesting areas or in nearby major coastal foraging grounds, where historically sea turtles have been reported as incidental catch in coastal fisheries.

We present data on the prevalence and expression of fibropapillomatosis in *Chelonia mydas* found stranded (i.e. washed ashore dead or alive, found floating dead or alive in coastal waters), or incidentally caught in fisheries. Data were collected along the Brazilian coast, from nine States (Santa Catarina – SC, São Paulo – SP, Rio de Janeiro – RJ, Espírito Santo – ES, Bahia – BA, Sergipe – SE, Rio Grande do Norte – RN and Ceará – CE), and from oceanic islands (Rocas Atoll and Fernando de Noronha).

Since 2000, TAMAR has defined a new field named "TUMORS" in its database. Each turtle was thoroughly examined for the detection of external tumors and a standardized protocol was developed for collecting biological data, which included date, species, sex, location, curved carapace length and width (CCL and CCW, respectively), tag number, general condition (dead or alive), and health status, among others. Tumor samples were classified according to size, aspect, shape, contour, and presence of ulceration, and tissue samples were collected for histopathological analysis in the Department of Pathology of the University of São Paulo – USP (Matushima et al., 2001). Decomposed carcasses were excluded from the analysis.

From 2000 to 2005, 10,170 sea turtles were found stranded, alive or dead. The animals were identified, measured, and examined for the presence or absence of tumors. Most records (82.2%; 8359) corresponded to green turtles (*Chelonia mydas*). Analyses were performed only for *Chelonia mydas* (Baptistotte, 2007). Affected animals varied from juvenile (30 cm minimum recorded CCL) to adults (112 cm maximum recorded CCL).

Higher prevalence of FP was recorded in the State of Ceará, followed by Rio Grande do Norte, Espírito Santo, and Sergipe. The disease demonstrated a decreasing trend during the sampled period, and juvenile turtles (40 - 60 cm CCL) were the predominant age/size class among affected individuals. There was no evident seasonality in FP distribution. A total of 501 green turtles from Fernando de Noronha and 486 from Rocas Atoll (where regular in-water surveys are conducted) were examined, and no evidence of the disease was found.

The mean FP prevalence among the species was 15.4% (1,288/8,359) (0% – 36.9% \pm 13.3). Mean CCL for turtles with FP was 47.9 cm (30 – 112 \pm 10.8cm); apparently healthy individuals measured 45.7 cm (8 – 140 \pm 15.8 cm). Tumor prevalence and size-class distribution (Fig. 2 and

Fig. 3) were significantly different among States (Kruskall-Wallis: H = 910.66; DF = 9; p = 0.000), with smaller individuals and lower tumor prevalence in southern States (SP, SC, RJ). On the other hand, the states with higher proportions of turtles with CCL between 40 and 60 cm (CE, SE e BA) showed higher tumor prevalence (Fig. 1).

Tumors were recorded primarily in eyes, neck, flippers, axillary and inguinal areas, and cloaca – rarely in carapace and plastron; no tumors were recorded in the oropharynx.

Sea turtle fibropapillomatosis in Brazil was detected only in coastal regions, which are most affected by human activities and are constantly exposed to pollutants from different origins, such as domestic, agricultural, and industrial effluents (Santos et al., 2010). The disease has shown low to moderate frequencies in Brazilian populations when compared to studied populations elsewhere. Results demonstrate that there is an increase in FP prevalence up to sub adult stages, followed by a decrease in adult turtles documented within the affected size classes.

Comparisons of FP frequency between stranded and intentionally captured turtles (through either cast nets or set nets) in ES showed significant differences, with higher tumor proportion in turtles captured in the industrial discharge area (chi-square, p < 0.05); groups did not differ in size-class (Mann-Whitney U-test, p > 0.05) During a study in this area carried out from August 2000 to July 2006, a total of 640 individual green turtle were captured and 34.4% had tumors (Torezani et al., 2010). The region is densely populated and suffers from several environmental problems, such as solid wastes, domestic wastewater, and industrial wastes (Jesus et al., 2004).

The average prevalence of FP in sea turtles in Brazil between 2000 and 2014 is presented in Fig. 2. These data were collected by TAMAR, NEMA (Nucleo de Educação e Monitoramento Ambiental, located in Rio Grande do Sul state), and Guajiru located in Paraíba state (Mascarenhas and Iverson, 2008). Data from Uruguay were published by Ferrando et al., (2015) and data from Argentina were obtained through personal communication with Laura Prodoscimi (Programa Regional de Investigación y Conservación de Tortugas Marinas de la Argentina – PRICTMA).

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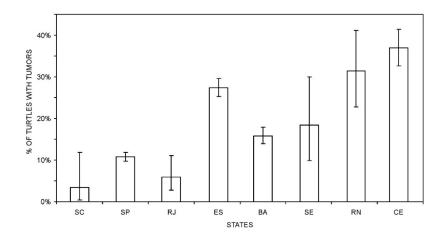


Figure 1.--Percentage of turtles with tumors in each Brazilian State between 2000 and 2005.

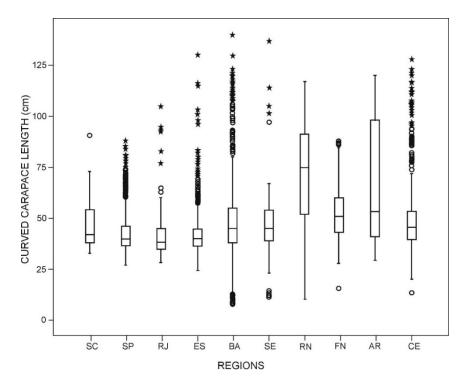


Figure 2.--Curved carapace length (CCL) of both healthy and sick turtles in each State, between 2000 and 2005.

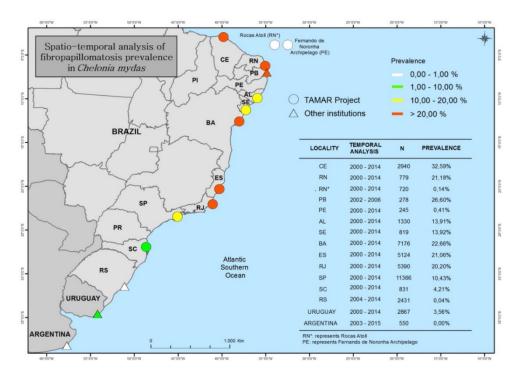


Figure 3.--Average prevalence of FP in green turtles for all Brazilian states, as well as for Uruguay and Argentina. TAMAR 2000-2014; Uruguay 2000-2014; Guajiru (PB) 2002-2006; NEMA (RS) 2004-2014; Uruguay 2000-2014; Argentina 2003-2015.

Fibropapillomatosis in Africa - Point-Data and Data Sets Available for FP in Africa; Knowledge Gaps; Lesions and Trends in a Well-Documented Area: Republic of Congo

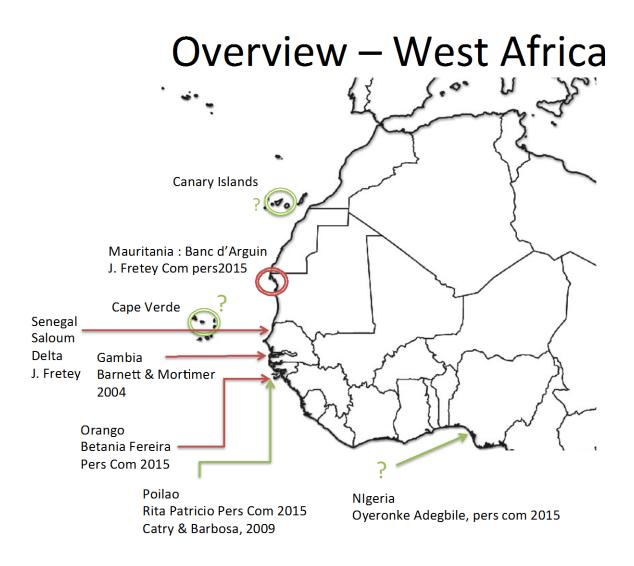
Alexandre Girard E-mail: agirard6@gmail.com Rastoma - Central African Network for Sea Turtle Conservation Professionals Renatura - www.renatura.org

Africa is still poorly documented for many aspects of sea turtle biology and conservation, including FP occurrence and trend. Published results are rare and available information is frequently based on personal communication from the local NGO managers or researchers working in a particular place.

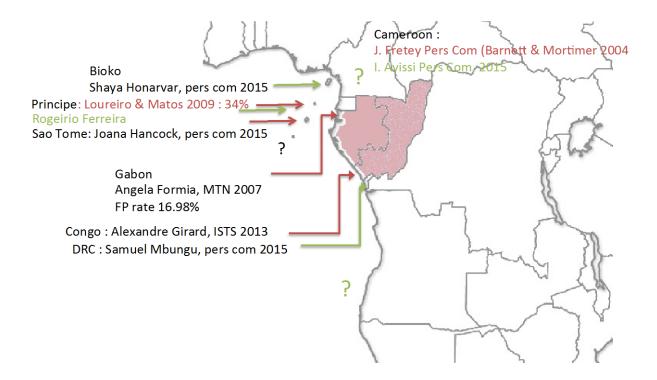
In West Africa, the most northerly FP cases have been observed in Mauritania. In 2015, 7 (20%) green turtles were observed with FP out of 35 dead strandings (Fretey, pers. comm., 2015) inside the Banc d'Arguin National Park. This represents a sharp increase since only 4 green turtles were recorded with skin masses among sea turtle stranding and by-catches observed from 2009 until 2014 in Mauritania. To my knowledge, no FP case has been reported in the green turtle feeding grounds of Canary Islands and in Cape Verde to date. One case of FP has been observed on a green turtle in the Northern part of the Senegal (Saloum Delta) (Jacques Fretey, pers. comm. reported by Barnett et al., in 2004) and one case on a stranded green turtle in Gambia (Barnett et al., 2004). In Guinea Bissau, two FP cases have been reported (out of a total of 7 observations): one in the northern part of the Bijagos archipelago (Unhocomozinho Island) and one in Bissau (Betania Ferreira, pers. comm., 2015). Three cases were also recorded in the year 2000 (Catry et al., 2009). In Nigeria, FP-like lesions have not been observed from green juveniles stranding (Oyeronke Adegbile, pers comm., 2014).

In central Africa, FP is more comprehensively documented, with three studies providing reliable FP rates. In Principe (Sao Tome & Principe), Loureiro and Matos (2009) published results revealing high FP rate: 34.04% CI-95% [22.17–48.33]. In Corsico bay, a feeding ground at the border between North Gabon and South Equatorial Guinea, a survey (1998–2006) revealed an average FP prevalence of 16.98% CI-95%: [13.89–20.58] with no apparent trend in the yearly FP rate. In the Republic of Congo, the current FP rate in Loango Bay is approximately 10%. The 2008–14 FP rate trend in Congo shows that a 20% peak occurred in 2009. The global 7 year trend shows a slight decrease over the years (Girard et al., 2015). No case of FP has been observed in either the Democratic Republic of Congo (Mbungu, pers. comm., 2015) or in Angola (Morais, pers. comm., 2015).

In South Africa and Mozambique, there are no data on FP available to my knowledge. In the Mozambique Channel, FP has been well documented in Barren Islands on the western coast of Madagascar where FP rates monitored in 2010–2012 ranged from 9 to 13% (Campillo, 2011, 2012; Leroux et al., 2010). Anecdotal FP cases were recorded in Comoros Island (Ballorain et al., unknown year), in Tanzania (1 case out of thousands of observations, Boniventure Mchomvu, pers. comm., 2015) and in Kenya (1 case out of 1,422 releases, Zanre, 2005).



Overview – Central Africa



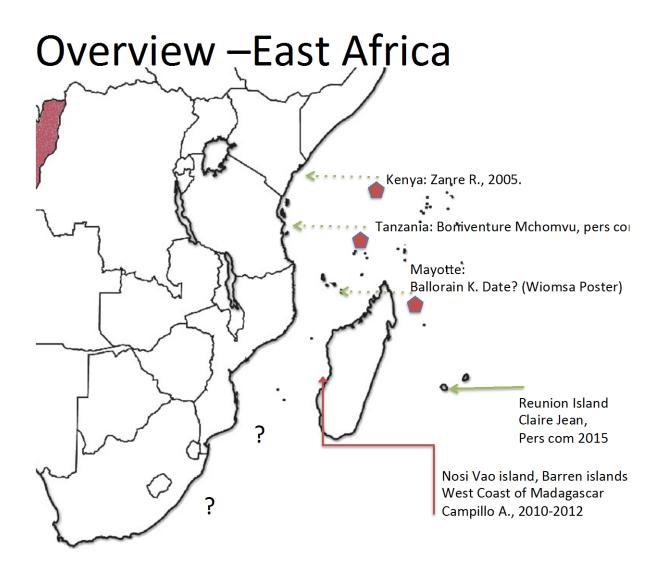


Figure 1.--Overviews of the documented FP status on major green turtle feeding grounds in West Africa, Central Africa, and East Africa. Red = FP present. Green = FP absent.

Results of the FP monitoring in Congo (2005–2014)

In Congo, the first FP lesions were observed in the framework of a field program launched in 2005, by the NGO Renatura. The Renatura Release program was designed to release sea turtles incidentally caught in artisanal fishing gears and thanks to this initiative, thousands of turtles have been released every year. In the framework of the release program, all the information about by-catch observation has been recorded in the Renatura database. The consolidated data set available is made of 12,432 observations of green turtles and it covers a 7-year period, from Jan 2008 until Oct 2014. The study site is centered on Loango Bay, located 20 km north of the town of the economic capital of the country, Pointe Noire. The rocky sea ground of 'Pointe Indienne', the cape delimiting the bay at its southern end, is an important feeding ground for green turtles. The bay is also the most important site for artisanal fishing. It is thus a place of strong interaction between sea turtle and fisheries. Every year, hundreds to thousands of turtles are incidentally caught in artisanal fishing nets, primarily dormant gillnets. Juvenile green turtles represent 90% of the bycatches.

FP prevalence Trend in Congo

Since 2008, 806 FP cases have been recorded, representing an average prevalence rate of 9%. The FP monthly rate trend in Congo over 82 months, nearly 7 years, shows a peak of approximately 20% in 2009. Nevertheless, when we adjust for trend, we obtain a slight decrease over the years.

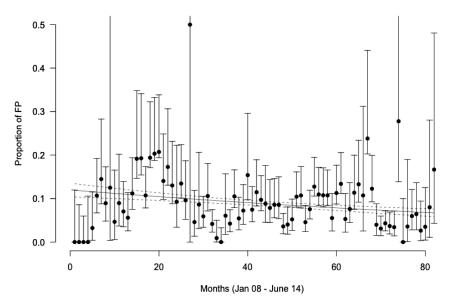


Figure 2.--FP monthly rate over 82 months (Jan 2008–Oct 2014) in Loango Bay, Republic of Congo, Central Africa.

Impact of FP on population in Congo

The FP presence in 10 to 20% of the juvenile green turtles over at least 7 years had no impact, locally since the abundance of green turtles on the feeding ground increased after the FP peak in 2009. The conservation effort or another phenomenon reinforcing the population counterbalanced the potential impact – if any – of the FP epizootic. A proper way to really assess the potential impact of FP would be to compare survival rate between FP and non-FP and to get access to the nesting trends on related rookeries. But connection of the Loango Bay feeding ground with regional green rookeries remains unknown.

Green turtle by-catch trend (2008-13)

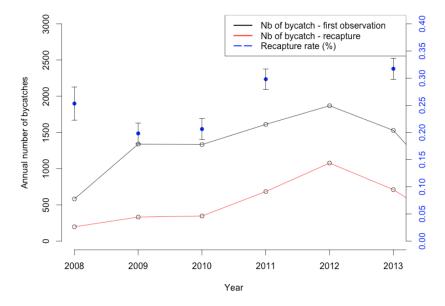


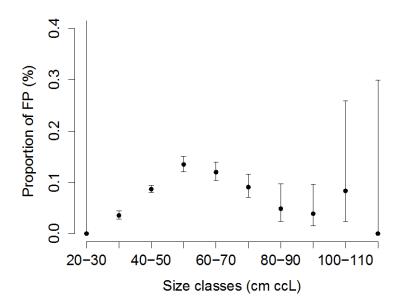
Figure 3.--Bycatch trend and recapture rates (2008–2013) in Loango Bay, Republic of Congo, Central Africa.

Lesions' aspect and localization in Congo

In Congo, lesions have been observed in green turtles only. None of the other species observed among by-catch, such as leatherback, olive ridley, and hawksbill turtles were seen to exhibit skin masses suggestive of FP. The FP lesions observed in green turtles in Congo are cutaneous masses of various shapes and sizes. Most of the lesions are located by decreasing order of frequency in the following locations: proximal parts of hind limbs, frequently at the Monel tag insertion site, around the neck, around the eyes, on the proximal parts of hind limbs, and around the cloaca. The possible occurrence of internal or oral FP lesions was not assessed since neither oral examination nor necropsy was undertaken. To date, skin mass sampling for histopathological analysis has not been implemented to confirm FP in Congo. However, the localization and aspects of the skin masses are strongly evocative. Fibropapilloma has been confirmed by histopathological analysis in Central Africa in the Corisco bay, located approximately 1000 km north of Congo (Formia, 2007).

FP rate according to the size classes in Congo

The FP rate observed among size classes in Congo is consistent with the common pattern observed in other FP study sites. A significant shift of the FP rate is observed between the 30–40, 40–50, and the 50–60 cm CCL classes: from less than 5% of FP in the 30–40 size class, to 10% for the > 40-50 cm interval, and up to nearly 15% in average for the > 50-60 cm interval. FP rate then decrease regularly for larger sizes. Average FP rates calculated for individuals larger than 100cm have to be discarded given the small number of individual in this size class and the consecutively large confidence intervals.



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The Direction of Research on Fibropapillomatosis on the Great Barrier Reef

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Although significant progress has been made in understanding fibropapillomatosis (FP) in green turtles (*Chelonia mydas*), there are still several knowledge gaps surrounding various aspects of this disease. Research being conducted at James Cook University in the College of Public Health, Medical and Veterinary Sciences is attempting to fill some of these knowledge gaps, with a particular focus on the epidemiology of FP on the Great Barrier Reef (GBR). Chelonid herpesvirus 5 (ChHV5) has been identified as the likely aetiological agent of FP and variants of this virus have been described in both Florida and Hawaii (Ene et al., 2005; Herbst et al., 2004). This research will describe the phylogeny of ChHV5 on the GBR, identifying any variants of this virus on the Queensland coast. Sensitive polymerase chain reaction (PCR) assays, which are specific to this virus, are currently being designed and optimised in the laboratory. Sample collection has been ongoing at six locations spread along the GBR. Building on the findings from this study, the relationship of the virus and host lineage will be assessed. The results may provide new clues about the transmission of this virus. The samples collected for these studies will also be screened for other oncogenic viruses using quantitative PCR assays.

This project will also explore the possible correlation between reduced water quality and high incidence of FP (Adnyana et al., 1997; dos Santos et al., 2010; Foley et al., 2005; Herbst, 1994; Van Houtan et al., 2010). The Great Barrier Reef Marine Park Authority (GBRMPA) and the Department of Environment and Heritage Protection (DEHP) are collaborating with James Cook University to identify which specific water quality parameters may be responsible for this trend. This study will examine green turtles with and without FP lesions at an array of sites spanning the GBR. We will gain an understanding of the viral variants present at each site. The results from this project will fill significant knowledge gaps surrounding FP in Australia and possibly the epidemiology of FP.

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Fibropapilloma Disease in Marine Turtles in Eastern Indian Ocean – South Western Pacific Ocean

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Comprehensive marine turtle studies expanded in Queensland via the Queensland Turtle Research Project, which progressively commenced mark-recapture studies of nesting and foraging marine turtles at multiple study sites in Queensland, starting in1968. Turtles with fibropapillomatosis (FP) tumors were identified as studies began at each long-term study site: commencing with nesting loggerhead turtles, *Caretta caretta*, at Mon Repos (24°S) in 1968; with immature green turtles, *Chelonia mydas*, foraging in Hervey Bay (25°S) in 1971 and Moreton Bay (27°S) in 1972; foraging loggerhead and green turtles at Heron Island Reef (23oS) in 1976 and 1977, respectively; foraging green turtles at Shoalwater Bay (22°S) in 1986 and Repulse Bay (20°S) in 1987. It is concluded that FP was widely occurring in marine turtle foraging populations in eastern Australia, pre-dating the commencement of this turtle research program in 1968. When green turtles were first studied in detail in SE Asia, Hendrickson (1958) found FP tumors on the nesting green turtles at the Sarawak Turtle Islands in Malaysia.

Commercial fishers reported what we now interpret as the first Australian record of an FP epidemic on foraging green turtles in 1974–1975, at Airlie Beach (20°S), central Queensland. No evidence of an elevated frequency of green turtles with FP tumors was found in the same area during the 2000s.

The history of FP disease identification in Australia summarizes as follows.

- **1972**: University of Queensland School of Veterinary Science identified the "growths" on an immature green turtle from Moreton Bay and nesting female loggerhead turtles from Mon Repos as "fibro-epithelial growths" as defined by Smith & Coates (1938).
- **1998**: Aguirre et al., (2000) concluded that histologically, FP tumors sampled from foraging loggerhead and green turtles on the Eastern Banks of Moreton Bay in 1998, were similar to the tumors that have been observed on Hawaiian green turtles.
- **1998**: Based on viral genetic samples collected from the foraging turtles in eastern Moreton Bay in 1998, Quackenbush et al., (2001) concluded that there was a high degree of relatedness among new herpesvirus sequences from Australia, Barbados, and Pacific Mexico with those previously identified (from all turtle species) and that FPTHV sequences amplified from tumors from Hawaii and Australia are very similar to each other, differing only by one amino acid substitution.

FP tumors have been encountered on foraging green turtles at numerous sites from Cocos-Keeling Island in the Indian Ocean, Kimberley Coast, Exmouth Coast, and Shark Bay in Western Australia, Wellesley Islands in the Gulf of Carpentaria, multiple sites within Torres Strait, numerous sites in eastern Queensland from Princess Charlotte Bay to Moreton Bay, and Byron Bay in northern New South Wales (Fig. 1). There is no evidence of a latitudinal north-south cline in increasing frequency of turtles with tumors from temperate waters of northern New South Wales SE Queensland to tropical waters of Torres Strait. The highest frequency of green turtles with FP tumors was recorded on the Eastern Banks of Moreton Bay. FP tumored green turtles were recorded with the commencement of systematic biological studies of foraging green turtles in New Caledonia in 2012-2015 (T. Read, pers. comm.). Many green turtles transported from widely scattered locations from throughout Indonesia presented with FP tumors when examined in slaughter houses in Bali in 1994 (Adnyana et al., 1997). However, the frequency of occurrence has uncertainty because these turtles were transhipped via a number of other sites before arrival in Bali with the possibility of diseased turtles being preferentially forwarded on to Bali, resulting in probable elevation in proportion of FP turtles recorded at Bali.

FP tumors have been recorded on foraging loggerhead turtles in Exmouth Gulf and Shark Bay in Western Australia in the eastern Indian Ocean and Moreton Bay, south Queensland, and Byron Bay, northern New South Wales in the SW Pacific Ocean (Fig. 2). The frequency of FP among loggerhead turtles is lower than among green turtle populations. The highest frequency of loggerhead turtles with FP tumors was recorded on the Eastern Banks of Moreton Bay.

Although numerous foraging hawksbill turtle (*Eretmochelys imbricata*) populations have been examined in the eastern Indian Ocean and SW Pacific Ocean (Fig. 3), hawksbill turtles with FP tumors have only be recorded at a very low frequency at one site – Eastern Banks of Moreton Bay.

FP tumors have been recorded at very low frequencies on nesting female green, loggerhead, hawksbill, and flatback (*Natator depressus*) turtles on widely scattered Australian nesting beaches (Table 1).

Long term mark-recapture studies of green turtles dominated by the same genetic stock have been conducted at multiple foraging study sites in central and southern Queensland, using standard turtle rodeo and beach jumping capture methods and flipper tagging with titanium flipper tags in studies lead by the same research team:

- **Moreton Bay** (27°S): 1990–2014, elevated and variable frequencies of turtles with FP tumors; annually recorded frequencies ranged 5–20% when averaged across all age classes and all study sites within the Bay. Moreton Bay is a large coastal bay receiving large outflows from 5 rivers, with catchments substantially altered by agricultural, pastoral, urban, and industrial developments.
- Western Shoalwater Bay (22°S): 1986–2012, low but variable frequencies of turtles with FP tumors; annually recorded frequencies ranged 2–5% when averaged across all age classes and all study sites within the Bay. Shoalwater Bay is a large coastal bay with no substantial rivers and whose catchments are relatively unaltered.by European development.

Heron and Wistari Reefs (23°S): 1984–1999, trivial frequency of turtles with FP tumors; annually recorded frequencies ranged < 1% when averaged across all age classes and all study sites within these reefs on the outer Great Barrier Reef, ~ 80 km offshore from the mainland. These reefs are bathed by oceanic water with little direct influence from coastal river outflows.

The differences in frequency of occurrence of turtles with FP tumors have remained relatively similar within each of these study sites, but markedly different among these study sites across decades of monitoring. A qualitative generalization made from examination of FP tumor frequency at all study sites in eastern Queensland, irrespective of the length of the study period, is that FP tumor frequency will be highest in coastal embayments with reduced water quality associated with altered catchments, and lower in coastal embayments with relatively unaltered catchments. The frequency of FP tumored turtles has been trivial at all coral reef study sites offshore from the mainland coast.

There have been two extensive veterinary pathology studies conducted by University of Queensland School of Veterinary Science to determine cause of strandings and death of green turtles foraging in Moreton Bay:

- **1990-1996**: 108 green turtles examined; 7% mortality attributed to FP (Anita Gordon, PhD study).
- **2006-2009**: 153 green turtles examined, 0.7% mortality attributed to FP (Mark Flint, PhD study).

There have been two records of turtles with internal FP tumors and no records of turtles with tumors within the buccal cavity in eastern Australia. Corneal FP tumors are commonly encountered, will impede vision, and are expected to be associated with reduced survivorship of these turtles. There is a lower frequency of turtles with nasal passages blocked by FP tumors, which are expected to impede olfaction and negatively impact feeding.

An examination of the population structure of marine turtles foraging in the eastern Australian study sites has shown that FP tumors occur with all age classes of turtles, except for those that have very recently recruited from open ocean pelagic foraging to benthic foraging in coastal waters. From these observations, it is concluded that turtles are infected with FP after recruitment to residency in coastal foraging areas.

A comprehensive Capture-Mark-Recapture analysis of green turtles of all age classes, from small recently recruited juveniles to large adults, of both sexes and dominated by green turtles from the sGBR genetic stock, foraging on the Eastern Banks of Moreton Bay during 1990–2014, concluded:

• Prevalence has been variable across the decades. For juveniles: increasing during the 1990s, from ~ 2% in 1991–1992, reaching a peak of ~ 20% in the mid-2000s, and declining to ~ 10% by 2014. For large immature turtles and adults: declining from ~ 14% in the early 1990s to approaching zero in 2014.

- Apparent survival probability was age class and disease-state dependent.
 - The apparent survival probability was high, as expected, with the respective age classes not presenting with FP tumors, with adults having the highest survival probability.
 - Turtles that presented with FP tumors had lower apparent survival probabilities by ~ 0.07 for both juveniles and the large immature-adult age classes.
- Prevalence rate is age class dependent, with the higher prevalence recorded among juvenile turtles, CCL < 65.0 cm.
- Recovery rate following being recorded with FP tumors as not age class dependent. A good recovery rate was recorded across all age classes.
- This green turtle population, which as has the highest frequency of turtles recorded with FP in Queensland, has been increasing robustly across the 25 years of the CMR study, with an approximate tripling of the foraging population on these banks.

There has been a comparable increase in the size of the annual nesting populations at the index beaches for this sGBR genetic stock over recent decades.

Studies in Queensland have explored the hypothesis that the toxic blue-green alga (*Lyngbya majuscula*) produces a toxic cofactor (Lyngbyatoxin A) which promoted the formation of FP tumors in green turtles (Arthur et al., 2006a, b, 2008):

- Major *Lyngbya majuscula* blooms in eastern Moreton Bay (2000) and western Shoalwater Bay (2002).
- Green turtles reduced their feeding on seagrass that was overgrown with Lyngbya.
- Green turtles did ingest small quantities of *Lyngbya* when blooms were present.
- Lyngbyatoxin A was detected in green turtle tissues even though only small quantities of the alga were being consumed.
- There were no spikes of increase of turtles with FP in the years that immediately followed the very large *Lyngbya* blooms in either eastern Moreton Bay or western Shoalwater Bay.

From these studies, there is no clear evidence supporting the hypothesis that the toxic algae *Lyngbya majuscula* produces a toxic cofactor (Lyngbyatoxin A) which promotes FP.

Given that the virus has been detected in ozobranchid leeches and given the very high incidence of these leeches in foraging areas with turtles with elevated frequency of turtles with FP, we would encourage experimental work to test how ChHV5 virus can be transmitted via ozobranchid leeches between turtles within a single foraging area, and between foraging areas with leeches travelling on migrating turtles.

Current research at JCU is focusing on the viral activity within the turtles examining, among other issues, CHHV5 infection and tumor development.

Summary conclusions

- FP has been present for a long time in eastern Australia, long before the commencement of the Queensland Turtle Research project in 1968.
- Elevated levels of frequency of turtles with FP have occurred at very few localized foraging areas in recent decades.
- Many turtles show recovery from FP.
- While FP can result in death of some turtles, mortality is low.
- The foraging area with the highest prevalence of FP in eastern Australia, eastern Moreton Bay, supports a robustly increasing population of green turtles in spite of an FP epidemic in the area during the late 1990s–2000s
- FP has, at worst, been a minor threat to population recovery.
- Management response should be based on sound science.
- The broad scale project promoted by federal and state Government conservation agencies to improve coastal water quality in eastern Queensland via the "Paddock to Reef Program" is expected to contribute to reduction in poor water quality conditions that could be conducive to proliferation of this disease in our turtles.

Collaborating partners in data sharing for this report:

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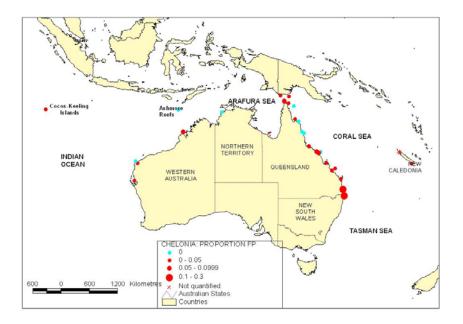


Figure 1.--Frequency of occurrence of green turtles, *Chelonia mydas*, with FP tumors in foraging populations sampled in mark-recapture studies in the eastern Indian Ocean and South West Pacific Oceans.

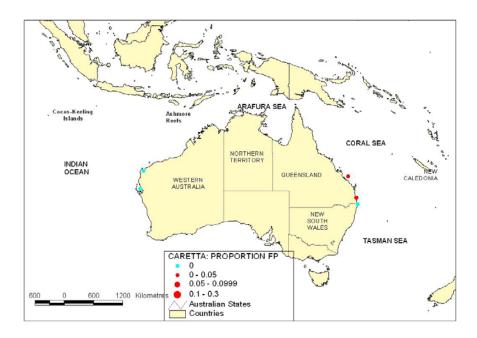


Figure 2.--Frequency of occurrence of loggerhead turtles, *Caretta caretta*, with FP tumors in foraging populations sampled in mark-recapture studies in the eastern Indian Ocean and South West Pacific Oceans.

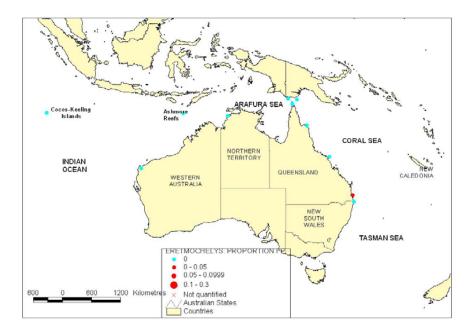


Figure 3. Frequency of occurrence of hawksbill turtles, *Eretmochelys imbricata*, with FP tumors in foraging populations sampled in mark-recapture studies in the eastern Indian Ocean and south West Pacific Oceans.

Table 1.--Summary of frequency of turtles recorded with FP tumors at Australian marine turtle nesting beaches by species and genetic stocks. Stock identification follows FitzSimmons and Limpus (2014).

| Species | Genetic stock | Rookery | FP frequency |
|--------------|-------------------|-----------------|--------------|
| Caretta | SW Pacific | WOONGARRA COAST | 0.02 |
| | | HERON ISLAND | ~ 0.002 |
| | | WRECK ROCK | ~ 0.002 |
| | | WRECK ISLAND | ~ 0.01 |
| Chelonia | sGBR | HERON ISLAND | ~ 0.005 |
| | | WRECK ISLAND | ~ 0.005 |
| | nGBR | RAINE ISLAND | 0.0001 |
| Eretmochelys | East Indian Ocean | ROSEMARY ISLAND | 0.0002 |
| Natator | East Australian | WOONGARRA COAST | ~ 0.005 |
| | | CURTIS ISLAND | ~ 0.005 |
| | Arafura Sea | CRAB ISLAND | ~ 0.001 |
| | | FLINDERS BEACH | ~ 0.001 |

Hawaiian Archipelago Fibropapillomatosis Data

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Information on fibropapillomatosis (FP) in Hawaii is collected by the Pacific Islands Fisheries Science Center's (PIFSC) Marine Turtle Biology and Assessment Program (MTBAP). The data collected are diverse, ranging from isolated observations of incidental encounters with turtles on the high-seas to behavioral data gathered in systematic surveys of nesting beaches or inshore habitats. Due to the different types of data, the information is opportunistically and periodically collected. Data come from the Hawaiian Archipelago, American Samoa, the U.S. Pacific Remote Island Areas, and the Mariana Archipelago (Pooley, 2013).

These data are essential to support timely population assessments and recovery monitoring managed in a comprehensive Turtle Data Processing System (TDPS). The TDPS is modular and supports data of various types including strandings (14.8%), nearshore encounters (62.4%), and nesting turtles (22.8%). Cross-referencing and multiple-encounter analysis are enabled through a core database file of individual turtle identifications, based on inscribed flipper and passive integrative transponder tags or by unique identifiers such as the date, location, and size for those that are not tagged. There are many fields and types of data collected, but only an extracted portion of what is entered into TDPS will be presented (Tokunaga, 1992).

TDPS provides for the systematic storage, retrieval, and summarization of data collected in the three source categories: data pertaining to turtles found stranded on beaches or incidentally caught in nearshore fishing gear, data collected by MTBAP or partners during surveys of nearshore turtle habitat, and data on nesting activity collected by MTBAP, U.S. Fish and Wildlife Service (USFWS), or collaborating biologists from turtle nesting beaches. In all three categories, most turtles involved are uniquely identified through the application of flipper tags. Thus, a key feature of the database is the ability to trace the history of encounters with individual turtles and cross-reference information among data sets. A central turtle identification file facilitates this (Tokunaga, 1992). For this report, the focus will be on the data collected within the Hawaiian Archipelago. The large demographic data streams (from 1973 to 2014) of the strandings, ocean capture, and nesting turtles related to FP will be extracted from TDPS and presented.

Strandings

Most of the data on FP occurrence come from the turtles that wash up or haul out on the beach and are reported by the public. MTBAP tracks the data from a turtle stranding and salvaging program that began in 1982 (Murakawa et al., 2000), which allowed the public to provide information on dead, alive, or injured turtles. In 1990, additional staff were hired and a stranding hotline number added, increasing the amount of reports received by MTBAP (Fig. 1). Reports jumped from 159 reports in 1990, to a high of 308 in 2011. The number of strandings has remained steady, averaging between 234 and 254 per year since 1996.

Overall demographics

Stranding data will be summarized by species, age class, gender, and cause of strandings. Unlike other sources of turtle data from nearshore ocean capture and nesting surveys, the stranding data allow the collection of additional morphometrics, performance of health assessments and necropsies, and collection samples.

There are five sea turtle species that have stranded in Hawaiian waters (Fig. 2). The majority of the stranding data are collected from the greens, with a minority from hawksbills, olive ridleys, and sporadically from leatherbacks and loggerheads. The unknown turtles are those that we were unable to confirm or visually inspect.

Categorizing turtles into specific age classes is based upon using the straight carapace length (SCL, cm) measurement. The age classes are: < 6.0 cm SCL hatchling, 6.0-9.9 cm SCL post-hatchling, 10.0-64.9 cm SCL juvenile, 65.0-79.9 cm SCL subadult, and 80.0 cm+ SCL adult. Based upon the 6,960 strandings in the database, there were < 1% hatchlings and post-hatchlings, 37.6% juveniles, 9.6% sub-adults, 5.7% adults, and 46.5% unknown SCL (Fig. 3). The high number of the unknown age class is due to the fact that some of the SCLs cannot be determined because the live turtle may have swum away, the carcass washed away, or there is damage to the carapace.

Necropsies are performed on collected carcasses and the gonads are visually inspected (Fig. 4). Of course, this is dependent on the decomposition of the carcass since severely decomposed turtles have internal organs that are indistinguishable. Throughout the years, there appears to be nearly a 1:1 sex ratio as the trend for females and males follow a very similar pattern. There is a high number of turtles with unknown gender as not all turtles are recovered, necropsied, or in good body condition to examine the gonads.

Most turtles that are retrieved are assessed for any diseases, injuries, or abnormalities, providing treatment, care and/or release for live turtles, and necropsying dead turtles. There are 146 cause of stranding categories in the database and the top 7 categories (in order from highest to lowest occurrence) are unknown, FP, miscellaneous, fish hook and line, gillnet, boat impact, and shark attack (Fig. 5). FP cases were the most common in a moderate to severe stage of affliction. Miscellaneous cases were a consolidation of the remaining 100+ categories. The fish hook and line category represents nearshore recreational fishing gear. Coastal net entanglements are usually due to discarded nets. Boat impact cases were turtles that had evidence of linear or parallel gashes indicative of a propeller strike. A shark attack is represented by a semi-circular pattern on the carapace or plastron, or having tattered skin with a missing limb.

Fibropapillomatosis (FP) effect

As the dominant cause of strandings, there appears to be a shift in the number of tumored turtles in recent years (Fig. 6). Overall, there were 39.7% tumored turtles, 41.7% non-tumored turtles, and 18.6% with unknown tumor presence as we were unable to visually inspect the turtle. Tumored turtles comprised less than 40% of those seen from 1982 to 1987. Then from 1987 to 2004 (with the exception of 1990), the number peaked at 52% of total turtles examined. However, in 2005, the number of tumored turtles declined to the current level of 32%. To evaluate these trends, fibropapillomatosis was examined within each of these categories: geographic location, age class, gender, tumor severity.

In 2006, President George W. Bush created the Papahānaumokuākea Marine National Monument, making this the largest marine wildlife reserve in the world (Federal Register, 2006, Fig. 7). Access to the monument is limited through a permit system. The uninhabited sandy atolls of the Northwestern Hawaiian Islands (NWHI) present a different type of habitat than the main Hawaiian Islands (MHI). These islands are not populated by humans except during the field seasons, used mainly for monk seal surveying during the summer months. Data collected on stranded turtles are secondary, not consistent, and opportunistically collected throughout the year during research cruises that transverse the archipelago. Overall, not many strandings are recorded in the NWHI. There was a total of 143 strandings (1976–2014) with the highest number of 21 in 1996. Of this small sample, 7.0% were tumored turtles, 83.2% non-tumored, and 9.8% with unknown tumor presence.

In contrast to the NWHI, the MHI are populated with some very developed areas. There is more coverage of the MHI as the public and other Federal, State, and city agencies provide information to the stranding hotline. In addition, there are collaborators on several islands that respond to strandings. Therefore, the strandings database primarily reflects the MHI and that area is our best insight to the prevalence of fibropapillomatosis (Fig. 8).

The MHI is made up of 8 islands, 7 of which are populated. Oahu had more than half of the total strandings followed by Maui, Hawaii island, Kauai, Molokai, and Lanai and Kahoolawe (Fig. 9). There were 41.6% tumored, 38.7% non-tumored, and 19.7% with unknown tumor presence. We think the percent of strandings is the consequence of accessible coastline and density of people to encounter strandings. Overall turtles with FP were found mostly on Maui, followed closely by Oahu. On Maui, there were 56.9% tumored, 41.6% on Oahu, and 19.6% tumored on Hawaii Island. On Hawaii Island, there is a unique situation where the division of the island into east and west sections provides different scenarios. On the east side, there were 37.6% tumored, but on the west side of Hawaii Island there were just 2.8% tumored. More interestingly, the west side of the island is considered a tumor-free area, but there have been 9 cases of fibropapillomatosis which appear to be anomalies. On Kauai, Molokai, and Lanai there were very few strandings which lead to erratic data patterns due to no consistent stranding collection. Although Kahoolawe is uninhabited, a live post-hatchling was found washed ashore in lethargic condition in 2011.

Of all the age classes, the sub-adults had the highest incidence of FP. There was a minimal amount of hatchlings and post-hatchlings, none of which were found with fibropapillomatosis. In juveniles, there were 42.2% with tumors, 56.3% non-tumored, and 1.5% with unknown tumor evidence (Fig. 10). From 1988, juvenile tumored turtles increased before starting a decline in 2004. In contrast, for sub-adults, there were a total of 71.1% with tumors, 24.6% non-tumored, and 4.3% unknown tumor evidence (Fig. 11). Tumored sub-adults peaked in 1992, then declined from 1994 to 1995, then hit a peak in 2006. These numbers then decreased from 2009 to 2013, but slightly increased in 2014. For adults, the incidence of FP declined, 53.5% with tumors, 39.2% non-tumored, and 7.3% with unknown tumor presence (Fig. 12). The peak was in 1993, and then declined until 1999 (there were only 3 adults total). Tumored adults peaked again in 2010, and decreased dramatically until 2014.

FP incidence was roughly equal in females and males. For the females there were 53.8% tumored turtles, 44.0% non-tumored turtles, and 2.2% with unknown tumor presence. Tumored female turtles peaked at 41% in 2004, started declining until 2013, and then did a slight increase in 2014 (Fig. 13). For the males, there were 50.8% tumored turtles, 47.5% non-tumored turtles, and 1.7% with unknown tumor presence. Tumored male turtles peaked at 37 in 2005, then started declining to a low of 12 in 2013 (Fig. 14).

The presence of a tumor does not reflect the stage of the disease so the tumors were scored to determine how afflicted the turtle is with fibropapillomatosis. To calculate the overall tumor score, the turtle is first examined visually for any presence of tumors. Additionally, if the turtle is necropsied, an internal examination is performed to visually inspect for tumors found in the body cavity or in the internal organs. If a tumor is found, it is counted and assigned a number based on its size. A tumor that is 1-3 cm (size of a pinky nail) is categorized as a 1, 4-6 cm (when joining the thumb tip to the pointer tip, the area of the circle) is a 2, 6-10 cm (smaller than a fist) is a 3, and >10 cm (larger than a fist) is a 4. Once all the tumors are counted and sized, an overall score is given to determine the severity of the tumor affliction. An overall score of 0 means that there is no presence of fibropapillomatosis, 1 is mildly afflicted, 2 is moderately afflicted, and 3 is severely afflicted (prior to 2000, a score of 4 would be the worst case, but after 2000, Fig. 15).

Nearshore Ocean Capture

Approximately 62% of the database is from hand capturing sea turtles in nearshore waters to take morphometrics, perform health assessments, apply tags for identification, and collect samples (Balazs et al., 2000).

Overall demographics

For the nearshore ocean capture of foraging turtles, there were 82.3% captured in the MHI and 17.7% captured in the NWHI. Breaking it down by age class, there were 11.5% hatchlings, 5.0% post-hatchlings, 43.4% juveniles, 7.2% sub-adults, 2.6% adults, and 30.3% unknown age class (Fig. 16).

Nearly all the turtles captured were greens, but there were also 49 hawksbills.

The tumor prevalence in nearshore ocean captures peaked at 23.1% in 2001, but was then followed by a steep decline until 2006. The tumor prevalence remained under 5% until 2014 (with only 394 total turtles captured, Fig. 17).

Although the juveniles represent the largest age class encountered, it was the sub-adult age class which had the most tumored turtles at 21.1%, followed by the juveniles at 8.3% (Fig. 16). The nearshore encounters in the Hawaiian Archipelago are mainly non-tumored turtles (92.8%), followed by mildly afflicted (3.2%), moderately afflicted (2.2%), severely afflicted (1.6%), and unknown tumor affliction (0.2%). In the mid-90s, Palaau, Molokai was one of the study sites where nearly 50% of all turtles captured were tumored. The first tumored turtle was seen in 1982, the second one in 1987 (Balazs et al., 1998). There was a high of 51.5% (N = 101) tumored turtles in 1992, then it declined to a low of 8.5% (N = 6) in 2010 (Fig. 18). In comparison, the Kaneohe Bay, Oahu study site had a high of 81% (N = 21) tumored turtles in 2001, which then declined to 50% (N = 2) in 2011 (Fig. 19).

Nesting

Nesting for Hawaiian greens occurs mainly at French Frigate Shoals in the Northwestern Hawaiian Islands (Balazs, 1980). The nesting data comprise 22.8% of the database. Usually 1 or 2 nesting technicians take about 1 month during the peak nesting season to identify/mark each nester, record the nesting activity, collect morphometrics and samples, scan/apply flipper and/or passive integrated transponder tags, examine the turtle for disease, injuries, or abnormalities, and sometimes apply satellite transmitters. Turtle nesting surveys are performed, mainly at East Island, with some data collected at Tern Island and Midway Atoll. A comprehensive nesting survey at East Island has been collected for 42 seasons (Fig. 20). The trend for nesting females has increased considerably with 2012 and 2014 being extremely high. There was no comprehensive survey in 2013, due to limited personnel and time, but brief site surveys were performed on 12 of the NWHI. Tumored turtles are seen during the nesting season, but the turtles are found mildly or moderately afflicted with FP. Overall, there was a total of 7.0% tumored turtles, 93.0% non-FP, and < 1.0% unknown tumor presence. Tumored turtles peaked at 18% in 2000, and have remained under 10% since then, with the exception of 2006, when there were 13% tumored turtles. Main Hawaiian Island nests are slowly increasing but monitoring has not been initiated.

Discussion

The overall trend of tumor prevalence in the Hawaiian Archipelago appears to be on a decline for the three data streams of strandings, nearshore ocean capture, and nesting turtles. There is a probable bias for stranded turtles as they are sick and, therefore, likely will have a higher FP prevalence. The nearshore ocean captured turtles appear to provide the best numbers to calculate FP prevalence as we hand capture most turtles sighted. But the nearshore data are site specific as the west side of Hawaii Island is predominantly non-tumored. There is light affliction to turtles found in the NWHI. Also, FP has not been documented in newly recruited turtles from pelagic waters, which suggests the disease is related to their coastal environment. The nesting surveys in the NWHI have shown only lightly afflicted turtles, as most turtles must be in good condition to

travel the long distance from neritic to nesting habitats. Considering that most of them return to the MHI after nesting, it is curious as to why more nesters are not tumored as they interact with tumored turtles in the neritic waters.

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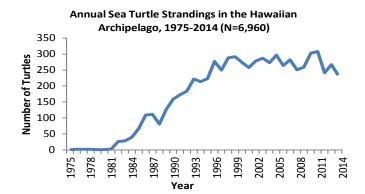


Figure 1.--Annual sea turtle strandings in the Hawaiian Archipelago from 1975 to 2014 (N = 6,960).

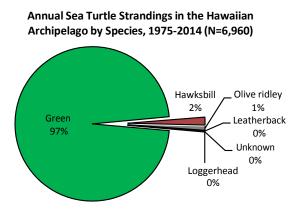


Figure 2.--Annual sea turtle strandings in the Hawaiian Archipelago based on species from 1975 to 2014 (N = 6,960).

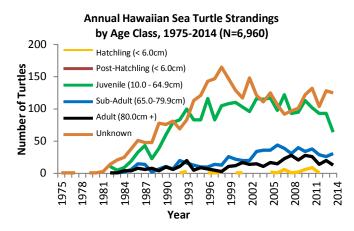


Figure 3.--Annual Hawaiian sea turtle strandings based on age class from 1975 to 2014 (N = 6,960).

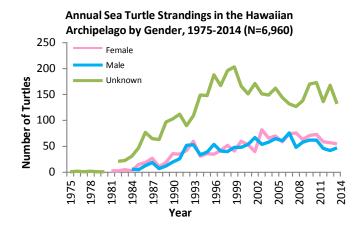


Figure 4.--Annual Hawaiian sea turtle strandings based on gender from 1975 to 2014 (N = 6,960).

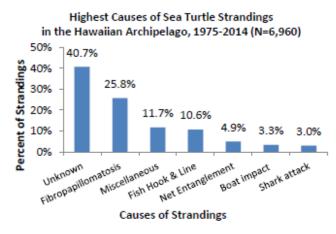


Figure 5.--Highest causes of Hawaiian sea turtle strandings from 1975 to 2014 (N = 6,960).

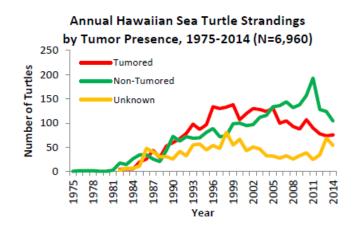


Figure 6.--Annual Hawaiian sea turtle strandings based on tumor presence from 1975 to 2014 (N = 6,960).

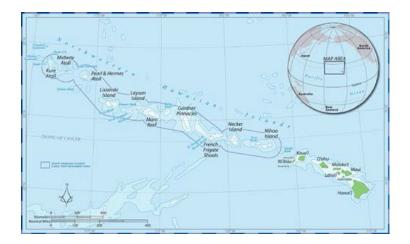


Figure 7.--Map of the Hawaiian Archipelago including the Papahānaumokuākea Marine National Monument of the Northwestern Hawaiian Islands (white islands surrounded by the dark blue line) and the main Hawaiian Islands (green islands on the bottom right).

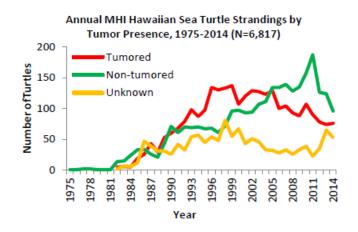


Figure 8.--Annual MHI sea turtle strandings from 1975 to 2014 (N = 6,817).

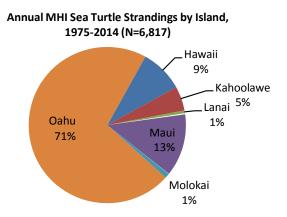


Figure 9.--Annual MHI sea turtle strandings by island from 1975 to 2014 (N = 6,817).

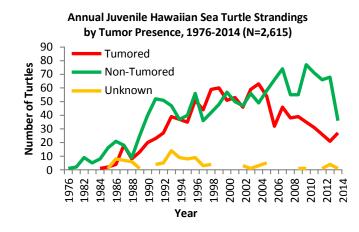


Figure 10.--Annual juvenile Hawaiian sea turtle strandings based on tumor presence from 1976 to 2014 (N = 2,615).

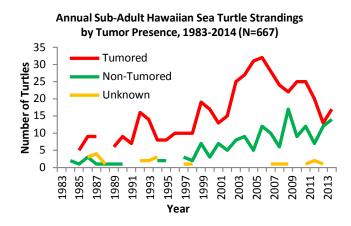


Figure 11.--Annual sub-adult Hawaiian sea turtle strandings based on tumor presence from 1983 to 2014 (N = 667).

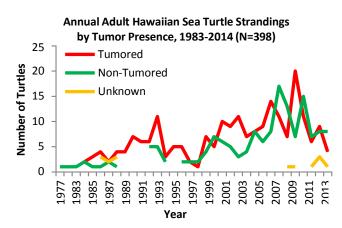


Figure 12.--Annual adult Hawaiian sea turtle strandings based on tumor presence from 1983 to 2014 (N = 398).

Annual Female Hawaiian Sea Turtle Strandings by Tumor Presence, 1981-2014 (N=1,473)

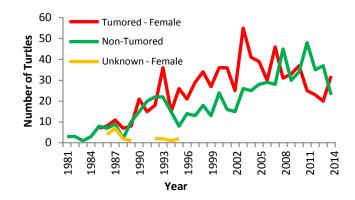


Figure 13.--Annual female Hawaiian sea turtle strandings by tumor presence from 1981 to 2014 (N = 1,473).

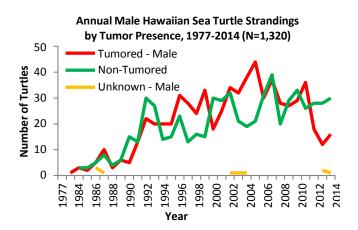


Figure 14.--Annual male Hawaiian sea turtle strandings by tumor presence from 1977 to 2014 (N = 1,320).

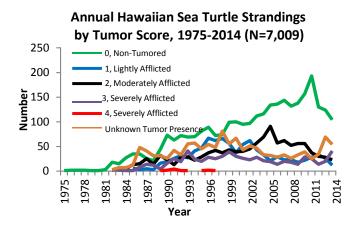


Figure 15.--Annual Hawaiian sea turtle strandings based on tumor score from 1975 to 2014 (N = 7,009).

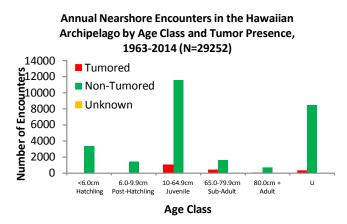


Figure 16.--Nearshore ocean captures of Hawaiian sea turtles based on age class and tumor presence from 1963 to 2014 (N = 29,252).

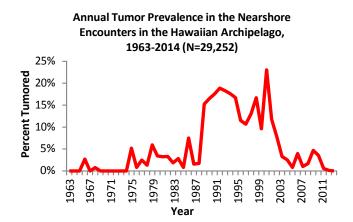


Figure 17. Annual tumor prevalence in nearshore encounters in the Hawaiian Archipelago from 1963 to 2014 (N = 29,252).

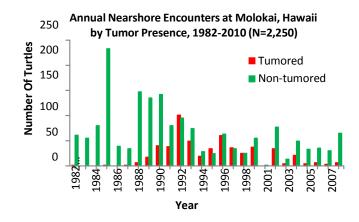


Figure 18.--Annual nearshore ocean captures at Molokai, Hawaii, based on tumor presence from 1982 to 2010 (N = 2,250).

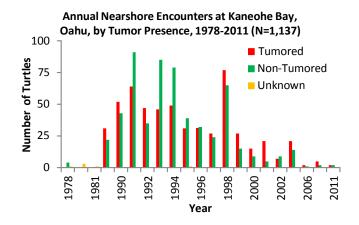


Figure 19.--Annual nearshore encounters at Kaneohe Bay, Oahu based on tumor presence from 1978 to 2011 (N = 1,137).

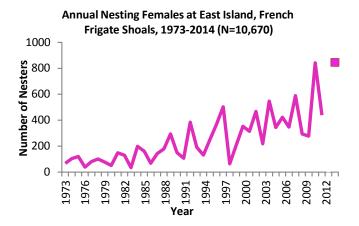


Figure 20.--Annual nesting females at East Island, French Frigate Shoals from 1973 to 2014 (N = 10,670).

Chronic Disease Impacts on the Population Dynamics of a Marine Megaherbivore

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Prepared for the Western Pacific Regional Fisheries Management Council, Honolulu, Hawaii, USA; April 2015

Summary

Most studies of wildlife disease ecology focus on infectious diseases for terrestrial species with few studies on marine species or chronic diseases. Marine megafauna such as whales, sharks, and marine turtles are particularly suitable for the study of chronic disease effects on wildlife population dynamics because they are long-lived, hence the disease can be fully expressed. So a 29-yr monitoring and surveillance program was used to explore the impact of a major cancerous disease (fibropapillomatosis) on the population dynamics of a green sea turtle population resident in coastal waters near Molokai (Hawaii), which is considered the main global enzootic hotspot for this disease. A size class-structured multistate capture-mark-recapture modelling approach was used to derive epidemiologic parameters based on capture-mark-recapture histories for 1,904 uniquely tagged immature turtles sampled since 1982. Disease status of each turtle was assessed at each encounter using a 4-level tumor severity score but recoded as disease presence or absence to simplify analysis. Significant pathogen-induced mortality was found with the annual apparent survival probability lower for FP-diseased immature turtles (0.78, 95% CI: 0.68–0.85) than for disease-free immatures (0.88, 95% CI: 0.81–0.93), irrespective of size class. The recapture probabilities were also independent of size class but time-varying and disease-state-dependent, suggesting sampling bias or behavioral differences for the diseased turtles. Annual abundance estimates derived from disease-state-dependent recapture probabilities suggests a stable longterm population size trend of ca. 1,860 immature green sea turtles. So despite exposure to a virulent disease, this population of turtles has shown no sign of any decline over the past 3 decades. The estimated FP disease prevalence curve shows a rapidly increasing prevalence rate following the disease outbreak in the early-1980s, followed by a significant and gradual decline from the mid-1990s as the disease ran its course. At the peak of the epidemic in the mid-1990s, it was estimated that prevalence was at least 46%. The annual disease infection rate, or force-ofinfection, was size class-dependent with larger turtles having a higher probability of infection (0.26, 95% CI: 0.15–0.42) than smaller turtles (0.18, 95% CI: 0.11–0.29). The annual disease recovery rate was independent of time and very similar for both size classes (small: 0.16, 95% CI: 0.07–0.34, large: 0.15, 95% CI: 0.07–0.29). Recapture probabilities were low and so some model parameters or model-derived outputs, like population size and prevalence, were estimated with low precision. Nonetheless, this is the first comprehensive study of the impact of a chronic and virulent disease on the long-term population dynamics of a large long-lived marine species.

APPENDIX A—Terms of Reference

2015 International Summit on Fibropapillomatosis: Global Status, Trends, and Population Impacts June 11-14, 2015 Honolulu, Hawaii

Purpose

The 2015 Fibropapillomatosis Summit is being held to provide a forum to assess the status and trends of the disease globally and its demographic impact on sea turtles.

The aims of the Summit are:

- To identify areas where substantial status and trend data exist for fibropapillomatosis in sea turtles.
- Convene an expert working group to evaluate data and identify data gaps.
- Identify priority regions where status and trends data would be desirable.
- To exchange ideas, strengthen skills, and share examples of good practice.
- Develop recommendations for standardized monitoring of fibropapillomatosis.

Steering Committee

George Balazs – Summit Chair – NOAA, Pacific Islands Fisheries Science Center Allen Foley – Florida Fish and Wildlife Conservation Commission Thierry Work – USGS National Wildlife Health Center-Honolulu Field Station Stacy Hargrove* – NOAA, Southeast Fisheries Science Center Shandell Brunson – NOAA, Pacific Islands Fisheries Science Center

* Replacing Yonat Swimmer who resigned with regrets due to other compelling agency duties. Yonat continues to serve as a Special Consultant to the Steering Committee.

Participants

The Summit is open to those who have data on status and trends of fibropapillomatosis. This includes, but is not limited to, invitational expert participants from five regions. In addition, participation can include representatives from other organizations that also promote and support research on fibropapillomatosis.

Products

At the conclusion of the Summit, the Steering Committee in collaboration with invitational participants and Quantitative Specialists will draft a paper summarizing global trends of

fibropapillomatosis its demographic impacts, future research needs, and guidelines for standardized monitoring of the disease.

Regions to be Represented

Eastern Indian Ocean and Southwest Pacific Brazil and Adjacent South Atlantic Congo – West Africa Florida and Southeast USA Puerto Rico and wider Caribbean Hawaiian Islands

Summit Facilitator

Shandell Brunson - NOAA, Pacific Islands Fisheries Science Center

Keynote Address Speaker

Brian Stacy - NOAA Office of Protected Resources

Broad View Wildlife Disease Perspective

Daniel Walsh – United States Geological Survey

Fibropapillomatosis Etiology Research Sub Chairs

Thierry Work – United States Geological Survey Jennifer Lynch – National Institute of Standards and Technology

Quantitative Specialists

Milani Chaloupka – Ecological Modelling Services Kyle Van Houtan – NOAA, Pacific Islands Fisheries Science Center Daniel Walsh – United States Geological Survey

Workshop Dates

June 11-14, 2015

Past and Future Time-Lines for the Workshop

January-August 2014 – Conceived and Formulated Summit conduction and ideas through discussion involving G. Balazs, T. Work and A. Foley.

September 2014 – Submitted Summit proposal for Pacific Islands Fisheries Science Center FY2015 Milestone.

October 2014 – Acceptance of Summit proposal as an approved Pacific Islands Fisheries Science Center Milestone.

October – December 2014 – Summit Steering Committee appointed and meetings convened to exchange ideas, draft actions, and conduct global polling and communications to identify regions

and persons of particular prominence to fibropapillomatosis Steering Committee sets Summit dates of 11-14 June 2015.

February 2015 – Invitations sent to identified individuals from key regions.

January – June 2015 – Steering Committee meets every 2-3 weeks.

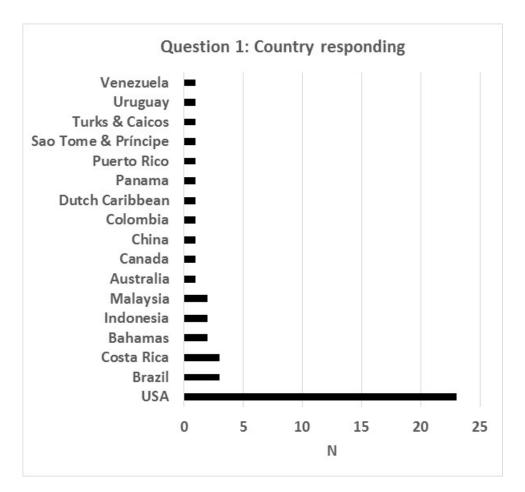
June 2015 – Convene Summit

December 2015 – Draft manuscript prepared suitable for review under the Pacific Islands Fisheries Science Center publications approval process.

APPENDIX B—Monkey Survey Poll and Results

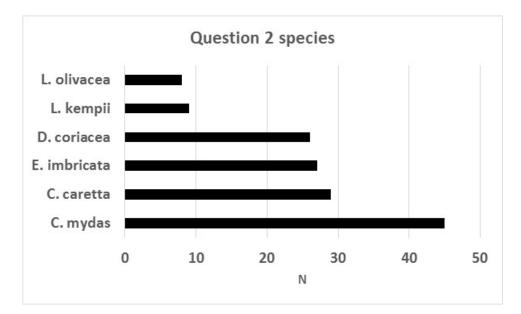
Dear members of C-Turtle. Thank you for filling out the FP survey. We had 47 responses.

Question 1: Please write in the country (and the coastline if there is more than one) in which you monitor sea turtles.



| you monitor sea turtles. Site Country Australia Australia, Moreton Bay, Queensland Australia Abaco, Bahamas Bahamas Bahamas Bahamas Bahamas Bahamas Angra dos Reis Coastline, Rio de Janeiro State, Brazil Brazil Brazil Brazil BRAZIL Brazil BRAZIL Brazil Canada Canada Canada Canada Costa Altan Colombia Colombia Costa Altante Costa Rica Costa Rica Costa Rica St Eustatius, Duch Caribbean Indonesia Indonesia Indonesia Malaysia (Borneo: Sabah & Sarawak) Malaysia (cast coast Sabah) Malaysia (cast coast) Uruguay Murica Sates, Sabah Broward County, Florida (cast coast) USA Pain Beach County, Florida (cast coast) USA Malaysia USA United States, Atlantic Coast of Florida USA USA USA USA USA USA USA USA | Responses to Question 1: Please write in the country (and the c | oastline if there is more than one) in which | | |
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| USA, Florida east and west coastsUSAUSA, Northeast FloridaUSAVenezuelaVenezuela | | | | |
| USA, Northeast FloridaUSAVenezuelaVenezuela | | | | |
| Venezuela Venezuela | | | | |
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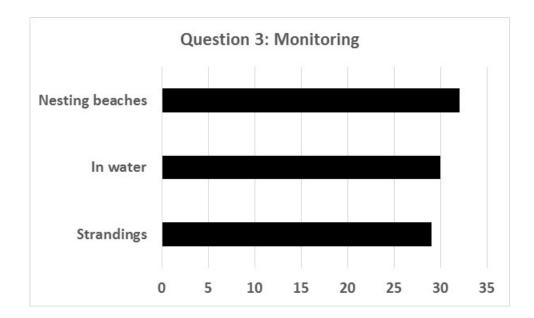
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Question 2: What species do you monitor (check all that apply)?

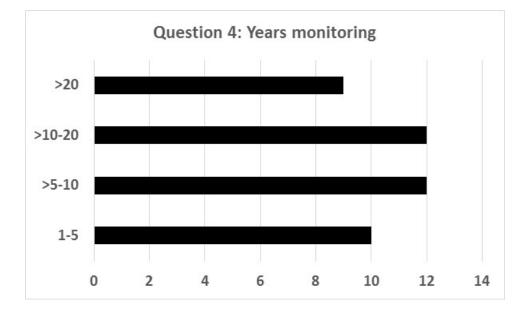
| Responses to Question 2: What species do you monitor? | | | | | | | |
|---|---------|-------|----------|-----------|--------|----------|--|
| Row Labels | C. | C. | D. | E. | L. | L. | |
| | caretta | mydas | coriacea | imbricata | kempii | olivacea | |
| Australia | 1 | 1 | | | | 1 | |
| Bahamas | | 2 | | 1 | | | |
| Brazil | 1 | 3 | 1 | 2 | | 1 | |
| Canada | 1 | 1 | 1 | | 1 | | |
| China | 1 | 1 | | 1 | | | |
| Colombia | 1 | 1 | 1 | 1 | | 1 | |
| Costa Rica | 1 | 2 | 3 | 1 | | | |
| Dutch Caribbean | | 1 | 1 | 1 | | | |
| Indonesia | | 2 | | 1 | | 1 | |
| Malaysia | | 2 | | 2 | | | |
| Panama | 1 | 1 | 1 | 1 | | | |
| Sao Tome & | | 1 | | 1 | | | |
| Principe | | | | | | | |
| Turks & Caicos | | 1 | | 1 | | | |
| Uruguay | 1 | 1 | 1 | 1 | | 1 | |
| USA | 20 | 24 | 16 | 12 | 7 | 2 | |
| Venezuela | 1 | 1 | 1 | 1 | | 1 | |

Question 3: Where does your monitoring occur?

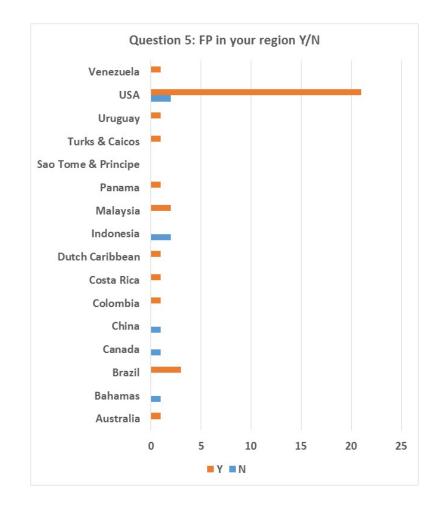


| Responses to Question 3: Where does your monitoring occur? | | | | |
|--|----------|-----------------|------------|-------------|
| Row Labels | In water | Nesting beaches | Strandings | Grand Total |
| Australia | | | 1 | 1 |
| Bahamas | 2 | | | 2 |
| Brazil | 3 | | | 3 |
| Canada | 1 | | 1 | 2 |
| China | | 1 | | 1 |
| Colombia | 1 | | | 1 |
| Costa Rica | | 3 | | 3 |
| Dutch Caribbean | | 1 | | 1 |
| Indonesia | | 2 | | 2 |
| Malaysia | 2 | | | 2 |
| Panama | 1 | | | 1 |
| Sao Tome & Principe | 1 | | | 1 |
| Turks & Caicos | 1 | | | 1 |
| Uruguay | 1 | | | 1 |
| USA | 16 | 11 | 1 | 28 |
| Venezuela | 1 | | | 1 |
| (blank) | | 1 | | 1 |
| Grand Total | 30 | 19 | 3 | 52 |

Question 4: How many years have you monitored sea turtles in your region?



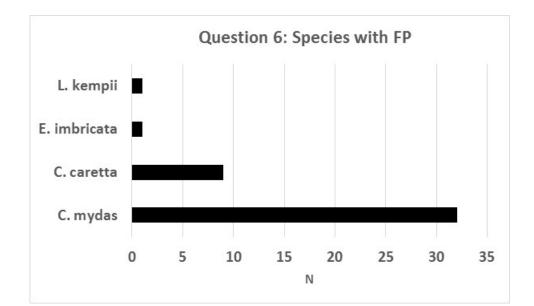
| Responses to Question 4: How many years have you monitored sea turtles in your region? | | | | |
|--|---------|------|--------|-----|
| Row Labels | > 10-20 | > 20 | > 5-10 | 1–5 |
| Australia | | | 1 | |
| Bahamas | | | | 1 |
| Brazil | | | 2 | 1 |
| Canada | 1 | | | |
| China | | | | 1 |
| Colombia | | 1 | | |
| Costa Rica | 1 | | | |
| Dutch Caribbean | 1 | | | |
| Indonesia | | | 1 | 1 |
| Malaysia | 1 | | | 1 |
| Panama | | 1 | | |
| Sao Tome & Principe | | | 1 | |
| Turks & Caicos | | | 1 | |
| Uruguay | 1 | | | |
| USA | 6 | 7 | 6 | 4 |
| Venezuela | 1 | | | |



Question 5: Have you ever encountered fibropapillomatosis during your monitoring?

| Responses to Question 5: Have you ever encountered | | | |
|--|---|----|--|
| fibropapillomatosis during your monitoring? | | | |
| | N | Y | |
| Australia | | 1 | |
| Bahamas | 1 | | |
| Brazil | | 3 | |
| Canada | 1 | | |
| China | 1 | | |
| Colombia | | 1 | |
| Costa Rica | | 1 | |
| Dutch Caribbean | | 1 | |
| Indonesia | 2 | | |
| Malaysia | | 2 | |
| Panama | | 1 | |
| Sao Tome & Principe | | | |
| Turks & Caicos | | 1 | |
| Uruguay | | 1 | |
| USA | 2 | 21 | |
| Venezuela | | 1 | |

Question 6: In what species have you seen fibropapillomatosis?



| Responses to Question 6: In what species have you seen fibropapillomatosis? | | | | |
|---|------------|----------|--------------|-----------|
| Row Labels | C. caretta | C. mydas | E. imbricata | L. kempii |
| Australia | | 1 | | |
| Bahamas | | | | |
| Brazil | | 3 | | |
| Canada | | | | |
| China | | | | |
| Colombia | | 1 | 1 | |
| Costa Rica | | 1 | | |
| Dutch Caribbean | | 1 | | |
| Indonesia | | | | |
| Malaysia | | 2 | | |
| Panama | | 1 | | |
| Sao Tome & Principe | | | | |
| Turks & Caicos | 1 | 1 | | |
| Uruguay | | 1 | | |
| USA | 7 | 19 | | 1 |
| Venezuela | 1 | 1 | | |
| (blank) | | | | |
| Grand Total | 9 | 32 | 1 | 1 |

Question 7: For each species affected, please give us an overall impression of the estimated percent of animals affected.

| Responses to Question 7: For each species affected, please give us an overall impression of the estimated percent of animals affected. | | | | |
|--|----|-------|----------|-------|
| | 0% | 1–10% | > 10–30% | > 30% |
| C. caretta | 6 | 4 | 1 | |
| C. mydas | 3 | 12 | 9 | 6 |
| D. coriacea | 7 | | | |
| E. imbricata | 8 | 1 | | |
| L. kempii | 6 | 1 | | |
| L. olivacea | 5 | 1 | | |
| N. depressus | 5 | | | |

Question 8: How would you characterize the longer term trend of fibropapillomatosis for each species affected above?

| Responses to Question 8: How would you characterize the longer term trend of fibropapillomatosis for each species affected above? | | | | |
|---|------------|------------|------------|--------|
| Row Labels | Decreasing | Don't know | Increasing | Stable |
| C. caretta | | 4 | | 5 |
| C. mydas | 6 | 9 | 2 | 13 |
| D. coriacea | | 5 | | 2 |
| E. imbricata | | 6 | | 4 |
| L. kempii | | 4 | 1 | 2 |
| L. olivacea | | 4 | | 2 |
| N. depressus | | 4 | | 2 |

APPENDIX C—1997 Honolulu Workshop on Marine Turtle Fibropapillomatosis, Priority Recommendations of the Workshop Participants

1997 HONOLULU WORKSHOP ON MARINE TURTLE FIBROPAPILLOMATOSIS

Priority Recommendations of the Workshop Participants

National Marine Fisheries Service Southwest Fisheries Science Center Honolulu Laboratory 2570 Dole Street Honolulu, Hawaii 96822-2396

Priorities for Research

Evaluate the disease in other sea turtle species.

Identify modes of transmission.

Determine impacts at the population level.

Identify causative agent(s).

Isolate herpesvirus.

Identify and examine toxic effects (biotoxins/pollutants).

Develop diagnostic test.

Understand relationships and interface between epidemiology, epizoology, and ecologic geography of different turtle species and the disease.

Examine similarities and differences of habitats and turtles where the disease does and does not occur.

Determine long-term effects on turtles using tag recapture data.

Develop a strategy for determining and differentiating causative agent(s) from other "symptoms".

Determine and identify the presence of anatomically specific viruses (such as in glottal tumors).

Priorities for Epidemiology

Conduct field studies on immunosuppression and associations of the disease.

Conduct basic studies on mortality and morbidity rates.

Identify methods for monitoring the prevalence and incidence of the disease worldwide.

Priorities for Transmission Studies

Identify virus(es).

Development experimental designs.

Priorities for Management Options

Promote international cooperation.

Identify possible scenarios and related actions that can be taken.

Insure that research activities do not spread the disease.

APPENDIX D—Agenda for 2015 International Summit on Fibropapillomatosis: Global Status, Trends, and Population Impacts



Convened by the NOAA Pacific Islands Fisheries Science Center JUNE 11-14, 2015 HONOLULU, HAWAII

| | , | |
|------------------------|--|--------------------|
| Thursday, June 1 | , | |
| Daniel K. Inouye | Regional Center (IRC), Ford Island Pearl Harbor, Honolulu | , Hawaii |
| **Please note there an | re security restrictions for entry onto Ford Island IRC - please contact th | he Summit Chair*** |
| 1100 am - 100 pm | Arrival of Regional Representatives and Steering Committee | |
| | Lunch on-site with NMFS Center/Region personnel | |
| | Short tour of IRC facility | |
| 100 - 115 pm | Hawaiian Blessing and recognition of King Kamehameha Day | |
| 115 - 130 pm | Formal Opening by Summit Chair, George.Balazs@noaa.gov | |
| | • Introduction of Michael Seki, Science Center Director, and | |
| | Frank Parrish, Protected Species Division Chief, for | |
| | welcoming remarks | |
| | • Introduction of Devon Francke, Joint Institute for Marine | |
| | and Atmospheric Research, for welcoming remarks | |
| | • Introduction of Summit Steering Committee members: | |
| | Thierry Work, Allen Foley, Shandell Brunson, Stacy | |
| | Hargrove | |
| | • Introduction of Quantitative Specialists: Milani | |
| | Chaloupka, Kyle Van Houtan, Daniel Walsh | |
| | Introduction of Regional Representatives and Other | |
| | Presenters: Colin Limpus, Alexandre Girard, Cecilia | |
| | Baptistotte, Carlos Diez, A. Foley, Llew Ehrhart, Jennifer | |
| | Lynch, Shawn Murakawa, Brian Stacy | |
| | • Introduction of Rapporteur, <i>Stacy Hargrove</i> , for welcome | |
| | and instruction | |
| | • Turn over to Summit Moderator/Facilitator, S. Brunson, for | |
| 120 220 pm | opening remarks and Summit goals and procedures Keynote: <i>"Fibropapillomatosis in 2015: a historical review</i> | Drion Stoory |
| 130 - 230 pm | and modern perspective on why it remains an important | Brian Stacy |
| | disease" | |
| 230 - 315 pm | "Overview of Fibropapillomatosis Disease Etiology" | Thierry Work |
| 230 313 pm | "Role of environmental pollution in fibropapillomatosis of | Jennifer Lynch |
| | marine turtles" | benniner Lynen |
| 315 - 330 pm | Moderated audience comments and questions | Shandell Brunson |
| 330 - 345 pm | Break | |
| 345 - 430 pm | Non-fibropapillomatosis Epidemiology Presentation: | Daniel Walsh |
| × | "Wildlife Epidemiological Investigations: Moving from | |
| | Observation to Understanding Processes" | |
| 430 - 445 pm | Moderated audience comments and questions | Shandell Brunson |
| 445 - 545 pm | Puerto Rico/Caribbean Regional Data Presentation | Carlos Diez |
| 545 - 600 pm | Questions and preliminary comments by Quantitative | Milani Chaloupka |
| | Specialists | Daniel Walsh |
| | | Kyle Van Houtan |
| 600 pm | Adjourn | |

Agenda for 2015 International Summit on Fibropapillomatosis: Global Status, Trends, and Population Impacts



Convened by the NOAA Pacific Islands Fisheries Science Center JUNE 11-14, 2015 HONOLULU, HAWAII

Friday, June 12, 2015 – DAY 2

Daniel K. Inouye Regional Center (IRC), Ford Island Pearl Harbor, Honolulu, Hawaii ***Please note there are security restrictions for entry onto Ford Island IRC - please contact the Summit Chair***

| 830 - 845 am | Opening remarks and agenda overview for Day 2 | Shandell Brunson George Balazs |
|-------------------|---|-----------------------------------|
| 845 - 945 am | Florida/Southeast USA Regional Data Presentation | Allen Foley |
| 945 - 1030 am | Florida Ocean-Capture Data Presentation | Llew Ehrhart |
| 1030 - 1045 am | Moderated audience comments and questions | Shandell Brunson |
| 1045 - 1100 am | Break | |
| 1100 am - 1200 pm | Brazil/South Atlantic Regional Data Presentation | Cecilia Baptistotte |
| 1200 - 1215 pm | Questions and preliminary comments by Quantitative | Milani Chaloupka |
| | Specialists | Daniel Walsh |
| | | Kyle Van Houtan |
| 1215 - 130 pm | Lunch | |
| 130 -230 pm | Congo/West Africa Regional Data Presentation | Alexandre Girard |
| 230 - 245 pm | Moderated audience comments and questions | Shandell Brunson |
| 245 - 345 pm | Eastern Indian Ocean/Southwest Pacific Regional Data Presentation | Colin Limpus |
| 345 - 445 pm | Hawaiian Islands Regional Data Presentation | Shawn Murakawa |
| 445 – 500 pm | "Chronic Disease Impacts on the Population Dynamics of a Marine Megaherbivore" | Milani Chaloupka |
| 500 - 530 pm | Questions and preliminary comments by Quantitative | Milani Chaloupka |
| | Specialists | Daniel Walsh |
| | | Kyle Van Houtan |
| 530 pm | Adjourn | |
| | | |

Agenda for 2015 International Summit on Fibropapillomatosis: **Global Status, Trends, and Population Impacts**



Convened by the NOAA Pacific Islands Fisheries Science Center JUNE 11-14, 2015 HONOLULU, HAWAII

| Saturday, June 13 | . 2015 – DAY 3 | | | | |
|--|---|------------------|--|--|--|
| Conference Room of the Western Pacific Regional Fishery Management Council | | | | | |
| | Suite 1400, 1164 Bishop Street in Downtown Honolulu | | | | |
| Suite 1100, 1101 D | | | | | |
| 830 - 845 am | Opening remarks and agenda overview for Day 3 including | Shandell Brunson | | | |
| | any adjustments needed | George Balazs | | | |
| 845 - 1130 am | Round-Table discussions involving all Presenters, | | | | |
| | Quantitative Specialists, and Steering Committee focusing on | | | | |
| | Summit Terms of Reference, including: | | | | |
| | • Development of a framework for determining whether or | | | | |
| | not demographic effects of fibropapillomatosis can be | | | | |
| | assessed for each region based on data and findings | | | | |
| | presented in the previous two days; | | | | |
| | • For those regions where this is not possible, identify data | | | | |
| | gaps and potential actions to modify monitoring to | | | | |
| | address those gaps; | | | | |
| | • For those regions where this is possible, explore specific | | | | |
| | collaborations between particular quants and regional | | | | |
| | representatives, as appropriate, to do further post- | | | | |
| | workshops analytics and assess demographic effects of | | | | |
| | Fibropapillomatosis; | | | | |
| | • Draft outline of paper summarizing the process above for | | | | |
| | publication in Endangered Species Research or other | | | | |
| | comparable journal. | | | | |
| 1130 am - 1200 pm | Moderated open discussion with all attendees on any topic | | | | |
| | relating to Fibropapillomatosis | | | | |
| 1200 pm | Formal Closing of the Summit Chair | George Balazs | | | |
| 1200 - 115 pm | Lunch | | | | |
| 115 - 400 pm | Special post-Summit session for deliberations, collaborative | | | | |
| | future-planning and partnership building. Session limited to | | | | |
| | Presenters, Quantitative Specialists, and Steering Committee | | | | |
| 100 | Members | | | | |
| 400 pm | Adjourn | | | | |

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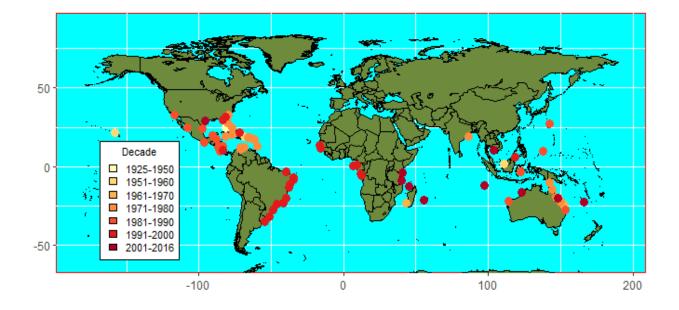
| | HONOLULU, HAWAII |
|--|--|
| Sunday, June 14 Guided field trip t | , 2015 – DAY 4 to Oahu green turtle foraging and basking habitats |
| 1000 am – 430 pm | Designed primarily for presenters and others visiting from outside the Hawaiian Islands. Availability dependent upon number of authorized vehicles arranged. |

APPENDIX E—Summit Participants



Alexandre Girard – France Allen Foley – USA Brian Stacy – USA Carlos Diez – Puerto Rico Cecilia Baptistotte – Brazil Cheryl King – USA Colin Limpus – Australia Daniel Walsh – USA Devon Franke – USA Frank Parrish – USA George Balazs – USA Irene Kelley – USA Isabela Domiciano – Brazil Jennifer Homcy – USA Jennifer Lynch – USA Jessica Jacob – USA Julia Smith – USA Karina Jones – Australia Llewellyn Ehrhart – USA Michael Seki – USA Milani Chaloupka – Australia Renee Breeden – USA Sarah Alessi – USA Shandell Brunson – USA Shawn Murakawa – USA Shawn Murakawa – USA Stacy Hargrove – USA Thierry Work – USA Tommy Cutt – USA Not Shown – Kyle Van Houtan – USA

APPENDIX F—Locations where Fibropapillomatosis was First Documented in Green Turtles Color Coded by Decade



APPENDIX G—Bibliography of Fibropapillomas in Marine Turtles

The Bibliography of Fibropapillomatosis in Marine Turtles is available through the PIFSC Marine Turtle Biology and Assessment Program's website and will be updated quarterly.

http://www.pifsc.noaa.gov/library/pubs/murakawa_balazsbibliography_of_fibropapillomas_in_marine_turtles-2016.pdf

Availability of NOAA Technical Memorandum NMFS

Copies of this and other documents in the NOAA Technical Memorandum NMFS series issued by the Pacific Islands Fisheries Science Center are available online at the PIFSC Web site http://www.pifsc.noaa.gov in PDF format. In addition, this series and a wide range of other NOAA documents are available in various formats from the National Technical Information Service, 5285 Port Royal Road, Springfield, VA 22161, U.S.A. [Tel:.703.-605-6000]; URL: http://www.ntis.gov. A fee may be charged.

Recent issues of NOAA Technical Memorandum NMFS-PIFSC are listed below:

NOAA-TM-NMFS-PIFSC-51 R Stock Assessment Updates of the Bottomfish Management Unit Species of American Samoa, the Commonwealth of the Northern Mariana Islands, and Guam in 2015 Using Data through 2013. A. YAU, M. NADON, B. RICHARDS, J. BRODZIAK, and E. FLETCHER (March 2016)

- 52 Status Review Report: Orange Clownfish (Amphiprion percula).K. A. MAISON and K. S. GRAHAM (April 2016)
- 53 Design and Implementation of a Bottomfish Fishery-independent Survey in the Main Hawaiian Islands.
 B. RICHARDS, S. G. SMITH, J. S. AULT, G. T. DINARDO, D. KOBAYASHI, R. DOMOKOS, J. ANDERSON, J. TAYLOR, W. MISA, L. GIUSEFFI, A. ROLLO, D. MERRITT, J. C. DRAZEN, M. E. CLARKE, and C. TAM (June 2016)