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Marine Turtles as Sentinels of Ecosystem Health: Is Fibropapillomatosis an Indicator?

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Abstract: Marine turtle fibropapillomatosis (FP) is a disease primarily affecting green turtles (*Chelonia mydas*) that is characterized by multiple cutaneous masses. In addition, the condition has been confirmed in other species of sea turtles. The disease has a worldwide, circumtropical distribution and has been observed in all major oceans. Although reported since the late 1930s in Florida, it was not until the late 1980s that it reached epizootic proportions in several sea turtle populations. Long-term studies have shown that pelagic turtles recruiting to near shore environments are free of the disease. After exposure to these benthic ecosystems, FP manifests itself with primary growths in the corner of the eyes spreading to other epithelial tissue. One or more herpesviruses, a papillomavirus, and a retrovirus have been found associated with tumors using electron microscopy and molecular techniques; however, the primary etiological agent remains to be isolated and characterized. Field observations support that the prevalence of the disease is associated with heavily polluted coastal areas, areas of high human density, agricultural runoff, and/or biotoxin-producing algae. Marine turtles can serve as excellent sentinels of ecosystem health in these benthic environments. FP can possibly be used as an indicator but correlations with physical and chemical characteristics of water and other factors need to be made. Further research in identifying the etiologic agent and its association with other environmental variables can provide sufficient parameters to measure the health of coastal marine ecosystems, which serve not only as ecotourism spots but also as primary feeding areas for sea turtles.

Key words: *Chelonia mydas*, ecosystem health, green turtle, fibropapillomatosis, herpesvirus, papillomavirus, pollution, sea turtles, sentinel species

Introduction

It is widely agreed that while tropical coastal marine ecosystems are under severe anthropogenic stresses, the causes and effects are poorly understood. There is consequently an urgent need to identify sentinel species that will accurately serve as environmental health indicators for warm water coastal marine habitats. For many reasons, sea turtles are excellent candidates for this role. These large, long-lived, air-breathing, marine vertebrates operate at the air/water interface and can therefore receive environmental burdens not only from their marine food, but also through inspiration of toxic volatiles. With the exception of the leatherback turtle (*Dermochelys coriacea*), juvenile sea turtles spend many years maturing, with high site fidelity to near-

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coastal feeding habitats, and thus are good representatives of the health of their local ecosystems. Indeed, the high public awareness of sea turtles is such that they can serve not only as effective sentinels of ecosystem health but also as ''flagship species'' to attract public attention (Caro and O'Doherty, 1999).

Marine Turtles as Sentinel Species

Although physically robust and able to accommodate severe physical damage, marine turtles appear surprisingly susceptible to biological and chemical insults (Lutcavage et al., 1997). For example, a short exposure to crude oil in the green turtle (*Chelonia mydas*) may produce necrosis of epidermal epithelium and loss of cellular architectural organization of the skin layers, opening routes for infection (Lutcavage et al., 1995). Harmful effects from such anthropogenic insults include compromised physiology, chronic stress, impaired immune function, and an increase in disease susceptibility (Aguirre et al., 1995; Lutz, 1998).

Unfortunately, our lack of understanding of marine turtle physiology poses a major difficulty for establishing criteria to measure the health of sea turtle populations. To be able to determine the health status of an individual or a population, it is necessary to be able to distinguish between ''normal'' (i.e., functional) physiology and ''disturbed'' (i.e., pathological or nonfunctional) physiology. Much of what we know of reptilian physiology, however, cannot easily be applied to sea turtles. In many respects, marine turtles are unique reptiles with unique problems associated with their life history and physiology, including pulmonary and systematic adaptations for prolonged physiological apnea, and diving reflex and specialized adaptations for osmoregulation in seawater. Furthermore, our knowledge of sea turtle physiology is basic at best (see recent reviews by Bjorndal, 1995; Lutz, 1997).

Marine Turtle Fibropapillomatosis

There is one unambiguous and dramatic indicator of sea turtle health: marine turtle fibropapillomatosis (FP). This is a metaplastic disease of recent epidemic proportions, which is found in tropical coastal ocean waters worldwide, but linked to environmentally challenged habitats. It is a severely debilitating disease externally characterized by epizootic tumors of the skin, flippers, periocular tissues, carapace, and plastron. It also can produce nodules in all internal organs including lungs, heart, liver, spleen, kidneys, gastrointestinal tract, and gonads (Campbell, 1996). The tumors, which can grow to more than 30 cm in diameter, can interfere with the animal's biological fitness and may ultimately prove fatal (George, 1997). Although most tumors appear to be benign and tolerated for many years, the larger tumors can severely mechanically hamper the ability of turtles to swim and dive, locate, capture, and swallow food, and avoid predators. Additionally, the internal tumors may interfere with systemic function. Approximately 25–30% of turtles with external tumors present internal tumors diagnosed as fibromas, fibrosarcomas of low-grade malignancy, and myxofibromas, primarily in the lungs, kidneys, and heart. In addition, tumors are commonly identified in the glottal area and mouth in Hawaiian green turtles (Aguirre et al., 2001; Balazs et al., 1997; Norton et al., 1990; Work and Balazs, 1998). The epidemiology of FP could serve as an effective tool to monitor ecosystem health in local warm-water, near-shore marine habitats.

Marine turtle fibropapillomatosis was first recorded in the 1930s in green turtles caught in Key West, Florida (Smith and Coates, 1938). Following an apparent lull, there has been a great increase in the distribution and incidence of disease since the 1980s. It now has a worldwide, circumtropical distribution and has reached epidemic proportions in many habitats; for example, prevalences of the disease as high as 92% have been reported in Kaneohe Bay, Oahu, Hawaii (Balazs and Pooley, 1991). Initially, it was thought that FP was confined to green turtles, but recent studies have found the disease in all other species, including loggerhead (*Caretta caretta*) (Herbst, 1994), olive ridley (*Lepidochelys olivacea*) (Aguirre et al., 1999), Kemp's ridley (*L kempii*) (Harshbarger, 1991), and leatherback (Huerta et al., 2000). Tumors have been histologically documented in hawksbill turtles (*Eretmochelys imbricata*) hatched and raised in captivity (D'Amato and Moraes-Neto, 2000). Also, tumors have been grossly observed in flatback turtles (*Natator depressus*) but never histopathologically confirmed (Limpus and Miller, 1994).

Distribution and Prevalence

Florida

Smith and Coats reported FP for the first time from captive green turtles in Florida. The following year, the condition was reported in the wild in Key West (Smith and Coats,

1938) and Cape Sable (Lucké, 1938). Perhaps the most complete records of the increase in FP are from the Atlantic coast of Florida. Since 1975, green turtles have been monitored along east central Florida at Mosquito Lagoon during cold-water stunning events (Hirama and Ehrhart, 1999). These long-term studies found no evidence of the disease during the 1970s; however, few animals were handled at the time. During 1985, FP was recorded in 29% of 145 turtles stunned at Mosquito Lagoon. Since 1982, the prevalence of FP in turtles in the central region of the Indian River System (about 120 km south of Mosquito Lagoon) has been 28–65%. At a field site in the Indian River, no FP was observed prior to 1996 but, during 1997, about 5% of turtles presented FP, and this rose to 20% by 2001. The work by Ehrhart et al. (2000) suggests the following pattern of infection: immature green turtles free of FP first migrate to the neritic zone along the Florida coast and remain free of tumors while residing there, but turtles that enter the lagoon system may become infected. During a survey of the distribution of stranded sea turtles in the eastern U.S. from Texas to Massachusetts from 1980 to 1998, it was found that reports of FP were restricted to central and southern Florida. The occurrence of the disease was also correlated with the extent of shallow-water areas (Fick et al., 2000).

Hawaii

The first documented case of FP for the Hawaiian Islands based on gross observations occurred in 1958 in Kaneohe Bay, Island of Oahu, Hawaii. Since then, FP has been recorded from turtle strandings and long-term monitoring studies in these foraging pastures (Aguirre et al., 1994b, 1998, 2002; Balazs and Pooley, 1991). Between 1989 and 1997, mild-to-severe FP was reported in 44% of the turtles sampled, of which 17% presented oral tumors. The annual prevalence of the disease in this region ranges from 42–65% with no consistent trend. Growth rates for turtles with severe tumors were statistically significantly lower than turtles free of FP (1.0 vs. 2.2 cm per year carapace length) (Balazs et al., 2000). However, there has been no detection of population impacts caused by FP in Hawaiian green turtles nesting at French Frigate Shoals. On the contrary, the population has increased since its protection 18 years ago. Interestingly, the disease is absent along the entire west (Kona) coast of the island of Hawaii (Balazs et al., 2000). Previous studies in the Hawaiian Islands demonstrated that turtles approximately 35-cm straight carapace length (SCL) recruit to coastal habitats where they spend most of their lives (Balazs, 1980). Turtles captured in Kaneohe Bay, averaging < 40-cm SCL were free of FP and green turtles averaging > 45-cm SCL had FP. Furthermore, pelagic turtles have been observed free of FP. Apparently, turtles become infected with the infectious agent following movement to near-shore environments (Aguirre et al., 1994b, 1998).

Mexico

La Escobilla Beach, Oaxaca, Mexico, is the most important sanctuary for olive ridley turtles nesting in Mexico. Since federal protection in 1991, the number of turtles nesting has increased considerably, with close to 1 million nests laid in 1997. The presence of FP was first reported during the late 1980s when harvesting of sea turtles was still legal. A recent field survey by gross observation of tumors was performed during the fifth ''arribada'' (massive nesting) of the 1997 nesting season occurring at La Escobilla Beach. Out of a total sample of 9201 nesting females, 140 (1.5%) presented one or more tumors (Aguirre et al. 2000b; Vasconcelos et al., 2000). More recently, Huerta et al. (2000) confirmed FP in a leatherback turtle sampled while nesting in Michoacan, Mexico in 1994.

Costa Rica

The first report of fleshy tumors on the head, neck, and front flippers of nesting olive ridley females in Costa Rica occurred in 1982 (Cornelius and Robinson, 1983). FP was first identified in the Ostional olive ridley population during the ''arribada'' of October 1987. A nesting female was photographed demonstrating grossly multiple cutaneous tumors with the largest measuring 30 mm in diameter. Since that time, based on field observations, both the prevalence and the size of skin tumors in individual animals have increased. An average of 300,000 turtles nest at Ostional, Costa Rica each month. Fibropapillomatosis has recently been histologically confirmed in olive ridley turtles from Costa Rica. It is calculated that approximately 6–10% of these nesting females present cutaneous FP, with 1% being severely affected (Aguirre et al., 1999).

Australia

Marine turtle fibropapillomatosis was first diagnostically confirmed in a stranded, juvenile green turtle in Baba

Head, Shark Bay, Western Australia in 1995 (Raidal and Prince, 1996). More detailed investigations by Aguirre et al.(2000) in Eastern Australia at Moreton Bay, Queensland in 1998 described FP in specimens collected from 50 green and 12 loggerhead turtles demonstrating a prevalence of 16 and 6%, respectively. Tumors from both species of sea turtles were histologically similar to those previously described in sea turtles of the Hawaiian Islands, Florida, Indonesia, and Costa Rica. The presence of tumors has been reported in Australian waters through visual observations since 1988 with a prevalence ranging from 2 to 22% in green turtles and 1% in loggerheads (Limpus and Miller, 1994).

Cosmopolitan Disease

Perhaps due to limited funding or human resources, FP has gone unnoticed in other parts of the world until current times. A recent note documents the possible presence of FP in one green turtle in St. Croix, U.S. Virgin Islands since 1971 (Eliazar et al., 2000). The disease has been reported in all oceans and many tropical countries, including countries in the Caribbean, making it a panzootic (Williams et al., 1994). Marine turtle fibropapillomatosis was observed in green turtles in Barbados around 1982 or 1983, then grossly diagnosed in 1990 (Gamache and Horrocks, 1992). More recently, FP has been diagnostically confirmed in harvested green turtles from Bali, Indonesia (Adnyana et al., 1997) and green turtles (Matsushima et al., 2000) and captive-raised hawksbill turtles in Brazil (D'Amato and Moraes-Neto, 2000). Gross diagnosis has occurred in green turtles in Cuba (Moncada and Prieto, 2000), Nicaragua (Lagueux et al., 1998), and Panama (Herbst, 1994).

Etiology

While FP has been investigated intensively, little has been conclusively ascertained as to its cause. There are currently two main etiological hypotheses of FP. The nontumor hypothesis purports that the growths are a cellular reaction to scar tissue or may represent hyperplastic tissue. The tumor hypothesis regards the growths as neoplasia caused by one or several of a wide variety of agents. It is generally accepted that FP is a disease of ''multifactorial'' etiology, in which parasites, including spirorchid trematodes and their ova (Aguirre et al., 1994a, 1998; Dailey and Morris, 1995; Herbst et al., 1998), bacteria (Aguirre et al., 1994b), environmental pollutants (Aguirre et al., 1994a; Herbst and Klein, 1995a; Sakai et al., 2000), UV light, changing water temperatures (Herbst and Klein, 1995b), and biotoxins (Landsberg et al., 1999) may all be contributing factors. Chronic stress, immunologic status, physiologic status, and genetic factors have also been discussed as potential contributing factors of tumor formation (Aguirre, 1991; Aguirre et al., 1995; Balazs and Pooley, 1991; Landsberg et al., 1999; Lutz et al., 2001; Work and Balazs, 1999; Work et al., 2000, 2001). The causative agents, the potential contributing factors, and the natural transmission of the disease remain unknown.

Viruses

Two groups of viruses have been associated with the tumors. The enveloped viruses, which are sensitive to environmental factors, include herpesvirus and retroviruses. The nonenveloped viruses, which are environmentally resistant, include papillomavirus and polyomavirus. A herpesvirus was found in over 95% of the tumors studied in Florida (Herbst, 1994). Experimental transmission studies using cell-free tumor extracts were performed implicating a filterable, chloroform-sensitive, subcellular, infectious agent, most likely a virus. Direct experimental inoculation of turtles has consistently demonstrated horizontal transmission; tumors have developed within a year post- inoculation at the site of inoculation in turtles previously free of the disease (Herbst et al., 1995, 1996). Herpesvirus-like intranuclear inclusions were found in 50% of the experimentally induced tumors (Herbst et al., 1999). A herpesvirus, probably belonging to the subfamily *Alphaherpesvirinae* was found associated with FP (Herbst et al., 1999; Lackovich et al., 1999). Electromicroscopic evidence of a herpes virus has been reported on several occasions (Aguirre and Spraker, 1996; Herbst, 1994; Herbst et al., 1995; Jacobson et al., 1989). The presence of a herpesvirus was also confirmed by using a consensus-primer Polymerase Chain reaction (PCR) for a highly conserved region of the herpesviral DNA-polymerase gene (Quackenbush et al., 1998; VanDevanter et al., 1996), and by conventional and inverse PCR (Lu et al., 2000b; Yu et al., 2000). Tissue specimens from different marine turtle species from Australia, Barbados, and Mexico demonstrated by real-time PCR and sequence analysis of the DNA polymerase (pol) DNA from the fi-

bropapillomatosis-associated turtle herpesvirus that the sequences were highly related to each other regardless of species and geographic location (Quackenbush et al., 2001). The virus has not been cultured in vitro (Lackovich et al., 1999) and there is no proof of causality for FP either in laboratory or field conditions.

A study by Casey et al., (1997) demonstrated that retroviral infections are widespread in the Hawaiian green turtle population, but did not find association with FP. More recently, tumor specimens from Hawaiian green turtles were screened for infectious agents using representational difference analysis. Previously unknown viral sequences were detected including a terminase and a major capsid protein, both related to the alphaherpesvirus subfamily, and a reverse transcriptase, related to endogenous retroviruses. The herpesvirus DNA sequences were found almost exclusively in tumor tissue and in absence of RNA expression. In contrast, the retroviral reverse transcriptase DNA was present in both tumor and skin apparently free of tumors (Kurz and Aguirre, 2000).

Finally, Lu et al. (2000a) identified a small, naked virus in tumor-like aggregates in cell lines derived from a green turtle. This virus was identified as a papilloma-like virus using electron microscopy. However, research by Brown et al. (1999) utilizing PCR on a large number of freshly isolated tumor samples failed to detect papillomavirus suggesting that papillomavirus is not the etiological agent of FP. The molecular identification of one or more herpesviruses (Aguirre and Spraker, 1996; Herbst et al., 1995, 1996; Lackovich et al., 2000, Lu et al., 2000b,c), a papilloma-like virus (Lu et al. 2000a) and retrovirus sequences (Casey et al., 1997) in Hawaiian green turtles has complicated the scenario for determining the primary infectious agents of tumor formation.

Immunosuppression

There appears to be a link between the development of FP and the immune system. Immunosuppression is strongly correlated with FP in captive green turtles (Cray et al., 2002), and in a study of wild green turtles from different habitats in East Florida, Sposato et al., (2002) found that compromised immune function is associated with tumor development. Similarly, turtles in Hawaii with advanced FP were immunosuppressed and chronically stressed (Aguirre et al., 1995; Work et al., 2000, 2001). Immunosuppression could be the cause or consequence of conditions that lead to FP expression.

Pollutants

Aguirre et al. (1994a) tested several turtle tissues from Hawaiian waters for environmental pollutants, but no toxic levels were detected nor was any correlation with the presence of FP found. A recent review found that persistent chlorinated compounds, such as polychlorinated biphenyls (PCBs) and chlorinated pesticides, tend to be at the low end of the range of values reported for sea birds and marine mammals; however, loggerhead and Kemp ridley turtles had higher heavy metal burdens than other sea turtles (Pugh and Becker, 2001).

Marine Biotoxins

Landsberg et al. (1999) reported an association between the distribution of toxic benthic dinoflagellates (*Prorocentrum* sp.) and the occurrence of FP in green turtles of the Hawaiian Islands. The finding is of particular interest because *Prorocentrum* has been documented to produce a tumor-promoting toxin, okadaic acid. The dinoflagellates are epiphytic on microalgae and sea grasses and would therefore be ingested by foraging green sea turtles. During the same study, okadaic acid was also found in the tissues of Hawaiian green turtles.

Water Temperature

Several environmental factors may be involved in the progression or regression of FP (Herbst, 1994). An important parameter linked to tumor formation and spread of the infectious agent is water temperature. Apparently, warm water temperatures and seasonality have been related to tumor growth and metastasis in Lucké renal adrenocarcinoma of leopard frogs (*Rana pipiens*) and papillomas of Japanese newts (*Cynops pyrrhogaster*). Tumor regression of FP has been observed in green turtles from Australia and Hawaii, and in olive ridley turtles from Costa Rica and Mexico. A common factor is that most samples came from adult nesting females. However, more studies are underway to characterize regression and identify its relationship with the role of the host immune response (Aguirre et al., 2000a).

General Health

It is difficult to define health parameters for sea turtles using clinical, chemical and hematological workups for avian and mammalian medicine since sea turtles appear to regulate most blood chemistry parameters over a much wider range than do endotherms (Sposato et al., 2002). For example, in healthy (non-FP) juvenile green turtles from the Trident basin in Florida, the blood glucose level ranged from 29–245 mg/dl and the lactate dehydrogenase (LDH) ranged from 141–1987 U/L (Sposato et al., 2002). Aguirre and Balazs (2000) found that Hawaiian turtles with large numbers of tumors were hypoproteinemic, hypoferremic, and anemic, and in an advanced stage of acidosis with imbalanced calcium/phosphorus ratios and severe emaciation. However, they found no significant differences in most parameters between healthy turtles and those with light or moderate FP. On the other hand, Sposato et al. (2002) found that turtles from FP-prevalent Indian River Lagoon had altered blood chemistries compared to turtles from nearby FP-free habitats even if the turtle showed no outward sign of the disease. The differences included lower blood glucose, calcium, phosphorous, uric acid, alkaline phosphatase, aspartate aminotransferase, and LDH.

Conclusions

The advantages of utilizing the sea turtle as a sentinel species are clear: sea turtles have a high public profile and are especially vulnerable to anthropogenic degradation of environmental health. Sea turtles are valid sentinels of specific habitats since they are long lived, and juveniles demonstrate a high degree of coastal habitat site fidelity. In particular, FP is a dramatic indicator of sea turtle health. In such impacted habitats as Indian River, Florida and Kaneohe Bay, Hawaii, extensive sea turtle monitoring programs and the biodiversity and chemical monitoring programs already in place offer effective platforms for long-term monitoring of the health status of sea turtle populations. The health is monitored through the spread, pathogenesis, and impacts of FP on foraging aggregations and, eventually, the population. Indeed, recent evidence indicates that sea turtles may be particularly vulnerable to a variety of environmental insults such as high water temperature, infectious agents, pollutants, and marine biotoxins. Harmful effects include compromised physiology, impaired immune function, and an increase in the incidence of disease.

We should continue the development and refinement of environmental and physiological health assessment and monitoring protocols for sea turtles worldwide. The first step has been undertaken by conducting current health assessments at some sites (i.e., Australia, Florida, Hawaii). These long-term assessments will provide basic information, which, when combined with other species information, can be utilized to evaluate the ecological system and determine the responses to naturally fluctuating environmental variables (Aguirre et al., 2002; Tabor et al., 2001).

Sea turtles represent ideal sentinel species that increase monitoring efficiency at the ecosystem level. The manifestation of FP can be utilized during rapid risk assessments to provide information on the environmental conditions of an area. Sea turtles clearly are a unique health indicator species because of their ability to reflect environmental perturbations. Based on their life history and physiological attributes, sea turtle species can provide insightful information about environmental changes at various spatial, temporal, and trophic scales (Aguirre et al., 2002; Tabor et al., 2001).

In conclusion, although there is evidence of viral involvement in FP, the identity of the causative factor is yet to be determined. Several agents could be involved in the expression of the disease in a predisposing or multifactorial manner. Infectious agents, chemical carcinogens, environmental pollutants, biotoxins, and immunosuppression may play roles in the etiology of FP (Adnyana, 1997). While it is clearly vital to identify the causative factor(s), from a utilitarian point of view, a practical diagnostic tool is needed that will identify the disease and ascertain its progress and regression.

The potential for applying existing diagnostic capacity to health monitoring of sea turtles is immeasurable. Particularly, the limitation of financial resources has been a major barrier. Laboratory support for performing diagnostic testing is minimal, occurring in limited laboratories with other primary research activities in Hawaii, Florida, and New York, among others. Cross-species diagnostic testing, in which one laboratory can do comparative assessment of disease between populations and species, is sorely lacking. Yet diagnostic laboratory testing plays an important role in monitoring health and diseases such as FP. The results of diagnostic testing are most useful when used to complement thorough field-based ecological research. These will represent the next steps to address ecosystem health in the marine environment.

References

Adnyana W, Ladds PW, Blair D (1997) Observations of fibropapillomatosis in green turtles (*Chelonia mydas*) in Indonesia. *Australian Veterinary Journal* 75:737–742

Aguirre AA (1991) Green turtle fibropapilloma: an epidemiologic perspective. In: *Research Plan for Marine Turtle Fibropapilloma*, Balazs GH, Pooley SG (editors), U.S. Department of Commerce, NOAA Technical Memorandum NMFS-SWFSC-156, pp 107–113

Aguirre AA, Balazs GH (2000) Plasma biochemistry values of green turtles (*Chelonia mydas*) with and without fibropapillomas in the Hawaiian Islands. *Comparative Haematology International* 10:132–137

Aguirre AA, Spraker TR (1996) Pathology associated with cardiovascular trematodes and fibropapillomas in juvenile green turtles (*Chelonia mydas*) from the Hawaiian Islands. U.S. Department of Commerce, NOAA-NMFS Administrative Report H-95-01C, Honolulu, Hawaii, 20 pp

Aguirre AA, Balazs GH, Zimmerman B, Galey FD (1994a) Organic contaminants and trace metals in the tissues of green turtles (*Chelonia mydas*) afflicted with fibropapillomas in the Hawaiian Islands. *Marine Pollution Bulletin* 28:109–114

Aguirre AA, Balazs GH, Zimmerman B, Spraker TR (1994b) Evaluation of Hawaiian green turtles (*Chelonia mydas*) for potential pathogens associated with fibropapillomas. *Journal of Wildlife Diseases* 30:8–15

Aguirre AA, Balazs GH, Spraker TR, Gross TS (1995) Adrenal and hematological responses to stress in juvenile green turtles (*Chelonia mydas*) with and without fibropapillomas. *Physiological Zoology* 68:831–854

Aguirre AA, Spraker TR, Balazs GH, Zimmerman B (1998) Spirorchidiasis and fibropapillomatosis in green turtles from the Hawaiian Islands. *Journal of Wildlife Diseases* 34:91–98

Aguirre AA, Spraker TR, Chaves-Quiroz A, du Toit LA, Balazs GH (1999) Histopathology of fibropapillomatosis in olive ridley turtles *Lepidochelys olivacea* nesting in Costa Rica. *Journal of Aquatic Animal Health* 11:283–289

Aguirre AA, Limpus CJ, Spraker TR, Balazs GH (2000a) Survey of fibropapillomatosis and other potential diseases in marine turtles from Moreton Bay, Queensland, Australia. In: *Proceedings of the 19th Annual Symposium on Sea Turtle Conservation and Biology*, Kalb H, Wibbels T (editors), U.S. Department of Commerce, NOAA Technical Memorandum NMFS-SEFSC-443, p 36

Aguirre AA, Vasconcelos Perez J, Spraker TR, Hernandez Saldafia P, Principe B, Albavera Padilla E, et al. (2000b) Studies of marine turtle fibropapillomatosis in Mexico: an international collaboration of research and training. In: *Proceedings of the 20th Annual Symposium on Sea Turtle Biology and Conservation*, Mosier A, Foley A, Brost B (editors), U.S. Department of Commerce, NOAA Technical Memorandum NMFS-SEFSC-477, p 50

Aguirre AA, Spraker TR, Morris R, Powers B, Zimmerman B (2001) Low-grade fibrosarcomas in green turtles (*Chelonia mydas*) from the Hawaiian Islands. In: *Proceedings of the 21st Annual Symposium on Sea Turtle Biology and Conservation*, Philadelphia, Pennsylvania. U.S. Department of Commerce NOAA Technical Memorandum NMFS-SEFSC (in press)

Aguirre AA, O'Hara TM, Spraker TR, Jessup DA (2002) Monitoring the health and conservation of marine mammals and sea turtles and their ecosystems. In: *Conservation Medicine: Ecological Health in Practice*, Aguirre AA, Ostfeld RS, Tabor GM, House CA, Pearl MC (editors), New York: Oxford University Press, pp 79–94

Balazs, GH (1980) Synopsis of biological data on the green turtle in the Hawaiian Islands. U.S. Department of Commerce, NOAA Technical Memorandum NMFS-SWFSC-7, Honolulu, Hawaii, 141 pp

Balazs, GH, Pooley, SG (editors) (1991) Research plan for marine turtle fibropapilloma. U.S. Department of Commerce, NOAA Technical Memorandum NMFS-SWFSC-156, Honolulu, Hawaii, 113 pp

Balazs GH, Aguirre AA, Murakawa SKK (1997) Occurrence of oral fibropapillomas in the Hawaiian green turtle: differential disease expression. *Marine Turtle Newsletter* 76:1–2

Balazs GH, Murakawa SKK, Ellis DM, Aguirre AA (2000) Manifestation of fibropapillomatosis and rates of growth of green turtles at Kaneohe Bay in the Hawaiian Islands. *Proceedings of the 18th Annual Symposium on Sea Turtle Biology and Conservation*, Abreu-Grobois FA, Briseño-Dueñas R, Márquez R, Sarti L (editors), U.S. Department of Commerce, NOAA Technical Memorandum NMFS-SEFSC-436, pp 112–113

Bjorndal KA (1995) *Biology and Conservation of Sea Turles,* Washington, DC: Smithsonian Institution Press

Brown DR, Lackovich JK, Klein PA (1999) Further evidence for the absence of papillomaviruses from sea turtle fibropapillomas. *Veterinary Record* 145:616–617

Caro TM, O'Doherty GO (1999) On the use of surrogate species in conservation biology. *Conservation Biology* 13:805–814

Campbell TW (1996) Sea turtle rehabilitation. In: *Reptile Medicine and Surgery*, Mader DR (editor), Philadelphia: W.B. Saunders, pp 427–436

Casey RN, Quackenbush SL, Work TM, Balazs GH, Bowser PR, Casey JW (1997) Evidence for retrovirus infections in green turtles *Chelonia mydas* from the Hawaiian Islands. *Diseases of Aquatic Organisms* 31:1–7

Cornelius SE, Robinson DC (1983) Abundance, distribution, and movements of olive ridley sea turtles in Costa Rica, III. U.S. Fish and Wildlife Service Endangered Species Report 13, Albuquerque, New Mexico, 52 pp

Cray C, Varella R, Bossart GD, Lutz PL (2002) Altered in vitro immune responses in green turtles with fibropapillomatosis. *Journal of Zoo and Wildlife Medicine* 32:436–440

Dailey MD, Morris R (1995) Relationship of parasites (Trematoda: Spirorchidae) and their eggs to the occurrence of fibropapillomas in the green turtle (*Chelonia mydas*). *Canadian Journal of Fisheries and Aquatic Sciences* 52:84–89

D'Amato AF, Moraes-Neto M (2000) First documentation of fibropapillomas verified by histopathology in *Eretmochelys imbricata*. *Marine Turtle Newsletter* 89:12–13

Ehrhart LM, Redfoot WE, Bagley AB (2000) Green turtles in three developmental habitats of the Florida Atlantic Coast: population structure, fibropapillomatosis and post-juvenile migratory destinations. In: *Proceedings of the 18th Annual Symposium on Sea Turtle Biology and Conservation*, Abreu-Grobois FA, Briseño-Dueñas R, Màrquez R, Sarti L (editors), U.S. Department of Commerce, NOAA Technical Memorandum NMFS-SEFSC-436, p 32

Eliazar PJ, Bjorndal KA, Bolten AB (2000) Early report of fibropapilloma from St. Croix, USVI. *Marine Turtle Newsletter* 89:16

Fick K, Redlow T, Foley A, Singel K (2000) The distribution of stranded green turtles with fibropapillomas in the Eastern United States, 1980–1998. In: *Proceedings of the 19th Annual*

Symposium on Sea Turtle Conservation and Biology, Kalb H, Wibbels T (editors), U.S. Department of Commerce, NOAA Technical Memorandum NMFS-SEFSC-443, p 236

Gamache N, Horrocks J (1992) Fibropapilloma disease in green turtles, *Chelonia mydas*, around Barbados, West Indies. In: *Proceedings of the 11th Annual Workshop on Sea Turtle Biology and Conservation*, Salmon M, Wyneken J (editors), U.S. Department of Commerce, NOAA Technical Memorandum NMFS-SEFSC-302, pp 158–160

George RH (1997) Health problems and diseases of sea turtles. In: *The Biology of Sea Turtles*, Lutz PL, Musick JA (editors), Boca Raton, FL: CRC Press, pp 363–385

Harshbarger JC (1991) Sea turtle fibropapilloma cases in the registry of tumors in lower animals. In: *Research Plan for Marine Turtle Fibropapilloma*, Balazs GH, Pooley SG (editors), U.S. Department of Commerce, NOAA Technical Memorandum NMFS-SWFSC-156, pp 63–70

Herbst LH (1994) Fibropapillomatosis of marine turtles. *Annual Review of Fish Diseases* 4:389–425

Herbst LH, Klein PA (1995a) Green turtle fibropapillomatosis: challenges to assessing the role of environmental cofactors. *Environmental Health Perspectives* 103(Suppl 4):27–30

Herbst LH, Klein PA (1995b) Monoclonal antibodies for the measurement of class-specific antibody responses in the green turtle, *Chelonia mydas*. *Veterinary Immunology and Immunopathology* 46:317–335

Herbst LH, Jacobson ER, Moretti R, Brown T, Sundberg JP, Klein PA (1995) Experimental transmission of green turtle fibropapillomatosis using cell-free tumor extracts. *Diseases of Aquatic Organisms* 22:1–12

Herbst LH, Moretti R, Brown T, Klein PA (1996) Sensitivity of the transmissible green turtle fibropapillomatosis agent to chloroform and ultracentrifugation conditions. *Diseases of Aquatic Organisms* 25:225–228

Herbst LH, Jacobson ER, Klein PA, Balazs GH, Moretti R, Brown T, et al. (1998) Comparative pathology and pathogenesis of spontaneous and experimentally induced fibropapillomas of green turtles (*Chelonia mydas*). *Veterinary Pathology* 36:551–564

Hirama S, Ehrhart LM (1999) Prevalence and severity of green turtle fibropapillomatosis in the Indian River lagoon. *Florida Scientist* 62:35

Huerta P, Pineda H, Aguirre AA, Spraker TR, Sarti L, Barragan A (2000) First confirmed case of fibropapilloma in a leatherback turtle (*Dermochelys coriacea*). In: *Proceedings of the 20th Annual Symposium on Sea Turtle Biology and Conservation*, Mosier A, Foley A, Brost B (editors), U.S. Department of Commerce, NOAA Technical Memorandum NMFS-SESFC-477, p 193

Jacobson ER, Mansell JL, Sundberg JP, Hajarr L, Reichmann ME, Ehrhart LM, et al. (1989) Cutaneous fibropapillomas of green turtles, *Chelonia mydas*. *Journal of Comparative Pathology* 101:39–52

Kurz SK, Aguirre AA (2000) Representational difference analysis reveals tumor-specific viral sequences in *Chelonia mydas* with fibropapillomatosis. In *25th International Herpesvirus Workshop*, Oregon Health Science University, Portland, Oregon

Lackovich JK, Brown DR, Homer BL, Garber RL, Mader DR, Moretti RH, et al. (1999) Association of herpesvirus with fibropapillomatosis of the green turtle *Chelonia mydas* and the loggerhead turtle *Caretta caretta* in Florida. *Diseases of Aquatic Organisms* 37:889–897

Lackovich JK, Brown DR, Homer BL, Garber RL, Mader DR, Moretti RH, et al. (2000) Association of a new chelonid herpesvirus with fibropapillomas of the green turtle, *Chelonia mydas*, and the loggerhead turtle, *Caretta caretta*. In: *Proceedings of the 18th Annual Symposium on Sea Turtle Biology and Conservation*, Abreu-Grobois FA, Briseño-Dueñas R, Márquez R, Sarti L (editors), U.S. Department of Commerce, NOAA Technical Memorandum NMFS-SEFC-436, pp 273–274

Lagueux CJ, Campbell CL, Herbst LH (1998) Characterization of fibropapilloma occurrence in a Nicaraguan green turtle fishery. In: *Proceedings of the 16th Annual Symposium on Sea Turtle Biology and Conservation*, Byles R, Fernandez Y (editors), U.S. Department of Commerce, NOAA Technical Memorandum NMFS-SEFC-412, p 90

Landsberg JH, Balazs GH, Steidinger KA, Baden DG, Work TH, Russel DJ (1999) The potential role of natural tumor promoters in marine turtle fibropapillomatosis. *Journal of Aquatic Animal Health* 11:199–210

Limpus CJ, Miller JD (1994) The occurrence of cutaneous fibropapillomas in marine turtles in Queensland. In: *Proceedings of the Australian Marine Turtle Conservation Workshop, 14–17 November 1990*. Queensland Department of Environment and Heritage and The Australian Nature Conservation Agency, Brisbane, Australia, pp 186–188

Lu Y, Aguirre AA, Work TM, Balazs GH, Nerurkar VR, Yanagihara R (2000a) Identification of a small, naked virus in tumor-like aggregates in cell lines derived from a green turtle, *Chelonia mydas*, with fibropapillomas. *Journal of Virological Methods* 86:25–33

Lu Y, Wang Y, Yu Q, Aguirre AA, Balazs GH, Nerurkar VR, et al. (2000b) Detection of herpesviral sequences in tissues of green turtles with fibropapilloma by polymerase chain reaction. *Archives of Virology* 145:1–9

Lu YN, Yu QG, Zamzow JP, Wang Y, Losey GS, Balazs GH, et al. (2000c) Detection of green turtle herpesviral sequence in saddleback wrasse *Thalassoma duperrey*: a possible mode of transmission of green turtle fibropapilloma. *Journal of Aquatic Animal Health* 12:58–63

Lucké B (1938) Studies on tumors in cold-blooded vertebrates. *Annual Report of the Tortugas Laboratory of the Camegie Institute* 1937:92–94

Lutcavage ME, Lutz PL, Bossart GD, Hudson DM (1995) Physiologic and clinicopathologic effects of crude oil on loggerhead sea turtles. *Archives of Environmental Contamination and Toxicology* 28:417–422

Lutcavage M, Plotkin P, Witherington B, Lutz PL (1997) Human impacts on sea turtle survival. In: *The Biology of Sea Turtles*, Lutz PL, Musick JA (editors), Boca Raton, FL: CRC Press, pp 387–410

Lutz PL (1997) Salt, water and pH balance in sea turtles. In: *The Biology of Sea Turtles*, Lutz PL, Musick JA (editors), Boca Raton, FL: CRC Press, pp 343–362

Lutz PL (1998) Health related sea turtle physiology. *Report of the Sea Turtle Health Assessment Workshop*, Fair P, Hansen LJ (editors), U.S. Department of Commerce, NOAA Technical Memorandum NOS-NCCOS-CCEHBR-0003, pp 45–49

Lutz PL, Cray C, Sposato P (2001) Studies on the association between immunosuppression and fibropapillomatosis within three habitats of *Chelonia mydas*. U.S. Department of Commerce, NOAA Technical Memorandum H-01-01C 2001

Matsushima ER, Filho AL, di Loretto C, Kanamura CT, Gallo B, Baptisotte C (2000) Cutaneous papillomas of green turtles: A morphological and immunohistochemical study in Brazillian specimens. In: *Proceedings of the Nineteenth Annual Symposium*

on Sea Turtle Conservation and Biology, Kalb, H, Wibbels, T (compilers), U.S. Department of Commerce, NOAA Technical Memorandum NMFS-SEFCS-443, pp 237–239

Moncada F, Prieto A (2000) Incidence of fibropapillomas in the green turtle (*Chelonia mydas*) in Cuban waters. In: *Proceedings of the 19th Annual Symposium on Sea Turtle Conservation and Biology*, Kalb H, Wibbels T (editors), U.S. Department of Commerce, NOAA Technical Memorandum NMFS-SEFSC-443, pp 40–41

Norton TM, Jacobson ER, Sundberg JP (1990) Cutaneous fibropapillomas and renal myxofibroma in a green turtle, *Chelonia mydas*. *Journal of Wildlife Diseases* 26:265–270

Pugh RS, Becker PR (2001) Sea Turtle Contaminants: a Review with Annotated Bibliography. National Institute of Standards and Technology, Technology Administration, U.S. Department of Commerce, NISTIR 6700, 144 pp

Quackenbush SL, Work TM, Balazs GH, Casey RN, Rovnak J, Chaves A, et al. (1998) Three closely related herpesviruses are associated with fibropapillomatosis in marine turtles. *Virology* 246:392–399

Quackenbush SL, Casey RN, Murcek RJ, Paul TA, Work TM, Limpus CJ, et al. (2001) Quantitative analysis of herpesvirus sequences from normal tissue and fibropapillomas of marine turtles with real-time PCR. *Virology* 287:105–111

Raidal SR, Prince RIT (1996) "First confirmation of multiple fibropapillomas in a western Australian green turtle (*Chelonia mydas*)" Marine Turtle Newsletter 74:7–9

Sakai H, Saeki K, Ichihashi H, Suganuma H, Tanabe S, Tatsukawa R (2000) Species specific distribution of heavy metals in tissues and organs of loggerhead turtle (*Caretta caretta*) and green turtle (*Chelonia mydas*) from Japanese coastal waters. *Marine Pollution Bulletin* 40:701–709

Smith GM, Coates CW (1938) Fibro-epithelial growths of the skin in large marine turtles, *Chelonia mydas* (Linneaus). *Zoologica (NY)* 23:93–98

Sposato PL, Lutz PL, Cray C (2002) A comparative approach in determining health status of chelonians with respect to avian and mammalian medicine. In: *Proceedings of the 21st Annual Symposium on Sea Turtle Biology and Conservation*, U.S. Department of Commerce, NOAA Technical Memorandum NMFS-SEFSC (in press)

Tabor GM, Ostfeld RS, Poss M, Dobson AP, Aguirre AA (2001) Conservation biology and the health sciences: defining the research priorities of conservation medicine. In: *Research Priorities in Conservation Biology*, *2nd edition*. Soulé ME, Orians GH (editors), Washington, DC: Island Press, pp 165–173

VanDevanter DR, Warrener P, Bennett L, Schultz ER, Coulter S, Garber RL, et al. (1996) Detection and analysis of diverse herpes-viral species by consensus primer PCR. *Journal of Clinical Microbiology* 34:1666–1671

Vasconcelos J, Albavera E, Lopez EM, Hernandez P, Peñaflores C (2000) First assessment on tumors incidence in nesting females of olive ridley sea turtle *Lepidochelys olivacea*, at La Escobilla Beach, Oaxaca, Mexico. In: *Proceedings of the 18th Annual Symposium on Sea Turtle Conservation and Biology*, Abreu-Grobois FA, Briseño-Dueñas R, Márquez-Millán R, Sarti-Martinez L (editors), U.S. Department of Commerce, NOAA Technical Memorandum NMFS-SEFSC-436, pp 276–278

Williams EH Jr, Bunkley-Williams L, Peters EC, Pinto-Rodriguez B, Matos-Morales R, Mignucci-Griannoni AA, et al. (1994) An epizootic of cutaneous fibropapillomas in green turtles *Chelonia mydas* of the Caribbean: part of a panzootic? *Journal of Aquatic Animal Health* 6:70–78

Work TM, Balazs GH (1998) Causes of green turtle (*Chelonia mydas*) morbidity and mortality in Hawaii. In: *Proceedings of the 17th Annual Symposium on Sea Turtle Biology and Conservation*, U.S. Department of Commerce, NOAA Technical Memorandum NMFS-SEFSC-415, pp 291–292

Work TM, Balazs GH (1999) Relating tumor score to hematology in green turtles with fibropapillomatosis in Hawaii. *Journal of Wildlife Diseases* 35:804–807

Work TM, Balazs GH, Rameyer RA, Chang SP, Berestecky J (2000) Assessing humoral and cell-mediated immune response in Hawaiian green turtles, *Chelonia mydas*. *Veterinary Immunology and Immunopathology* 74:179–194

Work TM, Rameyer RA, Balazs GH, Cray C, Chang SP (2001) Immune status of free-ranging green turtles with fibropapillomatosis from Hawaii. *Journal of Wildlife Diseases* 38:574–581

Yu Q, Lu Y, Nerurkar VR, Yanagihara R (2000) Studies on the turtle tumor susceptibility gene TSG101: full-length cDNA sequence, genomic structural analysis, and role in green turtle fibropapilloma. *Journal of Aquatic Animal Health* 12:274–282