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14.1 INTRODUCTION

Assessing the health of free-ranging sea turtles has become increasingly important as evidence grows that environmental and animal health are intrinsically linked and sea turtle numbers continue to decline. Dr. George's Chapter 14 of The Biology of Sea Turtles, Volume I (George 1997) has an excellent review of the known nutritional anomalies, diseases, parasites, and environmental health problems affecting sea turtles at the time. Dr. Herbst's and Professor Jacobson's Chapter 15 of Volume II (Herbst and Jacobson 2003) also has an excellent comprehensive review of the systematic approaches and pitfalls to conducting a sea turtle disease investigation as at the turn of the new millennium. Since then, there have been advances in our understanding of the ecology and pathogenesis of certain diseases, such as fibropapillomatosis, as well as the development of more sophisticated and standardized tools to assess the health of wild and captive turtles. Further, in this field of veterinary conservation medicine, there has been a movement toward Dr. Schwabe's 1960s principle of One Medicine (Schwabe 1969), with the proposal that sea turtles are sentinel indicators of environmental health (Aguirre and Lutz 2004). Now known as One World One Health One Medicine, this concept, defined by the American Veterinary Medical Association as "the collaborative effort of multiple disciplines-working locally, nationally, and globally-to attain optimal health for people, animals and our environment" (http://www.avma. org/onehealth/responding.asp), appears to well suit the objectives and needs of a free-ranging sea turtle disease investigation.

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While clinical management of sea turtles in captive situations will continue to play an important role, there is an increasing need, through epidemiological research, to understand causes of decline if many of these species are to recover successfully. As many diseases of sea turtles are complex, understanding their drivers and how to manage them will require that veterinarians collaborate with biologists, environmental scientists, and other professions. This management strategy will need to proceed in concert with efforts to develop innovative field and laboratory tools and robust baseline reference intervals to assess the health of wild turtles. Reptile medicine generally, including sea turtle medicine, has come a long way in recent years with comprehensive texts examining medicine, surgery, and pathology (Jacobson 2007a, Mader 2006); however, these advances in understanding clinical management of sea turtles have not significantly increased our understanding of wild turtle health. There is still a need to continue and refine the research currently being undertaken.

Using a veterinary approach to analyze sea turtle health within a collaborative team of investigators working in complimentary areas may increase our overall understanding of health and ill-health etiology and transmission in sea turtles, in addition to the health of the marine environment (Wilcox and Aguirre 2004). Findings may help mitigate further challenges to both sea turtle populations and the ecosystem. It is through this philosophy that recent sea turtle disease investigations have been undertaken, leading to the proposal that sea turtles and other marine vertebrates may act as a (proxy) sentinel indicator of environmental health due to (1) association of occurrence of certain diseases such as fibropapillomatosis and poor environmental conditions (Aguirre and Lutz 2004, Aguirre and Tabor 2004) and (2) their long-lived site fidelity in some species, such as green turtles that can reside in a single foraging site for decades, only leaving for courtship and/or nesting.

This chapter outlines factors affecting sea turtle survivorship, design of free-ranging sea turtle disease investigations, the current diseases of concern, and the employment of sea turtle population modeling in predicting environmental health.

14.2 SEA TURTLE SURVIVORSHIP

Sea turtles are an integral component of our oceanic ecosystems, and many populations continue to decline (Chaloupka and Limpus 2001, Chan 2006, Jackson et al. 2001) with all seven extant species continuing to be of conservation concern (IUCN/SSC 2008). Loss of nesting habitat, nest depredation, and fisheries by catch are well-documented causes of wild turtle mortality and probably play an important role in declines of some populations (Dobbs and Pierce 2005, Limpus 2008a,b, 2009), particularly those that come in contact with human development or activities, but other populations that are not directly influenced by humans also continue to decline. Other than some isolated or opportunistic postmortem surveys and case reports producing some baseline references (Flint et al. 2010e, Glazebrook and Campbell 1990a, Gordon 2005, Limpus et al. 2009, Oros et al. 2005, Raidal et al. 1998, Work and Balazs 2010, Work et al. 2004), veterinary-orientated populationbased analyses or monitoring of free-ranging turtles has not been used to determine the influence disease has on turtle survivorship. More information on the effects of diseases, their prevalence, route of transmission, infectivity, and potentiating factors is needed on the basic physiology and health status of free-ranging turtles as well as improved diagnostic techniques to gain a greater understanding of the interplay between the environment and their health (Aguirre and Lutz 2004, Herbst and Jacobson 2003, Morton et al. 2009, Ward and Lafferty 2004, Whiting et al. 2007). Potential reasons for continued population decline could include effects of climate change that has been proposed to cause disease emergence, skewed sex ratios of emerging hatchlings, or declines in available nesting habitat (Habib et al. 2010, Wallace et al. 2011). These environmental factors may act on the local or global level. Regional variation places individuals under different stressors, suggesting that diseases may be unique to a specific area (Jackson et al. 2001), but the migratory capacity of individuals, and as such the capability to transmit disease, suggests disease investigation and subsequent population trends should also be addressed on a global scale.

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14.2.1 FACTORS AFFECTING SEA TURTLE SURVIVORSHIP

Surveillance programs have concentrated on establishing the essential basic parameters of biology and ecology. These global population surveillance programs identified sea turtle numbers were experiencing long-term declines and subsequent intervention strategies were developed (Dobbs and Pierce 2005). Recovery plans, such as the Recovery Plan for Marine Turtles in Australia (RMPTA 2003, 2006) and the recovery plans for U.S. Pacific and Atlantic populations of the green and loggerhead turtles (NMFS and USFWS 2008, NOAA 2008a,b), have synthesized data and issued mandates to various environmental protection and management agencies to design protocols to decrease mortality and increase the survivorship of sea turtles. With a focus on human impacts, one of the significant deficits in the recovery plans is a lack of understanding of the effects of environmental impacts on morbidity and mortality, the role of disease, and how to verify whether a turtle population was clinically healthy (Herbst and Jacobson 2003).

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From a management perspective, the majority causes of death in sea turtles can be broken down into human impacts (e.g., boat strike, harvest, disorientation, fishing-related effects, malicious mischief, and drowning), natural (e.g., parasites, disease, nesting [trauma and thermal stress] and predation), and unknown (no cause of death determined due to advanced decomposition or unclear findings).

14.2.1.1 Human Impacts

Potential causes of anthropogenic population declines in sea turtles include (1) bycatch of turtles in trawl nets or longline fisheries resulting in turtle drownings; (2) commercial (large-scale) harvesting of turtles for saleable products such as jewelry, oils, meat, and ornaments; (3) traditional harvest of breeding females or eggs by indigenous peoples for food; (4) marine debris such as discarded/lost/ unmonitored fishing gear (crab nets, lobster or crab pots, and hook and fishing line) causing entrapment resulting in drowning or intestinal disorders such as torsion caused by linear foreign bodies and/or gastric ruptures; (5) boat strike by recreational watercraft in high-traffic zones; (6) predation by terrestrial predators including native high-density predators such as raccoon and varanids and nonnative pigs (e.g., Sus scrofa in Australia) and foxes (e.g., Vulpes vulpes in Australia) raiding nests to consume eggs; (7) environmental contamination caused by agricultural pesticide and herbicide runoff such as DDTs and pollutants including sewage and oils entering shallow waters via commercial and public drains causing loss of feeding grounds such as sea grass beds, primary toxicosis in individuals, and, in the case of oils, the formation of tar balls (e.g., nonvolatile petroleum or sand conglomerated by oil) that subsequently causes gastrointestinal impaction; (8) environmental degradation caused by habitat removal and/or flooding events; and (9) human-induced climate change, causing damage to oceanic systems such as coral reefs, sea grass species and survivorship, changing water and ambient temperature that affects sea level, as well as altering the sex ratio of hatchlings, and potentially creating previously absent niches for the emergence of new diseases (Dobbs and Pierce 2005, Habib et al. 2010, Limpus 2008a,b, Limpus 2009).

Strategies that have been implemented to combat these threats include (1) protected habitats (national parks, marine protected areas, and green zones), (2) turtle exclusion devices (TEDs) and bycatch reduction devices (BRDs), (3) regulations to cease the legal commercial harvesting of sea turtles, (4) indigenous involvement in conservation, (5) substantial fines for littering marine reserves, (6) "go-slow" zones (speed-limits) for boats in areas known to have turtles, (7) feral animal control strategies, (8) environmental monitoring and mitigation, and (9) unsuccessful global attempts to limit impacts or production of climate-change-inducing carbon dioxide (Dobbs and Pierce 2005, Habib et al. 2010, Limpus 2008a,b, 2009). The response of sea turtle populations to these single or multiple strategies have been variable, with some populations continuing to decline.

14.2.1.2 Natural Factors

With population numbers continuing to decline and adverse environmental events, both natural and anthropogenic, managers in many regions have increased efforts to determine the cause of death. It

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is becoming apparent that an increasing number of sea turtle deaths cannot be fitted into the human impacts identified earlier, nor is it sufficient with respect to making management decisions to group other causes of death under the umbrella of "natural." Collaboration between biologists, ecologists, toxicologists, and veterinarians is starting to tease out the undisclosed "natural" causes using a structured, holistic approach to investigating disease (Flint et al. 2010e).

14.3 SEA TURTLE DISEASE INVESTIGATION

Disease investigation is the characterization of disease processes, sources, and contributing factors that result in abnormal findings in individuals and populations. This contrasts health assessment programs that describe and establish normal and abnormal values within a population (Herbst and Jacobson 2003). In the case of sea turtles, the investigator often needs to establish baseline parameters while simultaneously characterizing disease processes using both live and dead turtles to gather the required information to gain a comprehensive understanding of the diseases affecting sea turtle populations.

The first foray into researching the diseases of sea turtles arose with commercial green turtle farming on the Grand Cayman Island, British West Indies, in 1968 and the *head start* programs in the 1970s (Jacobson 1993). Recently, attention has turned to the concept of disease emergence as a major cause of population decline (George 1997, Ward and Lafferty 2004). Attempts are under way to understand etiologies and pathogenesis of specific diseases within turtle populations and to develop and refine diagnostic methods to identify and characterize these processes (Flint et al. 2009b, 2010c,d, Herbst and Jacobson 2003, Valente et al. 2006, 2007a,b).

Specific primary diagnostic tools such as reference intervals for hematology and blood biochemistry are an effective way of assessing the health of live free-ranging sea turtles. However, these reference intervals often do not exist for the cohort or population of turtle being examined and need to be derived. Absent or inadequate tools may be due to reference intervals derived from limited replicates or from captive sea turtles, which are known to vary from free-ranging sea turtles (Herbst and Jacobson 2003). Further, variations are known to be due to differences in the geographical location, gender, breeding status, age, and diet (Hamann et al. 2002, 2003, Jessop et al. 2004, Spotila 2004, Whiting et al. 2007).

To advance the field of sea turtle disease investigation, studies should be designed to incorporate components of health assessments and disease investigation. This requires examining sufficient numbers of clinically functional (sufficiently healthy live animals to not strand or die but may still carry subclinical disease) and nonfunctional (dead) sea turtles within a population to derive statistically sound comparisons. To achieve this, sea turtle disease investigations may require morphometric data, external examination, clinical examination, primary diagnostic screening tests, derivation of differential diagnoses, secondary diagnostic tests, and postmortem examinations before definitive diagnoses of the diseases processes within a population may be made (Herbst and Jacobson 2003).

14.3.1 APPROACH TO A DISEASE INVESTIGATION

Health assessments and disease investigations have advantages and disadvantages. Assessing the health of live turtles lends itself to collecting blood samples for hematology and blood biochemistry, collecting fresh samples for microbial cultures, and performing radiographs, ultrasounds, and other advanced imaging. While these tools can indicate if the turtle is clinically healthy or not, they seldom give the exact cause of how any identified abnormalities were contracted or if this abnormality is going to affect the turtle's chances of surviving or breeding. By contrast, conducting disease investigations on dead turtles allows for gross and microscopic pathological examination and assessment of parasitism, which will more often provide a cause of death. However, this strategy biases sample collection toward the critically ill or dead subsets of the population and may miss subclinical diseases or nonfatal diseases of the functional population, reducing our understanding of the range of diseases within a population.

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14.3.1.1 Live Sea Turtles

14.3.1.1.1 Body Condition

Morphometric examination allows for the collection of fundamental biological information such as body condition that can indicate the health status of the individual. Determined as the ratio of straight or curved carapace length and weight (or girth), body condition can be expressed empirically providing a quantitative value (Bjorndal et al. 2000).

Other morphometric data collected should include species identification, presence of applied tags and/or microchips for individual identification, head dimensions, standard depth, plastron length, and tail length (Figure 14.1) to gain an understanding of the population structure, to allow



(d)

FIGURE 14.1 Landmarks for the recommended morphometric measurements taken as part of the preliminary external examination: (a) curved carapace length, measured dorsally from the midline cranial suture of soft tissue and carapace and the caudal midline (blue line); and curved carapace width, measured dorsoventrally at the widest point of the carapace from the lateral edge of the marginal scutes (red line); (b) head width, measured dorsally at the widest part of the skull; (c) standard depth, measured dorsoventrally from the highest point of the carapace; (d) plastron length, measured ventrally midline from the cranial most to caudal most section of the plastron; and (e) tail length, measured from the caudal most point of the carapace to the tip of the tail.

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managers the ability to confirm recorded data, and to provide cohort data that may be used to predict disease predilection for a specific age group, sex, or species (Flint et al. 2009b). In addition, it is advantageous if the sex of the animal, its breeding status, and relative age can be determined by laparoscopic examination (Limpus and Limpus 2003, Limpus and Reed 1985) based on gonad development (Miller and Limpus 2003).

14.3.1.1.2 External Examination

External examination provides the initial subjective assessment of the health and well-being of a sea turtle and may provide clues as to what factors (e.g., fresh boat strike wounds) impacted on the animal's health. In addition to body condition, it should entail assessment for abnormalities, asymmetry, epibiont load and growths, such as tumors (Bjorndal et al. 2000, Herbst and Jacobson 2003, Wolke and George 1981, Work 2000, Wyneken 2001). For example, a high epibiont load in a loggerhead turtle (*Caretta caretta*) may be normal, but in a green turtle (*Chelonia mydas*), it could indicate ill-health such as a buoyancy disorder causing the animal to spend extended periods of time at the water's surface.

14.3.1.1.3 Clinical Examination

In addition to the external examination, clinical assessment should entail, but not be limited to, cranial nerve examination, mentation, cloacal temperature (particularly in cases of suspected hypothermia), respiratory rate, heart rate, symmetrical use of head and limbs, and assessment of the accessible internal organs (Chrisman et al. 1997, Deem et al. 2006, 2009, Flint et al. 2010c, Herbst and Jacobson 2003).

14.3.1.1.4 Screening Tests

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Screening tests should be the same basic assessment techniques as those used in domestic animal veterinary medicine. These may include hematology, blood biochemistry, microbiology, parasitology, toxicology, and histology. Selection of which test may be used or what order tests should be prioritized is based on a case-by-case basis in individual medicine. In population health assessments, tests can be selected by those which will achieve the objectives (or test the hypotheses) of the study. In most cases, assessments should prioritize hematology and blood biochemistry as these tools can provide disease process information at the turtle's system level, which can provide direction for the selection of follow-up tests.

14.3.1.1.4.1 Hematology and Blood Biochemistry Blood obtained from the external jugular veins (Owens and Ruiz 1980) can be used to derive hematological and biochemical values (Arthur et al. 2008, Bolten and Bjorndal 1992, Jacobson et al. 2007) as well as identify clinical ill-health once normal reference intervals have been established using clinically healthy representatives of a turtle population (Deem et al. 2006, 2009, Flint et al. 2009a, 2010c, Perrault et al. 2012, Stamper et al. 2005, Whiting et al. 2007, Work and Balazs 1999).

With well-detailed descriptions of blood cytology and morphology (Casal 2007, Casal and Oros 2007, Samour et al. 1998, Work et al. 1998) and improved techniques for the establishing of reference intervals(Flint et al. 2010c, Horn et al. 1998, Pesce et al. 2005, Solberg 1987), risk factors such as geographical location, habitat, genetics (Herbst and Jacobson 2003), maturity, sex (Hamann et al. 2006), breeding status (Deem et al. 2006), migratory status (Stamper et al. 2005), and diet (Whiting et al. 2007) can be grouped to produce a robust and functional diagnostic tool that may be applicable to multiple sea turtle cohorts (Flint et al. 2010c). With these improvements, hematology and blood biochemistry reference intervals are becoming an increasingly useful and necessary tool in health assessments for determining conservation management strategies (Hamann et al. 2006) and provide a less invasive alternative to postmortem-based disease investigations.

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14.3.1.1.4.2 Microbiology, Parasitology, Toxicology, and Histology Microbiology, parasitology, and histology are important tools in refining the diagnoses of disease in animals within a population. Numerous species of bacteria and fungi have been identified in sea turtles (Work et al. 2003); however, the pathogenic nature of these microbes should be interpreted with caution as many may be present without causing significant pathology. Parasite infections have been proposed as a significant cause of clinical disease and mortality in sea turtles (Aguirre et al. 1998, Cribb and Gordon 1998, Dailey et al. 1991, Flint et al. 2010a,c, Glazebrook et al. 1989, Gordon et al. 1998, Jacobson et al. 2006, Raidal et al. 1998), but the association between parasitism and stranding/mortality is not straightforward, rather a part of a multifactorial disease process (Gordon et al. 1998, Jacobson et al. 2006). Researchers should view studies on parasites as an integral component of any disease investigation. Morphological identification (Platt 2001, Smith1997) and characterization of the genetic sequences (Blair 2006, Nolan and Cribb 2005, Work et al. 2005) of the prevalent parasite species affecting sea turtles can be employed to identify which species are in selected populations or if body organ predilection (and secondary disease) is occurring. Collecting biopsies of tissues (soft tissue organs, blood, skeletal muscle, subcutaneous tissue, or bone) allows for the assessment of contaminants (toxins, heavy metals, PCBs, and DDTs) (Jacobson et al. 2006, Keller et al. 2004, 2006), for histological diagnoses (Flint et al. 2009b) and research investigations such as ageing (Klinger and Musick 1992). Currently under investigation is the development of bulk screening kits to cheaply screen multiple potential contaminants using relatively noninvasive whole blood or hematologic sampling (C. Gaus, personal communication).

14.3.1.1.5 Secondary Diagnostic Tests

The use of secondary tests in sea turtle diagnostics allows for the refinement of the diagnoses, and is often selected based on the clinical examination and results of the primary tests, such as hematology and blood biochemistry, in the same manner as for other species. Secondary tests include radiography (contrast or plain), ultrasonographic investigation, endoscopy, exploratory laparoscopy, exploratory surgery, computerized tomography scanning (CT scan), and magnetic resonance imaging (MRI) (Norton 2005, Nutter et al. 2000, Valente et al. 2006, 2007a,b, 2008. Although guidelines for the use of these tools are in the early stages of development, their application to individual patient care and the nonlethal approach is allowing easy collection of information that may be applicable at the population level if multiple sea turtles from within a population are examined to provide health extrapolation to the population as a whole.

14.3.1.2 Dead Sea Turtles

14.3.1.2.1 Postmortem Examination

Necropsies form the basis of disease investigations and are often the most useful tool for diagnosing causes of mortality required at the population level to indicate what appropriate actions, if any, should be taken to mitigate mortalities or morbidity (Flint et al. 2009b). Information derived from isolated single individuals compiled over time can contribute substantially to understanding what is causing mortality in wild turtles, thereby allowing managers to prioritize whether or not particular causes of mortality need to be mitigated or reduced. Like any other technique, necropsies have their limitations in that the population sample comprised only animals that are found dead and/or stranded and does not take into account animals with less severe or subclinical diseases. For each local area, any fresh dead turtles should be necropsied to determine the prevalent local causes of death and disease to contribute to the body of knowledge.

In the absence of medical history, one looks to environmental clues that could explain the mortality, for example, assessing the local food resources for depletion or change, determining if known foraging habitats or migratory paths have undergone anthropogenic environmental (dredging or redirecting) or usage (new shipping lanes or mining activity) changes, and factoring in occurrence of unusual events such as droughts or floods causing alteration of freshwater runoffs or

concentrations of contaminants; all of which may contribute to interpretation of postmortem findings (Flint et al. 2009b, Herbst and Jacobson 2003). Similarly, one should also use the environmental clues to exclude potentially erroneous differential diagnoses. For example, multiple superficial abrasions on the carapace and soft tissue of a turtle carcass found at the low tide mark of a rocky beach may be due to wave action on a hard substrate and could have occurred postmortem.

Postmortem examination should consist of complete external and internal exams along with collections of tissues for microscopic examination (Flint et al. 2009b,c, Jacobson 1999, Wolke and George 1981, Work 2000, Wyneken 2001).

14.4 DISEASES OF CONCERN IN FREE-RANGING SEA TURTLES

14.4.1 PARASITISM

Professor Greiner provides a comprehensive review in Chapter 16 of this volume concentrating on parasites found in sea turtles in Florida. As such, this section will present findings in sea turtles in Australia where two predominant parasite groups have been associated with diseases: Spirorchiidae (digenetic trematode parasites) and *Caryospora cheloniae* (coccidia). Under certain circumstances, high death rates in sea turtles have been associated with both parasites (Flint et al. 2010a, Gordon et al. 1993, 1998).

Spirorchiid trematode (cardiovascular or heart fluke) infection has been proposed as a significant cause of clinical disease and mortality in turtles in some regions (Flint et al. 2010e). Eggs and the lesions they cause are found in all organs, most significantly affecting the cardiovascular, gastrointestinal, lymphatic, and central nervous systems (Flint et al. 2010e, Gordon et al. 1998, Jacobson et al. 2006, Patterson-Kane et al. 2009, Stacy et al. 2008). Postmortem examinations show multiple granulomas and adult parasites throughout each organ with clinical pathology including complete occlusion of the primary vasculature, gastrointestinal stasis, lymphatic congestion, and space-occupying lesions of the brain (Figure 14.2; Flint et al. 2010e, Patterson-Kane et al. 2009, Stacy et al. 2

Globally, stranding investigations report a spirorchiid infection rate of up to 100% (Dailey et al. 1991, 1992, Gordon et al. 1998). Despite at least 14 species being reported in turtles, only 4 of the spirorchiid species have had their pathology of disease in sea turtles characterized (*Carettacola*, Hapalotrema, Neospirorchis, and Learedius spp). Similarly, the life cycles of these parasites have not been described, resulting in a poor understanding of how transmission occurs and the role of intermediate hosts in their life cycle (Flint et al. 2009b). A Hawaiian study demonstrated spirorchiid antibody could be detected by a crude ELISA test (Work et al. 2005), but it did not identify what species were infecting turtles nor did it give an indicator of parasite burden, thereby complicating our understanding of the effects of these parasites on sea turtles. To date, much of the parasite investigations have been conducted during postmortem examinations. Using molecular biological techniques on blood would provide a technique applicable to antemortem animals. Adjunct research into this area indicates the ITS region, which can be used to code for the Spirorchiid parasites of interest in terms of causing pathology in sea turtles (Platt 2001, Snyder 2004, Tkach et al. 2009), and mitochondrial DNA are showing promise as an accurate way to identify the known trematode parasite species found in sea turtles. When refined, these techniques may be employed to determine parasite status on initial examination of live sea turtles, response to treatment and prevalence of infection in a population. Similarly, as part of this study, a comparison is being made of the species of spirorchiid parasites and their associated pathology in sea turtles of Florida, Hawaii, and Queensland to determine what role, if any, regional mortality and environmental factors are having on this complex disease process.

Coccidial infection by the species *Caryospora cheloniae* has been reported to cause epizootic mortality in Australian green turtles in Moreton Bay, Queensland (Gordon 2005, Gordon et al. 1993). Infection rates and severities, as for those of Spirorchiid parasites, may be related to environmental

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FIGURE 14.2 (a) Adult spirorchiid parasites causing occlusion of the aorta; (b) spirorchiid parasite granulomas, formed around the spirorchiid parasite eggs, causing occlusion of the right descending aorta; and (c) histological micrograph (100×; H&E stain) adult spirorchiid parasite and eggs within a granuloma in the brain of a large immature green sea turtle in Australia.

factors including temperature-related efficiency of turtle immune responses (Gordon et al. 1993). Sporadic fatalities due to *C. cheloniae* infection are still being observed in the Moreton Bay area but at a much reduced rate (Flint et al. 2010e). Reasons for these changes in these associated mortality rates and why Moreton Bay was a "hot spot" for such an outbreak are still unknown.

14.4.2 FIBROPAPILLOMATOSIS

Fibropapillomatosis (FP) is a common cutaneous, apparently infectious condition in all species of sea turtles, with gross and histological appearance similar to that in domestic species. Sea turtle FP was first reported in Florida in 1938 and has now caused numerous epidemics in green, loggerhead, and olive ridley (*Lepidochelys olivacea*) sea turtles worldwide (Greenblatt et al. 2005, Herbst 1994, Limpus and Miller 1990, Smith and Coates 1938, Stacy et al. 2008, Work et al. 2004). Juvenile turtles are most often affected, but not during or immediately after the pelagic life phase. Indeed, molecular evidence suggests that sea turtles acquire infection with the virus after recruitment to neritic habitat (Ene et al. 2005). Low levels of disease are observed in adult sea turtles (George 1997, Limpus and Miller 1990). Papillary or smooth, flat or nodular masses, ranging in size from <1 to >30 cm diameter, occur on soft tissues including the oral, ocular, orbital adnexa, neck, limbs, and tail, as well as the sutures of the plastron and carapace (Figure 14.3; Flint et al. 2009b). They may also involve the internal organs (Jacobson 2007b, Milton and Lutz 2003, Wyneken et al. 2006) depending on the biogeographic region, where in Florida and Hawaii it is seen more commonly than in places like Australia. Similarly, they may invade the corneal surface of the eye as has been

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FIGURE 14.3 Cutaneous fibropapillomatosis of the left axilla in a small immature green sea turtle in Australia. Tumors range in presentation from smooth to cauliflower, white to dark gray and from less than 1 cm in diameter to greater than 8 cm.

recorded in several parts of the world at different times (Flint et al. 2010b, Jacobson et al. 1989, Norton et al. 1990). Spontaneous regression of tumors has been observed in turtles in Hawaii, Florida, and Queensland, although the frequency of regression and the duration of FP in sea turtles appear to vary at each location. Further, significance of body part affected by the FP also influences potential survivorship. Animals with internal, glottal, or corneal FP are thought to have a poor prognosis, whereas in Hawaii approximately 30% of animals with cutaneous FP, and near 100% in Australia, are believed to make a full recovery without the need for medical or surgical intervention (Chaloupka and Balazs 2005).

Fibropapilloma masses are benign, although they often cause severe debilitation due to spaceoccupying effects or interference with systemic function (Aguirre and Lutz 2004). Advanced FP has been linked to lymphopenia, chronic inflammation, immunosuppression, and systemic Gramnegative bacterial infections (Norton et al. 1990, Work and Balazs 1999, Work et al. 2000, 2003). These factors may contribute to the overrepresentation of turtles with moderate to severe FP in strandings when compared with the FP frequency of the functional population.

The etiology and pathogenesis of FP in sea turtles are only partly understood. An alpha-herpesvirus (chelonid FP-associated herpesvirus; CFPHV) has been isolated from naturally occurring tumor masses (Greenblatt et al. 2004), but to date attempts to isolate and sequence the virus have not been successful (Herbst et al. 1995, Work et al. 2009). In the natural environment, the marine leech *Ozobranchus margoi* and cleaner fish saddleback wrasse *Thalassoma duperrey* are suspected mechanical vectors (Greenblatt et al. 2004, Lu et al. 2000). Progression to tumor development is likely to be multifactorial and involve environmental cofactors. For example, seasonal elevation of water temperature, high levels of anthropogenic activity in the immediate environment, and exposure to potential tumor-promoting compounds including okadaic acid produced by the benthic dinoflagellate *Prorocentrum* species and products of *Lyngbya majuscula* (toxic cyanobacterium) algal blooms are thought to be implicated (Aguirre et al. 1994, Arthur et al. 2006, Greenblatt et al. 2004, Herbst 1994, Work et al. 2005).

14.4.3 INFECTIOUS DISEASES

Reported bacterial infections in sea turtles have included Vibrio, Aeromonas, Salmonella, Pseudomonas, Bacteroides, Fusobacterium, Flavobacterium, Clostridium, and Mycobacterium spp. (Figure 14.4; Clary and Leong 1984, Glazebrook and Campbell 1990a,b, Greer et al. 2003, McArthur 2004, Obendorf et al. 1987, Sinderman 1977, Stewart 1990, Wiles and Rand 1987). A few infectious diseases with zoonotic potential have been identified in sea turtles. These include Vibrio, Campylobacter, Salmonella, and atypical Mycobacterium spp. (Glazebrook and Campbell 1990a).

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FIGURE 14.4 Secondary infiltration of a spirorchiid parasite granuloma with bacteria in a large immature green sea turtle in Australia.

These isolates are of particular importance to indigenous communities who hunt these turtle species and to veterinarians, who are likely to come in close contact with fluids and discharges during examination and treatment. Vigilance for zoonotic diseases should be maintained, and any occurrence reported to monitoring authorities to ensure the information may be circulated.

Fungal infections in free-living sea turtles are considered rare but may manifest as systemic mycoses, gastrointestinal disorders, or pulmonary airway diseases under stressful conditions such as captive environments or cold shock. Fungal infections including *Colletotrichum acutatum, Candida, Penicillium lilacinum, Cladosporium, Sporotrichum, Paecilomyces, Aspergillus*, and *Fusarium* spp. have been identified, predominantly in captive turtle populations (Flint et al. 2009b, Glazebrook and Campbell 1990a, Oros et al. 2004).

14.4.4 Toxins

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The encroachment of urbanization, with associated runoffs of herbicides, pesticides, and storm-water debris, pathogens, toxins, and wastes (Chilvers et al. 2005), can affect sea turtles either directly or indirectly by affecting food sources and breeding cycles. Covered comprehensively by Dr Keller in an earlier chapter of this volume, environmental pollutants including heavy metals and organochlorine pesticides have been identified in blood and tissues of various sea turtle populations, and are associated with subtle negative effects on immune function and other health parameters. However, until recently, most resulting impacts on the effects of contaminants on sea turtles were subject to conjecture (Day et al. 2005, 2007, Hermanussen et al. 2006, Hochscheid et al. 2004, Keller et al. 2004, 2006, Perrault et al. 2011). It is assumed the full suite of contamination effects acting on sea turtle populations have not yet been comprehensively measured due to the cost-inhibitory nature of mass toxin screening and consequently our understanding of their exact role in disease contribution and manifestation is limited.

14.4.5 GASTROINTESTINAL ANOMALIES

In wild populations of sea turtles, gastrointestinal anomalies may include linear foreign bodies and perforations, obstructions, obstipation, or gastrointestinal ileus (Figure 14.5). Although there is substantial literature highlighting techniques to treat impactions and devitalized gastrointestine, the causes of these nonmechanical anomalies are poorly understood. In cases such as linear foreign bodies caused by fishing lines and hooks, which may result in plication of the gut or coelomitis with intestinal perforation, there is a clear etiology. Other types of foreign body material that may cause obstruction include fiber, metal, plastic, rubber, and atypical food types. These are a common cause of pathology in some cohorts of stranded or sick sea turtles (Boyle and Limpus 2008), but are not

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FIGURE 14.5 (a) Gastrointestinal torsion with associated natural-fiber fecal balls and secondary inflammation of the intestinal mucosa in a mature green sea turtle; and (b) a common gross presentation of fishing line ingestion (linear foreign body) causing intestinal plication in a small green sea turtle.

noted in others (Chaloupka et al. 2008b, Flint et al. 2010e, Oros et al. 2005). Also with a straightforward etiology, obstructions can be due to torsions as seen in other species, or by digesta that occlude or reduce the patency of the lumen of the intestine. By contrast, it is the etiology of obstipation that are less obvious. It is often associated with fecal balls comprised solely of natural food products (e.g., crushed shells in loggerheads and compressed sea grasses in green turtles), but whether the impaction is secondary to a primary pathology such as ileus is unknown. A theory, which is yet to be supported or rejected, is denervation of the vagal and associated nerves, which innervate and regulate/control the peristaltic movement of the gut that enables ingesta and digesta to move. Viral, bacterial, or parasitic agents may be implicated, rendering the gut functionally static.

14.5 POPULATION MODELING

Population modeling is becoming an important tool in wildlife management (Ward and Lafferty 2004). Since the first deterministic models were used to predict survivorship of sea turtles (Crouse et al. 1987), modeling has improved to include population response to a wide range of parameters (Bjorndal et al. 2000, Chaloupka 2002, Chaloupka and Balazs 2005, 2007, Wallace et al. 2011) that take into account predicted mortality and recruitment within the population, but do not necessarily account for the effects of disease. While these models allow for overall changes in mortality or survivorship to be investigated, they do so irrespective of the origin of the change. Modeling using meta-analyses has concentrated on using published studies to elicit trends in populations providing retrospective feedback to an environment's management plan (Dethmers et al. 2006, Jackson 1997, Jackson et al. 2001). This resulted in a delayed feedback to implemented changes (i.e., they were not predictive of the effect on environment). Population models, especially those implemented in Marine Area Protection management, are now capable of dynamic predictive monitoring (Chaloupka and Limpus 2005, Chaloupka et al. 2008a). This allows small adjustments to be incorporated into both the model and the management plan in "real time," creating an adaptive management plan (Gerber et al. 2005).

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Given the versatility of this analysis tool, disease modeling could be directly applied to sea turtles with minor modifications of principles used in existing models. Models could be improved and modified as a better understanding of the risk factors and diseases affecting sea turtles in each locale are gained. Such an approach would provide near-instant survivorship feedback under a range of disease and environmental conditions and allow the real-time adjustments to management plans required to mitigate negative effects on these threatened animals.

14.6 CONCLUSIONS

Disease in sea turtles has shown to be a multifactorial, understudied area of epidemiology and veterinary medicine. Efforts to address this have increased greatly in recent years. If the impact of disease on wild populations of sea turtles is to be mitigated, then disease investigations incorporating turtle biology and ecology, clinical assessment, postmortem examination to definitively diagnose disease (at a population level through collaborative efforts), environmental assessment, and disease modeling need to be conducted. This will allow prediction of the effects of environment on disease occurrence and prevalence in a timely manner that may allow for active intervention strategies to be employed.

REFERENCES

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- Aguirre AA, G Balazs, B Zimmerman, and FD Galey. Organic contaminants and trace metals in the tissues of green turtles (*Chelonia mydas*) afflicted with fibropapillomas in the Hawaiian islands. *Marine Pollution Bulletin* 1994; 28: 109–114.
- Aguirre AA and P Lutz. Sea turtles as sentinels of marine ecosystem health: Is fibropapillomatosis an indicator? *EcoHealth* 2004; 1: 275–283.
- Aguirre AA, TR Spraker, GH Balazs, and B Zimmerman. Spirorchidiasis and fibropapillomatosis in green turtles from the Hawaiian Islands. *Journal of Wildlife Diseases* 1998; 34: 91–98.
- Aguirre AA and GM Tabor. Introduction: Marine vertebrates as sentinels of marine ecosystem health. *EcoHealth* 2004; 1: 236–238.
- Arthur KE, CJ Limpus, and JM Whittier. Baseline blood biochemistry of Australian green turtles (*Chelonia mydas*) and effects of exposure to the toxic cyanobacterium *Lyngbya majuscula*. Australian Journal of Zoology 2008; 56: 23–32.
- Arthur K, G Shaw, CJ Limpus, and JW Udy. A review of the potential role of tumour-promoting compounds produced by *Lyngbya majuscula* in marine turtle fibropapillomatosis. *African Journal of Marine Science* 2006; 28: 441–446.
- Bjorndal KA, AB Bolten, and M Chaloupka. Green turtle somatic growth model: Evidence fordensity dependence. *Ecological Applications* 2000; 10: 269–282.
- Blair D. Ribosomal DNA variation in parasitic flatworms. In: Maule A, ed. *Parasitic Flatworms: Molecular* AQ2 *Biology, Biochemistry, Immunology and Control.* Oxfordshire, U.K.: CABI, 2006, pp. 96–123.
- Bolten AB and KA Bjorndal. Blood profiles for a wild population of green turtles (*Chelonia mydas*) in the southern Bahamas: Size-specific and sex-specific relationships. *Journal of Wildlife Diseases* 1992; 28: 407–413.
- Boyle MC and CJ Limpus. The stomach contents of post-hatchling green and loggerhead sea turtles in the southwest Pacific: An insight into habitat association. *Marine Biology* 2008; 155: 233–241.
- Casal AB, F Freire, G Bautista-Harris, A Arencibia, and J Oros. Ultrastructural characteristics of blood cells of juvenile loggerhead sea turtles (*Caretta caretta*). Anatomia, Histologia, Embryologia 2007; 36: 332–335.
- Casal AB and J Oros. Morphologic and cytochemical characteristics of blood cells of juvenile loggerhead sea turtles (*Caretta caretta*). *Research in Veterinary Science* 2007; 82: 158–165.
- Chaloupka M. Stochastic simulation modelling of southern Great Barrier Reef green turtle population dynamics. *Ecological Modelling* 2002; 148: 79–109.
- Chaloupka M and G Balazs. Modelling the effect of fibropapilloma disease on the somatic growth dynamics of Hawaiian green sea turtles. *Marine Biology* 2005; 147: 1251–1260.
- Chaloupka M and G Balazs. Using Bayesian state-space modelling to assess the recovery and harvest potential of the Hawaiian green sea turtle stock. *Ecological Modelling* 2007; 205: 93–109.
- Chaloupka M, N Kamezaki, and CJ Limpus. Is climate change affecting the population dynamics of the endangered Pacific loggerhead sea turtle? *Journal of Experimental Marine Biology and Ecology* 2008a; 356: 136–143.

()

- 390
- Chaloupka M and CJ Limpus. Trends in the abundance of sea turtles resident in southern Great Barrier Reef waters. *Biological Conservation* 2001; 102: 235–249.
- Chaloupka M and CJ Limpus. Estimates of sex- and age-class-specific survival probabilities for a southern Great Barrier Reef green sea turtle population. *Marine Biology* 2005; 146: 1251–1261.
- Chaloupka M, T Work, G Balazs, S Murakawa, and R Morris. Cause-specific temporal and spatial trends in green sea turtle strandings in the Hawaiian Archipelago (1982–2003). *Marine Biology* 2008b; 154: 887–898.
- Chan EH. Marine turtles in Malaysia: On the verge of extinction? *Aquatic Ecosystem Health and Management* 2006; 9: 175–184.
- Chilvers BL, IR Lawler, F Macknight, H Marsh, M Noad, and R Paterson. Moreton Bay, Queensland, Australia: An example of the co-existence of significant marine mammal populations and large-scale coastal development. *Biological Conservation* 2005; 122: 559–571.
- Chrisman CL, M Walsh, JC Meeks, H Zurawka, R LaRock, and LH Herbst. Neurological examination of sea turtles. *Journal of American Veterinary Medical Association* 1997; 211: 1043–1047.
- Clary JC and JK Leong. Disease studies aid Kemp's ridley sea turtle headstart research. *Herpetological Review* 1984; 15: 69–70.
- Cribb TH and AN Gordon. Hapalotrema (Digenea: Spirorchidae) in the green turtle (*Chelonia mydas*) in Australia. *Journal of Parasitology* 1998; 84: 375–378.
- Crouse DT, L Crowder, and H Caswell. A stage-based population model for loggerhead sea turtles and implications for conservation. *Ecology* 1987; 68: 1412–1423.
- Dailey MD, ML Fast, and GH Balazs. Carettacola hawaiiensis n. sp. (Trematoda: Spirorchidae) from the green turtle, Chelonia mydas, in Hawaii. Journal of Parasitology 1991; 77: 906–909.
- Dailey MD, ML Fast, and G Balazs. A survey of the Trematoda (Platyhehninthes: Digenea) parasitic in green turtles, *Chelonia mydas* (L.) from Hawaii. *Bulletin of the Southern Californian Academy of Science* 1992; 91: 84–91.
- Day RD, SJ Christopher, PR Becker, and DW Whitaker. Monitoring mercury in the loggerhead sea turtle, *Caretta caretta. Environmental Science and Technology* 2005; 39: 437–446.
- Day RD, AL Segars, MD Arendt, AM Lee, and MM Peden-Adams. Relationship of blood mercury levels to health parameters in the loggerhead sea turtle (*Caretta caretta*). *Environmental Health Perspectives* 2007; 115: 1421–1428.
- Deem SL, ES Dierenfeld, GP Sounguet, AR Alleman, C Cray, RH Poppenga, TM Norton et al. Blood values in free-ranging nesting leatherback sea turtles (*Dermochelys coriacea*) on the coast of the Republic of Gabon. *Journal of Zoo and Wildlife Medicine* 2006; 37: 464–471.
- Deem SL, TM Norton, MA Mitchell, AL Segars, AR Alleman, C Cray, RH Poppenga et al. Comparison of blood values in foraging, nesting, and stranded loggerhead turtles (*Caretta caretta*) along the coast of Georgia, USA. *Journal of Wildlife Diseases* 2009; 45: 41–56.
- Dethmers KE, D Broderick, C Moritz, NN Fitzsimmons, CJ Limpus, S Lavery, S Whiting et al. The genetic structure of Australasian green turtles (*Chelonia mydas*): Exploring the geographical scale of genetic exchange. *Journal of Molecular Ecology* 2006; 15: 3931–3946.
- AQ3 Dobbs K and S Pierce. Marine reptiles. In: Chin A, ed. *The State of the Great Barrier Reef On-line*. Townsville, Queensland, Australia: Great Barrier Reef Marine Park Authority, 2005. http://www.gbrmpa.gov.au/ corp_site/info_services/publications/sotr/marine_reptiles/index.html
 - Ene A, M Su, S Lemaire, C Rose, S Schaff, R Moretti, J Lenz et al. Distribution of chelonid fibropaillomatosisassociated Herpesvirus variants in Florida: Molecular genetic evidence for infection of turtles following recruitment to neritic developmental habitats. *Journal of Wildlife Diseases* 2005; 41: 489–497.
 - Flint M, D Blair, JC Patterson-Kane, M Kway-Tanner, and PC Mills. Blood flukes (Spirorchiidae) as a major cause of marine turtle mortality in Queensland. XII International Congress of Parasitology 2010a; 57–61.
 - Flint M, CJ Limpus, JC Patterson-Kane, PJ Murray, and PC Mills. Corneal fibropapillomatosis in green sea turtles (*Chelonia mydas*) in Australia. *Journal of Comparative Pathology* 2010b; 142: 341–346.
 - Flint M, JM Morton, CJ Limpus, JC Patterson-Kane, PJ Murray, and PC Mills. Development and application of biochemical and haematological reference intervals to identify unhealthy green sea turtles (*Chelonia* mydas). The Veterinary Journal 2010c; 185: 299–304.
 - Flint M, JM Morton, JC Patterson-Kane, CJ Limpus, and PC Mills. Reference intervals for plasma biochemical and hematological measures in loggerhead sea turtles (*Caretta caretta*) from Moreton Bay, Australia. *Journal of Wildlife Diseases* 2010d; 46: 731–741.
 - Flint M, JM Morton, JC Patterson-Kane, CJ Limpus, PJ Murray, and PC Mills. Using plasma biochemistry and haematological blood reference ranges as a tool in diagnosing disease for green turtles (*Chelonia* mydas) in Queensland Australia. Proceedings of the 29th International Sea Turtle Symposium, February 17–19, 2009. Brisbane, Queensland, Australia, 2009a.

K13384_C014.indd 390

Flint M, JC Patterson-Kane, CJ Limpus, and PC Mills. Health surveillance of stranded green turtles in southern Queensland, Australia (2006–2009): An epidemiological analysis of causes of disease and mortality. *EcoHealth* 2010e; 7: 135–145.

Flint M, JC Patterson-Kane, CJ Limpus, TM Work, D Blair, and PC Mills. Post mortem diagnostic investigation of disease in free-ranging marine turtle populations: A review of common pathological findings and protocols. *Journal of Veterinary Diagnostic Investigation* 2009b; 21: 733–759.

Flint M, JC Patterson-Kane, PC Mills, and CJ Limpus. A veterinarian's guide to sea turtle post mortem examination and histological investigation, 2009c. http://www.uq.edu.au/vetschool/index.html?page=102248

George RH. Health problems and diseases of sea turtles. In: Lutz PL and JA Musick, eds. *The Biology of Sea Turtles*. Boca Raton, FL: CRC Press, Inc., 1997.

Gerber LR, M Beger, MA McCarthy, and HP Possingham. A theory for optimal monitoring of marine reserves. *Ecology Letters* 2005; 8: 829–837.

Glazebrook JS and RSF Campbell. A survey of the diseases of marine turtles in northern Australia I. Farmed turtles. *Disease of Aquatic Organisms* 1990a; 9: 83–95.

Glazebrook JS and RSF Campbell. A survey of the diseases of marine turtles in northern Australia II. Oceanarium-reared and wild turtles. *Disease of Aquatic Organisms* 1990b; 9: 97–104.

Glazebrook JS, RS Campbell, and D Blair. Studies on cardiovascular fluke (Digenea: Spirorchiidae) infections in sea turtles from the Great Barrier Reef, Queensland, Australia. *Journal of Comparative Pathology* 1989; 101: 231–250.

Gordon AN. A necropsy-based study of green turtles (*Chelonia mydas*) in South-East Queensland. PhD thesis, School of Veterinary Science, The University of Queensland, Brisbane, Queensland, Australia, 2005, p. 234.

Gordon AN, WR Kelly, and TH Cribb. Lesions caused by cardiovascular flukes (Digenea: Spirorchidae) in stranded green turtles (*Chelonia mydas*). Journal of Veterinary Pathology 1998; 35: 21–30.

Gordon AN, WR Kelly, and RJ Lester. Epizootic mortality of free-living green turtles, *Chelonia mydas*, due to coccidiosis. *Journal of Wildlife Diseases* 1993; 29: 490–494.

Greenblatt RJ, TM Work, GH Balazs, CA Sutton, RN Casey, and JW Casey. The Ozobranchus leech is a candidate mechanical vector for the fibropapilloma-associated turtle herpesvirus found latently infecting skin tumors on Hawaiian green turtles (*Chelonia mydas*). Virology 2004; 321: 101–110.

Greenblatt RJ, TM Work, P Dutton, CA Sutton, TR Spraker, RN Casey, CE Diez et al. Geographic variation in marine turtle fibropapillomatosis. *Journal of Zoo and Wildlife Medicine* 2005; 36: 527–530.

Greer LL, JD Strandberg, and BR Whitaker. Mycobacterium chelonae osteoarthritis in a Kemp's Ridley sea turtle (*Lepidochelys kempii*). Journal of Wildlife Diseases 2003; 39: 736–741.

Habib RR, K El Zein, and J Ghanawi. Climate change and health research in the Eastern Mediterranean Region. *EcoHealth* 2010; 7: 156–175.

Hamann M, CJ Limpus, and JM Whittier. Patterns of lipid storage and mobilisation in the female green sea turtle (*Chelonia mydas*). Journal of Comparative Physiology 2002; 172: 485–493.

Hamann M, CJ Limpus, and JM Whittier. Seasonal variation in plasma catecholamines and adipose tissue lipolysis in adult female green sea turtles (Chelonia mydas). *General and Comparative Endocrinology* 2003; 130: 308–316.

Hamann M, CS Schäuble, T Simon, and S Evans. Demographic and health parameters of green sea turtles *Chelonia mydas* foraging in the Gulf of Carpentaria, Australia. *Endangered Species Research* 2006; 2: 81–88.

Herbst L. Fibropapillomatosis of marine turtles. Annual Review of Fish Diseases 1994; 4: 389-425.

Herbst LH and ER Jacobson. Practical approaches for studying sea turtle health and disease. In: Lutz P, JA Musick, and J Wyneken, eds. *The Biology of Sea Turtles*, Vol. II. New York: CRC Press, 2003, pp. 385–410.

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

Hermanussen S, CJ Limpus, O Papke, DW Connell, and C Gaus. Foraging habitat contamination influences green turtle PCDD/F exposure. Organohalogen Compounds 2006; 68: 592–595.

Hochscheid S, F Bentivegna, and JR Speakman. Long-term cold acclimation leads to high Q10 effects on oxygen consumption of loggerhead sea turtles *Caretta caretta*. *Physiological and Biochemical Zoology* 2004; 77: 209–222.

Horn PS, AJ Pesce, and BE Copeland. A robust approach to reference interval estimation and evaluation. *Clinical Chemistry* 1998; 44: 622–631.

IUCN/SSC. The IUCN Red List of Threatened Species. *IUCN Red List*. Cambridge, U.K. 2008. www.redlist.org Jackson JB. Reefs since Columbus. *Coral Reefs* 1997; 16: S23–S32.

Jackson JB, MX Kirby, WH Berger, KA Bjorndal, LW Botsford, BJ Bourque, RH Bradbury et al. Historical overfishing and the recent collapse of Coastal Ecosystems. *Science* 2001; 293: 629–638.

Jacobson ER. Implications of infectious diseases for captive propogation and introduction programs of threatened/endangered reptiles. *Journal of Zoo and Wildlife Medicine* 1993; 24: 245–255.

- Jacobson ER. Sea turtle biopsy and necropsy techniques. Gainesville, FL: University of Florida, 1999. http:// www.vetmed.ufl.edu/college/departments/sacs/research/SeaTurtleBiopsyandNecropsyTechniques.html
- Jacobson ER. Infectious Diseases and Pathology of Reptiles: Color Atlas and Text. Boca Raton, FL: CRC Press, 2007a.

Jacobson ER. Viruses and viral diseases of reptiles. In: Jacobson ER, ed. *Infectious Diseases and Pathology of Reptiles: Color Atlas and Text*. Boca Raton, FL: CRC Press, 2007b, pp. 395–460.

- AQ4 Jacobson ER, K Bjorndal, A Bolten, R Herren, G Harman, and L Wood. Establishing plasma biochemical and hematocrit reference intervals for sea turtles in Florida. 2007.
 - Jacobson ER, BL Homer, BA Stacy, EC Greiner, NJ Szabo, CL Chrisman, F Origgi et al. Neurological disease in wild loggerhead sea turtles *Caretta caretta*. *Disease of Aquatic Organisms* 2006; 70: 139–154.
 - Jacobson ER, JL Mansell, JP Sundberg, L Hajjar, ME Reichmann, LM Ehrhart, M Walsh et al. Cutaneous fibropapillomas of green turtles (*Chelonia mydas*). Journal of Comparative Pathology 1989; 101: 39–52.
 - Jessop TS, JM Sumner, CJ Limpus, and JM Whittier. Interplay between plasma hormone profiles, sex and body condition in immature hawksbill turtles (*Eretmochelys imbricata*) subjected to a capture stress protocol. *Journal of Comparative Biochemistry and Physiology* 2004; 137: 197–204.
 - Keller JM, JR Kucklick, MA Stamper, CA Harms, and PD McClellan-Green. Associations between organochlorine contaminant concentrations and clinical health parameters in loggerhead sea turtles from North Carolina, USA. *Environmental Health Perspectives* 2004; 112: 1074–1079.
 - Keller JM, PD McClellan-Green, JR Kucklick, DE Keil, and MM Peden-Adams. Effects of organochlorine contaminants on loggerhead sea turtle immunity: Comparison of a correlative field study and in vitro exposure experiments. *Environmental Health Perspectives* 2006; 114: 70–76.
 - Klinger RC and JA Musick. Annular growth layers in juvenile loggerhead turtles (*Caretta caretta*). Bulletin of Marine Science 1992; 51: 224–230.
 - Limpus CJ. A Biological Review of Australian Marine Turtles. 1. Loggerhead Turtle, Caretta caretta (Linnaeus). Brisbane, Queensland, Australia: Queensland Environmental Protection Agency, 2008a.
 - Limpus CJ. A Biological Review of Australian Marine Turtles. 2. Green Turtle, Chelonia mydas (Linnaeus). Brisbane, Queensland, Australia: Queensland Environmental Protection Agency, 2008b.
 - Limpus CJ. A Biological Review of Australian Marine Turtles. 6. Leatherback Turtle, Dermochelys coriacea (Vandelli). Brisbane, Queensland, Australia: Queensland Environmental Protection Agency, 2009.
 - Limpus CJ and DJ Limpus. The biology of the loggerhead turtle, *Caretta caretta*, in Western South Pacific Ocean foraging areas. In: Witherington B and A Bolten, eds. *Biology and Conservation of Logerhead Turtles*. Washington, DC: Smithsonian Institution Press, 2003, pp. 93–113.
 - Limpus C and J Miller. The occurrence of cutaneous fibropapillomas in marine turtles in Queensland. *Proceedings of Australian Marine Turtle Conservation Workshop*, Canberra, ACT, Australia, 1990, pp 186–188.
 - Limpus CJ, JD Miller, and DJ Limpus. The occurrence of ectopic cloaca deformity in the green turtle in eastern Australia. *Chelonian Conservation and Biology* 2009; 8: 100–101.
 - Limpus CJ and P Reed. The green turtle, *Chelonia mydas*, in Queensland: A preliminary description of the population structure in a coral reef feeding ground. In: Grigg G, R Shine, and H Ehmann, eds. *Biology* of Australasian Frogs and Reptiles. Sydney, Australia: Royal Zoological Society of New South Wales, 1985, pp. 47–52.
 - Lu Y, Y Wang, Q Yu, AA Aguirre, GH Balazs, VR Nerurkar, and R Yanagihara. Detection of herpesviral sequences in tissues of green turtles with fibropapilloma by polymerase chain reaction. Archives of Virology 2000; 145: 1885–1893.

Mader DR. Reptile Medicine and Surgery, 2nd edn. St. Louis, MO: Saunders Elsevier, 2006.

- McArthur S. Infectious agents. In: McArthur S, R Wilkinson, and J Meyer, eds. *Medicine and Surgery of Tortoises and Turtles*. Ames, IA: Blackwell Publishing, 2004, pp. 31–34.
- Miller JD and CJ Limpus. Ontogeny of marine turtle gonads. In: Lutz P, JA Musick, and J Wyneken, eds. *The Biology of Sea Turtles*, Vol. II. Boca Raton, FL: CRC Press, 2003, pp. 199–224.
- Milton SL and P Lutz. Physiological and genetic response to environmental stress. In: Lutz P, JA Musick, and J Wyneken, eds. *The Biology of Sea Turtles*, Vol. II. Boca Raton, FL: CRC Press, 2003, pp. 163–198.
- Morton SR, O Hoegh-Guldberg, DB Lindenmayer, M Harriss Olson, L Hughes, MT McCulloch, S McIntyre et al. The big ecological questions inhibiting effective environmental management in Australia. *Austral Ecology* 2009; 34: 1–9.
- NMFS and USFWS. Recovery Plan for the Northwest Atlantic Population of the Loggerhead Sea Turtle (Caretta caretta), Second Revision. Silver Spring, MD: National Marine Fisheries Service, 2008.

()

NOAA. Green Turtle (Chelonia mydas). NOAA Fisheries Office of Protected Resources, 2008a.

- NOAA. Loggerhead turtle (Caretta caretta). NOAA Fisheries Office of Protected Resources, 2008b.
- Nolan MJ and T Cribb. The use and implications of ribosomal DNA sequencing for the discrimination of digenean species. Advances in Parasitology 2005; 60: 101–163.
- Norton TM. Chelonian emergency and critical care. *Seminars in Avian and Exotic Pet Medicine* 2005; 14: 106–130.
- Norton TM, ER Jacobson, and J Sundberg. Cutaneous and renal fibropapilloma in a green turtle, *Chelonia* mydas. Journal of Wildlife Diseases 1990; 26: 212–216.
- Nutter FB, DD Lee, and MA Stamper. Hemiovariosalpingectomy in a loggerhead sea turtle (*Caretta caretta*). *Veterinary Record* 2000; 146: 78–80.
- Obendorf DL, J Carson, and TJ McManus. Vibrio damsela infection in a stranded leatherback turtle (*Dermochelys coriacea*). Journal of Wildlife Diseases 1987; 23: 666–668.
- Oros J, A Arencibia, L Fernandez, and HE Jensen. Intestinal candidiasis in a loggerhead sea turtle (*Caretta caretta*): An immunohistochemical study. *Veterinary Journal* 2004; 167: 202–207.
- Oros J, A Torrent, P Calabuig, and S Deniz. Diseases and causes of mortality among sea turtles stranded in the Canary Islands, Spain (1998–2001). *Diseases of Aquatic Organisms* 2005; 63: 13–24.
- Owens DW, and GJ Ruiz. New methods of obtaining blood and cerebrospinal fluid from marine turtles. *Herpetologica* 1980; 36: 17–20.
- Patterson-Kane JC, M Flint, PC Mills, CJ Limpus, and D Blyde. A retrospective study of histological lesions in stranded sea turtles in the Gold Coast region, Queensland. *Proceedings of the 29th International Sea Turtle Symposium*, February 17–19, 2009. Brisbane, Queensland, Australia, 2009.
- Perrault JR, D Miller, E Eads, C Johnson, A Merrill, LJ Thompson, and J Wyneken. Maternal health status correlates with nest success of leatherback sea turtles (*Dermochelys coriacea*) from Florida. *PLoS One* 2012; 7: e31841. doi:31810.31371/journal.pone.0031841.
- Perrault JR, J Wyneken, LJ Thompson, C Johnson, and D Miller. Why are hatching and emergence success low? Mercury and selenium concentrations in nesting leatherback sea turtles (*Dermochelys coriacea*) and their young in Florida. *Marine Pollution Bulletin* 2011; 62: 1671–1682.
- Pesce AJ, PS Horn, and D Lewis. Reference Interval. Draft ed, 2005.
- Platt TR. Family Spirorchiidae Stunkard, 1921. In: Gibson DI, A Jones, and RA Bray, eds. Keys to the Trematoda, Vol. 1. Wallingford, Oxfordshire, U.K.: CABI Publishing, 2001, pp. 453–467.
- Raidal SR, M Ohara, RP Hobbs, and RI Prince. Gram-negative bacterial infections and cardiovascular parasitism in green sea turtles (*Chelonia mydas*). Australian Veterinary Journal 1998; 76: 415–417.
- RMPTA. *Recovery Plan for Marine Turtles in Australia*. Canberra, ACT, Australia: Department of Environment and Heritage. Commonwealth of Australia, 2003.
- RMPTA. *Recovery Plan for Marine Turtles in Australia*. In: MSS, ed: Canberra, ACT, Australia: Commonwealth of Australia, 2006.
- Samour JH, JC Howlett, C Silvanose, CR Hasbun, and SM Al-Ghais. Normal Haematology of Free-Living Green Sea Turtles (*Chelonia mydas*) from the United Arab Emirates. *Comparative Haematology International* 1998; 8: 102–107.
- Schwabe CW. Veterinary Medicine and Human Health, 1st edn. Baltimore, MD: The Williams & Wilkins Company, 1969.
- Sinderman CJ. Aeromonas disease in loggerhead turtles. In: Sinderman CJ, ed. Disease Diagnosis and Control in North American Marine Aquaculture, Developments in Aquaculture and Fisheries Sciences, Vol. 6. New York: Elsevier North-Holland, 1977, pp. 292–293.
- Smith JW. The blood flukes (Digenea: Sanguinicolidae and Spirorchidae) of cold-blooded vertebrates: Part 2. Appendix 1: Comprehensive parasite-host list; Appendix 2: Comprehensive host- parasite list. *Helminthological Abstracts* 1997; 66: 329–344.
- Smith GM and CW Coates. Fibro-epithelial growths of the skin in large marine turtles, *Chelonia mydas* (Linnaeus). *Zoologica (NY)* 1938; 23: 93–98.
- Snyder SD. Phylogeny and paraphyly among tetrapod blood flukes (Digenea: Schistosomatidae and Spirorchiidae). *International Journal for Parasitology* 2004; 34: 1385–1392.
- Solberg HE. International Federation of Clinical Chemistry (IFCC). Scientific Committee, Clinical Section. Expert Panel on Theory of Reference Values (EPTRV) and Internation Committee for Standardization in Haematology (ICSH) Standing Committee on Reference Values. Approved recommendation (1987) on the theory of reference values. Part 5. Statistical treatment of collected reference values. Determination of reference limits. *Clinica Chimica Acta* 1987; 170: S13–S32.
- Spotila JR. Sea Turtles: A Complete Guide to their Biology, Behavior, and Conservation. Baltimore, MD: The Johns Hopkins University Press and Oakwood Arts, 2004.

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- Stacy BA, AM Foley, EC Greiner, LH Herbst, AB Bolten, PA Klein, CA Manire et al. Spirorchiidiasis in stranded loggerhead Caretta caretta and green turtles *Chelonia mydas* in Florida (USA): Host pathology and significance. *Disease of Aquatic Organisms* 2010; 89: 237–259.
- Stacy BA, JF Wellehan, AM Foley, SS Coberley, LH Herbst, CA Manire, MM Garner et al. Two herpesviruses associated with disease in wild Atlantic loggerhead sea turtles (*Caretta caretta*). Veterinary Microbiology 2008; 126: 63–73.
- Stamper MA, C Harms, SP Epperly, J Braun-McNeill, and MK Stoskopf. Relationship between barnacle epibiotic load and hematologic parameters in loggerhead sea turtles (*Caretta caretta*), a comparison between migratory and residential animals in Pamlico Sound, North Carolina. *Journal of Zoo and Wildlife Medicine* 2005; 36: 635–641.
- Stewart JS. Anaerobic bacterial infections in reptiles. *Journal of Zoo and Wildlife Medicine* 1990; 21: 180–184.
- Tkach VV, SD Snyder, and JA Vaughan. A new species of blood fluke (Digenea: Spirorchiidiae) from the Malayan box turtle, *Cuora amboinensis* (Cryptodira: Geomydidae) in Thailand. *Journal of Parasitology* 2009; 95: 743–746.
- Valente ALS, R Cuenca, ML Parga, S Lavin, J Franch, and I Marco. Cervical and coelomic radiology of loggerhead sea turtle (*Caretta caretta*). *Canadian Journal of Veterinary Research* 2006; 70: 285–290.
- Valente ALS, R Cuenca, M Zamora, ML Parga, S Lavin, F Alegre, and I Marco. Computed tomography of the vertebral column and coelomic structures in the normal loggerhead sea turtle (*Caretta caretta*). *The Veterinary Journal* 2007a; 174: 362–370.
- Valente ALS, ML Parga, Y Espada, S Lavin, F Alegre, I Marco, and R Cuenca. Ultrasonographic imaging of loggerhead sea turtles (*Caretta caretta*). Veterinary Record 2007b; 161: 226–232.
- Valente ALS, ML Parga, Y Espada, S Lavan, F Alegre, I Marco, and R Cuenca. Evaluation of Doppler ultrasonography for the measurement of blood flow in young loggerhead sea turtles (*Caretta caretta*). Veterinary Journal 2008; 176: 385–392.
- Wallace BP, AD DiMatteo, AB Bolten, M Chaloupka, BJ Hutchinson, FA Abreu-Grobois, JA Mortimer et al. Global conservation priorities for marine turtles. *PLoS One* 2011; 6: e24510. doi:24510.21371/journal. pone.0024510.
- Ward JR and KD Lafferty. The elusive baseline of marine disease: Are diseases in ocean ecosystems increasing? PLoS Biology 2004; 2: 542–547.
- Whiting SD, ML Guinea, CJ Limpus, and K Fomiatti. Blood chemistry reference values for two ecologically distinct population of foraging green turtles, eastern Indian Ocean. *Comparative Clinical Pathology* 2007; 16: 109–118.
- Wilcox B and AA Aguirre. One ocean, one health. EcoHealth 2004; 1: 211–212.
- Wiles M and TG Rand. Integumental ulcerative disease in a loggerhead turtle, *Caretta caretta*, at the Bermuda Aquarium: Microbiology and histopathology. *Disease of Aquatic Organisms* 1987; 3: 85–90.
- Wolke RE and A George. *Sea Turtle Necropsy Manual*. Panama City, FL: National Oceanic and Atmospheric Administration, 1981.
- Work TM. Sea Turtle Necropsy Manual for Biologists in Remote Refuges. Honolulu, Hawaii: USGS, 2000.
- Work TM and GH Balazs. Relating tumor score to hematology in green turtles with fibropapillomatosis in Hawaii. *Journal of Wildlife Diseases* 1999; 35: 804–807.
- Work T and G Balazs. Pathology and distribution of sea turtles landed as bycatch in the Hawaii-based North Pacific pelagic longline fishery. *Journal of Wildlife Diseases* 2010; 46: 422–432.
- Work TM, GH Balazs, RA Rameyer, SP Chang, and J Berestecky. Assessing humoral and cell-mediated immune response in Hawaiian green turtles, *Chelonia mydas. Veterinary Immunology and Immunopathology* 2000; 74: 179–194.
- Work TM, GH Balazs, RA Rameyer, and RA Morris. Retrospective pathology survey of green turtles *Chelonia* mydas with fibropapillomatosis in the Hawaiian Islands, 1993–2003. *Disease of Aquatic Organisms* 2004; 62: 163–176.
- Work TM, GH Balazs, JL Schumacher, and M Amarisa. Epizootiology of spirorchiid infection in green turtles (*Chelonia mydas*) in Hawaii. *Journal of Parasitology* 2005; 91: 871–876.
- Work TM, GH Balazs, M Wolcott, and R Morris. Bacteraemia in free-ranging Hawaiian green turtles Chelonia mydas with fibropapillomatosis. Diseases of Aquatic Organisms 2003; 53: 41–46.
- Work TM, J Dagenais, G Balazs, JL Schumacher, TD Lewis, JC Leong, RN Casey et al. In vitro biology of fibropapilloma-associated turtle herpesvirus and host cells in Hawaiian green turtles (*Chelonia mydas*). *Journal of General Virology* 2009; 90: 1943–1950.

394

Work TM, RE Raskin, GH Balazs, and SD Whittaker. Morphologic and cytochemical characteristics of blood cells from Hawaiian green turtles. *American Journal of Veterinary Research* 1998; 59: 1252–1257.

()

- Wyneken J. *The Anatomy of Sea Turtles*. Miami, FL: National Oceanic and Atmospheric Administration, 2001, p. 172.
- Wyneken J, DR Mader, ES Weber, and C Merigo. Medical care of sea turtles. In: Mader DR, ed. *Reptile Medicine and Surgery*, Second edition. St Louis, MO: Saunders Elsevier, 2006, pp. 972–1007.

AUTHOR QUERIES

- [AQ1] Hermanussen 2006, Keller 2004 has been changed to Hermanussen et al. 2006, Keller et al. 2004 as per reference list. Please check.
- [AQ2] Please provide the location for Blair et al. 2006.
- [AQ3] Please provide the accessed date for Dobbs et al. 2005, Flint et al. 2009c.
- [AQ4] Please provide the complete details for Jacobson et al. 2007, RMPTA 2003, 2006, Pesce 2005.

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