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Potential impacts of historical disturbance on green turtle health in the unique & protected marine ecosystem of Palmyra Atoll (Central Pacific)

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ABSTRACT

Palmyra Atoll, in the Central Pacific, is a unique marine ecosystem because of its remarkably intact food web and limited anthropogenic stressors. However during World War II the atoll was structurally reconfigured into a military installation and questions remain whether this may have impacted the health of the atoll's ecosystems and species. To address the issue we assessed green sea turtle ($n = 157$) health and exposure to contaminants at this foraging ground from 2008 to 2012. Physical exams were performed and blood was sampled for testosterone analysis, plasma biochemistry analysis, hematology and heavy metal exposure. Hematological and plasma chemistries were consistent with concentrations reported for healthy green turtles. Heavy metal screenings revealed low concentrations of most metals, except for high concentrations of iron and aluminum. Body condition indices showed that <1% of turtles had poor body condition. In this study, we provide the first published blood values for a markedly healthy sea turtle population at a remote Central Pacific Atoll.

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1. Introduction

Sea turtles are an integral part of diverse marine ecosystems and face numerous conservation challenges worldwide (Jackson et al., 2001; Chaloupka and Limpus, 2005; Hamann et al., 2010). The globally Endangered (IUCN, 2014) green sea turtle (*Chelonia mydas*) is generally found in tropical and subtropical waters in all major ocean basins. The Central Pacific supports several marine turtle stocks (Wallace et al., 2010), many of which are poorly studied with little known about their current or historical ecology and status (Balazs, 1995; Chaloupka et al., 2004). Demographic data from green turtles at foraging grounds in a few Pacific regions exist, but large parts of the Central Pacific are understudied. Green turtles spend a significant portion of their lives at foraging grounds, and studies of these areas are necessary for comprehensive population assessments, yet research lags behind that of more accessible nesting sites (Balazs et al., 1987; NRC, 2010).

Investigating the health status of protected species is important in population and conservation assessments, because health stressors and disease can be a threat to long-term persistence. Monitoring green turtle health status is of special relevance because fibropapillomatosis (FP), a debilitating herpes-virus linked disease characterized by benign tumors, has a circumtropical distribution and has reached epizootic concentrations in several sites across their range (Aguirre and Lutz, 2004). Further, baseline health information on green turtles may help to identify potential stressors. Exposure to contaminants has been acknowledged as a potential threat to sea turtles (Godley et al., 1999; Ikonomopoulou et al., 2009; Hamann et al., 2010), and as large bodied, long-lived marine vertebrates, green turtles are vulnerable to the effects of bioaccumulation (Caurant et al., 1999; Godley et al., 1999; Gardner et al., 2006; Barbieri, 2009; Todd et al., 2010). Numerous studies have found that while herbivorous green turtles feed at a lower trophic level than carnivorous sea turtles, adverse health impacts from contaminants still occur (Gardner et al., 2006; Komoroske et al., 2011). Collecting baseline information from hematology and metal screenings can advance our understanding of species health, and contribute to the development of conservation and management plans.

The Palmyra Atoll National Wildlife Refuge (PANWR), located approximately 1800 km south of Hawaii in the Central Pacific (Fig. 1a and b), is one of the most remote atolls in the world, but has a complex history. The atoll, among the most intact extant oceanic reef environments (Dinsdale et al., 2008; Sandin et al.,

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2008), is currently uninhabited except for limited research and management personnel, and free from most pervasive anthropogenic inputs. It has been described on several occasions as a unique ecosystem with a remarkably intact food web (Lafferty et al., 2008; Williams et al., 2008, 2011; Work et al., 2008).

However, during the Second World War (WWII), Palmyra was developed into a naval installation. Building on an undisturbed atoll made up of hundreds of small islets and distinct shallow water lagoons (Fig. 1b), the military increased land area by dredging, filling and constructing runways and causeways. Modifications also included dredging of a deep channel to allow ship access to the central lagoon (Dawson, 1959). The military base at Palmyra housed several thousand personnel from 1941 to 1945, thus generating sewage pollution and various toxic and hazardous chemicals, and leaving behind abandoned metal structures in the lagoons (Brainard et al., 2005).

While it is believed that most of the military equipment and debris was removed at the end of WWII, little is known about what contaminants may remain in the marine environment at Palmyra (Collen et al., 2009, 2011). Studies examining trace metal contamination there showed no evidence of lead or other heavy metals in bivalve shells (Collen et al., 2011). However, preliminary X-ray fluorescence (XRF) screening of sand and sediment samples from five marine sites within the lagoons indicated that, based on NOAA's Screening Quick Reference Tables (Buchman, 2008), chromium and strontium in particular may occur in moderately and highly hazardous concentrations for biota, respectively (Papoulias et al., 2010). In contrast, concentrations of most heavy metals known to adversely impact wildlife health (e.g. mercury, lead, arsenic, cadmium, etc.) were below detection levels (Papoulias et al., 2010).

One aim of this study was to contribute to the body of literature evaluating if Palmyra may be considered a relatively intact ecosystem (Dinsdale et al., 2008; Sandin et al., 2008). Given our observational data that Palmyra green turtles appear in relatively good health (see Sterling et al., 2013), the goal of this study was to further constrain or support this hypothesis with quantifiable data. Quantifiable clinical data can add to our understanding of turtle health at this regionally important feeding ground, and to the effectiveness of Palmyra as a marine protected area. Additionally, the US Fish & Wildlife Service (USFWS), which co-manages the refuge, was interested in the health of the sea turtles at Palmyra, especially in light of debris left by the military and consideration of restoration plans for the lagoons. Baseline health data are noteworthy in that they can determine if health issues are present in a population, and help assess changes in health status that may occur in response to environmental pressures. In this study we report on comprehensive health assessments, and compare our findings across stage classes, sex, or site of capture within the atoll.

2. Materials and methods

2.1. Study area

The Palmyra Atoll National Wildlife Refuge (05°52′ N, 162°06′ W) is one of the Northern Line islands (Fig. 1a) in the Central Pacific Ocean. The atoll was purchased by the Nature Conservancy (TNC) in 2000, and the USFWS manages some of the islets and surrounding 15,000 acres of atoll waters. In 2009, it was designated as part of the Pacific Remote Islands National Monument.

The connection of islets and dredging of a channel for ship access during WWII created three artificially deep lagoons with reduced amounts of tidal flow (Maragos, 1993; Maragos et al., 2008). These lagoons vary in depth between 35 and 65 m and are geographically described as the Western, Central, and Eastern lagoons (Fig. 1b). In the northern and southern parts of the atoll, habitat consists of wide, shallow-water reef flats, while there are broad submerged reef terraces on the western and eastern ends (Collen et al., 2009). These reef flats and terraces are characterized by turf and macroalgal assemblages (McFadden et al., 2010). Steeply-sloped fore reefs surround the reef break atoll edges and these areas, combined with the shallow water reef habitats, serve as foraging habitat for green sea turtles (McFadden et al., 2010; Sterling et al., 2013).

2.2. Sampling

We captured green turtles at Palmyra Atoll from 2008 to 2012 using scoop nets, tangle nets, or by hand. We measured weight (kg) straight carapace length (SCL), and tail length (TL) (see Sterling et al. (2013) for methodological details). A body condition index (BCI) was calculated based on the commonly used relationship between mass and SCL (BCI = [mass/SCL^3] × 10^4; Bjorndal et al., 2000). Each turtle was given a comprehensive physical examination including assessments of: plastron concavity/convexity; muscle tone and mass; weight; presence of ectoparasites or fibropapilloma tumors; bloody superficial lesions; and external abnormalities such as amputated flippers or injuries.

Approximately 20 ml of blood was collected from the dorso-cervical sinus using a 20-gauge 1.5-in. needle. Blood samples were visually examined for lymph clot contamination, and those with possible contamination were excluded. Samples were kept in a cooler with ice packs and were typically processed within 6 h of collection. Thin blood smears were made and fixed with 99% methanol. One hundred randomly selected blood smears were later examined by microscopy (100×) to assess potential hemoparasite infections. Heparinized whole blood mounts were made at the time of bleeding and later stained utilizing Diff Quick differential stain kits (Polysciences, Inc.). Leukocytes were then manually counted under 100× magnification and recorded as a percentage of the total leukocyte population. Estimated total leukocyte counts from a blood smear are not as reliable as manual counts using a hemocytometer, but they are far more practical for field situations. Packed cell volumes (PCV) were determined via hematocrit tubes and centrifugation. Serum total solids (g/100 ml) were determined by refractometer (Reichert VET 360) in the field.

Blood was dispensed directly into vacutainer tubes (Becton–Dickinson Diagnostics, Pre-Analytical Systems, Franklin Lakes, New Jersey, USA) containing no additive (for serum refractory analysis), lithium heparin (for hematology and plasma biochemistry analyses), or buffered citrate sodium (for heavy metal testing). Not all tests were performed on all individuals because the amount of material was limited for some of the turtles. Whole blood for heavy and trace metal analysis was collected from 2009 to 2012. Samples collected in the field were transferred to cryovials and stored frozen at −80 °C until analysis at RTI Laboratories (Research Triangle, NC). Plasma biochemistry samples were collected from 2008 to 2012. Samples from heparinized blood-containing tubes were centrifuged at approximately 3250 rpm (2078 g) for 5 min and 3–5 ml of plasma was subsampled and stored at −80 °C until analysis at IDEXX Laboratories (North Grafton, MA) for plasma chemistries analysis.

2.3. Sex determination

To assess sex ratios, subsamples of plasma were collected for testosterone radioimmunoassay procedures and androgen sexing (Owens et al., 1978; Wibbels, 1988; Wibbels et al., 1987, 1991). Radioimmunoassay procedures were conducted at Cornell University, and the testosterone assay's lower limit of detection was 3.12 pg. We used a size cutoff for adults of 80 cm SCL (Wibbels, 1988), and conservatively assigned as males turtles with: (1) >80 cm SCL; (2) tail length (TL) > 25 cm; and (3) testosterone concentrations >30 pg/ml. All other turtles with >80 cm SCL, <25 cm TL, and testosterone concentrations <20 pg/ml were con-