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Original Article

Blood Biochemistry Values of Green Turtles, *Chelonia Mydas*, With and Without Fibropapillomatosis

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Abstract. Baseline blood biochemistry values were obtained for two foraging aggregations of clinically healthy wild, juvenile green turtles (*Chelonia mydas*) inhabiting Kaneohe Bay, Island of Oahu, and the Kona Coast, Island of Hawaii. Mean reference values were compared to values obtained from green turtles of similar size affected with fibropapillomas (FP) collected at Kaneohe Bay. Statistically significant differences were identified for total protein values, blood urea nitrogen, and enzyme values between healthy turtles and turtles with FP. In addition, turtles with severe FP were hypoproteinaemic, hypoalbuminaemic, hypoferraemic, azotaemic, and presented inverse calcium/phosphorus ratios, low cholesterol and triglyceride values, indicating the chronicity and severity of FP. It is concluded that blood reference values should be established for green turtles at the population level and by geographic area considering disease status, age, sex, and seasonal variations.

Keywords: Blood chemistry; *Chelonia mydas*; Clinically normal; Fibropapillomatosis; Green turtle; Hawaiian Islands

Introduction

Green turtles, *Chelonia mydas,* in the Hawaiian Islands represent a geographically isolated marine turtle population protected under the United States Endangered Species Act and Wildlife Laws of the State of Hawaii. The species has demonstrated a gradual increase since 1978 in the Hawaiian Islands (Balazs 1991). A neoplastic disease known as fibropapillomatosis, however, has dramatically increased since 1982 in geographic reference interval and magnitude threatening the species in Hawaii and other parts of the world (Williams et al. 1994). Fibropapillomas (FP) in Hawaiian green turtles have been described as multiple cutaneous and internal tumours ranging from 0.1 to more than 30 cm in diameter (Aguirre et al. 1994a). The disease has a circumtropical distribution and has been observed in all major oceans; where present, prevalence varies among locations, ranging from as low as 1.4% to as high as 90% (Herbst 1994). The primary/aetiological agent of FP remains unknown despite intensive research efforts. Several viruses have been identified as being associated with the tumours, including herpes viruses (A. A. Aguirre and T. R. Spraker unpublished data 1996; Herbst et al. 1995; Quackenbush et al. 1998), retroviruses (Casey et al. 1997) and papilloma-like viruses (Lu et al. 2000). Aetiological factors including other infectious agents (Aguirre et al. 1994a), a response to trematode ova (Dailey and Morris 1994), environmental pollutants impairing the immune system (Aguirre et al. 1994b), and chronic stress (Aguirre et al. 1995) have also been evaluated.

Blood biochemistry represents a valuable diagnostic tool for monitoring the health and condition of free-ranging wildlife. Comparative studies of clinically normal and diseased turtles can provide insightful information for their management and conservation (Norton et al. 1990; Bolten and Bjorndal 1992; Aguirre et al. 1995; Hasbun et al. 1998). The objective of this study was twofold; first to characterise the baseline

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blood biochemistry values of two discrete foraging aggregations of juvenile, free-ranging clinically healthy green turtles on their nearshore habitats in the Hawaiian Islands, and second, to compare these clinically normal profiles to a group of FP-affected turtles within the same age class.

Materials and Methods

Between 15 September and 15 October 1991 to 1995, blood specimens of 53 clinically healthy, juvenile green turtles and 56 turtles with FP were collected from a foraging aggregation in Kaneohe Bay (21 N, 157 W), Island of Oahu. Blood specimens were also collected from a foraging aggregation of 37 clinically healthy, juvenile green turtles captured along the Kona Coast (19 N, 156 W), Island of Hawaii, at Keawa Nui Bay and Kiholo Bay.

Green turtles at Kaneohe Bay were captured by hand, alive and unharmed using snorkeling equipment. Green turtles from the Kona Coast were also captured alive and unharmed by hand and by using a closely monitored tangle net. A blood specimen from each turtle was taken by venepuncture from the dorsal postoccipital sinuses (Owens and Ruiz 1980) following manual restraint. Blood (3–10 ml) was collected using 21 gauge needles and 5 ml or 10 ml syringes. Blood specimens were transferred into Vacutainer® tubes containing lithium heparin (Becton, Dickinson, Rutherford, NJ, USA) and placed on blue ice until processing in the laboratory 4–6 h after capture. Plasma was separated by centrifugation at 2000 rpm for 10 minutes, and split in two or more vials. Vials were stored on solid CO_2 or in a freezer at −70 °C until tested at SmithKline Laboratory after each sampling period.

Following blood collection, turtles were measured, tagged, and weighed according to techniques previously described (Balazs et al. 1987). Turtles were categorised into the following age/size classes: pelagic, turtles under 35 cm straight carapace length (SCL); juveniles, turtles between 35 and 65 cm SCL; subadults, turtles between 65 and 85 cm SCL; and presumed adults, turtles above 85 cm SCL. All turtles were thoroughly examined for the presence of fibropapillomas and a description of their size, number, and location was recorded. Turtles were assigned a fibropapilloma severity score (FPS) on a scale of 0–4: FPS = 4, the most severe case; FPS = 3, heavily affected; FPS = 2, moderately affected; FPS = 1, lightly affected. Turtles without fibropapillomas were given a score of 0. Anatomic site influenced FPS when vision or ability to feed was impaired (Aguirre et al. 1998).

A total of 25 biochemistry analytes were measured at SmithKline Beecham Clinical Laboratories (Honolulu, Hawaii and Van Nuys, California). Blood biochemistry variables were determined using an automated random access analyser Olympus 5000 Series AU5061 (Olympus Corporation, Lake Success, New York, USA). This autoanalyser demonstrated excellent precision when measuring biochemistry values for loggerhead turtles (*Caretta caretta*) (Bolten et al. 1992). Samples that appeared haemolysed to the naked eye were discarded. The following plasma determinants were measured: total protein, albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (AP), gamma glutamyl transpeptidase (GGT), lactate dehydrogenase (LDH), blood urea nitrogen (BUN), creatinine, uric acid, calcium, phosphorus, cholesterol, triglycerides, glucose, iron, sodium, potassium and chloride. In addition, calculated values included globulin, albumin/globulin ratio, bilirubin and BUN/creatinine ratio.

Statistical analyses applying one-way ANOVA or Kruskal–Wallis test if data fit normality assumptions and equal variance. Post hoc multiple pairwise comparisons with the least significant difference test in an ANOVA analysis and Dunn's test for Kruskal–Wallis test were performed using SAS software (Schlotzhauer and Littell 1987). Differences were identified for mean values between the two clinically normal foraging aggregations, and among healthy turtles and turtles and their different FPS. Results were considered statistically significant for probabilities ≤ 0.05 (alpha = 0.05). Data were expressed as mean, standard deviation (SD), and reference interval of values for each blood parameter.

Results

Mean, SD, and reference interval of straight carapace length (SCL), weight and blood biochemistry values for 90 clinically healthy green turtles by collection site are summarised in Table 1. Similar data for 56 green turtles with FP and classified by their FPS 1 to 3, are presented for Kaneohe Bay (Table 2). Mean SCL and weights were significantly smaller for clinically healthy turtles in both aggregations when compared to turtles with FP in the same age/class group. In addition, FP severity scores increased with size and weight.

Blood enzymatic values were significantly different between both healthy turtle aggregations. The Kona Coast group presented higher AST and LDH values, whereas the Kaneohe Bay healthy turtle group had higher ALT and AP values. ALT decreased with FP severity, however, AST, AP and LDH demonstrated the opposite trend (Tables 1 and 2). Blood parameters for the two wild healthy aggregations were similar in general; however, significantly higher values of total protein and globulin were identified for the Kona Coast aggregation when compared to the Kaneohe Bay aggregation. Differences were also identified for calcium (Ca) and phosphorus (P) levels for the healthy aggregations. Significantly higher Ca and lower P levels were detected in the Kona Coast turtles when compared to the healthy, wild Kaneohe Bay population. The Ca/P ratios for Kona Coast and Kanehoe Bay turtles were 2:1 and 1:1, respectively (Fig. 1).

Comparisons between clinically healthy turtles of both aggregations and turtles with FP yielded significant results. Hypoproteinaemia and related protein deficien-

Table 1. Mean, standard deviation (SD), and reference interval of straight carapace length, weight and plasma biochemistry values for clinically healthy green turtles (*Chelonia mydas*), Hawaiian Islands, 1991–95

Variable	Kaneohe Bay, Oahu ($n = 53$)			Kiholo and Kona, Hawaii ($n = 37$)		
	Mean	± SD	Reference interval	Mean	± SD	Reference interval
Straight carapace length (cm)	45.3	4.8	37.4–55.2	47.7	7.2	38.5–62.0
Weight (kg>)	14.1	4.7	7.7–25.4	17.8	6.6	9–26
Protein (g/dl)	4.2	0.6	2.9–5.6	5.0	0.7	3.5–6.7
Albumin (g/dl)	1.7	0.4	0.6–2.2	1.7	0.2	1.3–2.0
Globulin (g/dl)	2.7	0.5	1.8–4.0	3.3	0.6	1.9–4.7
Albumin/globulin ratio	0.6	0.2	0.2–1.2	0.6	0.1	0.4–0.9
Total bilirubin (mg/dl)	0.2	0.1	0.0–0.4	0.2	0.04	0.0–0.3
Direct bilirubin (mg/dl)	0.05	0.1	0.0–0.5	0.02	0.05	0.0–0.2
Indirect bilirubin (mg/dl)	0.2	0.1	0.0–0.4	0.2	0.06	0.0–0.2
Alanine aminotransferase (U/l)	3.9	7.0	0.0–50.0	2.3	1.6	1–7
Asparate aminotransferase (U/l)	158.4	41.5	1.0–270	215.3	100.9	47–491
Alkaline phosphatase (U/l)	33.5	12.2	12–62	18.2	8.5	5–42
Gamma glutamyl transpeptidase (U/l)	2.7	1.5	0.0–5.0	1.0	0.0	1.0
Lactate dehydrogenase (U/l)	203.8	180.4	55–1286	316.7	165.0	67–769
Urea nitrogen (BUN) (mg/dl)	5.2	14.1	0.0–64.0	6.1	4.4	1–19
Creatinine (mg/dl)	0.2	0.1	0.1–0.5	0.2	0.1	0.1–0.3
BUN/creatinine ratio	25.8	70.7	0.0–320.0	32.9	31.1	3–160
Uric acid (mg/dl)	1.3	0.8	0.0–4.5	1.5	0.7	0.7–3.9
Calcium (mg/dl)	9.1	1.7	1.1–12.1	11.2	2.2	4.8–15.0
Phosphorus (mg/dl)	8.2	1.3	5.9–11.8	5.0	1.3	2.8–10.7
Cholesterol (mg/dl)	140.0	43.0	32–280	99.1	34.0	42–184
Triglycerides (mg/dl)	124.2	68.7	28–331	84.0	53.3	24–245
Glucose (mg/dl)	114.7	35.0	64–234	109.1	13.2	86–133
Iron (µg/dl)	46.3	64.8	9–321	43.5	25.5	23–91
Sodium (meq/l)	158.0	4.0	146–170	154.0	4.8	145–164
Potassium (meq/l)	5.2	0.9	3.9–8.6	4.8	0.5	4–6
Chloride (meq/l)	115.2	5.7	103–130	114.9	6.2	102–134

cies were evident for turtles with advanced FP. Pooled Ca/P ratios were reversed in turtles with advanced stages of the disease. Iron levels fluctuated sharply in turtles with FP, declining with the degree of fibropapilloma severity from 71 µg/dl to 24 µg/dl (Fig. 2). Likewise, blood urea nitrogen (BUN) increased with disease severity, producing highly disproportionate BUN/creatinine ratios (Fig. 3). Statistically significant changes in cholesterol and triglyceride levels were observed when comparing clinically healthy turtles to turtles with FP in Kaneohe Bay. A decline of these mean values was significant in turtles with severe FP.

Discussion

An attempt was made to obtain a homogeneous sample size at both sites considering similar size/age class, season, and feeding behaviour. All turtles in this study were considered juvenile based on the SCL (35–65 cm). We observed statistical differences in size and weight when we compared the different stages of FP within the same age/class group. Previous studies in the Hawaiian Islands have demonstrated that turtles approximately 35 cm SCL recruit to coastal habitats where they spend most of their lives. Turtles captured in Kaneohe Bay, averaging $\leqslant$40 cm SCL were free of FP and green turtles averaging $\geqslant$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 nearshore environments (Aguirre et al 1994a, 1998).

The establishment of baseline blood biochemistry profiles for healthy wild sea turtles is a priority for their conservation and management. Normal blood biochemistry values have not been established for most free-ranging marine turtle populations. Studies are limited to green turtles in southern Bahamas (Bolten and Bjorndal 1992), Hawaii (Aguirre et al. 1995) and the United Arab Emirates (Hasbun et al. 1998).

Blood biochemistry parameters have previously been reported for wild, maricultured or captive reared green turtles. Those values were not comparable to our study due to differences in techniques, conditions, units reported, and/or age (Holmes and McBean 1964; Berkson 1966; Dessauer 1970; Bonnet 1979; Frair and

Table 2. Mean, standard deviation (SD), and range of straight carapace length, weight and plasma biochemistry values by severity of fibropapillomas (GTFP) for green turtles (*Chelonia mydas*), Kaneohe Bay, Island of Oahu, Hawaii, 1991–95

Plasma biochemistry variable	Tumour score (TS) = 1 (n = 12)			TS = 2 (n = 16)			TS = 3 (n = 28)		
	Mean	± SD	Range	Mean	± SD	Range	Mean	± SD	Range
Straight carapace length (cm)	53.3	3.7	46.9–60.8	53.9	6.5	46.8–63.4	58.0	10.3	43.0–64.9
Weight (kg)	21.9	6.0	17.7–33.6	24.4	8.8	16.0–43.1	25.2	10.7	10.5–43.6
Protein (g/dl)	4.9	0.6	4.0–6.1	4.5	0.8	2.9–6.3	3.5	1.0	1.8–5.7
Albumin (g/dl)	1.9	0.2	1.6–2.2	1.7	0.3	0.8–2.0	1.2	0.4	0.4–1.8
Globulin (g/dl)	3.0	0.5	2.2–3.9	2.7	0.5	2.1–3.5	2.3	0.7	1.1–4.0
Albumin/globulin ratio	0.6	0.1	0.5–0.8	0.6	0.1	0.4–0.9	0.5	0.1	0.3–0.9
Total bilirubin (mg/dl)	0.2	0.1	0.0–0.2	0.2	0.1	0.0–0.3	0.1	0.1	0.0–0.4
Direct bilirubin (mg/dl)	0.02	0.1	0.0–0.2	0.02	0.04	0.0–0.1	0.02	0.1	0.0–0.3
Indirect bilirubin (mg/dl)	0.2	0.1	0.0–0.2	0.2	0.04	0.1–0.3	0.2	0.1	0.0–0.4
Alanine aminotransferase (U/l)	3.8	5.8	1.0–22	2.2	2.4	0.0–9.0	2.1	4.1	0.0–21.0
Aspartate aminotransferase (U/l)	152.7	12.4	134–171	148.2	39.6	94–260	168.2	47.2	88–295
Alkaline phosphatase (U/l)	41.2	12.1	17–61	25.2	9.0	1–39	24.3	8.0	10–48
Gamma glutamyl transferase (U/l)	2.8	1.7	0.0–5.0	2.8	1.3	1–4	1.1	1.7	0.0–5.0
Lactate dehydrogenase (U/l)	226.2	67.4	146–403	198.4	90.2	89–418	179.7	122.8	79–599
Urea nitrogen (BUN) (mg/dl)	1.9	2.1	0.0–8.0	6.5	14.2	0.0–56.0	14.8	19.6	1.0–78
Creatinine (mg/dl)	0.2	0.1	0.1–0.4	0.2	0.1	0.1–0.4	0.2	0.1	0.1–0.4
BUN/creatinine ratio	16.0	21.6	0.0–80	54.5	141.7	0.0–560	75.1	89.5	3.0–290
Uric acid (mg/dl)	1.0	0.2	0.6–1.5	1.1	0.6	0.5–2.8	1.6	2.9	0.1–16.2
Calcium (mg/dl)	9.9	0.7	8.3–11	9.0	1.4	6.6–12.0	7.5	1.7	2.9–10.9
Phosphorus (mg/dl)	8.4	1.0	6.7–10.6	7.7	1.0	6.5–9.3	8.0	2.0	5.3–13.4
Cholesterol (mg/dl)	143.2	31.2	93–208	139.4	43.3	71–233	106.8	38.7	26–181
Triglycerides (mg/dl)	141.8	49.2	78–231	124.7	87.6	25–300	84.1	54.5	32–278
Glucose (mg/dl)	115.6	23.2	84–174	120.6	19.0	80–157	111.9	23.4	83–166
Iron (µg/dl)	61.2	25.8	11–151	70.8	76.1	16–183	34.7	21.0	9–76
Sodium (meq/l)	158.6	4.1	151–164	158.0	5.4	152–171	154.5	4.7	141–165
Potassium (meq/l)	5.5	0.7	4.4–7.2	5.1	1.0	3.5–7.1	4.5	0.8	3.8–7.7
Chloride (meq/l)	113.6	4.6	109–125	113.9	6.5	103–126	113.5	5.9	101–130

Shah 1982; Norton et al. 1990). However, total protein, total bilirubin, glucose, uric acid, calcium and chloride were comparable to reference intervals reported for healthy, juvenile wild green turtles from southern Bahamas (Bolton and Bjorndal 1992) and United Arab Emirates (Hasbun et al. 1998).

Research by Aguirre et al. (1995) demonstrated low blood cholesterol values, low triglyceride values, hypoproteinaemia, hypoalbuminaemia, and hypoferraemia in an experimental group of wild green turtles with severe FP (FPS = 3) from Kaneohe Bay, Island of Oahu. Norton et al. (1990) presented comparable results indicating the severity and chronicity of fibropapillomatosis. The hypoferraemia observed in turtles with FP can be considered as a host defence mechanism in response to infection. Serum iron levels decrease in several reptile and mammal species during an infectious process (Aguirre et al. 1995).

Differences in enzyme activities between the two foraging aggregations of clinically healthy green turtles may be explained by their exposure to disease. Higher AST and LDH values in the Kona Coast turtles may reflect a good response to acute stress based on the fact that FP has not been diagnosed in that population. Evidence of chronic stress and immunosuppression in turtles with FP was previously described for wild green turtles in Kaneohe Bay (Aguirre et al. 1995).

Variables including age, sex, diet and seasonal changes will influence blood parameters (Lutz and Dunbar-Cooper 1987). During this study, we attempted to randomly capture turtles of similar age/size class, with similar feeding behaviour and during the same season. Sex was not determined in our study since all turtles were juvenile and not from the breeding cohort. Normal Ca/P ratios in sea turtles have not been established. Stamper and Whitaker (1994) noted improvement of Ca/P ratios following change in diet of captive sea turtles. Differences in Ca and P levels between the two clinically healthy aggregations could not be explained except for possible differences in their diet based on natural sea grasses and algae. Both minerals are important for normal physiological activity and their regulation requires integration of other hormones including vitamin D, calcitonin and parathormone. Decreases of Ca observed in turtles with FP may be related to hypoalbuminaemia or a rapid alkalinisation process for FP turtles. Inverse Ca/P ratios have been reported for clinically normal loggerhead turtles (Stamper and Whitaker 1994).

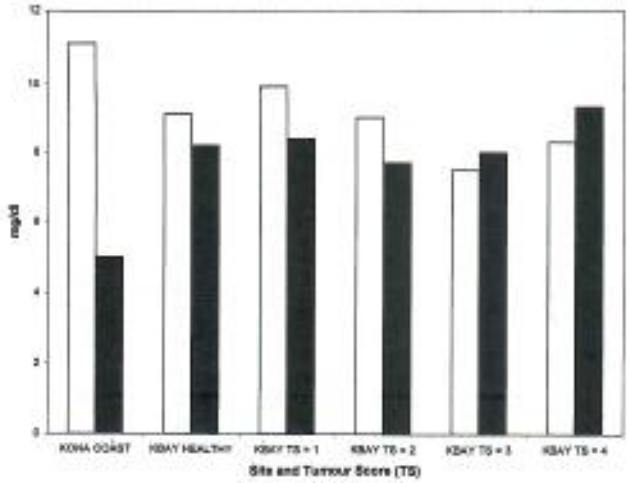

Fig. 1. Mean calcium (□) and phosphorus (■) values (mg/dl) for clinically normal juvenile green turtles (*Chelonia mydas*) captured at the Kona Coast and Kaneohe Bay (KBay), and turtles with fibropapillomas (FP) captured at KBay, 1991–1995.

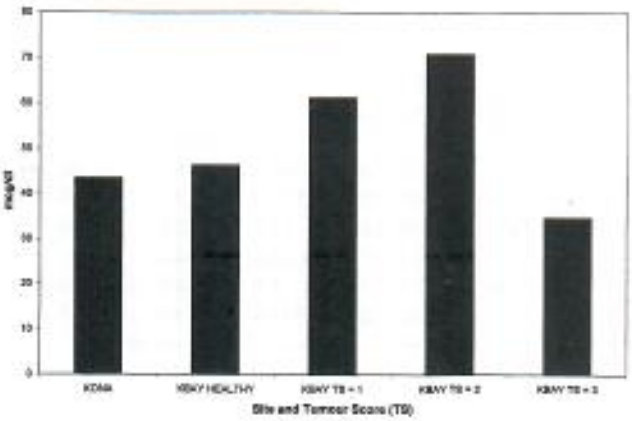

Fig. 2. Mean iron values (μg/dl) for clinically normal juvenile green turtles (*Chelonia mydas*) captured at the Kona Coast and Kaneohe Bay (KBay), and turtles with fibropapillomas (FP) captured at KBay, 1991–1995. Values were not measured for KBay TS = 4.

Azotaemia was clearly observed in turtles with FP during the present study and may be explained by the neoplastic process. The presence of internal FP in kidney, lung and liver is highly (30%) prevalent in turtles with external FP. Decline of cholesterol and triglyceride levels observed in turtles with severe FP may relate to loss of body condition and starvation. This chronic condition and related starvation syndrome may cause acidosis, azotaemia and eventually death (Aguirre et al. 1998).

The large variance in some of the parameters outlined in this study may indicate effects of population variability and lack of repeatability of sampling. More precise estimates can be achieved by increasing sample size and repeating our measurements with the same individuals over time. Also, sampling at different locations with different disease conditions is recommended to establish normal values of healthy individuals.

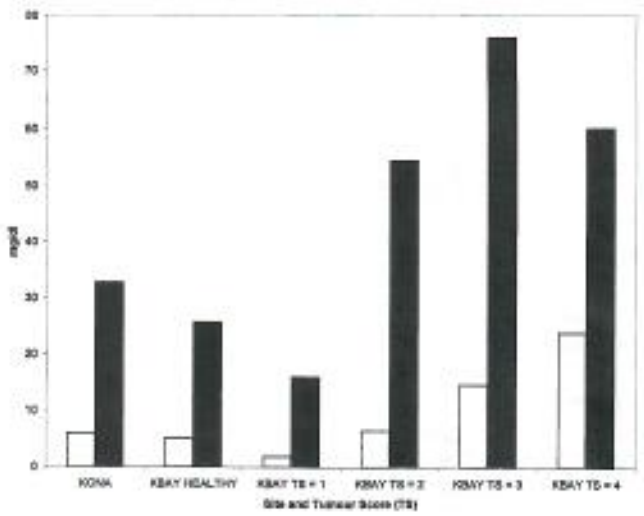

Fig. 3. Mean blood urea nitrogen (BUN) (□) values and BUN/creatinine (■) ratios for clinically normal juvenile green turtles (*Chelonia mydas*) captured at the Kona Coast and Kaneohe Bay (KBay), and turtles with fibropapillomas (FP) captured at KBay, 1991–1995.

Further research is warranted to identify and characterise blood chemistry differences among healthy and diseased green turtle populations and among sex and age cohorts. Also more studies are needed to provide insight into host susceptibility to FP in different populations as it relates to blood biochemistry including protein, mineral deficiencies (i.e. Ca/P ratios) and enzymatic factors.

The significant differences observed between foraging aggregations of Hawaiian green turtles confirmed that baseline data should be collected by geographic area, discrete population, foraging aggregation, seasonal variability, and presence of FP and other related disease conditions. The data generated from this study may be useful for clinical assessment of health and disease of two foraging aggregations of wild green turtles on nearshore habitats in the Hawaiian Islands. In addition, this study will contribute to the development of appropriate management and conservation tools for wild Hawaiian *C. mydas*.

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