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research paper

The impact of fibropapillomatosis on clinical characteristics, blood gas, plasma biochemistry, and hematological profiles in juvenile green turtles (*Chelonia mydas*)

[1] *National Museum of Marine Biology and Aquarium, Pingtung 94450, Taiwan*

[2] *Graduate Institute of Microbiology and Public Health, College of Veterinary Medicine, National Chung Hsing University, Taichung, 40227, Taiwan*

* *Corresponding author email: <changcc@dragon.nchu.edu.tw>, telephone: 886-4-2284-0894*

Tsung-Hsien Li [1]
Chao-Chin Chang [2 *]

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ABSTRACT.—Fibropapillomatosis (FP) is a tumor-forming disease that afflicts all marine turtles and is the most common in green turtles (*Chelonia mydas*). In this study, the morphometric characteristics, blood gas, biochemistry, and hematological profiles of 28 (6 FP-positive and 22 FP-negative) green turtles from the coast of Taiwan were investigated. The results indicated that body weight ($P < 0.001$) and curved carapace length (CCL; $P < 0.001$) in green turtles with FP were significantly higher than in turtles without FP. Furthermore, green turtles with FP had a significantly lower value of hemoglobin (HB; $P = 0.010$) and packed cell volume (PCV; $P = 0.005$) than turtles without FP. Blood cell counts of white blood cells (WBC; $P = 0.008$) and lymphocytes ($P = 0.022$) were observed with significant difference; green turtles with FP had lower counts than turtles without FP. In addition, turtles with FP had significantly higher pH ($P = 0.036$), base excess in extracellular fluid (BEecf; $P = 0.012$), bicarbonate (HCO_3^-; $P = 0.008$), and total carbon dioxide (TCO_2; $P = 0.025$) values than turtles without FP. The findings of this study provide valuable clinical parameters for the medical care of the species in sea turtle rehabilitation centers and help us to understand the physiological response of green turtles to different tumor-forming conditions.

Sea turtles can serve as sentinel indicators of marine ecosystem health (Aguirre and Lutz 2004). Lewbart et al. (2014) described how health assessments of green turtles can have implications for wildlife biology and species conservation. Peripheral blood biochemical and hematology parameters have been widely used in health assessment studies and death investigations for development of conservation strategies in wild populations of sea turtles worldwide (Lewbart et al. 2014, Page-Karjian et al. 2015).

Fibropapillomatosis (FP) is an infectious neoplastic disease that afflicts all sea turtle species and is the most common in green turtles (*Chelonia mydas*; Page-Karjian et al. 2014, 2015). FP has been documented in every major region inhabited by green turtles (Herbst 1994), however, reports regarding FP in green turtles in Asia are still

very limited. FP is considered a major threat to the survival of sea turtles (Jones et al. 2016). In diseased turtles, tumors caused by FP can appear on the eyes, lower jaw, neck, mouth, front flippers, armpits, carapace, plastron, groin, base of tail, and even internal organs (Work et al. 2004). It is well-recognized that FP can have influences on feeding, locomotion, vision, and organ function (Herbst 1994, Aguirre and Lutz 2004).

The body condition index (BCI; Bjorndal et al. 2000) is a calculated index to determine health status in sea turtles according to their body weight and length. However, Deus Santos et al. (2015) pointed out that BCI values of FP-positive sea turtles and FP-negative sea turtles are not significantly different. Rossi et al. (2019) further documented that FP-positive green turtles had significantly higher BCI values than turtles without FP. The previous literature has reported differences in hematologic and plasma biochemical parameters between FP-positive sea turtles and those without FP. Researchers have also sought to determine the significance of changes in hematologic and plasma biochemical profiles in sea turtles suffering from FP (Work and Balazs 1999, Aguirre and Balazs 2000, Rossi et al. 2009, Deus Santos et al. 2015). The relevant reports in Taiwan are still very limited; therefore, establishing and comparing base data for hematologic and plasma biochemical parameters in FP-positive and FP-negative sea turtles should enable veterinarians at rescue centers to gain a better understanding of the physiological state of sea turtles afflicted with FP. This should in turn allow affected sea turtles to be treated more effectively and facilitate veterinarians to establish treatment guidelines for sea turtles at risk of developing FP. In the present study, our primary objective was to compare FP-positive and FP-negative juvenile green turtles in terms of clinical characteristics, blood gas, biochemistry, and hematological profiles. The information from the comparison of these parameters could be applied to improve conservation work in sea turtles in Taiwan. These data could be beneficial to veterinarians to offer appropriate and timely clinical management in endangered sea turtle rehabilitation facilities, as well as to stimulate further studies to understand possible mechanisms.

Materials and Methods

Sample Collection.—Blood samples were collected from 28 unhealthy (i.e., weak or injured) green turtles that were admitted to the rehabilitation center at the National Museum of Marine Biology and Aquarium (NMMBA) in Checheng Township, Pingtung County, Taiwan during 2017–2018. Seven of the 28 turtles (25%) were discovered with buoyancy disorders and 21 (75%) were accidentally caught by fishermen (Fig. 1). Twenty-eight blood samples were collected from the 28 green turtles, including six (21%) with and 22 (79%) without external FP tumors. In the present study, we followed the green turtle tumor score system to record FP condition for each study individual (Work and Balazs 1999). The stranded animals were brought to the center for special clinical care under the authority of the Forestry Bureau, Council of Agriculture, Executive Yuan, Taiwan. Blood samples and gross observational data [body weight, curved carapace length (CCL), straight carapace length (SCL), cloacal temperature] were collected from each turtle to evaluate their health status upon admission to the rehabilitation center. BCI was calculated using the following formula [as suggested by Bjorndal et al. (2000)]:

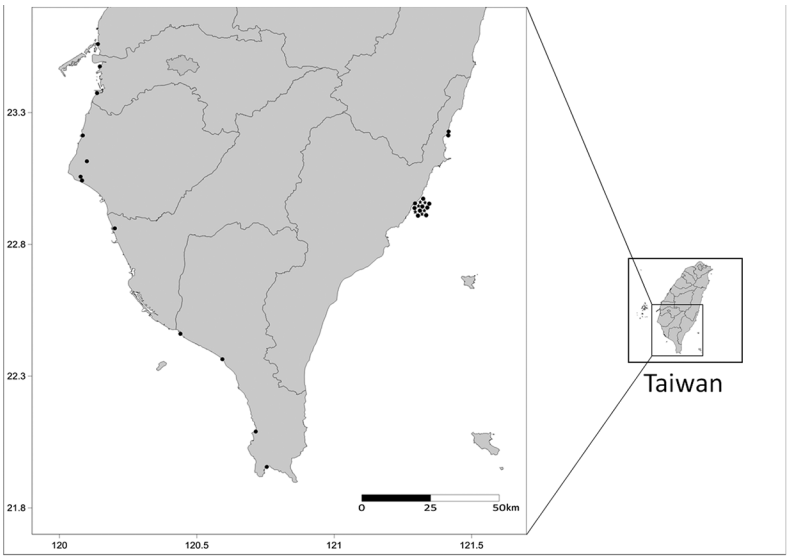

Figure 1. Map showing the sites where turtles were stranded and collected for the analysis in this study. Turtles without FP = black circles, turtles with FP = asterisks.

$$BCI = \text{body weight}/SCL^3 \times 10{,}000$$

Venous blood samples (maximum volume < 0.8 ml kg^{-1}) were collected by a veterinarian from the external jugular vein (Day et al. 2010) using a 12 ml syringe fitted with a 23-gauge needle. Each whole blood sample was aliquoted into several vials for use in creating blood smears and testing the erythrocyte sedimentation rate (ESR) and lactate values. The rest of the blood was aliquoted in tubes containing lithium heparin solution and stored at 4 °C prior to the analysis of blood gas, hematology, and blood/plasma biochemistry profiles. Plasma was separated from the whole blood samples after centrifugation at 3000 rpm for 5 min and was stored at −80 °C for further analysis.

Ethics.—Sea turtle rehabilitation at the NMMBA was approved by the Forestry Bureau, Council of Agriculture, Executive Yuan under project permits No. 1061701173, 1071700868, and 107AS-10.8.1-FB-e2. These cover the rescue, medical procedure, rehabilitation, collection, and processing of samples. All samples in this study were collected under humane procedures from an external jugular vein as described by Day et al. (2010) by a certified veterinarian (Dr. Tsung-Hsien Li). In the rehabilitation center, the collection of samples from the sea turtles for blood tests was conducted only during routine medical care but not for any specific research intension. Accordingly, data used for analysis in this study were obtained from the historical data bank in the rehabilitation center, and thus no specific approval from the Institutional Animal Ethics Committee was required.

Examinations of Hematology, Biochemistry, and Blood Gas Profiles.—Hematological examinations included quantitative analysis of hemoglobin (HB), white blood cells (WBC), heterophils, lymphocytes, monocytes, eosinophils,

basophils, thrombocytes, erythrocyte sedimentation rate (ESR), and lactate values. Blood smears were made on glass slides and stained with Liu's stain for counting leukocytes. The Liu's staining method includes Liu A and Liu B solutions (Yue et al. 2014). Briefly, the air-dried blood smear was directly stained with Liu A for 50 sec and then with Liu B for 80–100 sec. Then, the stain solution was rinsed out softly with tap water and the slide was air dried prior to examination. Differential WBC counts were performed manually using the blood smears to determine the percentage of each type of leukocytes and the absolute counts of heterophils, lymphocytes, monocytes, eosinophils, and basophils (Stamper et al. 2005). The HB, WBC, and thrombocyte values were measured using a hematology analyzer (Exigo eos, Boule Diagnostics, Spanga, Sweden). The ESR value was obtained using the standard Westergren method. The lactate level was assayed using a lactate analyser (EDGE Lactate Analyser, Woodley Equipment Company, Bolton, UK).

Biochemistry profiles were created for each turtle by testing plasma samples for total protein, albumin, total bilirubin, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, γ-glutamyltranspeptidase, creatinine kinase, lactate dehydrogenase, cholesterol, triglyceride, glucose, blood urea nitrogen, uric acid, phosphorus, calcium, and amylase concentrations. All of the above values were obtained using an automated dry chemistry analyzer (Spotchem EZ SP-4430, Arkray Inc., Kyoto, Japan) in accordance with the manufacturer's instructions within 1 d of sample collection.

The blood gas and blood biochemistry profiles, including potential hydrogen (pH), partial pressure of carbon dioxide (pCO_2), partial pressure of oxygen (pO_2), base excess in extracellular fluid (BEecf), bicarbonate (HCO_3^-), total carbon dioxide (TCO_2), saturated oxygen (sO_2), ionized calcium (iCa), potassium (K), sodium (Na), glucose (Glu), packed cell volume (PCV), and creatinine (CRE), were obtained by an iSTAT analyzer (Abbot Point of Care Inc., Princeton, NJ, USA) using CG8+ cartridges (Innis et al. 2014, Lewbart et al. 2014) within 10 min of sample collection (Abbot Point of Care Inc., Princeton, NJ). The CRE value was obtained using Crea cartridges (Abbot Point of Care Inc., Princeton, NJ).

Statistical Analysis.—Statistical comparison of gross observational characteristics, hematology, blood/plasma biochemistry, and blood gas values was conducted to identify which of these variables differed between FP-positive and FP-negative turtles. Either *t*-tests or Mann–Whitney *U* tests were used, depending on whether the data met the assumption of normality. A significant difference was declared when $P < 0.05$. No alpha adjustment was made to protect against Type I errors. All analyses were performed using SPSS for Windows, v18.0 (Chicago, Illinois).

Results

A total of 28 green turtles were sampled. The mean CCL for all turtles was 47.6 cm, with a range of 38.3–66.0 cm. All of the turtles were classified as juveniles (CCL < 67.0 cm; Ng et al. 2018b). Based on the gross observations when the animals were delivered to the rehabilitation center (Table 1), it was found that the body weight in green turtles with FP was significantly higher than the ones without FP ($P < 0.001$). A similar phenomenon was also reflected in the significantly longer CCL in green turtles with FP ($P < 0.001$). However, there was no significant difference in BCI between

Table 1. Comparison of gross observations between green turtles with and without FP.

Variable	Turtles with FP (n = 6)	Turtles without FP (n = 22)	P-value
Body weight [a] (kg)	22.82 (16.92–28.70)	9.32 (5.68–16.28)	<0.001
Curved carapace length [a] (cm)	57.45 (53.3–66.0)	44.65 (38.3–56.0)	<0.001
Body condition index [a]	1.28 (1.15–1.42)	1.27 (0.82–1.46)	0.822
Body temperature [b] (°C)	27.17 (2.78)	25.77 (2.22)	0.208

[a] Data with nonnormal distribution presented by median (range) and compared using Mann–Whitney U test.

[b] Data with normal distribution presented by mean (SD) and compared using Student's t-test.

green turtles with and without FP. In addition, there was no significant difference in body temperature between animals with and without FP.

Regarding the results of hematological profile comparison (Table 2), green turtles with FP had a significantly lower value of HB than the ones without FP (P = 0.010). Blood cell counts of WBC (P = 0.008) and lymphocytes (P = 0.022), which are relevant to the animal's immune status, were observed with significant difference; green turtles with FP had lower counts than turtles without FP. Among the green turtles with FP, significantly lower PCV levels were observed after comparison to the ones without FP (P = 0.005). The monocyte count in green turtles with FP was much lower than that in green turtles without FP, though not showing statistical significance. Other hematological profiles were similar between green turtles with and without FP.

There were no major significant findings for the comparison of blood/plasma biochemistry profiles between green turtles with and without FP (Table 3).

Regarding the comparison of blood gas profiles (Table 4), levels of pH (P = 0.036), BEecf (P = 0.012), HCO_3^- (P = 0.008), and TCO_2 (P = 0.025) were significantly higher in green turtles with FP than in turtles without FP.

Table 2. Comparison of hematological profiles between green turtles with and without FP.

Variable	Turtles with FP (n = 6)	Turtles without FP (n = 22)	P-value
HB [a] (g dl^{-1})	9.00 (2.00–11.00)	11.00 (4.00–16.00)	0.010
PCV [b] (%)	18.17 (6.52)	27.00 (6.26)	0.005
WBC [b] (μl^{-1})	12,750.00 (5,123.96)	18,781.82 (4,382.82)	0.008
Heterophils [a] (μl^{-1})	6,576.00 (5,015.00–9,246.00)	7,418.00 (2,948.00–20,713.00)	0.695
Lymphocytes [b] (μl^{-1})	4,692.67 (3,445.05)	7,886.23 (2,695.89)	0.022
Monocytes [a] (μl^{-1})	1,130.50 (558.00–2,100.00)	2,039.50 (0.00–6,195.00)	0.057
Eosinophils [a] (μl^{-1})	31.00 (0.00–630.00)	0.00 (0.00–1,712.00)	0.951
Basophils [a] (μl^{-1})	0.00 (0.00–0.00)	0.00 (0.00–224.00)	0.602
Thrombocytes [a] (μl^{-1})	2,000.00 (1,000.00–4,000.00)	2,000.00 (1,000.00–22,000.00)	0.362
ESR [a] (mm $1h^{-1}$)	7.50 (5.00–29.00)	6.00 (4.00–12.00)	0.127
Lactate [a] (mg dl^{-1})	14.00 (7.00–22.00)	15.00 (6.00–77.00)	0.483

[a] Data with nonnormal distribution presented by median (range) and compared using Mann–Whitney U test.

[b] Data with normal distribution presented by mean (SD) and compared using Student's t-test.

HB = Hemoglobin, PCV = Packed cell volume, WBC = White blood cell, ESR = Erythrocyte sedimentation rate.

Table 3. Comparison of plasma biochemistry profiles between green turtles with and without FP.

Variable	Turtles with FP (n = 6)	Turtles without FP (n = 22)	P-value
Total protein [b] (g dl^{-1})	3.03 (0.81)	3.42 (0.82)	0.311
Albumin [b] (g dl^{-1})	1.63 (0.41)	1.86 (0.59)	0.382
Total bilirubin [a] (mg dl^{-1})	0.25 (0.20–0.40)	0.30 (0.20–0.60)	0.350
Aspartate aminotransferase [a] (IU L^{-1})	181.50 (127.00–301.00)	226.50 (112.00–1,202.00)	0.281
Alanine aminotransferase [a] (IU L^{-1})	10.00 (10.00–10.00)	10.00 (10.00–113.00)	0.270
Alkaline phosphatase [a] (IU L^{-1})	115.00 (50.00–245.00)	83.50 (50.00–345.00)	0.275
γ-glutamyltranspeptidase [a] (IU L^{-1})	10.00 (10.00–14.00)	10.00 (10.00–15.00)	0.479
Creatinine kinase [a] (IU L^{-1})	4,506.00 (500.00–80,688.00)	8,200.50 (643.00–40,180.00)	0.576
Lactate dehydrogenase [a] (IU L^{-1})	356.50 (230.00–2,754.00)	430.50 (157.00–3,966.00)	0.955
Cholesterol [b] (mg dl^{-1})	142.67 (46.85)	163.64 (63.20)	0.458
Triglyceride [a] (mg dl^{-1})	49.50 (25.00–238.00)	33.50 (25.00–182.00)	0.066
Glucose [a] (mg dl^{-1})	82.00 (74.00–145.00)	90.00 (54.00–275.00)	0.911
Blood urea nitrogen [a] (mg dl^{-1})	8.50 (5.00–21.00)	16.50 (5.00–195.00)	0.245
Uric acid [a] (mg dl^{-1})	1.10 (1.00–1.60)	1.15 (1.00–10.80)	0.774
Phosphorous [a] (IU L^{-1})	6.70 (5.50–10.80)	6.75 (4.80–12.20)	0.978
Calcium [a] (mg dl^{-1})	11.55 (9.50–12.70)	11.05 (6.80–41.80)	0.911
Amylase [a] (mg dl^{-1})	676.50 (457.00–3,927.00)	658.50 (212.00–2,289.00)	0.520

[a] Data with nonnormal distribution presented by median (range) and compared using Mann–Whitney U test.

[b] Data with normal distribution presented by mean (SD) and compared using Student's t-test.

Table 4. Comparison of blood gas and profiles between green turtles with and without FP.

Variable	Turtles with FP (n = 6)	Turtles without FP (n = 22)	P-value
pH [b]	7.58 (0.14)	7.46 (0.10)	0.036
pCO_2 [a] (mm Hg)	46.00 (34.00–61.00)	43.00 (34.00–68.00)	0.822
pO_2 [a] (mm Hg)	41.50 (21.00–109.00)	33.00 (17.00–112.00)	0.355
BEecf [b] (mmol L^{-1})	21.00 (5.21)	10.77 (8.79)	0.012
HCO_3^- [b] (mmol L^{-1})	47.33 (7.55)	37.18 (7.68)	0.008
TCO_2 [b] (mmol L^{-1})	46.83 (2.85)	39.27 (7.55)	0.025
sO_2 [b] (%)	90.83 (8.79)	89.05 (8.05)	0.640
iCa [b] (mmol L^{-1})	0.93 (0.08)	0.92 (0.16)	0.875
K [a] (mmol L^{-1})	3.30 (2.80–3.70)	3.45 (2.00–5.40)	0.500
Na [b] (mmol L^{-1})	149.83 (2.63)	148.18 (4.58)	0.410
Glu [a] (mg dl^{-1})	82.50 (74.00–146.00)	91.50 (54.00–259.00)	0.933
CRE [a] (mg dl^{-1})	0.40 (0.30–0.70)	0.30 (0.20–2.40)	0.474

[a] Data with nonnormal distribution presented by median (range) and compared using Mann–Whitney U test.

[b] Data with normal distribution presented by mean (SD) and compared using Student's t-test.

pCO_2 = Partial pressure of carbon dioxide, pO_2 = Partial pressure of oxygen, BEecf = Base excess in extracellular fluid, HCO_3^- = Bicarbonate, TCO_2 = Total carbon dioxide, sO_2 = Saturated oxygen, iCa = Ionized calcium, K = Potassium, Na = Sodium, Glu = Glucose, CRE = Creatinine.

Discussion

Although green turtles are the most abundant sea turtle species in Taiwan (Cheng et al. 2019), knowledge regarding FP characteristics of green turtles within foraging areas in Taiwan remains very limited. The sample size (n = 28) in the present study is relatively small and thus the turtle size difference could be a confounding factor. Nevertheless, this was the first study to compare the physiological characteristics, hematology, blood/plasma biochemistry, and blood gas profiles in green turtles with and without FP in Taiwan. Although without major difference in blood/plasma biochemistry profiles identified in this study, it was found that FP could be more likely to be observed in large-size green turtles with lower inflammatory cell counts, such as WBC and lymphocytes. Furthermore, green turtles with FP were more likely to have higher levels of pH, BEecf, HCO_3^-, and TCO_2. The significantly lower levels of HB and PCV may suggest that green turtles with FP are more likely to be anemic. Our results did not identify a significant difference in BCI between FP-positive and FP-negative turtles. These findings suggest that green turtles with or without FP in this study were in equal healthy condition, similar to the previous study by Perrault et al. (2017). Additionally, Page-Karjian et al. (2014) also observed no significant difference between mean BCI of green turtles that developed FP during rehabilitation and individuals that already presented FP before rehabilitation. Nevertheless, it was reported that the BCI value decreased along with the increase in FP tumor scores (Deus Santos et al. 2015). Studies carried out in Brazilian juvenile green turtles revealed that turtles with FP tended to weigh less than turtles with same CCL in normal health condition (Torezani et al. 2010). On the other hand, a study conducted in Brazil demonstrated that FP-affected green turtles had a higher BCI than FP-free individuals (Rossi et al. 2019). It is possible that the higher BCI values found in FP turtles could be due to the presence of many and/or large tumors, which increases total body weight (Rossi et al. 2019). In the present study, we followed the green turtle tumor score system to record the tumor score for each study individual (an integer from 0 to 3, with a 0 score corresponding to the absence of tumors, while turtles assigned to score 1, 2, and 3 represent animals that are mildly, moderately, and heavily tumored, respectively (Work and Balazs 1999). In the current study, six of the 28 individuals had FP (n = 2 with tumor score 1; n = 1 with tumor score 2; n = 3 with tumor score 3) indicating that 50% of the FP-affected turtles are heavily afflicted. Furthermore, turtles with FP from our study were overall considered to be in good body condition (BCI ranged from 1.15 to 1.42; Bjorndal et al. 2000). The results would explain why there was no significant statistical difference in the mean BCI values between FP-postive and FP-negative green turtles in this study.

In the present study, the mean values for body weight and CCL in FP-positive turtles were significantly higher than those in FP-negative turtles. All six of the FP-positive turtles in this study (53.3–66.0 cm CCL) were designated as juveniles (CCL < 67.0 cm) in accordance with the size classifications established by Ng et al. (2018b). Previous research has indicated that the prevalence of FP is higher among juvenile green turtles than in other size/age groups (Jones et al. 2016). In Australia, it has been shown that hatchling green turtles remain in the pelagic phase (i.e., when sea turtles do not shift to a neritic foraging habitat) until the age of 5–10 yrs with CCL of 40–45 cm (Limpus et al. 1994). The mean (SD) CCL of FP-negative green turtles in this study was 44.65 cm (4.40). The average CCL (58.35 cm) of FP-positive turtles in this

study significantly exceeded that of FP-negative animals. It is reasonable to speculate that most of the FP-negative turtles in this study were newly recruited juveniles, and the FP-positive turtles were more likely to be resident animals. According to a study on sea turtle strandings/bycatch in Taiwan, the green turtle was the most common species in Taiwan and the majority of green turtles were identified to be juveniles to subadults (Cheng et al. 2019). The coastal waters of Taiwan are foraging areas for green turtles (Ng et al. 2018a). It has previously been established that green turtles develop FP only after recruiting to nearshore habitats (Jones et al. 2016), and this is consistent with previous reports that FP is more frequently observed in older (and therefore larger) turtles due to the fact that the tumors require time to grow (Adnyana et al. 1997).

In this study, PCV values were significantly lower in FP-positive turtles than in FP-negative turtles, similar to previous reports (Rossi et al. 2009). We also found that the mean PCV value of FP-positive green turtles identified in this study was lower than those reported in wild healthy green turtles (Lewbart et al. 2014, Page-Karjian et al. 2015). Our results also indicate that the HB levels were significantly lower in FP-positive turtles than in FP-negative turtles. This phenomenon has been previously reported in hematological studies of FP in green turtles (Aguirre et al. 1995, Rossi et al. 2009). After the comparison, it was found that the mean HB value for FP-positive turtles in our study was also lower than those reported for wild healthy sea turtles (Lewbart et al. 2014, Muñoz-Pérez et al. 2017). Lower HB and PCV values imply that FP-positive turtles are afflicted by a hematologic malady (e.g., anemia; Saggese 2009). This assertion is consistent with the findings in previous studies, which reported that green turtles with FP frequently suffered from anemia (Aguirre et al. 1995, Page-Karjian et al. 2014). Although FP itself has been reported as a cause of anemia (Aguirre et al. 1995), the reason for anemia in sea turtles with FP has also been speculated to be due to cardiovascular fluke (spirorchiid) infection (Adnyana et al. 1997). Hepatic haemosiderosis has been reported in loggerhead sea turtles [*Caretta caretta* (Linnaeus, 1758)] with spirorchiid infection, and which might be associated with hemolytic anemia (Wolke et al. 1982). In our study, spirorchiidiasis was 100% detected in the green turtles with FP tumors; this is similar to the previous study in which all stranded green turtles affected with FP are infected with spirorchiids (Aguirre et al. 1998). However, we found no significant difference in spirorchiid infection between FP-positive and FP-negative groups (100.0% vs 62.5%). A study conducted in Hawaii on the spirorchiid infection in green turtles also demonstrated no significant relationship between severity of FP and spirorchiid egg burden (Work et al. 2005). Work et al. (2005) hypothesized that immature green turtles become infected with spirorchiids shortly after recruiting into coastal foraging grounds from the pelagic environment. In our study, compared to turtles without FP, the FP-positive turtles had significantly longer CCL (57.45 vs 44.65 cm); it may imply that FP-affected turtles are more likely to be resident turtles and FP-free ones are newly recruited. This may possibly explain the higher proportion of spirorchiid infected turtles in FP-affected individuals in comparison with FP-free individuals found in our study.

Our results suggest that green turtles with FP may be under the condition of immune stress. The mean WBC value of FP-positive turtles in this study was lower than that of FP-negative turtles. Similar results have been previously observed in green turtles in Hawaii (Work and Balazs 1999). The WBC count in FP-positive turtles in this study was similar to that of debilitated green turtles with cutaneous FP

previously (Norton et al. 1990). We also found that lymphocyte counts in FP-positive turtles were significantly lower than those in FP-negative turtles. These findings are similar to the previous reports in green turtles with FP (Aguirre et al. 1995, Work and Balazs 1999, Cray et al. 2001). Changes in the relative numbers of lymphocytes in FP-positive turtles may be related to stress, nonspecific inflammatory responses, or neoplasia (Cray et al. 2001). In reptiles, leukopenia and lymphopenia could be considered nonspecific indicators of chronic inflammatory responses (Stacy et al. 2011).

In the present study, pH, BEecf, HCO_3^-, and TCO_2 levels were significantly higher among FP-positive turtles compared to FP-negative turtles. There have been many reports of blood gas and acid-base status in Kemp's ridley sea turtles [*Lepidochelys kempii* (Garman, 1880)] and loggerhead sea turtles (*C. caretta*; Innis et al. 2007, Keller et al. 2012, Camacho et al. 2015). However, relatively few studies have addressed this issue in green turtles (Lewbart et al. 2014, March et al. 2019). Factors such as temperature and digestion level have direct effects on reptile blood gas parameters (Adamovicz et al. 2018, March et al. 2019). Previous research has demonstrated that pH, BEecf, HCO_3^-, and TCO_2 levels had a negative association with temperature in free-living eastern box turtles (*Terrapene carolina carolina*; Adamovicz et al. 2018). It is unclear why the blood pH in the FP-positive turtles was higher than that in FP-negative ones in our study. Previous reports showed that sea turtles at cooler temperatures are known to have higher blood pH values (Lutz et al. 1989, Moon et al. 1997). Digestion has also been demonstrated to induce a postprandial metabolic alkalosis in a variety of reptiles (Hicks and Bennett 2004). Marked increases in postprandial levels of blood pH and HCO_3^- have also been documented in green turtles (March et al. 2019). Furthermore, as documented in previous studies (Stamper et al. 2005, Yang et al. 2019), there is a less active foraging pattern in migratory sea turtles. Interestingly, we found that most of the FP-negative turtles (CCL mean 44.65 cm) in this study were newly recruited juveniles (CCL 40–45 cm). This might explain the significantly lower levels of pH and HCO_3^- found in FP-negative turtles compared to FP-positive turtles in our study; those values were within the ranges reported in healthy green turtles from the Galapagos (Lewbart et al. 2014) and in post-hatchling green turtles (March et al. 2019). However, the mean pH and HCO_3^- values of FP-positive turtles in this study were higher than those of apparently healthy hawksbill [*Eretmochelys imbricata* (Linnaeus, 1766); Muñoz-Pérez et al. 2017] and wild leatherback turtles [*Dermochelys coriacea* (Vandelli, 1761); Innis et al. 2014]. In another study, it was reported that the pH and bicarbonate values were not significantly different between the initial and convalescent status in cold-stunned Kemp's ridley sea turtles (*L. kempii*; Innis et al. 2007). Several studies indicated that metabolic acidosis is a common acid/base disorder in hospitalized sea turtles (Keller et al. 2012, Camacho et al. 2015). Similarly, the mean blood pH in FP-negative turtles was 7.46, which may suggest that the FP-negative turtles had metabolic acidosis (pH < 7.5; Keller et al. 2012, Camacho et al. 2015).

The TCO_2 value for FP-positive turtles in our study was higher than that of wild hawksbill turtles (*E. imbricata*) in the Galápagos [46.82 mmol L^{-1} (SD 2.85) vs 37 mmol L^{-1} (SD 5); Muñoz-Pérez et al. 2017]. In the current study, BEecf levels in FP-positive turtles were significantly higher than in FP-negative turtles. There were no previous reports regarding BEecf levels in green turtles. After further comparison, we found that the mean blood BEecf level in green turtles with FP in our study was within the BEecf reference range in migrating loggerhead turtles (*C. caretta*; Yang et al. 2019).

In summary, according to our knowledge, this is the first study to present and compare physiological characteristics and profiles of hematology, blood/plasma biochemistry, and blood gas for green turtles with different health statuses (i.e., with and without FP) in Taiwan. The significant differences in body weight, CCL, HB, PCV, WBC, lymphocytes, pH, BEecf, HCO_3^- and TCO_2 levels identified between FP-positive and FP-negative green turtles not only provide valuable information for the medical care of sea turtles at rehabilitation centers, but also increase sea turtle conservation efficiency in Taiwan.

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