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Hematological and Blood Biochemical Variations, and Their Correlation with Body Condition in Wild-Foraging Sea Turtles of the Celebes Sea

(Variasi Hematologi dan Biokimia Darah serta Kaitannya dengan Keadaan Badan pada Penyu yang Mencari Makanan secara Liar di Laut Sulawesi)

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ABSTRACT

The Celebes Sea provides critical foraging and migratory habitats for sea turtles in Malaysian waters, yet health status data, including hematologic and blood biochemistry parameters, remain scarce. To address this gap, we examined the blood profiles of green turtles and hawksbill turtles in these foraging grounds. Physical assessments such as body condition scores and indices suggested overall clinical health, though one green turtle exhibited fibropapillomatosis, and a new occurrence of the burrowing barnacle *Chelolepas cheloniae* was observed on the carapace. Blood samples from 32 green turtles and 4 hawksbill turtles were analyzed using an iSTAT handheld blood analyzer, showing interspecific differences in blood values. Hawksbill turtles exhibited higher blood urea nitrogen concentrations, possibly due to their high-protein diet, while one green turtle showed abnormal blood profiles indicative of potential acidosis. This study provides baseline health data for foraging sea turtles in the Celebes Sea, emphasizing the importance of hematological and biochemical monitoring in assessing health status and informing targeted conservation strategies to address threats in their foraging habitats. These findings help us better understand sea turtle health and support efforts to protect these important species and their habitats.

Keywords: Blood gas; body condition index; Celebes Sea; health assessments; Southeast Asia

ABSTRAK

Laut Sulawesi merupakan habitat penting untuk pemakanan dan migrasi penyu di perairan Malaysia, namun data status kesihatan, termasuk parameter hematologi dan biokimia darah, masih kurang. Untuk menangani jurang ini, kami mengkaji profil darah penyu hijau dan penyu karah di kawasan pemakanan ini. Penilaian fizikal seperti skor dan indeks keadaan badan menunjukkan kesihatan klinikal secara keseluruhan, walaupun terdapat seekor penyu hijau yang menunjukkan fibropapillomatosis, serta penemuan baharu teritip pengorek *Chelolepas cheloniae* pada karapas. Sampel darah daripada 32 penyu hijau dan 4 penyu karah dianalisis menggunakan penganalisis darah genggam iSTAT yang mendedahkan perbezaan antara spesies dalam nilai darah. Penyu karah menunjukkan kepekatan nitrogen urea dalam darah yang lebih tinggi, kemungkinan disebabkan oleh diet protein yang tinggi, manakala seekor penyu hijau menunjukkan profil darah tidak normal yang menunjukkan kemungkinan asidosis. Kajian ini menyediakan data asas kesihatan untuk penyu di kawasan pemakanan di Laut Sulawesi, menekankan kepentingan pemantauan hematologi dan biokimia dalam menilai status kesihatan serta membentuk strategi pemuliharaan yang disasarkan untuk menangani ancaman di habitat pemakanan mereka. Penemuan ini membantu kita memahami kesihatan penyu dengan lebih baik dan menyokong usaha melindungi spesies penting ini dan habitat mereka.

Kata kunci: Asia Tenggara; darah; indeks keadaan badan; Laut Sulawesi; penilaian kesihatan

INTRODUCTION

Assessing the health of marine wildlife, such as sea turtles, is vital for understanding and monitoring their well-being in natural habitats. Such assessments offer crucial insights into the impacts of environmental factors, habitat degradation, and human activities on marine populations. Furthermore, evaluating wildlife health is crucial for measuring the effectiveness of conservation efforts and ensuring the long-term survival of species (Balfour, Macdonald & Catto 2007; Uphyrkina et al. 2001).

Sea turtles are keystone species with significant ecological importance and vulnerability, crucial for maintaining the health and balance of marine ecosystems (Bjorndal & Jackson 2003; Lutcavage, Spotila & Witherington 2003). Comprehensive health assessments are essential to understand and mitigate numerous threats they face, including habitat loss, pollution, climate change, and fisheries interactions (Laloë, Schofield & Hays 2023). Key parameters such as body condition (Joseph et al. 2023; Nishizawa & Joseph 2022), reproductive health (Chaves et al. 2013; Santoro & Meneses 2007), and screening blood or tissue samples for diseases and pollutant levels (Aguirre & Balazs 2000; Arthur, Limpus & Whittier 2008; Chaves et al. 2013) are crucial in these assessments.

Beside physical observations, evaluating hematology, blood gas analysis, and biochemistry is instrumental in assessing health status in sea turtles. For example, hematologic, plasma biochemical, and acid-base evaluations provide important information regarding the physiologic status of sea turtles (Anderson et al. 2011; Harms et al. 2003; Kelly et al. 2015), including prognostic information for ill and injured individuals (Innis et al. 2009; Keller et al. 2012; Li et al. 2015; Stacy, Innis & Hernandez 2013). These parameters are important indicators for fluid balance, homeostasis, tissue oxygenation, pulmonary function, renal performance, bacterial infection, stress levels, nutritional status, and overall health.

For accurate health assessments of sea turtles, it is essential to understand the interspecific, ontogenetic, and geographical variations in key blood parameters. Previous studies have established reference intervals for species like the green turtle (*Chelonia mydas*) and loggerhead turtle (*Caretta caretta*), which are vital for interpreting test results. For example, Kelly et al. (2015) and Trocini et al. (2013) provided hematological and biochemical reference intervals for loggerhead turtles, highlighting the importance of having species-specific baselines. Similarly, Samsol et al. (2020) outlined blood profiles for green turtles in Malaysia, emphasizing regional differences. Kophamel et al. (2022) focused on ontogenetic shifts in blood parameters, which are critical for accurate health assessments. Achieving these benchmarks implies that the sea turtles' physiological responses, as reflected in the blood parameters, fall within expected healthy ranges. This allows for the differentiation between normal and abnormal values, ensuring reliable interpretation and better understanding of their health status. However, variations in hematocrit values have been observed among different turtle groups between nesting and pre-nesting females, as well as among juveniles and adult turtles (Chaves et al. 2013; Reséndiz et al. 2019; Santoro & Meneses 2007). McNally et al. (2020) observed a positive correlation between body weight and blood urea nitrogen (BUN) levels in green turtle, suggesting healthy protein metabolism in larger turtles with higher BUN values. However, no significant correlation was found in Kemp's ridley turtle (*Lepidochelys kempii*), likely due to a small sample size and the influence of outliers among larger turtles. Generally, a positive correlation may reflect healthy physiological processes if BUN levels remain within the normal range, while a negative correlation could indicate malnutrition or chronic kidney stress, where reduced body weight is associated with elevated BUN due to impaired renal function or protein breakdown. Thus, it is important to assess blood profiles and to understand inter-specific and ontogenetic differences of parameters.

This study aims to address the knowledge gaps in sea turtle health assessments at Semporna, Celebes Sea. The objectives of this study were: (1) to examine inter-specific differences of green (*Chelonia mydas*) and hawksbill turtles (*Eretmochelys imbricata*), and (2) to examine relationship among body condition, body size, and blood parameters for both. Given the Celebes Sea's critical role as a foraging habitat for green and hawksbill turtles in Malaysian waters (Jeethvendra et al. 2023; Joseph et al. 2023, 2021; Nishizawa et al. 2024, 2018; Pilcher 2010; Samsol et al. 2020), these health assessments contribute valuable data to inform and enhance ongoing conservation initiatives for these ecologically significant species.

MATERIALS AND METHODS

FIELDWORK AND SAMPLE COLLECTION

Sampling activities were carried out between 19-21 November 2021 and 1-4 February 2022 at Pom Pom (4.5378° N, 118.5937° E), Timba Timba (4.4490° N, 118.6456° E) and Pandanan Islands (4.4296° N, 118.7844° E), Semporna, Sabah, Malaysia (Figure 1). These locations are part of the Celebes Sea region and have been previously identified as significant foraging grounds for green and hawksbill turtles (Haziq Harith 2018; Jeethvendra et al. 2023; Joseph et al. 2021).

Turtles were manually captured at random in shallow waters using SCUBA diving, at depths ranging from 5 to 10 meters, prioritizing diver safety during sampling. Subsequently, each turtle was brought onto a boat for species identification and measurements, including curved carapace length (CCL), curved carapace width (CCW), and body weight (BW). Measurements were obtained using a 1.5-meter flexible tape for CCL and CCW (accuracy ± 0.5 mm) and a spring scale for BW (accuracy ± 0.1 kg).

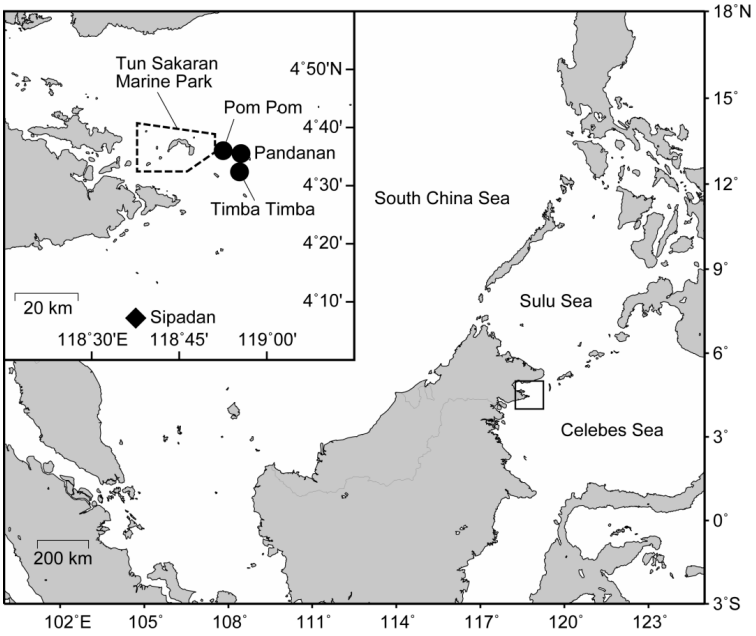

FIGURE 1. Map of sampling sites at Pom Pom, Timba Timba, and Pandanan Islands (indicated by black dot symbols) located east side of Tun Sakaran Marine Park within the Coral Triangle Area, Semporna, Sabah, Malaysia. Black filled diamond symbol indicates infamous diving site Sipadan Island

To reduce the turtles' stress, their eyes were covered with a cloth, and a modified net canvas was used for humane restraint to prevent aggressive movements during sample collection. Blood samples (approximately 5.0 mL) were collected from the dorsal cervical sinus using a heparinized 18-22 gauge needle attached to a 5.0 mL syringe (Joseph et al. 2016). Aseptic conditions were ensured by disinfecting the skin puncture site with iodine solution and 70% ethanol before and after blood collection (Li et al. 2015). The collected whole blood samples were immediately placed into lithium heparin tubes, divided into sub-samples, and stored in a portable ice box. Utilizing non-contact method, thermal infrared thermometer, we assessed body temperature from the head of sea turtles, mitigating stress-related effects during data collection (Burns, McCafferty & Kennedy 2015; McNally et al. 2020). On-board sample collections and physiological assessments were promptly conducted, ensuring the entire capture and release process for each individual took 9.2 min on average, ranging from 4 to 18 min. Underwater capture and transfer to the boat took less than 5 min. To avoid duplicate captures within the same sampling expedition, turtles were marked with waterproof paint following the sampling process. After sampling, the turtles were released back into the water.

PHYSICAL EXAMINATIONS

Physical health assessments comprised subjective and objective evaluations of body condition using the body condition score (BCS) numeric scale (1–5) (Tristan & Norton 2017), and body condition index (BCI). BCS relies on the assessor's experience, while BCI based on measurable data in which the former is quicker and easier to use in the field, making it suitable for rapid assessments, and the latter is more detailed and suitable for research and detailed health monitoring. The BCI, employing Fulton's K formula (BCI = [BW (kg) / SCL (cm)3] × 10,000; Bjorndal, Bolten & Chaloupka 2000), where SCL indicates straight carapace length, gauged the nutritional status and energy reserves of each turtle. CCL was converted to SCL using regression equations from Teas (1993): SCL = 0.294 + (0.937 × CCL) for green turtles and SCL = (-0.212) + (0.955 × CCL) for hawksbill turtles.

External health examinations included assessing the presence, description and characterization of epibionts, wounds/scars, and visible tumors. Tumor scores were assigned following the methods outlined in Work and Balazs (1999). Turtles were captured randomly, and their sex was determined for adults (mature individuals), while juveniles (immature turtles) could not be sexed visually as they had not yet developed sexual dimorphism. For this study, a CCL of >80 cm was used to classify individuals as 'mature,' with their sex identified based on morphological characteristics (Pilcher 2010). Juveniles were defined as immature turtles with a CCL of ≤80 cm.

EXAMINATIONS OF BLOOD GAS AND BIOCHEMISTRY, AND HEMATOLOGY PROFILES

Blood gas and biochemistry profiles, including pH, partial pressure of carbon dioxide (pCO_2), partial pressure of

oxygen (pO_2), base excess (BE), bicarbonate (HCO_3), saturated oxygen (sO_2), lactate (Lac), sodium (Na), potassium (K), chloride (Cl), ionized calcium (iCa), total carbon dioxide (TCO_2), glucose (Glu), blood urea nitrogen (BUN), creatinine (Crea), and anion gap (AnGap) were measured within 24 h of sample collection using an iSTAT Portable Handheld Blood Analyzer with Chem-8+ and CG-4+ iSTAT cartridges (Perrault et al. 2021; Samsol et al. 2020; Yang et al. 2019). Hematological assessments included quantifying hematocrit (Hct) and hemoglobin (Hb) values utilizing the iSTAT blood analyzer. While previous studies (Lewbart et al. 2014; Stacy & Innis 2017; Wolf, Harms & Beasley 2008) have debated the reliability of automated evaluations for sea turtles, it is generally agreed that these parameters are ideally determined manually by centrifugation. However, due to constraints of field conditions, the automated method was used as the best available option, and temperature auto-corrected values were reported to account for potential sensitivity (Harms et al. 2003; Lewbart et al. 2014; Li et al. 2015).

Manual hematological analyses were performed to determine total leukocyte counts, involving the examination of five replicate blood smears stained with Modified Wright's stain using estimation of White Blood Cell (WBC) count (Li et al. 2015). Differential leukocyte counts were conducted, categorizing absolute counts of heterophils, lymphocytes, monocytes, eosinophils, and basophils (Casal & Oros 2007; Stamper et al. 2005; Work et al. 1998).

STATISTICAL ANALYSIS

Statistical analyses utilized R ver. 4.1.3 (R Core Team 2022). Descriptive statistics were shown as mean ± standard deviation (SD). Correlations between CCL, BCI, and BCS were tested in green turtles, but not in hawksbill turtles due to small number of individuals. Multivariate data of blood gas, biochemistry, and hematology profiles were ordinated by non-metric multidimensional scaling (NMDS) using vegan package (Oksanen et al. 2022) as adopted by Arthur, Limpus and Whittier (2008). The standardized data was used to calculate Eucledian distance matrix. The NMDS was performed in two dimensions with maximum number of random starts as 100. First, to investigate inter-specific differences, NMDS was performed using data from immature green and hawksbill turtles because all sampled hawksbill turtles were immature (see Results). Effects of species, BCI, and BCS on blood parameters were tested using permutational analysis of variance (PERMANOVA). Then, NMDS was performed by using data of immature and mature green turtles. Effects of CCL and BCI on green turtles blood parameters were tested by PERMANOVA. In this analysis, because CCL was correlated with BCS in green turtles (see Results), BCS was not included in the analysis.

RESULTS AND DISCUSSION

PHYSICAL AND BODY CONDITION EVALUATIONS

This study presents the first comprehensive evaluation of body condition and blood parameters for foraging green and hawksbill turtles in the Celebes Sea, specifically in Semporna, Sabah, Malaysia. The Celebes Sea serves as a critical foraging ground, hosting green turtles originating from diverse populations in the Sulu and South China Seas, Micronesia, and New Guinea (Joseph et al. 2021; Nishizawa et al. 2018). Sea turtles in this area face significant health threats, including fibropapillomatosis associated with Chelonid Herpesvirus 5 (Loganathan, Palaniappan & Subbiah 2021), injuries from boat strikes (Phu & Palaniappan 2019), barnacle infestations, and physical trauma from fish bombing (Joseph et al. 2021). These stressors highlight the critical importance of conducting comprehensive health assessments to support effective conservation strategies.

Basic health assessments were conducted on 36 captured wild sea turtles (Table 1). Among the individuals sampled (Supplementary Table 1), 32 were identified as green turtles, while four were hawksbill turtles. Sampling was conducted across multiple locations in Semporna, comprising Pom Pom Island (North Tip: n = 18, Mandarin Playground: n = 5, Lobster Wall: n = 1), Timba Timba Island (n = 7), and Pandanan Island (n = 5). Notably, Mandarin Playground and Pandanan Island exclusively hosted two hawksbill turtles, while all other mentioned locations were solely inhabited by green turtles. Furthermore, six adults green turtles (five females and one male) could not be weighed due to their size exceeding the capacity of our modified net canvas and onboard manpower.

Out of the 32 green turtles, 21 were categorized as immature (CCL ranged from 41.0 to 71.2 cm; mean: 59.7 ± 8.7 cm), while 11 were categorized as mature (CCL ranged from 80.0 to 98.1 cm; mean: 86.8 ± 6.4 cm). Among the mature turtles, three males were exclusively captured at the North Tip, with CCL measurements of 83.5 cm, 82.5 cm, and 87.0 cm, and corresponding tail lengths of 35.5 cm, 34.4 cm, and 42.0 cm. Additionally, eight females had a mean CCL of 87.8 ± 7.2 cm. Hawksbill turtles, totalling four individuals in this study, exhibited a mean CCL of 48.0 ± 7.7 cm (ranged from 37.5 to 54.5 cm).

Of the 36 captured turtles, 16 green turtles were found to have barnacle attachments. These included *Chelonibia testudinaria* (Figure 2(a)) on the carapace, *Platylepas hexastylos* (Figure 2(b)) embedded in the skin of flippers, tail, and body, *Stomatolepas transversa* (Figure 2(c)) penetrating the median plastron and leading edges of flippers, and *Chelolepas cheloniae* (Figure 2(d)) boring into the skin and carapace. Additionally, one green turtle was diagnosed with a mild tumor (score 1), while all other green and hawksbill turtles appeared symptom-free.

One juvenile green turtle with a BCS of 1 (severely emaciated), captured at Mandarin Playground, was excluded from further clinical and blood assessments. This turtle received immediate first aid at the Tropical Research and Conservation Centre (TRACC) facility before being transferred to the Sabah Wildlife Department. Two green turtles, captured at the North Tip and Lobster Wall, exhibited a BCS of 2 (thinness but not emaciated). One of them was covered in algae, while the other was entangled in a fishing net, resulting in injuries to its neck and left jaw. The net was carefully removed, and the wounds were treated with iodine solution before a brief period of rest and subsequent return to the sea. All green turtles with a BCS of 5 (obese), including a male from the North Tip and a female from the Mandarin Playground, were a rare observation in the wild. In this investigation, most turtles exhibited a BCS within the range of 3 (a state of normalcy) to 4 (robust health). In this study, the BCS was significantly correlated with CCL ($r = 0.702$, $p < 0.0001$).

Six mature green turtles could not be weighed and thus BCI was not calculated. The mean BCI of 26 green turtles were 1.32 ± 0.17 kg/cm^3 (range: 0.86-1.57 kg/cm^3). The green turtle with ID SS36 (BCS = 1) showed relatively low BCI of 1.02, but BCI was not generally correlated with

TABLE 1. Physical examinations of 32 green turtles and four hawksbill turtles captured in November 2021 and February 2022 in Semporna, Celebes Sea

Parameters	*Chelonia mydas* (N = 32)		*Eretmochelys imbricata* (N = 4)	
	Mean ± SD	Min - Max	Mean ± SD	Min - Max
CCL (cm)	67.88 ± 14.81	41.00-98.10	48.03 ± 7.74	37.50-54.50
SCL (cm)	63.90 ± 13.87	38.72-92.22	45.30 ± 7.25	35.44-51.36
CCW (cm)	61.02 ± 13.18	40.00-87.00	41.15 ± 6.85	35.00-48.70
BW (kg)	38.75 ± 24.77	5.90-97.27	10.26 ± 5.21	6.30-17.40
Body temperature (°C)	31.85 ± 3.07*	26.90-35.90	32.83 ± 0.93	32.00-34.10
BCS	3.00 ± 0.78	1.00-5.00	3.00 ± 0.50	2.00-3.00
BCI (kg/cm^3)	1.31 ± 0.16	0.86-1.57	1.08 ± 0.32	0.74-1.42

*Body temperature was not measured in one Green Turtle

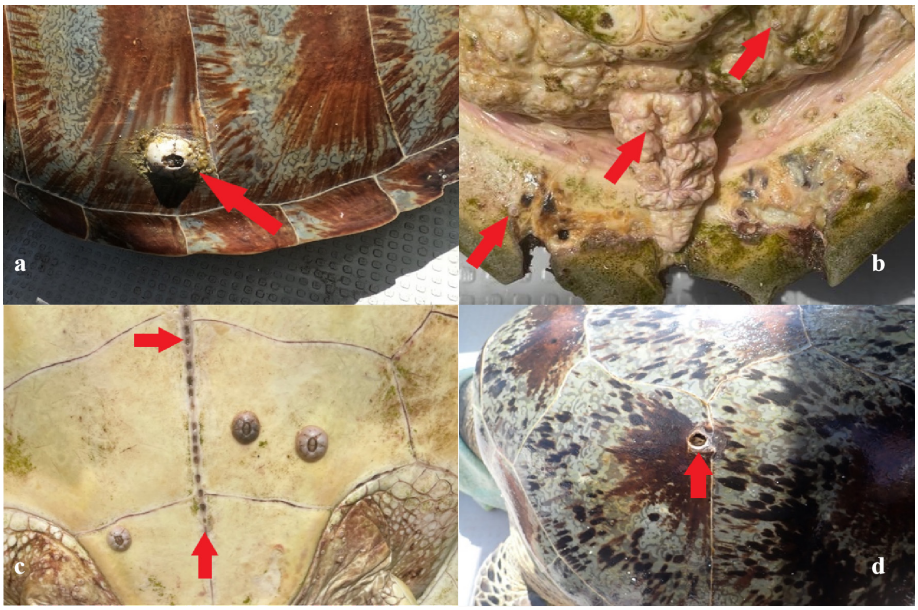

FIGURE 2. Barnacles attached to the turtles in this study indicated by red arrow (a) *Chelonibia testudinaria* attached to the turtle carapace, (b) *Platylepas hexastylos* embedded in the skin of flippers, tail, and body, (c) *Stomatolepas transversa* penetrating along the median plastron and leading edges of flippers, (d) *Chelolepas cheloniae* boring into the carapace

BCS (r = 0.262, p = 0.197). In fact, one green turtle with BCS = 2 had a BCI of 1.51 kg/cm³, whereas the lowest BCI (0.86 kg/cm³) was observed in the turtle with BCS = 3. The correlation between BCI and CCL of green turtles was also low (r = 0.026, p = 0.898). The mean BCI of hawksbill turtles was 1.08 ± 0.32 kg/cm³ (range: 0.74-1.42 kg/cm³). Two hawksbill turtles showed relatively low BCI values of 0.74 and 0.87 kg/cm³, but their BCI was scored as 3.

External observations indicated that most sea turtles foraging in Semporna were in good nutritional condition, as evidenced by a BCS of 3 or higher in 29 out of 32 green turtles and 3 out of 4 hawksbill turtles. This observation aligns with the BCI values obtained, where the mean BCI for green turtles was 1.32 ± 0.17 kg/cm³, exceeding the threshold of 1.2 kg/cm³ indicative of good body condition in green turtles, as reported by Nishizawa and Joseph (2022). Their study, conducted in Malaysian waters, found similar BCI values for juvenile and adult green turtles, supporting the reliability of this threshold for assessing health. Hawksbill turtles in our study exhibited a mean BCI of 1.08 ± 0.32 kg/cm³, which falls within the range previously reported for healthy hawksbill turtles by Nishizawa and Joseph (2022), despite the generally lower BCI values observed in this species due to inherent morphological differences. The BCI serves as a robust indicator of health, with higher values suggesting sufficient energy reserves and optimal nutritional status necessary for growth, reproduction, and immune functions. Conversely, lower BCI values may indicate malnutrition, poor health, or environmental stress. Thus, the BCI values observed in this study suggest that the majority of sea turtles foraging in Semporna are in apparently good health, consistent with regional benchmarks established in the literature.

The BCS and BCI of green turtles in this study were not significantly correlated, contrasting with the findings of Thomson et al. (2009), who reported consistency between rapid visual assessments and BCI. This discrepancy highlights the challenges of subjective evaluations, which can vary depending on the evaluator's experience and perspective. Furthermore, Thomson et al. (2009) primarily assessed subadult and adult green turtles, whereas this study included individuals spanning a broader size range. The significant positive correlation observed between BCS and CCL in this study suggests that visual assessments may be influenced by size, making it challenging to apply consistent evaluation criteria across turtles of varying sizes. To improve accuracy and reliability, it may be beneficial to provide training for evaluators and to refine or validate the assessment criteria specifically for turtles with a wide size range.

The three barnacle species identified in this study (*Chelonibia testudinaria, Platylepas hexastylos,* and *Stomatolepas transversa*) are commonly associated with sea turtles in the Indo-Pacific region (Zardus 2021), including Japan (Hayashi 2012; Hayashi & Tsuji 2008), Malaysia (Lim et al. 2021; Nishizawa et al. 2024), Taiwan (Chan, Prabowo & Lee 2009), and China (Liu & Ren 2007). Additionally, this study is the first to document the occurrence of *Chelolepas cheloniae* on green turtles in Semporna, Malaysia. While *C. cheloniae* is relatively uncommon in Southeast Asia (Zardus 2021), it has previously been reported from a green turtle in Talang-Talang Besar Island, Sarawak, Malaysia (Hayashi 2012). This finding enhances our understanding of the symbiotic relationships within marine ecosystems, underscores the rich biodiversity of Malaysian waters, and highlights the potential for previously unreported host-parasite associations as turtle populations migrate or adapt to shifting habitats. Such discoveries underscore the importance of continued biodiversity monitoring and ecological studies in the region.

The three commonly found barnacle species have long been associated with turtles due to their unique adaptations to live on mobile hosts, and their widespread presence emphasizes their resilience and ecological role in marine ecosystems. *C. testudinaria* is coined as a generalist species often found on various marine animals other than sea turtles as discussed by Zardus (2021), while *P. hexastylos* and *S. transversa* are more specialized turtle epibionts (Chan, Prabowo & Lee 2009; Lim et al. 2021; Liu & Ren 2007; Nishizawa et al. 2024). Their presence reflects the turtle's migration patterns and foraging behaviours, which intersect across the Indo-Pacific. In contrast, *Chelolepas cheloniae* is a rarely observed barnacle species in Southeast Asia. It bores into the soft skin and carapace of turtles, sometimes penetrating the body cavity and causing harmful effects (Flint et al. 2009; Herbert & Jacobson 1995). The frequent identification of these epibionts in the region underscores their potential as bioindicators, offering insights into turtle health and environmental conditions across habitats.

High levels of barnacle infestation can increase drag or affect buoyancy, potentially impairing turtles' swimming efficiency and overall health. Furthermore, their widespread occurrence on turtles from different locales provides valuable information on habitat overlaps and migratory routes. Their presence may also indicate environmental shifts impacting turtle habitats and health, contributing to a better understanding of regional ecological changes that influence both turtles and their symbionts.

In addition to barnacles, another health concern for sea turtles is fibropapillomatosis (FP), a debilitating disease characterized characterized by benign but potentially life-threatening tumors on the skin, eyes, and internal organs. These tumors can impair critical functions such as vision, mobility, and feeding, severely impacting the turtles' survival (Aguirre & Lutz 2004). While FP was not prevalent in this study, with only one green turtle exhibiting a mild case (score 1), its potential presence remains a concern.

FP is associated with the Chelonid FP-Associated Herpesvirus, which may be carried by apparently healthy turtles (Loganathan, Palaniappan & Subbiah 2021). This virus can proliferate under environmental conditions such

as warmer waters and increased pollution (Herbst 1994), both of which are exacerbated by climate change and anthropogenic activities. Evidence from previous studies has linked FP prevalence to environmental stressors that weaken turtles' immune systems, increasing their vulnerability to infections (Van Houtan, Hargrove & Balazs 2010). Given these risks, continuous monitoring for FP is crucial to understanding and mitigating its potential impacts on turtle populations, particularly as environmental changes may create conditions favorable for the disease to spread.

Monitoring FP is crucial for understanding the health of marine ecosystems, particularly coral reef habitats. Effective monitoring can utilize various technical approaches to collect both short- and long-term data on the disease's spread, potential triggers, and impacts. Sea turtles can be tagged with satellite transmitters and tracked using GPS devices equipped with environmental data loggers, as Van Houtan, Hargrove and Balazs (2010) demonstrated that variables such as temperature, salinity, and depth are associated with higher FP rates in certain areas. Periodic health assessments, including visual surveys and biometric measurements, can track health metrics over time. Additionally, blood and biopsy sampling provides in-depth insights into viral loads and immune functions, which are essential for understanding the progression of FP and documenting tumors (Herbst et al. 2008; Manes et al. 2023).

The prevalence of FP serves as a bioindicator of ecosystem stress, often linked to pollution, climate change, and habitat degradation (Van Houtan, Hargrove & Balazs 2010). Maintaining healthy sea turtle populations is vital for ecosystem balance, as turtles play a significant role in sustaining seagrass meadows and coral reef environments. Moreover, data from FP monitoring can inform policies on pollution control, sustainable fishing practices, and conservation strategies to address factors contributing to the disease. Conservation organizations and NGOs can leverage FP data to design targeted conservation programs, secure funding for projects, and advocate for stricter regulations to protect sea turtles and their marine habitats.

BLOOD PARAMETER EVALUATIONS

Investigating blood parameters identified an abnormal green turtle (SS5). Although BCI and BCS of SS5 indicated good condition, this individual was diagnosed as acidotic due to low pH, reduced HCO_3, and elevated partial pCO_2 levels (Innis et al. 2007; Mones et al. 2021; Stabenau, Heming & Mitchell 1991). There were no significant relations between blood parameters and physical body condition. Physical body condition evaluated by BCI and BCS is assumed to be related to relatively long-term nutritional condition, whereas blood parameters can fluctuate in response to short-term physiological and environmental stressors. Therefore, an in-depth examination of blood parameters would be important to clarify the individual's health status. Given that BCI and BCS reflect longer-term nutritional health, analyzing specific blood parameters can offer insights into short-term physiological responses to stress and environmental conditions. For instance, BUN and Crea can highlight kidney function, Glu may indicate recent nutritional intake or stress levels, and electrolytes such as Na and K levels help assess hydration and osmotic balance. By focusing on these variables, this study can provide a nuanced understanding of health beyond physical condition, enhancing the assessment of sea turtle health in response to immediate environmental challenges.

This study identified inter-specific differences in blood gas and biochemistry profiles between green and hawksbill turtles. The blood parameters were significantly different between green and hawksbill turtles ($F = 2.130$, df = 1, $p = 0.028$), but effects of BCI ($F = 0.874$, df = 1, $p = 0.519$) and BCS ($F = 1.015$, df = 1, $p = 0.427$) were not significant in immature turtles (Figure 3(a)). In fact, hawksbill turtles were characterized by high BUN, Glu, and Na values (Table 2). The NMDS of green turtles (Figure 3(b)) identified one outlying individual (SS5, mature female). The BCS of this SS5 was 4 (BCI was not calculated because BW could not be measured), but pH, BE, HCO_3, Na, and iCa were lowest, whereas pCO_2 and Crea were the highest in green turtles (Table 2). Mature turtles tended to be plotted to the right side in the NMDS (Figure 3(b)), but the effects of CCL ($F = 1.490$, df = 1, $p = 0.127$) and BCI ($F = 1.803$, df = 1, $p = 0.069$) on blood parameters of green turtles were not significant (data of SS5 was omitted due to missing BCI). The stress levels of NMDS of immature turtles and green turtles were 0.148 and 0.146, respectively.

The difference was obvious in BUN that was higher in hawksbill turtles probably due to their high-protein diet (sponges, corals, and crustaceans) in contrast with the herbivorous feeding habits of green turtles (McNally et al. 2020; Muñoz-Pérez et al. 2017). In comparison with previous studies, the hawksbill turtle BUN value in this study (62.25 ± 5.68 mg/dL, equal to 22.22 ± 2.03 mmol/L) was a little lower (31.0 ± 4.9 mmol/L; Stacy, Perrault & Wood 2023) or similar (18.3 ± 7.7 mmol/L; Muñoz-Pérez et al. 2017) values from in-water hawksbill turtles, but much higher compared to green turtles (Aguirre & Balazs 2000; Li et al. 2015; McNally et al. 2020). Thus, the difference in BUN between green and hawksbill turtles is generally supported.

Despite BUN values varying between green and hawksbill turtles mainly due to differences in diet, kidney function and physiology, evidence is yet to be established on the precise threshold values for kidney dysfunction in sea turtles with respect of specific sea turtle species. Limited studies such as Anderson et al. (2011) and Innis et al. (2007) documented the importance of setting species-specific baselines rather than relying on single threshold values, as these can vary significantly based on diet and environmental conditions.

The levels of Glu and Na in green turtles in this study was relatively lower than hawksbill turtles. In fact,

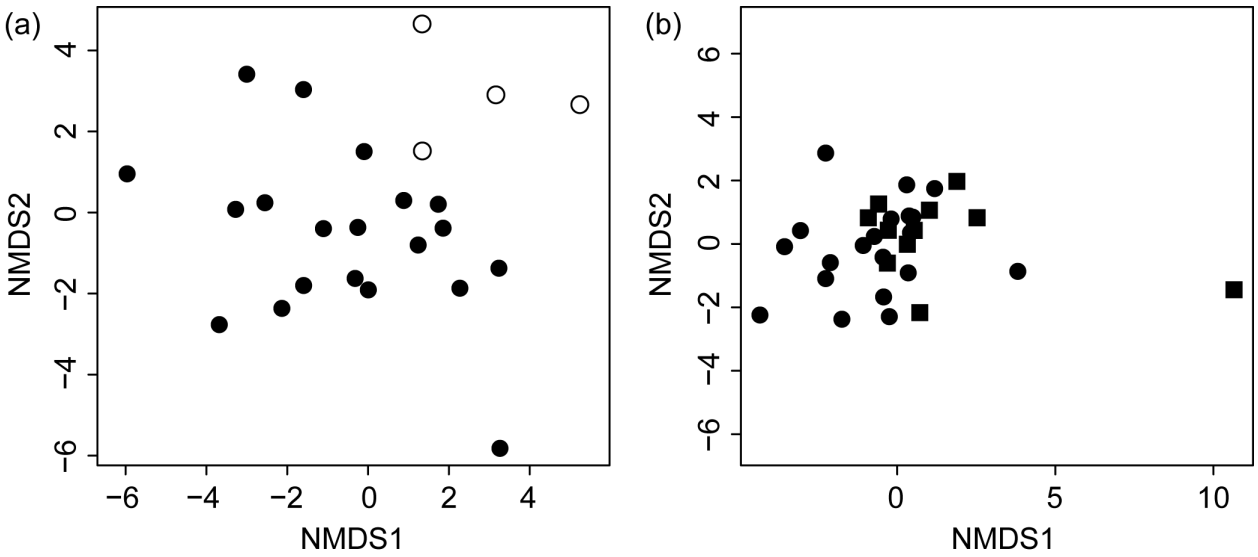

FIGURE 3. Two-dimensional non-metric multidimensional scaling ordination of blood parameters of (a) immature green (black circles) and hawksbill turtles (white circles) (stress = 0.148), (b) immature (black circles) and mature (black squares) green turtles where the outlying value indicates SS5 (stress = 0.146)

TABLE 2. Blood gas and biochemistry profiles of 31 green turtles (mean, SD, and range were calculated without outlying SS5, of which values were listed independently) and four hawksbill turtles captured in November 2021 and February 2022 in Semporna, Celebes Sea

Analytes	*Chelonia mydas*		*Eretmochelys imbricata* (N = 4)	
	Mean ± SD (N)	Min – Max (SS5)	Mean ± SD	Min – Max
Na (mmol/L)	145.60 ± 3.40 (30)	137.00–151.00 (118.00)	156.00 ± 3.60	153.00–161.00
K (mmol/L)	4.60 ± 1.10 (30)	3.10–9.00 (9.00)	5.20 ± 1.20	4.10–6.80
iCa (mmol/L)	1.06 ± 0.21 (30)	0.40–1.56 (0.25)	1.15 ± 0.37	0.59–1.31
Glu (mg/dL)	51.20 ± 13.60 (30)	20.00–94.00 (20.00)	73.30 ± 8.90	60.00–79.00
Lac (mmol/L)	8.43 ± 3.63 (30)	1.33–15.57 (12.91)	7.32 ± 2.65	4.67–10.12
pH*	7.34 ± 0.15 (29)	7.12–7.71 (6.93)	7.35 ± 0.08	7.24–7.41
pCO_2 (mmHg)*	67.40 ± 14.90 (29)	32.40–93.70 (117.10)	60.70 ± 14.70	43.40–74.00
pO_2 (mmHg)*	55.90 ± 20.40 (29)	28.00–116.00 (145.00)	76.80 ± 14.10	59.00–93.00
BE (mmol/L)	10.80 ± 8.40 (30)	-5.00–27.00 (-8.00)	7.50 ± 4.20	3.00–12.00
HCO_3 (mmol/L)	37.60 ± 6.60 (30)	25.30–51.80 (25.20)	34.30 ± 4.30	29.10–39.30
TCO_2 (mmol/L)	35.70 ± 4.70 (30)	26.00–48.00 (28.00)	33.30 ± 3.00	29.00–36.00
sO_2 (%)	87.60 ± 7.40 (30)	71.00–100.00 (97.00)	95.80 ± 2.50	92.00–97.00
Cl (mmol/L)	107.50 ± 6.20 (30)	97.00–124.00 (124.00)	118.80 ± 5.00	115.00–126.00
BUN (mg/dL)	8.50 ± 6.40 (30)	3.00–36.00 (22.00)	62.30 ± 5.70	56.00–68.00
Crea (mg/dL)	0.28 ± 0.07 (30)	0.20–0.40 (0.60)	0.20 ± 0.00	0.20
AnGap (mmol/L)	8.30 ± 2.60 (28)	4.00–13.00 (NA)	10.30 ± 3.30	7.00–14.00

*Analytes that are temperature corrected according to their body temperature

in Galápagos where in-water study was conducted for green turtles (Lewbart et al. 2014) and hawksbill turtles (Muñoz-Pérez et al. 2017) showed green turtle Glu (60 ± 9 mg/dL) and Na (148 ± 3 mmol/L) were relatively lower than those of hawksbill turtles (87 ± 10 mg/dL and 157 ± 3 mmol/L, respectively). However, Glu and Na may vary regionally, because higher values of Glu and Na have been reported from green turtles in other regions (Aguirre & Balazs 2000; Arthur, Limpus & Whittier 2008; Li et al. 2015; McNally et al. 2020). It is difficult to conclude the inter-specific differences of Glu and Na in Semporna from four individuals of hawksbill turtles; therefore, further information about blood profiles of sympatric green and hawksbill turtles is needed.

The levels of Na and Glu in sea turtles are influenced by several physiological and environmental factors, in particular stress, kidney function and salt gland activity. As for Glu levels, sea turtles can exhibit increased levels of production in response to stress as a part of their metabolic response. Similarly to other vertebrates, rise in glucose is an adaptive mechanism to provide additional energy (Harms et al. 2003; Stamper et al. 2005). Diet and metabolic rate are another factor contributing to such fluctuations depending on seasonal changes and food availability.

Whereas for Na levels, sea turtles possess specialized salt glands that help them excrete excess salt from ingested seawater. This is crucial in balancing Na levels, as dysfunction in these glands or dehydration led to altered Na levels in blood. As reported by Wyneken et al. (2001), elevated Na levels indicated dehydration or salt gland inefficiency that were seen in both wild and rehabilitated sea turtles. Stacy and Innis (2017) showed that renal issues are often indicated by abnormal Na levels alongside other blood chemistry parameters. They suggested that careful monitoring of Na levels is able to provide insights into kidney health in sea turtles.

Blood parameters of green turtles such as Lac, Glu, and BUN have been reported to change as the turtle develops (Labrada-Martagón et al. 2010; McNally et al. 2020), but this study did not support the significant relationship between blood parameters and CCL of green turtles. This may be partly attributable to the insufficient number of large mature green turtles in this study. In addition, Lac, Glu, and BUN as well as blood gases can vary with physiological stress and activity levels of sea turtles (Aguirre & Balazs 2000; Harms et al. 2003; Innis et al. 2007; McNally et al. 2020). However, these values can also fluctuate due to ontogenetic changes - natural shifts linked to age and metabolic rate. Thus, interpreting these variations without detailed discussion of age-related and metabolic influences could overlook how aging may mask these changes, making ontogenetic influences on blood parameters less apparent.

Physiological stress in sea turtles can be broken down into acute and chronic responses involving changes in blood chemistry, immune function and metabolic activity. During periods of stress, sea turtles like other vertebrates produce stress hormones primarily corticosterone that prompts several physiological responses. Parameters that are commonly monitored to evaluate stress are Glu, Lac and Hct, where increased Glu levels often associated with a stress-induced rise in corticosteroids as this provides the turtles with response of energy for a potential 'fight or flight' actions, yet prolonged high levels indicating chronic stress and negatively impact the health (Harms et al. 2003) (Table 3). Elevated Lac levels is due to stress-induced activity, handling or environmental factors as it is a byproduct of anaerobic metabolism that occur during high-energy responses or limited oxygen scenarios documented in Stamper et al. (2005). Whereas Hct levels is often used to assess stress indirectly, it can increase with dehydration that might be a stress response as osmotic regulation changes under stress.

TABLE 3. Hematology profiles of 31 green turtles (mean, SD, and range were calculated without outlying SS5, of which values were listed independently) and four hawksbill turtles captured in November 2021 and February 2022 in Semporna, Celebes Sea

Profiles	*Chelonia mydas*		*Eretmochelys imbricata* (N = 4)	
	Mean ± SD (N = 30)	Min – Max (SS5)	Mean ± SD	Min – Max
Hct (% / PCV)*	25.10 ± 4.50	17.00–35.00 (35.00)	20.80 ± 2.80	18.00–24.00
Hb (g/dL)*	8.50 ± 1.50	5.80–11.90 (11.90)	7.10 ± 1.00	6.10–8.20
Total leukocytes ($\times 10^9$/L)	16.00 ± 6.00	8.20–33.80 (14.80)	14.20 ± 1.20	12.60–15.60
Heterophils (%)	23.00 ± 7.40	8.00–36.00 (41.00)	24.80 ± 12.30	14.00–39.00
Eosinophils (%)	10.00 ± 4.80	2.00–22.00 (5.00)	14.30 ± 7.00	5.00–21.00
Basophils (%)	0.60 ± 0.90	0.00–4.00 (0.00)	0.30 ± 0.50	0.00–1.00
Lymphocytes (%)	56.10 ± 6.90	43.00–73.00 (48.00)	48.00 ± 12.60	34.00–61.00
Monocytes (%)	9.70 ± 4.40	1.00–17.00 (6.00)	12.50 ± 6.50	6.00–19.00

*Profile that was obtained from automated analysis using iSTAT

Activity levels also influence physiological parameters through increased metabolic demands or shifts in behaviour due to environmental factors such as physical and feeding activities. According to Perrault et al. (2021), changes in energy-related markers like Glu and Lac may indicate active swimming, foraging or migratory movements that are driven by environmental conditions like food scarcity. Activity levels also associated with feeding behaviours or prolonged fasting periods influence various parameters. Perrault et al. (2021) also reported that Glu levels may be lower during fasting while urea levels might increase because of protein catabolism in the absence of food with consideration of turtle's physiological phase. The combined effects of stress and activity levels highlight how the body responds under different conditions. While short-term activity might raise Glu or Lac levels, chronic stress or extended physical demands may also influence immune function and electrolyte balance. For example, high corticosterone is often linked to both increased Glu and altered immune cell counts in stressed turtles, while consistently high activity or stress levels might impair kidney function and over time affect Na levels.

CONCLUSIONS

This study provides essential baseline data on the health status of foraging green and hawksbill turtles in Semporna, Celebes Sea, offering a comprehensive assessment through body measurements, physical examinations, tumor scoring, external epibiota evaluations, and analysis of hematologic, blood gas, and biochemistry profiles. We identified distinct interspecific differences in blood parameters, with green turtles showing lower glucose and sodium levels compared to hawksbill turtles, consistent with findings from other regions. The occurrence of specific barnacle species and the low prevalence of fibropapillomatosis (FP) further emphasize the role of sea turtles as indicators of ecosystem health.

Although the sample size for hawksbill turtles was limited, the data provide critical insights into their health, contributing to a foundational understanding of sympatric green and hawksbill turtle populations. The findings highlight the importance of integrating subjective body condition assessments with quantitative blood parameter analysis to enhance health evaluations and conservation strategies. Notably, this study documented the occurrence of *Chelolepas cheloniae*, a barnacle species previously unreported in Semporna, underscoring the biodiversity and ecological dynamics in this region.

Sea turtles play a pivotal role in maintaining marine ecosystem balance, and their health status reflects the overall well-being of coastal and marine environments. This research underscores the necessity of continued monitoring and expanded studies to refine health metrics and address threats to these endangered and critically endangered species. The findings also serve as a valuable reference for developing conservation policies and management strategies aimed at safeguarding sea turtles and their habitats in the Celebes Sea and beyond.

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SUPPLEMENTARY TABLE 1. Detailed morphometric data for 36 individual sea turtles captured during the series of studies conducted in November 2021 and February 2022 in Pom Pom (North Tip, Mandarin Playground & Lobster Wall), Timba Timba and Pandanan Islands, Semporna, Sabah (Celebes Sea). The data includes the identification (ID), species (Sp), locations, curved carapace length (CCL), straight carapace length (SCL), curved carapace width (CCW), body weight, body condition score (BCS), body condition index (BCI), body temperature (Body Temp.), sex, size class, and external observations or remarks

ID	Sp	Location	CCL (cm)	SCL (cm)	CCW (cm)	Body Weight (kg)	BCS	BCI	Body Temp. (°C)	Sex	Size Class	External observations
SS 1	Cm	NT	69.00	64.95	64.00	34.00	3	1.24	32.00	N.D.	Juvenile	No injuries
SS 2	Cm	NT	81.00	76.20	77.50	65.00	4	1.47	32.00	F	Adult	Chip on left carapace
SS 3	Cm	NT	61.00	57.46	51.50	16.40	3	0.86	32.90	N.D.	Juvenile	Chip on right carapace; Barnacle on carapace
SS 4	Cm	NT	83.50	78.54	73.50	N.A.	5	N.A.	N.A.	M	Adult	Not weighed: Big-sized; Tail Length: 35.5 cm
SS 5	Cm	NT	88.50	83.22	81.00	N.A.	4	N.A.	35.00	F	Adult	Not weighed: Big-sized; 2 Fishing hooks in right fore-flipper; Chip on the right carapace
SS 8	Cm	NT	70.00	65.89	61.10	41.00	3	1.43	35.90	N.D.	Juvenile	Chip on right carapace
SS 9	Cm	NT	64.80	61.02	56.00	29.50	3	1.30	35.80	N.D.	Juvenile	Burrowing barnacle on the carapace
SS 10	Cm	NT	82.50	77.60	72.40	46.50	4	1.00	35.70	M	Adult	Tail length: 34.4 cm
SS 12	Cm	NT	71.20	67.01	63.00	36.30	3	1.21	34.80	N.D.	Juvenile	No injuries
SS 13	Cm	NT	79.00	74.32	71.00	58.00	4	1.41	35.30	F	Adult	No injuries
SS 14	Cm	NT	87.00	81.82	77.40	74.50	3	1.36	33.70	M	Adult	Chip on right carapace; Tail Length: 42 cm; Burrowing barnacle presented
SS 15	Cm	NT	50.00	47.15	47.00	15.80	2	1.51	33.50	N.D.	Juvenile	Chip on left carapace; Algae on shoulders & legs
SS 16	Cm	NT	47.00	44.34	44.50	13.70	3	1.57	33.60	N.D.	Juvenile	Barnacle on carapace
SS 17	Cm	NT	67.00	63.08	60.20	34.00	4	1.35	33.90	N.D.	Juvenile	No injuries
SS 18	Cm	NT	57.00	53.71	51.00	19.40	3	1.25	34.20	N.D.	Juvenile	No injuries

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SS 19	Cm	NT	79.50	74.79	73.00	64.50	4	1.54	34.30	F	Adult	Barnacle on plastron
SS 30	Cm	NT	70.30	66.17	62.10	38.00	3	1.31	26.90	N.D.	Juvenile	Acute injury at both hind flippers and shoulders
SS 31	Cm	NT	55.10	51.93	49.50	20.60	3	1.47	27.80	N.D.	Juvenile	Burrowing barnacles at hind flippers; 1 barnacle on the plastron
SS 6	Cm	MP	58.50	55.11	52.50	23.80	3	1.42	35.60	N.D.	Juvenile	Chip on left carapace; Signs of FP tumor: neck, shoulder, and eye
SS 36	Cm	MP	41.00	38.72	40.00	5.90	1	1.02	34.80	N.D.	Juvenile	Emaciated; No clinical pathology was performed
SS 7	Cm	MP	98.10	92.22	84.80	N.A.	5	N.A.	34.10	F	Adult	Not weighed: Big sized; Scar on carapace right; Barnacles on the plastron
SS 11	Cm	LW	60.70	57.17	55.40	22.10	2	1.18	34.50	N.D.	Juvenile	Fishing net around the neck and inside mouth; Injury on the neck and left jaw due to the net
SS 20	Cm	TT	53.20	50.15	47.80	15.45	3	1.23	32.30	N.D.	Juvenile	Acute injury at right hind flipper
SS 21	Cm	TT	93.80	88.19	82.00	N.A.	4	N.A.	27.90	F	Adult	Not weighed: Big-sized; 2 barnacles on plastron; Body with red algae; Acute injury on both hind flippers
SS 22	Cm	TT	52.50	49.49	46.20	15.60	3	1.29	27.70	N.D.	Juvenile	Acute injury on both hind flippers (possibility of bite marks)
SS 23	Cm	TT	93.20	87.63	87.00	N.A.	3	N.A.	30.30	F	Adult	Not weighed: Big-sized; 1 barnacle on plastron; Body with red algae; Burrowing barnacles at median plastron; White patches on tail
SS 24	Cm	TT	48.50	45.74	44.00	13.60	3	1.42	30.70	N.D.	Juvenile	Chip on left carapace; Small barnacles on the hind left flipper; Body with red algae
SS 25	Cm	TT	58.00	54.64	52.10	22.50	3	1.38	30.90	N.D.	Juvenile	1 barnacle on plastron
SS 26	Cm	TT	66.40	62.51	58.80	32.00	3	1.31	29.90	N.D.	Juvenile	3 barnacles on plastron; Burrowing barnacles at median plastron; Acute injury on hind left flipper

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SS 27	Cm	PP	64.60	60.83	60.90	34.20	4	1.52	32.70	N.D.	Juvenile	Chip on left carapace; 3 barnacles on plastron; Small barnacles on flippers
SS 28	Cm	PP	67.50	63.55	60.00	30.55	4	1.19	34.10	N.D.	Juvenile	Acute injury at both hind flippers
SS 29	Cm	PP	89.20	83.88	80.70	N.A.	4	N.A.	32.60	F	Adult	Small injury on both hind flippers; Burrowing barnacles on both fore-flippers
SS 32	Ei	MP	54.50	51.36	48.70	17.40	3	1.28	34.10	N.D.	Juvenile	Body with algae
SS 35	Ei	MP	37.50	35.44	35.00	6.30	2	1.42	32.00	N.D.	Juvenile	Chip carapace; Algae on carapace and plastron; Burrowing barnacles at soft tissue
SS 33	Ei	PP	47.00	44.34	35.70	6.45	3	0.74	32.30	N.D.	Juvenile	Algae on carapace and plastron; Acute tear on the hind left flipper; Small barnacles on soft tissues
SS 34	Ei	PP	53.10	50.05	45.20	10.90	3	0.87	32.90	N.D.	Juvenile	Algae on plastron; Chip on carapace; Small holes at fore flippers and burrowing barnacles inhabit the holes

Cm: *Chelonia mydas*
Ei: *Eretmochelys imbricata*
NT: North Tip, Pulau Pom-Pom
MP: Mandarin Playground, Pulau Pom-Pom
LW: Lobster Wall, Pulau Pom-Pom
TT: Pulau Timba-Timba
PP: Pulau Pandanan