



# Adipose tissue estimation of foraging and nesting green turtles *Chelonia mydas* using bioelectrical impedance analysis

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**ABSTRACT:** Adipose tissue is the main energy store in sea turtles and fluctuates in response to dietary conditions and external stressors. Monitoring programmes commonly use body condition indices (BCIs) to infer the nutritional and health status of sea turtle populations. However, BCIs have poor predictive power for estimating adipose tissue. We introduce the use of bioelectrical impedance analysis (BIA) as a portable technique to estimate adipose tissue in green turtles *Chelonia mydas*. The aims of this study were to estimate adipose tissue of green turtles on the Great Barrier Reef (Australia), and to examine whether adipose tissue is a more sensitive indicator than BCI. Turtles ( $n = 250$ ) were sampled at 3 foraging sites and at a nesting beach with differing levels of anthropogenic impact. Differences in adipose tissue, Fulton's BCI, and body mass across study sites and life stages were assessed by conducting linear mixed effects models. BIA estimates of mean adipose tissue revealed significant differences across life stages and sampling sites, that were not found using BCI data. Mean adipose tissue was estimated to be  $4.6 \pm 0.6\%$  (% body mass  $\pm$  SD) and was not correlated with mean BCI ( $1.2 \pm 0.1$ ). Adipose tissue was not reduced in turtles foraging at sites with a high level of anthropogenic impact. Adult turtles had significantly higher adipose tissue values than juveniles and subadults. Adult females measured during and shortly before nesting season had the highest adipose tissue values (%). BIA is a practical method for estimating adipose tissue, and we recommend this technique for consideration in sea turtle monitoring programmes.

**KEY WORDS:** Sea turtles · Body condition · Body fat · Nutritional status · Bioelectrical impedance analysis · Bioelectrical impedance spectroscopy · Body composition · Australia

## 1. INTRODUCTION

Green turtles *Chelonia mydas* are considered indicator species, as their behaviour, demography, reproductive success, and health status can help identify pressures on coastal ecosystems (Aguirre & Lutz 2004). The Great Barrier Reef (GBR) supports

some of the highest densities of green turtle foraging aggregations in the Western Pacific Ocean (Limpus et al. 2003). Four genetically distinct green turtle populations are found in north-east Australia: northern GBR (nGBR), southern GBR (sGBR), Coral Sea, and Gulf of Carpentaria (Bowen et al. 1992). Although considered migratory in their early life and

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during the nesting season, green turtles show strict site fidelity and long-term residency to foraging sites (Shimada et al. 2016). Degradation of foraging sites on the GBR may lead to reduced green turtle population viability (McCloskey & Unsworth 2015, Commonwealth of Australia 2017). Assessing the effects of anthropogenic impact on sea turtle habitats, population health, population size, and demographic data, as well as exploring mitigation methods, is therefore a global priority (Hamann et al. 2010, Wallace et al. 2011, Rees et al. 2016).

Population health assessments are particularly suitable for marine species research, which is often challenged by expensive and demanding field surveys; species rarity and distribution; limited funding and resources; and logistical, regulatory, and ethical constraints in threatened species (Mellin et al. 2011). The choice of method to assess health status should involve consideration of the accuracy and specificity (i.e. precision) of the method, as well as its temporal stability to detect changes, ease of use, and cost-effectiveness (Lindenmayer et al. 2015, Kophamel et al. 2022a). A typical example in sea turtle research is to use body condition indices (BCIs) to infer nutritional status, which is a surrogate of health status. Individuals in good health, physical, and reproductive condition are believed to have higher BCIs and therefore a better nutritional status than those exposed to chronic stressors (Hayes & Shonkwiler 2001, Speakman 2001, Rafferty et al. 2014, Birnie-Gauvin et al. 2017). BCIs are calculated from body mass and length measurements. The underlying principle of BCIs is that body mass is an indicator of energy or fat stores, which are primarily present as adipose tissue (Krebs & Singleton 1993, Warner et al. 2016, Bell et al. 2019). However, BCIs are considered insensitive predictors of adipose tissue in other species because they are at best semi-quantitative and do not correlate with adipose tissue (Schulte-Hostedde et al. 2001, Laaksonen et al. 2003, Labocha et al. 2014, Thornton 2016, Warner et al. 2016). An improved method for estimating the composition of the whole body, specifically the relative amount of adipose tissue, would greatly enhance our understanding of sea turtle population health and population viability (Stevenson & Woods 2006, Wilder et al. 2016).

A promising tool for sea turtle research is bioelectrical impedance analysis (BIA). BIA devices are used widely in human clinical studies and aquaculture to quantify body composition and assess nutritional status (Kyle et al. 2004, Stahn et al. 2012, Hafs & Hartman 2015, Ward 2019), and have also been piloted in vertebrate wildlife species (Bowen et al. 1999, Hwang

et al. 2005, Barthelmess et al. 2006, Pitt et al. 2006). This novel technique is more accurate and tissue-specific compared to existing metrics that estimate adipose tissue, such as BCI or body mass (Wilder et al. 2016, Kophamel et al. 2022c). BIA devices measure body impedance of biological tissues to a harmless electric current. Impedance is the opposition of body tissues to current flow. Measurements are typically made in the frequency range 5–1000 kHz (bioelectrical impedance spectroscopy [BIS] devices) or at a single fixed frequency of 50 kHz (single-frequency BIA [SFBIA] devices) (Stahn et al. 2012). The impedance (or more correctly its component, resistance) of the body is then used in prediction algorithms to estimate non-adipose tissue mass, which when subtracted from total body mass yields adipose tissue mass. For a detailed explanation on the physical processes involved in impedance measurements, see Van Marken Lichtenbelt (2001) and Ward et al. (2009).

We recently validated and calibrated a BIA device for adipose tissue estimation in sea turtles, using immature green turtles as model organisms for cheloniid sea turtles (Kophamel et al. 2022c, 2023). Estimates of adipose tissue mass by impedance measurements were highly precise (0.8 CV for repeated measurements) and over 50 times more accurate compared to using body mass as a predictor variable (Kophamel et al. 2022c). Furthermore, the BIA procedure takes less than 15 min per animal and does not require chemical or direct physical restraint.

The aims of this study were (1) to use BIA to predict mean adipose tissue (% body mass) of green turtles from 3 foraging sites with differing levels of anthropogenic impact and from a nesting beach; and (2) to determine if BIA predictions of adipose tissue are more robust than BCI for assessing differences in nutritional status across study sites and life stages. We hypothesised that, on average, predicted adipose tissue would be higher in turtles from the control site compared to turtles from sites with higher level of anthropogenic impact. Nesting females were also estimated to have higher adipose tissue mass compared to non-breeding turtles, and we expected adipose tissue to increase with life stage (i.e. size class).

## 2. MATERIALS AND METHODS

### 2.1. Study sites

This study was conducted on 3 major green turtle foraging grounds and 1 nesting beach on the Great

Barrier Reef (GBR, north-east coast of Australia) between August 2018 and October 2019 (Table S1 in the Supplement at [www.int-res.com/articles/suppl/n051p127\\_supp.pdf](http://www.int-res.com/articles/suppl/n051p127_supp.pdf)). The foraging sites examined represent: an industrial site experiencing a port expansion (Townsville Region), an agricultural site affected by high level of agricultural run-off (Upstart Bay), and a protected site with relatively little anthropogenic impact, which served as a control site (Howick Group of Islands). The green turtle nesting site (Milman Islet) was not considered to be directly affected by anthropogenic stressors and was visited at the end of the nesting season. The exact locations of the sites are (1) Combe Reef (14° 25' 48" S, 144° 54' 42" E) and Ingram Reef (14° 25' 03" S, 144° 52' 46" E), representing the coral cay Howick Group of Islands, which is subject to low anthropogenic impact and served as a control site; (2) Cleveland Bay (19° 13' 05" S, 146° 55' 19" E) and Toolakea Beach (19° 08' 40" S, 146° 34' 40" E), representing coastal bays in the industrialised Townsville Region; (3) Upstart Bay (19° 40' 02" S, 147° 36' 44" E), a coastal foraging site subject to agricultural run-off; and (4) Milman Islet (11° 10' 08" S, 143° 00' 55" E), a nesting beach for green turtles and hawksbill turtles *Eretmochelys imbricata* (Fig. 1). Anthropogenic impact was estimated based on available literature; see Section 4 for further details.

## 2.2. Animals and sampling protocol

Foraging turtles were captured from a boat using the rodeo technique (n = 218) as described by Limpus & Reed (1985) or hand-captured in shallow water (n = 12), and nesting females were examined on the beach (n = 20). Sampling was opportunistic, tide-dependent, and predominantly performed in the mornings for foraging turtles, which were immediately transported to shore after capture. Nesting females were examined at night after nesting. Tagging for identification purposes was conducted with approved titanium tags. Curved carapace length (CCL) was measured twice from the nuchal scute to the caudal tip of the supracaudal scute, using a measuring tape, to the nearest millimetre, with the average value being recorded. Turtles were allocated into life stages based on CCL as per Chaloupka & Limpus (2001), with juveniles CCL < 65 cm, subadults 65 cm < CCL < 90 cm, and adults CCL > 90 cm. Body mass was measured by suspending each turtle from a digital scale using a harness designed to be secured around the base of

each limb. The harness was removed immediately after measurements were completed. The scale was calibrated before each field trip and measured mass to the nearest 0.1 kg.

A general health assessment, which included a physical examination (n = 250) and blood sampling (n = 97), was performed by a qualified veterinarian (S.K.). The methodology related to the blood sampling and haematological and biochemical analyses is detailed in Kophamel et al. (2022b). Presence of lesions and ectoparasites was also noted. Body temperature was recorded by inserting the probe of a thermocouple (8402-20 Thermistor 237 Thermometer, Cole-Palmer Instruments) 5 cm into the cloaca (Flint 2013, Stacy & Innis 2017). The sex of a random selection of juvenile turtles from the control site (n = 33) was determined by laparoscopic examination as part of a longitudinal monitoring study; the methodology is detailed in Bell et al. (2019). Animals were released at their locations of capture.

Tagging, morphometric, and body mass measurements followed standard operating procedures outlined by the Department of Biodiversity Conservation and Attractions (2017) and the Conservation & Biodiversity Operations Branch, Department of Environment and Science (2018). Procedures were standardised and conducted in the following order: capture, physical examination, morphometric assessment (i.e. measurements of length and body mass), BIA procedure, blood sampling, laparoscopies (selected animals), and release. All procedures and protocols were conducted within 4 to 5 h of capture and were approved by the Great Barrier Reef Marine Park Authority (permit numbers G18/40749.1 and G19/42769.1) and the Department of Environment and Science, Queensland Government (permit numbers SPP18-001167 and SPP18-001167-1).

## 2.3. Body impedance measurements

Turtles were dried and placed on a non-conductive plastic surface. Visual stimuli and stress were minimised by covering the turtles' head with a non-cohesive bandage. Body impedance measurements were performed with a handheld bioelectrical impedance spectroscopy analyser (SFB7, ImpediMed) following procedures outlined by Kophamel et al. (2023). A tetrapolar arrangement of electrodes was applied to specific anatomic locations on the right front limb and hind limb as described by Kophamel et al. (2023). Resistance and reactance were measured at 256 logarithmically spaced frequencies in the range

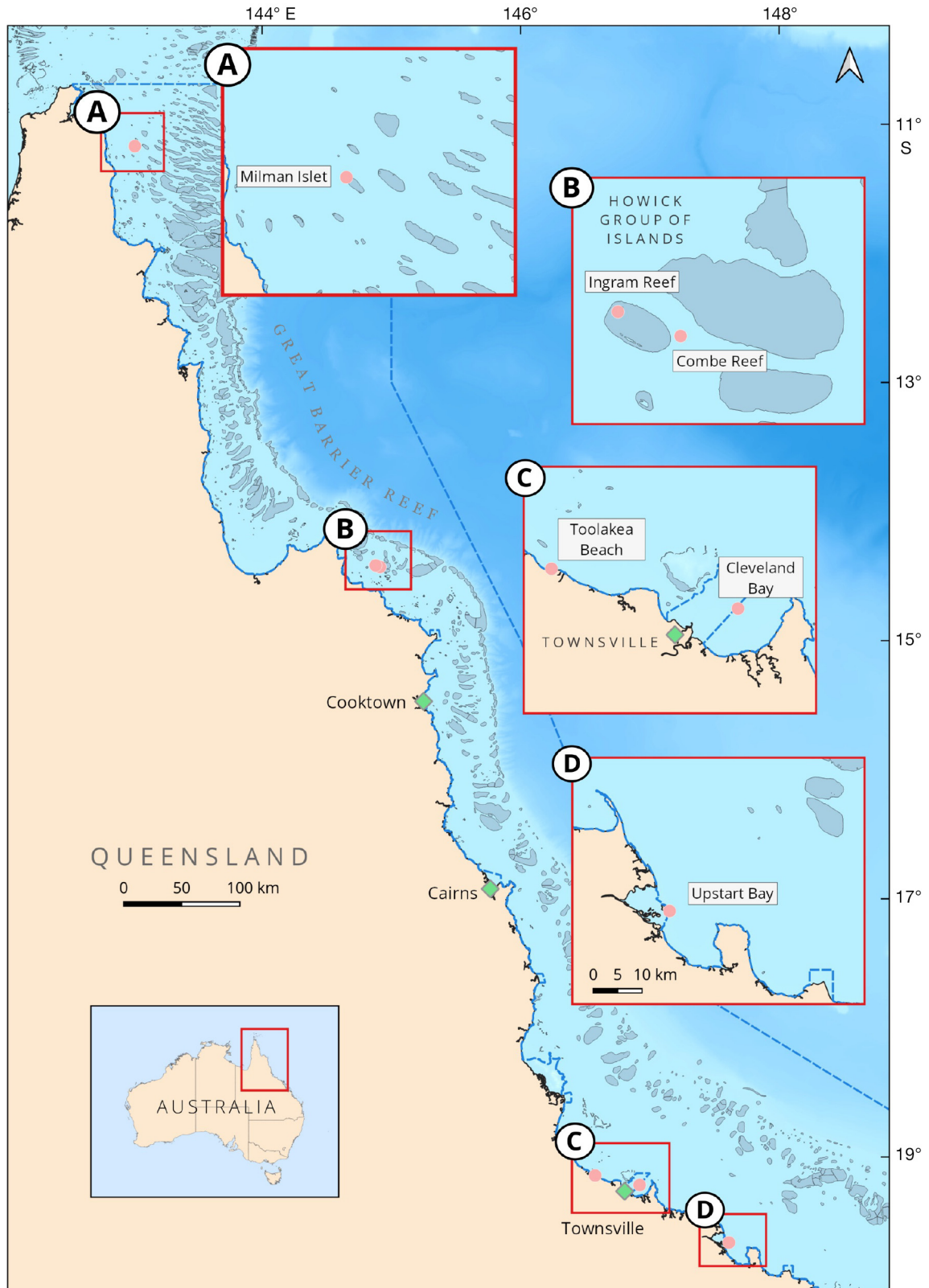


Fig. 1. Locations (pink dots) of the 4 examined green turtle *Chelonia mydas* sites on the north-east coast of Australia. Turtles ( $n = 250$ ) were examined at a nesting beach: (A) Milman Islet (fieldtrip conducted between January and February 2019); and at 3 foraging grounds: (B) Howick Group of Islands (Combe Reef and Ingram Reef; fieldtrips conducted in August 2018 and August 2019), (C) Townsville Region (Cleveland Bay and Toolakea Beach; fieldtrips conducted between June and October 2019), and (D) Upstart Bay (fieldtrip conducted in November 2018)



3–1000 kHz, and device calibration was verified daily. Ten replicate impedance measurements with an interval of 5 s were recorded to account for intra-animal variability (i.e. measurement precision), with each measurement taking 800 ms. The extracted data of interest were resistance at infinite frequency ( $R_{inf}$ , predictor of total body water and fat-free mass), resistance at zero frequency ( $R_0$ , predictor of extracellular water), intracellular resistance ( $R_i$ , an index of intracellular water); and, for comparison with studies using SFBIA devices, resistance at 50 kHz ( $R_{50}$ ), reactance at 50 kHz ( $X_{c50}$ ), and phase angle at 50 kHz ( $PhA_{50}$ ). Fig. 2 provides a summary of the adipose tissue estimation procedure in green turtles.

Nine animals were measured twice: 4 adult female turtles were repeatedly sampled on the nesting site during the 2019 nesting season, and 5 foraging turtles were sampled both in 2018 and 2019 at the control site (1 adult male, 1 adult female, and 3 juvenile females). These data were averaged before inclusion in the final model (non-significant differences observed across medians;  $p > 0.05$ ).

#### 2.4. Adipose tissue estimations

Impedance data were analysed using Bioimp software (v5.4.0.3, ImpediMed), which calculates resistance ( $R$ , ohm), reactance ( $X_c$ , ohm), and phase angle ( $PhA$ , arctangent  $X_c/R$ , degrees) at each frequency and uses Cole analysis to obtain estimated resistance at infinite frequency ( $R_{inf}$ ) and at zero frequency ( $R_0$ ) (Cornish et al. 1993). For further details, see the manufacturer's website: <https://www.impedimed.com/>. The extracted parameter of interest was resistance at 50 kHz ( $R_{50}$ ) for comparison with studies using the more affordable SFBIA devices. Adipose tissue (kg) for each individual turtle was estimated using the equation for predicting adipose tissue mass (kg) from single-frequency (50 kHz) body impedance measurements in green turtles derived by Kophamel et al. (2022c), where CCL was used as a measure for body length and  $CCL^2/R_{50}$  is the impedance index:

$$\begin{aligned} \text{Adipose tissue mass (kg)} = & \\ \text{body mass} - (-0.01 [\text{intercept}] - 0.28 & \\ \times CCL^2/R_{50} + 1.07 \times \text{body mass} - 0.10 & \\ \times \text{time after capture}) & \end{aligned} \quad (1)$$

Eq. (1) estimates adipose tissue from body mass (kg), impedance index  $CCL^2/R_{50}$  (i.e.  $\text{length}^2/R_{50}$ ), and time after capture (h). Time after capture was calculated based on the hours that had passed since cap-

turing the turtles. A previous study determined that body mass, body length, and time after capture influence impedance measurements in green turtles (Kophamel et al. 2023). For example, several hours of dry-docking after capture (a common fieldwork procedure) can potentially dehydrate the turtles and alter the intra- and extracellular fluid distributions that define the resistance values (Kophamel et al. 2023).

Predicted adipose tissue (kg) was then converted to percentage (%) by dividing it by total body mass, which was used as the response variable in the fitted models. BCIs were calculated based on the equation outlined by Bjorndal et al. (2000) and recommended by Harris et al. (2017), i.e. Fulton's condition factor  $K = \text{body mass}/\text{straight carapace length}^3 \times 10\,000$ .

#### 2.5. Statistical analyses

Sample size and power calculations were conducted using G\*Power (version 3.1.9.6 for Macintosh) (Erdfelder et al. 1996). A total sample size of 153 turtles was estimated to achieve a Power of 1 (effect size 0.25,  $\alpha = 0.05$ , 5 predictors). Outlier identification and exclusion were performed with residual plotting using the R package 'DHARMA' (Hartig 2020). Adipose tissue predictions for turtle aggregations were conducted with linear mixed effects models (LMMs) fitted by maximum likelihood. Model assumptions and validation, e.g. normality and variance homoscedasticity, were visually and statistically confirmed. The final model selection was based on diagnostic residual plots (e.g. DHARMA residual plotting), on the fit of the data to the selected model, and on Akaike's information criterion (Barton & Barton 2015, Hartig 2020). The response variable (adipose tissue in %) was logged, and predictions were back transformed. Tukey post hoc multiple comparison tests ( $\alpha = 0.05$ ) were conducted to assess the differences between study sites and life stages (R package 'emmeans',  $\alpha = 0.05$ ) (Lenth 2016). Repeated measurements of the 9 turtles sampled twice ( $n = 5$  from Howick Group of Islands and  $n = 4$  from Milman Island) were statistically compared with a permutation test (1000 iterations). Correlation between BCI and predicted adipose tissue was assessed using Pearson's correlation coefficient (strong correlation assumed when  $p < 0.05$  and  $r > 0.5$ ). Statistical analyses were completed in R (version 4.2.0, R Core Team 2019). All data are reported as mean  $\pm$  SD unless otherwise stated. Data sets (.xlsx, .ods, and .csv formats) are available at James Cook University Data Repository under the following link: <https://doi.org/10.25903/73jy-ky07>. Results of the post hoc multiple

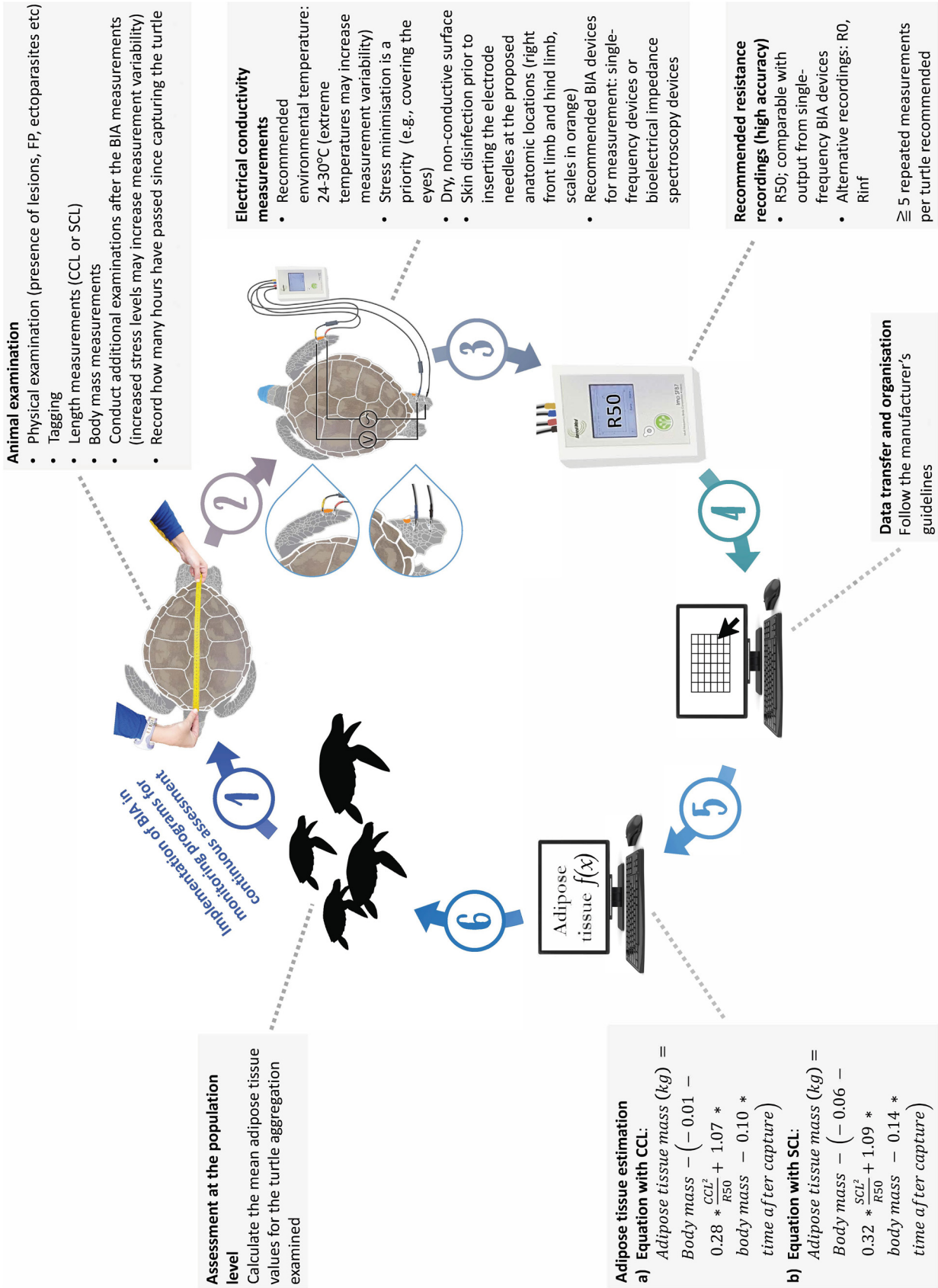


Fig. 2. Protocol for adipose tissue estimation in green turtles *Chelonia mydas*. Details on best practices and equations sourced from Kophamel et al. (2023) and Kophamel et al. (2022c). BIA: bioelectrical impedance analysis; FP: fibropapillomatosis; CCL: curved carapace length; SCL: straight carapace length; AT: adipose tissue; R50: resistance at 50 kHz; R0: resistance at zero frequency; Rinf: resistance at infinite frequency

comparison tests and the descriptions of parameters examined and codes used in the data set are provided in the Supplement.

### 3. RESULTS

#### 3.1. Animal characteristics

A total of 250 wild turtles were examined, of which 97 turtles were classified as mature (i.e. adults) and 153 as immature (i.e. juveniles and subadults). Life stages were distributed differently across study sites, with the industrial site consisting only of juvenile turtles ( $n = 40$ ) and the nesting site only of adult females ( $n = 20$ ). Morphological characteristics of the turtles sampled at each study site are detailed in Table S1. Most turtles ( $n = 248/250$ ) appeared clinically healthy upon physical examination, were in good body condition (visual assessment), and had no apparent external lesions. Exceptions were a juvenile turtle captured at the agricultural site, which was floating and appeared lethargic (ID number QA62317), and an adult female captured at the control site, which was apparently healthy but whose left front limb was missing (ID number QA94686). The lethargic turtle was taken to the nearest sea turtle rehabilitation centre on the same day. The BIA data from these 2 turtles were excluded, as altered hydration status and body shape could affect the impedance measurements if not accounted for in the predictive models (see Kophamel et al. 2022c, 2023 for details). The results of the haematological and biochemical analyses are detailed in Kophamel et al. (2022b); no abnormal findings were recorded. Laparoscopic examination of immature turtles at the control site identified 27 females and 6 male turtles. Across study sites, 77 adult females and 20 adult males were sampled, with adult males only captured at the control site (Table S1). The turtles examined in this study were presumed to originate from nGBR genetic stock (Milman Island), a mix of sGBR and nGBR genetic stocks (Howick Group of Islands), and sGBR genetic stock (Townsville Region and Upstart Bay) (Jensen et al. 2016, 2018, Jones et al. 2018).

#### 3.2. Adipose tissue estimation from body impedance measurements

The model that explained the data best accounted for study site (location), life stage (i.e. size class), body mass, and impedance index  $CCL^2/R50$  as fixed

effects, and time after capture (h) as a random effect (1|time after capture):

$$\begin{aligned} \log(\text{adipose tissue mass [\%]}) \sim & \text{location} \\ & + \text{life stage} + \text{body mass} \times CCL^2/R50 \\ & + (1|\text{time after capture}) \end{aligned} \quad (2)$$

Mean predicted adipose tissue (as % body mass) calculated from body impedance measurements for all turtles was  $4.6 \pm 0.6\%$  (95% CI: 1.8–9.9%). The following factors were identified to potentially alter adipose tissue: site (i.e. level of anthropogenic impact and available food resources), life stage, and reproductive status. A complete list and visual representation of post hoc multiple comparison tests across sites and life stages are provided in Tables S2 & S3 and Fig. S1.

##### 3.2.1. Effect of site and diet

Turtles from the agricultural site had significantly higher predicted adipose tissue than turtles at the control site (LMM,  $p < 0.05$ ,  $df = 95.4$ ,  $t$ -ratio =  $-3.4$ , Fig. 3), but there were no other significant differences between sampled sites. Turtles mainly feeding on seagrass (industrial and agricultural sites) had 23–55% higher adipose tissue values than turtles that were mainly feeding on seagrass and algae (control site) (Fig. 4). Haematological and biochemical parameters measured from a subset of the turtles were also not significantly different across sites (Kophamel et al. 2022b). Opportunistic repeated impedance measurements between 2018 and 2019 (control site) and between nesting events in 2019 (nesting site) were not significantly different ( $p > 0.05$ ).

##### 3.2.2. Effect of life stage

The models revealed that body mass was significantly related to predicted adipose tissue at all sites (LMM,  $p < 0.05$ ,  $df = 213$ ,  $t$ -ratio = 18.59). Adipose tissue therefore increased with life stage, and adult turtles had higher predicted adipose tissue values compared to juveniles and subadults (LMM,  $p < 0.05$ ,  $df = 214$  and  $215$ ,  $t$ -ratio = 2.58 and 3.22) (Fig. 3; Table S4). For example, adult turtles at the control site had 47% more predicted adipose tissue than juveniles at the same site (Fig. 4). Subadult turtles also had higher mean predicted adipose tissue values compared to juveniles; however, the differences were not significant across study sites (LMM,  $p > 0.05$ ,  $df = 216$ ,  $t$ -ratio =  $-1.08$ ). Statistical differences in predicted

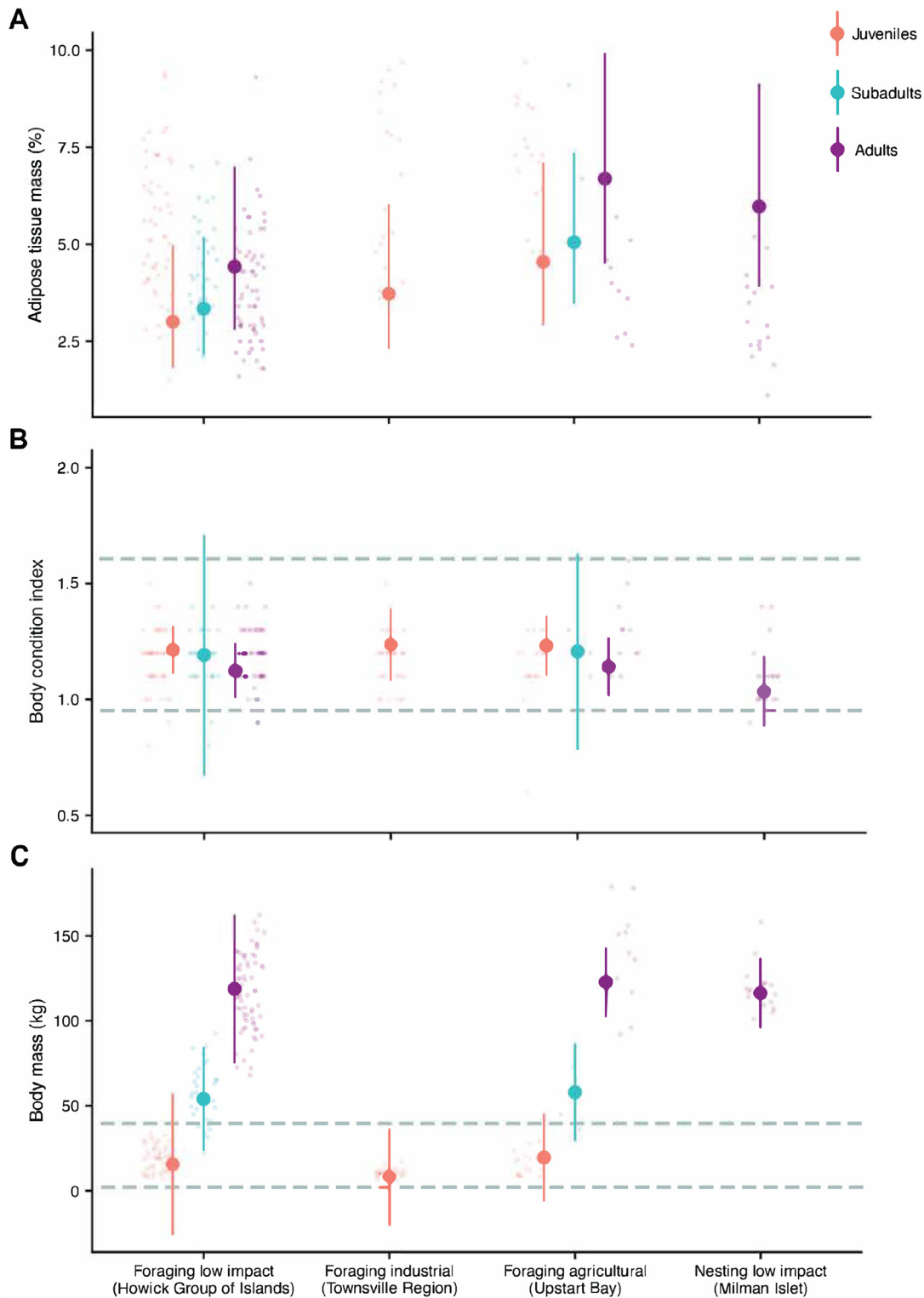


Fig. 3. (A) Predicted adipose tissue values (percentage), (B) Fulton's body condition index (BCI), and (C) body mass of green turtle *Chelonia mydas* aggregations on the north-east coast of Australia, categorised by study site and life stage ( $n = 250$ ). Turtles were examined at 3 foraging sites with differing levels of anthropogenic impact: a control site (Howick Group of Islands), an industrial site (Townsville Region), and an agricultural site (Upstart Bay); and at a nesting beach with a low level of anthropogenic impact (Milman Islet). Continuous lines display the mean values and 95% confidence levels; raw data are overlaid as dots; dotted lines represent the BCI and body mass ranges that were used for calibrating the bioelectrical impedance analysis device for adipose tissue estimation (see Kophamel et al. 2023). Fulton's BCI was calculated following the equation detailed in Bjørndal et al. (2000), i.e. Fulton's condition factor  $K = \text{body mass}/\text{straight carapace length}^3 \times 10\,000$



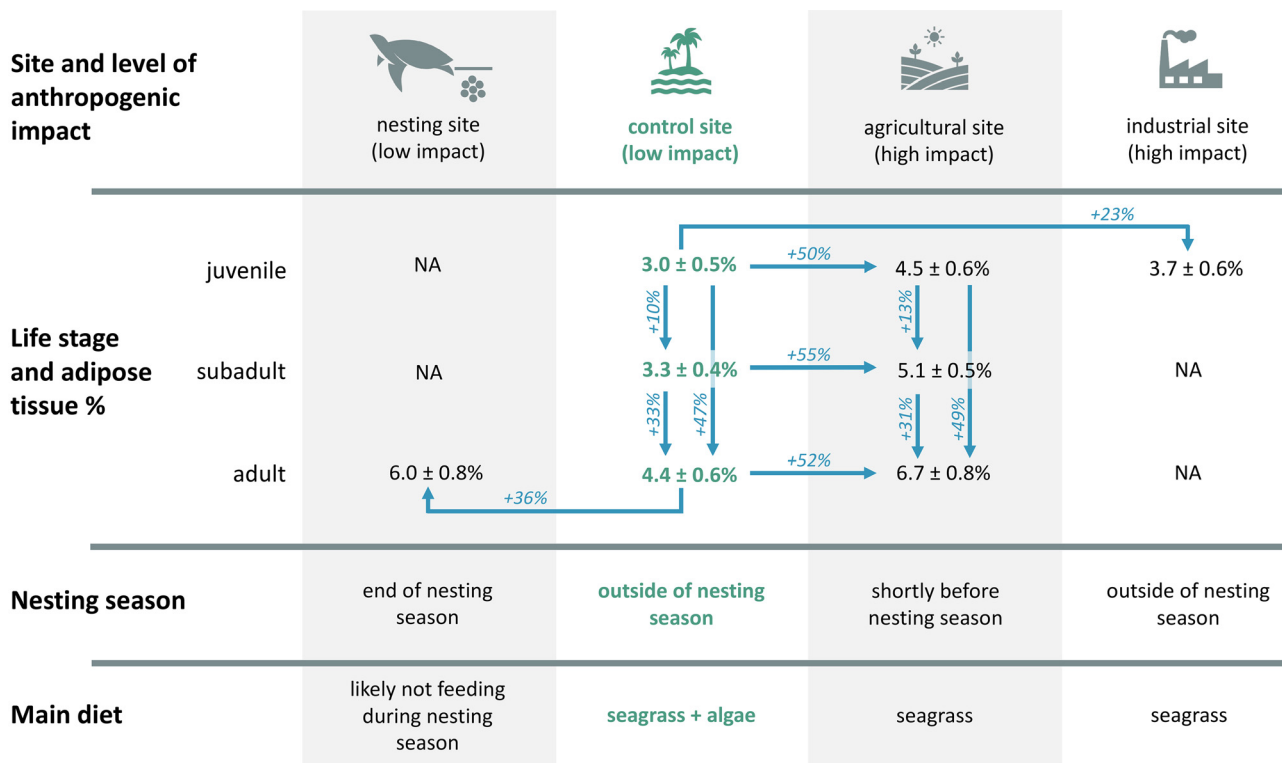


Fig. 4. Differences in predicted adipose tissue values (%; mean  $\pm$  SD) for green turtle *Chelonia mydas* aggregations on the north-east coast of Australia across study site and life stage (n = 250). Turtles were examined at 3 foraging sites with differing levels of anthropogenic impact: a control site (Howick Group of Islands), an industrial site (Townsville Region), and an agricultural site (Upstart Bay); and at a nesting beach with low level of anthropogenic impact (Milman Islet)

adipose tissue across sexes could not be assessed due to the low number of males captured (Table S1).

### 3.2.3. Effect of reproductive status

Adult female turtles sampled shortly before or at the end of the nesting season had the highest predicted adipose tissue values ( $6.7 \pm 0.8\%$  and  $6.0 \pm 0.8\%$  for agricultural and nesting sites, respectively), and had 36–52% more adipose tissue than turtles sampled outside of the nesting season (control site) (Figs. 3 & 4).

### 3.3. Body condition indices and correlation with adipose tissue

No correlation was found between BCI and predicted adipose tissue ( $r = -0.042$ ,  $p > 0.05$ ,  $t$ -ratio =  $-0.65$ ,  $df = 237$ ) (Fig. 5). As an example, a BCI score of  $>1.2$  (very good) was found in 144/250 turtles (58% of turtles); however, their predicted adipose tissue values ranged from 0.5 to 14.6%. The BCI across

sites and life stages ranged from normal (1.0–1.1) to very good ( $>1.2$ ; Fig. 3; Table S1). BCIs were not significantly different across sites, except for the control site and the nesting site (LMM,  $p < 0.05$ ,  $df = 176.3$ ,  $t$ -ratio = 2.64). Interestingly, no significant differences in BCI were found between the turtles from the agricultural site and the turtles from the control site, as had been identified for adipose tissue. Juveniles had slightly higher BCI ( $1.2 \pm 0.03$ , mean  $\pm$  SE) than subadults ( $1.18 \pm 0.04$ , with large 95% CIs due to small sample size), and adults had the lowest BCI ( $1.12 \pm 0.03$ ) (Fig. 3; Table S1). No significant differences in BCI across life stages were identified (Table S3).

## 4. DISCUSSION

In this study, predicted adipose tissue data were provided for foraging and nesting green turtles on the Great Barrier Reef World Heritage Area. BIA was identified as a more sensitive alternative to BCI and body mass for adipose tissue estimation and nutritional status assessment in green turtles. BIA met all of the

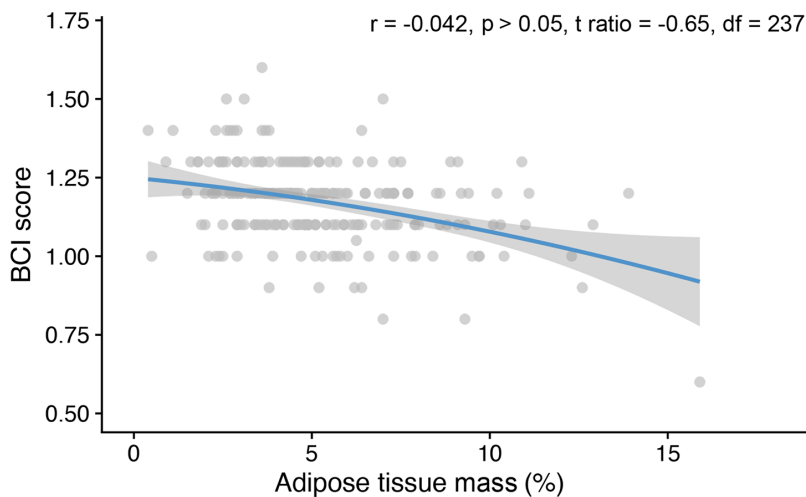


Fig. 5. Correlation, with 95% confidence intervals around the regression line, between body condition index (BCI) and adipose tissue mass (%) for green turtle *Chelonia mydas* aggregations on the north-east coast of Australia (n = 250)

specifications needed for an effective, field-usable method; it is portable, more accurate and precise than conventional methods, easy to use, affordable (US \$2000–4000), safe for both the operator and the animal, minimally invasive, provides quick results, and, importantly, is validated and standardised on the target species (Deem & Harris 2017, Harris et al. 2017, Page-Karjian et al. 2020, Page-Karjian & Perrault 2021, Kophamel et al. 2022a,c, 2023). Furthermore, BIA does not require extensive training, which broadens its potential for use by a diverse range of professionals, including biologists, ecologists, and veterinarians.

#### 4.1. Body condition indices for nutritional status assessment

We compared 2 indicators for nutritional status and population health, i.e. BCI and adipose tissue, and found that they were not correlated (Fig. 5). An increasing trend as turtles age was found for predicted adipose tissue but not for BCI, and BCI showed a smaller range across life stages (Fig. 3). Similar results have been reported in other vertebrate species, and the use of BCI in health studies remains controversial (Schulte-Hostedde et al. 2001, Laaksonen et al. 2003, Labocha et al. 2014, Thornton 2016, Warner et al. 2016). BCIs assume that changes in body mass, length, or volume are related to changes in adipose tissue and non-adipose tissue, even though the changes might be related to other causes (e.g. undigested food, increased water storage, or diseases) (Schulte-Hostedde et al. 2001, Laaksonen et al. 2003, Labocha et al. 2014, Thornton 2016, Warner et al.

2016). We therefore consider BCIs insensitive for inferring adipose tissue in green turtles and urge caution when using BCIs to inform conservation efforts, particularly if used in isolation. These results underscore the importance of accounting for multiple health, demographic, and environmental parameters when assessing population health, and to work with a multidisciplinary team to gather as much information as possible.

#### 4.2. Effect of site, diet, and life stage

Our results did not support the initial hypothesis that predicted adipose tissue would be higher in turtles from the control site compared to turtles from sites with higher level of anthropogenic impact. The predicted adipose tissue values indicate that the current levels of anthropogenic impact did not negatively affect nutritional status, although measurable changes in adipose tissue were found across sites and life stages. No indication of a negatively impacted nutritional status was found for turtles inhabiting areas subject to coastal expansion or agricultural run-off. As a result, the examined turtle aggregations were not considered to be at risk from immediate anthropogenic impact, and further investigations of dietary availability, population abundance, and demographic parameters are warranted.

The significantly higher adipose tissue values at the agricultural site, in comparison to the control site, raise questions about whether (1) the level of anthropogenic impact was not strong enough to negatively impact nutritional status; (2) relative grazing pressure and dietary availability might have led to the differences in adipose tissue between sites; (3) adipose tissue estimation should be combined with other health indicators to timely identify the experienced level of anthropogenic impact; (4) baseline adipose tissue ranges of clinically healthy sea turtles are naturally wide; (5) the differences in adipose tissue between study sites may be more strongly associated with other stressors, and less with decreased water quality (industrial site) and agricultural run-off (agricultural site); and (6) the control site may have been negatively impacted by anthropogenic and/or environmental stressors, which might have led to a reduced seagrass density, quality, or abundance.

The higher adipose tissue values at the agricultural site occurred across all life stages (Figs. 3 & 4), indicating that the agricultural site might have had a higher productivity than the control site. In fact, multi-year flows and sediment loads from the Burdekin River have led to a decline in seagrass meadows at the agricultural and industrial sites (Lambert et al. 2019); however, seagrass density at those sites was still higher than at the control site (Bell et al. 2019). The higher adipose tissue values at the agricultural site are unexpected and challenging to explain due to paucity of long-term routine monitoring at the agricultural site (McKenzie et al. 2021) and temporally matched comparative studies of seagrass meadows at the 3 sites. The interaction of multiple stressors on seagrass density and the subsequent impact on turtle nutritional status is also poorly understood. Mapping and monitoring programmes have identified that over a third of the seagrass meadows (38%) that are regularly monitored on the GBR are shrinking in area, biomass, and nutritional content, and a large number of meadows are declining in abundance (McKenzie et al. 2015). This decline has been attributed to anthropogenic stressors, such as climate change, agricultural run-off, and coastal development (Brodie 2021). Coastal development, such as the port expansion in Townsville city, has further hampered the recovery of seagrass meadows on the GBR due to reduced water quality (van Katwijk et al. 2016, Grech et al. 2018). A decline in seagrass meadows may likely affect the sea turtle aggregations that rely on these foraging sites. Although green turtles also use food sources other than seagrass (Brand-Gardner et al. 1999, Lemons et al. 2011, Burrett et al. 2018, Esteban et al. 2020), a significant loss in seagrass abundance may affect the demography and health status of foraging aggregations that have a predominantly herbivorous diet (Meylan et al. 2022).

The level of relative grazing pressure was similar across sites. Green turtle density at the control site has been estimated to be between 356 and 598 juvenile turtles (Bell et al. 2019), which aligns with predictions from other foraging sites in North Queensland (Hof et al. 2017; data up to 2014). Interestingly, turtles from the control site had the slowest growth rates across the examined sites (Bell et al. 2019), which correlates with the lower adipose tissue values found at that site. Possible explanations for the reduced growth rates at the control site are dietary differences, differences across genetically distinct populations, the lower productivity of nutrient-poor waters, density-dependent processes, annual vari-

ability, or other, non-described factors inhibiting physiological functions (Bjørndal 1996, Bjørndal et al. 2017, Bell et al. 2019). Turtles from the control site forage on both seagrass and red macro-algae (*Gelidium* and *Laurencia* spp.), whereas turtles from the industrial and agricultural sites mainly forage on seagrass (*Cymodocea serrulata* and *Halodule uninervis*) (Bell et al. 2019). A seagrass-based diet leads to higher growth rates than an algae-based diet (Bjørndal et al. 2017), which would support the higher adipose tissue values estimated at the industrial and agricultural sites. Algal abundance at the control site and seagrass abundance in north-east Australia have a very high within-year and location variation (Coles et al. 2007, 2015, McKenzie et al. 2021). This variation is supported by the dietary dichotomy (i.e. consumption of distinct dietary items) between seagrass and algae at the control site, and the predominant seagrass consumption at the industrial and agricultural sites (Bell et al. 2019). Dietary dichotomy has also been reported in other studies and may be due to spatial and temporal variations in environmental conditions and/or available food sources and their related nutritional content (Bjørndal 1996, Brand et al. 1999, André et al. 2005, Arthur 2005, Esteban et al. 2020). A thorough nutritional analysis of the seagrass meadows from which the turtles were sampled, and a comparison with the algal species that green turtles consume, might have provided further insight on the differences in predicted adipose tissue observed across sites.

The data presented in this study also indicate that adipose tissue increases as turtles age, with adult turtles having the highest proportion of adipose tissue (Figs. 3 & 4). This finding is not surprising, since adult turtles allocate higher proportions of their energy budget to storage and reproduction rather than to growth (Carr & Goodman 1970, Bjørndal 1982, Kwan 1994). This finding is further supported by the somatic growth rates of turtles at the examined sites, which peak in juvenile individuals (CCL <60 cm) and gradually decrease as they reach maturity (Bell et al. 2019). The same growth patterns were reported in other foraging aggregations within the GBR (Limpus & Chaloupka 1997, Chaloupka et al. 2004) and for turtles in the West Atlantic (Bjørndal et al. 2017).

### 4.3. Adipose tissue estimations in nesting females

Assuming that green turtles are capital breeders, adult females will have to store sufficient fat to migrate to their nesting beaches, produce enough

follicles and eggs to maximise reproductive output, and migrate back to the foraging site (Kwan 1994, Hamann et al. 2002, Hays et al. 2002). Vitellogenesis in green turtles takes approximately 9 mo, and deposition of fat reserves occurs before they are used for reproduction (Kwan 1994). Bjørndal (1985) estimated that a nesting female would store up to 8.8% of body mass as adipose tissue for a successful nesting period. Interestingly, this percentage is very similar to the predicted adipose tissue values of adult females sampled shortly before and at the end of the nesting season ( $6.7 \pm 0.8\%$  and  $6.0 \pm 0.8\%$  mean adipose tissue, respectively) (Figs. 3 & 4). Adult females at the control site had, comparatively,  $4.4 \pm 0.6\%$  mean adipose tissue, which is 52 and 36% lower than turtles sampled shortly before and at the end of the nesting season (Fig. 4). Females sampled shortly before the nesting season might have been ready to breed and might have had increased fat stores to prepare for reproduction. In comparison, sampling at the control site occurred outside of the nesting season (August, Southern Hemisphere), when fat stores were likely decreasing. This finding underscores the potential for BIA to assess adipose tissue changes in adult females during and across nesting seasons, which is also supported by the high precision of the impedance measurements (Kophamel et al. 2023). Future studies might benefit from exploring the links between reproductive output and adipose tissue.

#### 4.4. Study limitations

Our study was subject to several limitations, which were assumed not to affect the observations of the study and are further discussed in this section.

##### 4.4.1. Calibration on immature turtles

Immature green turtles were used in a previous study (Kophamel et al. 2022c) to calibrate the BIA device using computed tomography scans, and to develop the equation for adipose tissue estimation (Eq. 2). The calibration could not be performed on adult turtles due to size limitations of the computed tomography scanner. We assumed that the parametrisation of the model would not be altered if adult turtles were examined. In human-based studies, calibration across a wide range of body shapes and sizes is recommended to best represent the general population and to avoid potential inaccuracies in prediction in individuals at the extremes of body size (Kyle

et al. 2004). However, the positive linear relationship between predicted adipose tissue and life stage (Fig. 3) would support applying the calibration equation to adult turtles. Further investigation into the validity of the calibration equations on adult turtles is warranted.

##### 4.4.2. Seasonality effects

Field sites were visited at different times during the year due to logistical and time constraints and to avoid travelling during the cyclone season. Site effects were confounded by season, which may impact adult females more than adult males or immature turtles. The degree to which site vs. season affects adipose tissue across life stages and sexes is still unclear. Ideally, sampling should be seasonally matched to make comparisons across sites.

##### 4.4.3. Environmental and physiological confounders

A previous BIA validation study conducted on green turtles identified that impedance measurements were affected by increased environmental temperatures (which were particularly high at the agricultural site, see Table S1), time postprandial, and handling stress, although the relative differences (%) were found to be small in magnitude (Kophamel et al. 2023). Avoiding environmental confounders, such as high environmental temperatures ( $>30^{\circ}\text{C}$ ), is often not possible in field-based studies, as the timing of field trips is dependent on staff availability, planning, and financial constraints. Prandial state could not be assessed in this study without gastric lavage. We therefore assumed that the foraging animals had recently eaten. Effects of feeding have also been examined as a potential confounder in bioimpedance measurements in humans, with studies observing no significant impact on whole body impedance measurements (Hirsch & Smith-Ryan 2022). Acute stress (i.e. alterations in cardiovascular function and blood flow distribution) might also affect the impedance measurements and increase inter-animal variability to an unknown extent (Kophamel et al. 2023). Although handling stress was minimised by covering the turtles' eyes and by regularly cooling the turtles with water (Goodman et al. 2013, New South Wales Government, Department of Primary Industries 2020), the turtles sampled in this study were not used to human handling and likely experienced higher levels of acute stress than cap-

tive turtles used to handling. Nevertheless, high inter-animal variability in reptiles is not surprising, since sea turtles, and reptiles in general, have wider physiological tolerances than other taxa, are poikilothermic, have an intermittent and labile breathing pattern, and some species can tolerate prolonged fasting (Munns 2000, 2013, Hartzler et al. 2006a,b, Sacchi et al. 2020). Increased variability might lead to wider confidence intervals in the predictions and should therefore be accounted for when designing research studies on reptile species. It is worth mentioning that impedance measurements on non-fresh turtle carcasses are not recommended, since these will be in a state of decomposition. Fluid shifts related to decomposition (Payne 1965, Payne et al. 1968, Vass et al. 1992) will affect impedance measurements and will result in misleading body composition estimates. Thawed carcasses will also be affected by chemical and physical changes to body tissues, and only fresh (e.g. recently euthanised) specimens might be suitable for BIA examination. Should researchers still be interested in using the BIA technique in deceased animals, a separate validation and calibration study will be required.

These limitations should be acknowledged in future studies, and we recommend following species-specific BIA standardisation protocols (such as the one provided by Kophamel et al. 2023) to minimise the impact of confounding factors on impedance measurements.

#### 4.5. Future directions

Future studies should expand the BIA technique to other sea turtle species. The anatomy of sea turtles, and particularly the distribution of adipose tissue within the body, is very similar across species (with the exception of the leatherback turtle *Dermochelys coriacea*) (Wyneken 2001). Consequently, the high precision and accuracy levels of BIA for adipose tissue prediction in green turtles might be very similar in other chelonid species.

The BIA technique should also be tested, in combination with other health assessment methods, on emaciated and sick turtles. Future studies could then include these turtles in sampling regimes, thereby ensuring a robust reflection of sea turtle population health. In nesting females, BIA could be used to further explore the link between adipose tissue and reproductive output; in rehabilitating turtles, adipose tissue estimation might shed light on treatment efficacy and/or dietary supplementation; and in captive

animals, diets could be adjusted accordingly by assessing changes in adipose tissue values.

Foraging ground assessments in combination with demographic analyses and adipose tissue assessments may help identify whether foraging sites contain the resources needed to meet the nutrient requirements of sea turtles. Conservation efforts should evaluate the methods used in monitoring programmes to timely identify changes in population size and viability. Conservationists, and stakeholders in general, could use these data to advocate for habitat protection actions.

**Data availability.** The supplementary material contains additional tables on animal characteristics, predicted adipose tissue values, statistical analyses, and a description of the parameters examined and codes used in the data sets. Data sets (.xlsx, .ods., and .csv formats) are available at James Cook University Data Repository under the following link: <https://doi.org/10.25903/73jy-ky07>.

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