Surfacers change their dive tactics depending on the aim of the dive: evidence from simultaneous measurements of breaths and energy expenditure

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Air-breathing divers are assumed to have evolved to apportion their time between surface and underwater periods to maximize the benefit gained from diving activities. However, whether they change their time allocation depending on the aim of the dive is still unknown. This may be particularly crucial for ‘surfacers’ because they dive for various purposes in addition to foraging. In this study, we counted breath events at the surface and estimated oxygen consumption during resting, foraging and other dives in 11 green turtles (Chelonia mydas) in the wild. Breath events were counted by a head-mounted acceleration logger or direct observation based on an animal-borne video logger, and oxygen consumption was estimated by measuring overall dynamic body acceleration. Our results indicate that green turtles maximized their submerged time, following this with five to seven breaths to replenish oxygen for resting dives. However, they changed their dive tactic during foraging and other dives; they surfaced without depleting their estimated stores of oxygen, followed by only a few breaths for effective foraging and locomotion. These dichotomous surfacing tactics would be the result of behavioural modifications by turtles depending on the aim of each dive.

1. Introduction

For air-breathing divers, underwater activity is constrained by the available oxygen stored in the body. Under such physiological constraints, they are assumed to have evolved to apportion their time between surface and underwater periods to maximize the benefit gained from activities such as feeding, predator avoidance and mating [1]. Therefore, from an ecological point of view, it is essential to understand dive-induced respiratory patterns in air-breathing divers. Hence, a number of studies have considered how air-breathing divers maximize the rate of oxygen/energy gain (optimal diving/foraging theory; [2,3]) or how often diving animals stay within or exceed their aerobic dive limit (ADL) in nature [4,5]. In optimal foraging theory, however, completely depleting the available stored oxygen during a dive does not always maximize the rate of energy gain. Indeed, Thompson & Fedak [6] predicted that the decision to terminate a dive would yield a net benefit if no prey were encountered early during shallow dives, suggesting that air-breathing divers use different dive tactics with respect to the amount of oxygen consumed during a dive and consequently, the time spent breathing for the subsequent dive depending on the situation of each dive. This idea may be particularly crucial for so-called ‘surfacers’, such as
elephant seals (*Mirounga leonina* and *Mirounga angustirostris*), grey seals (*Halichoerus grypus*), and all species of sea turtle, which spend most of their time underwater and surface only briefly for gas exchange [7], because surfacers are assumed to maximize not only the foraging benefits of their dives but also benefits related to various other purposes (e.g. resting, mating and migration).

To understand such diving strategies/tactics of air-breathing animals, it is essential to measure both the energy expenditure during a dive and subsequent respiratory performance at the surface. A wide variety of approaches have been developed to determine energy expenditure during a voluntary dive in the wild, including heart rate [8,9], doubly labelled water (DLW) [10], and overall dynamic body acceleration (ODBA) [11]. Meanwhile, respiratory performance has been investigated using spectral analysis based on heart rate [12], an acoustic device [13], a hall sensor [14], an acceleration sensor [15] and direct observation based on animal-borne video data [16]. Unfortunately, these measurement techniques for energy expenditure and respiratory performance have not yet been used simultaneously to reveal the energy expended during a dive and subsequent respiratory performance at the surface.

Sea turtles are ectothermic marine animals and have well-adjusted physiological functions for prolonged dives (reviewed by Lutcavage & Lutz [17] and Williard [18]). Briefly, they have a considerably slower metabolism than do diving mammals and birds, but it is affected by water temperature and activity level [19–24]. They begin a voluntary dive with a level of oxygen in their body that approaches saturation, and they complete it at a level approaching depletion [19,25,26]. Their highly elastic reinforced lungs and high lung oxygen diffusion capacity allow them to reduce the time spent at the surface [26]. Their respiration frequency is related to the duration and activity level of a preceding dive and to water temperature [16,20–23]. However, all of these previous studies, except Reina et al. [16], were conducted under experimental conditions in and limited space.

In this study, we simultaneously counted breath events at the surface and estimated the oxygen consumption during limited space. Following Okuyama et al. [27], we classified dives into three groups based on depth and acceleration data, namely resting, foraging and other dives. Resting dives corresponded exactly to U-dives, which are characterized by a long flat-bottom phase with low dynamic acceleration. Foraging dives contained feeding events that were detected by head motion of the turtles at 30 frames s\(^{-1}\). Their respiration frequency is related to the duration and activity level of a preceding dive and to water temperature [16,20–23]. However, all of these previous studies, except Reina et al. [16], were conducted under experimental conditions in and limited space.

In this study, we simultaneously counted breath events at the surface and estimated the oxygen consumption during various types of dive in green turtles (*Chelonia mydas*) in the wild. Following Okuyama et al. [27], we classified dives into three groups based on depth and acceleration data, namely resting, foraging and other dives. Resting dives corresponded exactly to U-dives, which are characterized by a long flat-bottom phase with low dynamic acceleration. Foraging dives contained feeding events that were detected by head acceleration or direct observation from video loggers. Other dives included all remaining dives that represented active behaviours, such as shuttling between a resting place and a foraging ground or searching. For more details, see Okuyama et al. [27]. Breath events were counted using a head-mounted acceleration logger or direct observation based on an animal-borne video logger, and oxygen consumption was estimated by measuring ODBA and using equations developed by Enstipp et al. [23] and Halsey et al. [24] to convert ODBA to oxygen consumption. The objectives of this study were to clarify how green turtles manage their energy expenditure during a dive and subsequent respiratory patterns based on the purpose of the dive (resting, foraging and other) under natural conditions and to determine which dive tactics they use.

2. Material and methods

(a) Study area and experimental animals

This study was conducted around Irinomote Island, Okinawa, Japan (24°20′N, 123°50′E). We used 11 juvenile green turtles (straight carapace length: 62.4 ± 7.3 cm, body weight: 34.2 ± 9.1 kg; electronic supplementary material, table S2) that were hand captured by a local fisherman with the permission of the Okinawa Prefecture. The turtles were identified as CM1 through to CM11 (electronic supplementary material, table S2). After taking physical measurements of the turtles, a 1 to 4 day holding period in a 500 l tank at the Research Centre for Subtropical Fisheries, Seikai National Fisheries Research Institute, Fisheries Research Agency, Japan, was used to test for any negative impacts that might have been caused by the equipment. During this period, the turtles were not fed. Data for CM1–5 were also used in the analysis by Okuyama et al. [27].

(b) Instruments and experimental protocol

We used various types of data loggers (electronic supplementary material, table S2). To monitor breathing behaviour in turtles CM1 and CM2, we used a small acceleration data logger (M190L-D2DG: 15 mm diameter, 53 mm length, 17 g, Little Leonardo, Tokyo, Japan) that recorded two-axis acceleration at 1/32 s intervals. For the other turtles, we used a video data logger (GoPro HD, Woodman Labs, Half Moon Bay, CA, USA) with a custom-made waterproof case (Logical Product, Fukuoka, Japan: 72 × 56 × 88 mm, 266 g weight in air) that recorded the head motion of the turtles at 30 frames s\(^{-1}\). To measure depth and ODBA, multi-sensor data loggers (W1000-3MPD3GT, Little Leonard: 26 mm in diameter, 175 mm in length, 140 g in air for CM1–2 or LP-KUBL1101, Logical Product: 30 mm in diameter, 170 mm in length, 108 g in air for CM3–11) that recorded depth and temperature at 1 s intervals and three-axis acceleration at 1/16 s intervals were used. For CM3–7, only depth was measured and recorded using a Mk10-F, Wildlife Computers, WA, USA (86 × 83 × 32 mm, 180 g in air), with depth and temperature recorded at 10 s intervals. The frontal areas of the tags were 21, 54 and 66 cm\(^2\) for turtles CM1–2, CM3–7 and CM8–11, respectively.

To retrieve the data loggers from released turtles, we used a time-scheduled release system. Each data logger was attached to a float made of copolymer foam (Nichiyu Kiken Kogyo, Saitama, Japan) in which a VHF transmitter (130 BB, Advanced Telemetry Systems, Isanti, MN, USA) and a time-scheduled release mechanism (Little Leonardo) were embedded. A logger unit was affixed to the centre of the carapace and the head of a turtle using epoxy putty (Konishi, Osaka, Japan), two-component epoxy resin (ITW Products, Osaka, Japan) and a plastic cable tie connected to the time-scheduled release mechanism. The logger units were designed to be less than 4% of a turtle’s body weight. Although the logger units increased drag [28], no behavioural changes were observed during visual observations in preliminary attachment tests in the holding tank.

Turtles equipped with logger units were released at their capture point. The time-scheduled release mechanisms were programmed to activate 8–168 h after release, at which time an electric charge would incite the plastic cable. The logger units then detached from the turtles and floated to the sea surface. All of the logger units were located by radio telemetry using radio receivers and a four-element Yagi antenna (FT-290mk-II/AR; Yaesu Musen, Tokyo, Japan).

(c) Data analysis

The time-series data obtained were analysed using IGOR Pro v. 6.2 (WaveMetrics, Inc., Lake Oswego, OR, USA) and ETHROGRAPHER v. 2.00 software (K. Q. Sakamoto, Hokkaido University, Sapporo,
Japan; for details, see [29]). Dive parameters were extracted for each dive. Dive duration was defined as the time period between two consecutive breaths. However, two-thirds of the observed breath intervals were less than or equal to 30 s (see Results; electronic supplementary material, figure S1). In addition, as air-breathing divers such as birds [30], seals [31] and whales [32,33] manage their oxygen use and lactate metabolism during multiple cycles and not a single dive cycle, we investigated the appropriate duration of a single breath bout and found that a 30 s duration gave the best relationship between the number of breaths (NB) and the total oxygen consumption during a dive (electronic supplementary material, figure S2). Therefore, consecutive breaths that occurred within 30 s were regarded as a single breath bout, and the time period between them was not considered a dive. Moreover, dives with a maximum dive depth of less than 0.5 m were excluded from the analysis because recorded ODBA values appeared to be higher than usual when turtles were at the surface owing to the effect of surface waves (figure 1). Post-dive surface duration (PDSD) was defined as the period when turtles stayed at a depth of less than 0.5 m after a dive.

(d) Extraction of breaths

Breath events were extracted from head acceleration data and video data. With head acceleration, breath events can be extracted by detecting head angle, which was calculated from static acceleration [15]. Briefly, static acceleration was extracted using a low-path filter (IFDL v. 4; WaveMatrics) using a value defined from observations of breathing behaviours in the tank prior to the experiments because static acceleration is present as a low-frequency acceleration signal. We defined breathing behaviour as occurring when the pitch was greater than 30° and the water depth was less than 0.15 m. A detailed description of the method used to extract breaths from head acceleration data is given in Okuyama et al. [15]. From the video data, the NB was visually counted. Additionally, breath duration was estimated only from video data by calculating the time period during which the turtle’s head rose above the sea surface during frame-by-frame observation to determine differences in breath duration among breaths during a single breath bout.

(e) Extraction of dynamic body acceleration and estimation of energy expenditure

Recent studies have suggested that acceleration can serve as a proxy for the rate of energy expenditure in free-living animals [11]. This is because energy is required for mechanical work, and activity can account for a large proportion of the variation in metabolic costs. In this study, we calculated ODBA (defined in [11]) as an index of activity during a dive. ODBA was calculated from the arithmetic sum of dynamic acceleration extracted from three-axis acceleration data. Dynamic acceleration was calculated by subtracting static from raw acceleration [15].

We estimated oxygen consumption rate during dives from ODBA using conversion equations given in previous reports by Halsey et al. [24] and Enstipp et al. [23]. These studies demonstrated that ODBA was useful for estimating oxygen consumption rate ($V_{O_2}$) in green turtles. The conversion equations were given by:

\[
V_{O_2,Halsey} (\text{ml } \text{min}^{-1}) = 506.14 \times \text{ODBA(g)} + 14.64
\]

and

\[
V_{O_2,Enstipp} (\text{ml } \text{min}^{-1} \text{kg}^{-0.83}) = 12.17 \times \text{PDBA(g)} + 0.03 T_w (\text{C}) - 0.46,
\]

where PDBA is partial dynamic body acceleration, which is almost the same as ODBA but is calculated from two-axis acceleration (surge and heave) [23]. The equation of Enstipp et al. [23] included $T_w$ (water temperature) in the model, whereas the equation of Halsey et al. [24] did not.

(f) Linear mixed model

Using the dataset of NB and estimated energy expenditure (CM1, CM2 and CM8–11) measurements, we investigated the factors regulating NB in turtles using a linear mixed model (LMM). The NB was treated as an explanatory variable and estimated energy expenditure during the preceding dive, activity state (resting, foraging or other) and water temperature were dependent variables. LMM analysis was carried out for each of the estimations with the equations of Halsey et al. [24] and Enstipp et al. [23]. Water temperature was only included when using the equation of Halsey et al. [24] because the equation of Enstipp et al. [23] already included an effect of temperature (see above).

We used a mixed model because the repeated sampling of dive parameters from the same individuals creates a hierarchical structure [34]. Therefore, individual was treated as a random effect. The significance of the fixed effects on the dependent variables was assessed by a likelihood ratio test using the log-likelihood of the test model (including fixed effects) and the null model (without fixed effects) [34]. We used the ‘lme4’ package in the software R v. 3.0.1 [35] to run the LMM analyses.

3. Results

(a) Breath events

A total of 684 dive datasets (dive duration, ODBA and PDSD), including 2237 breaths by 11 immature green turtles, were extracted (electronic supplementary material, table S3); of these, 555 datasets included both NB and ODBA. Typical examples of breaths and dive profiles are shown in figure 1. The interval between successive breaths was 2.6 ± 5.8 min, but 68% (1522 out of 2237) of events occurred within 30 s (electronic supplementary material, figure S1). The time duration which a turtle’s head rose above the sea surface in each breathing event was 1.46 ± 0.30 s, which did not differ significantly according to NB or different types of dive (electronic supplementary material, table S4). NB was significantly related to PDSD (ANOVA, $n = 684$, $F = 1098$, $p < 0.001$), but several breaths fell outside the 95% prediction interval based on the linear regression (figure 2). All of these outliers from the prediction interval were higher PDSD values, and acceleration data revealed that most were associated with respiratory behaviour, although some were related to surface swimming at depths less than 0.5 m.

The mean NB following each dive was 3.3 ± 2.0, and two significant outliers (22 and 24 breaches) were recorded (Smirnov–Grubbs test, $p < 0.05$). The NB was significantly different among resting dives ($n = 177$, mean ± s.d. = 5.9 ± 2.5), foraging dives ($n = 113$, 2.1 ± 1.6) and other dives ($n = 394$, 2.3 ± 2.2) (ANOVA, $F = 171.0$, $p < 0.001$; figure 3). Video observation and depth profiles showed that during resting dives, individuals took breaths without continuing to swim, although they often rotated. During this rotating behaviour, the turtles kept their heads down and appeared to be engaging in locating/searching behaviours for a resting place before surfacing (figure 1; electronic supplementary material, movie S1). Indeed, turtles generally returned to the same resting place (electronic supplementary material, movie S1). During foraging and other dives, meanwhile, the turtles took breaths while continuing to feed or swim (figure 1; electronic supplementary material, movies S2 and S3). Two significant outliers occurred in the series, one before and one after a resting dive, between morning and midday foraging trips to a seagrass meadow [27].
Figure 1. Typical profiles of dive, ODBA, and head pitch during (a) resting, (b) foraging and (c) other dives in a green turtle (CM2). The horizontal bars at the bottom represent the timing of breath, surface and dive events, respectively. (d–f) Enlarged views of the time periods enclosed by dashed boxes in (a–c), respectively. (Online version in colour.)
(b) Estimated oxygen consumption
The water temperature that the turtles experienced during the experiments was 28.5 ± 0.9 °C (electronic supplementary material, table S2). The dive durations during resting, foraging and other dives were 21.9 ± 7.2, 4.4 ± 2.0 and 5.2 ± 5.1 min, respectively, which was significantly different among the dive types (ANOVA, F = 665.4, p < 0.001; electronic supplementary material, table S3). The values for oxygen consumption rate ($V_O$), estimated using the Halsey et al. [24], were 0.69 ± 0.19, 0.95 ± 0.27 and 1.02 ± 0.38 ml min$^{-1}$ kg$^{-1}$ during resting, foraging and other dives, respectively. Using the equation of Enstipp et al. [23], these values were 0.30 ± 0.05, 0.38 ± 0.04, and 0.39 ± 0.06 ml min$^{-1}$ kg$^{-1}$, respectively (electronic supplementary material, table S3; figure 4). There were significant differences among the dive types in the estimates using the equations of Halsey et al. (ANOVA, F = 61.9, p < 0.001) and Enstipp et al. (ANOVA, F = 31.4, p < 0.001).

As for the total oxygen consumption during each dive ($V_O$), values were estimated at 14.54 ± 3.70, 4.01 ± 2.31 and 5.10 ± 4.57 ml kg$^{-1}$ using the equation of Halsey et al. during resting, foraging and other dives, respectively. Using the equation of Enstipp et al., these values were 6.34 ± 1.69, 1.56 ± 0.82 and 2.07 ± 1.91 ml kg$^{-1}$, respectively (electronic supplementary material, table S3). The greater energy expenditure during resting dives was due to the longer dive duration than for the other two types of dive. There were significant differences in the total oxygen consumption during each dive among the dives estimated using both the equations of Halsey et al. (ANOVA, F = 34.8, p < 0.001) and Enstipp et al. (ANOVA, F = 38.4, p < 0.001).

(c) Relationship between the number of breaths and dive performance
The LMM analysis using the 555 datasets that included measures of both NB and dive performance revealed that NB increased significantly with larger energy expenditures as estimated by both the equations of Halsey et al. ($\chi^2 = 114.7, p < 0.0001$; figure 5a) and Enstipp et al. ($\chi^2 = 94.6, p < 0.0001$; figure 5b), but not with water temperature ($\chi^2 = 0.00, p = 1$, for the equation of Halsey et al. only). The activity state (i.e. resting, foraging or other dive) also significantly affected NB using both the equations of Halsey et al. ($\chi^2 = 17.7, p < 0.001$) and Enstipp et al. ($\chi^2 = 13.8, p < 0.01$).

4. Discussion
(a) General discussion
Here, we quantitatively demonstrated how NB in sea turtles changed with energy expenditure during the preceding dive and how respiratory patterns differed among dive types in the wild. In most previous studies conducted under limited experimental conditions, turtles took only a few breaths (e.g. [23,25]). However, our study in the wild revealed that NB usually varied within the range of 1–12, indicating that it is important to conduct an experiment under natural conditions to correctly understand the respiratory physiology of sea turtles.

The NB was significantly correlated with PDSD and most values fell within the 95% prediction intervals of the linear regression, indicating that PDSD could be regarded as a proxy for NB, that is, for respiratory behaviour. During resting dives, turtles appeared to locate or search for the resting place at the surface (electronic supplementary material, movie S1). If this behaviour was the reason for the increment in PDSD, no clear relationship between NB and energy expenditure would appear. Thus, this searching at the surface would be an incidental behaviour with breathing.

The period during which a turtle’s head rose above the sea surface during each breath did not differ significantly with NB. Magellanic penguins (Spheniscus magellanicus) change their beak angle, representing tidal volume, systematically over the surface, pause, initially starting with the beak high and then decreasing the height before raising it again to a maximum just before diving [37]. Sea turtles change the volume of air inspired into their lungs to adjust their buoyancy for each dive [38,39]. Therefore, our results suggest that the difference in tidal volume in each breath is not reflected in the period during which the turtle’s head rises above the sea surface. Further study is needed to determine how turtles change the volume of air inspired during the breath sequence at the sea surface, although turtles took only a few breaths under limited experimental conditions (e.g. [23,25]).

(b) Energy expenditure during dives
The ODBA allows us to estimate oxygen consumption during each dive, which is not possible with DLW. Estimated oxygen consumption rates during each dive were 0.38, 0.95 and 2.04 ml min$^{-1}$ kg$^{-1}$, respectively (electronic supplementary material, table S2). The greater energy expenditure was significantly different among the dive types (ANOVA, F = 61.9, p < 0.001; electronic supplementary material, table S3). The greater energy expenditure was significantly different among the dive types (ANOVA, F = 61.9, p < 0.001) and the equation of Enstipp et al. [23], these values were 0.30 ± 0.05, 0.38 ± 0.04, and 0.39 ± 0.06 ml min$^{-1}$ kg$^{-1}$, respectively (electronic supplementary material, table S3; figure 4). There were significant differences among the dive types in the estimates using the equations of Halsey et al. (ANOVA, F = 61.9, p < 0.001) and Enstipp et al. (ANOVA, F = 31.4, p < 0.001).

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consumption rates for our study fell within the range of the results of previous studies that used respirometry ([22], 0.46–0.49 ml min\(^{-1}\) kg\(^{-1}\) at 26°C; [36], fasted turtle = 0.47 ml min\(^{-1}\) kg\(^{-1}\) at 25.1°C, fed turtle = 0.99 ml min\(^{-1}\) kg\(^{-1}\) at 25.8°C; figure 4), indicating that our estimate is reliable. These conversion equations were developed under limited experimental conditions (e.g. small tank), which may have led to incorrect estimates during this study. The confidence of these two formulae for the estimation of oxygen consumption rate in our turtles is reinforced by comparing the conditions of turtles and the experimental environment between our study and these two studies. Halsey et al. [24] used turtles of similar size to those in our study. Although the equation of Halsey et al. [24] did not include the effect of water temperature, the water temperature in our study fell within the range of that of the equation of Halsey et al. (22–30°C). Therefore, these factors would not affect the estimation result derived from the equation of Halsey et al., indicating the confidence of this equation for our turtles. The difference in the estimation result between the two formulae appears to be caused mainly by the low metabolic rate of the turtles used in Enstipp et al. [23]. They reported that the metabolic rate of their turtles was lower than that in other studies, although the oxygen consumption rate of these turtles was expected to be higher because they continued feeding during the experiments (see below) [23]. Therefore, the estimation results derived from ODBA using the equation of Enstipp et al. [23] would also be low. Moreover, the turtles used in Enstipp et al. seemed to be fatter than normal turtles owing to long-term rearing, which might have resulted in an underestimation of energy expenditure per unit body weight. Consequently, the confidence of the equation of Enstipp et al. for our turtles might not be high. Although the values estimated using the equations of Halsey et al. [24] and Enstipp et al. [23] differed, the linear relationship between NB and the estimated total oxygen consumption during each dive in this study is reasonable. A similar linear relationship was observed between the oxygen consumption rate and breath rate in the hatchlings of leatherback (*Dermochelys coriacea*) and olive ridley (*Lepidochelys olivacea*) turtles under laboratory conditions [40].

The ODBA can estimate only energy expenditure from mechanical work by body movements and cannot estimate internal energy expenditure, such as from digestion. The oxygen consumption rate of fed green turtles is approximately twice that of fasted turtles [36] (figure 4). Indeed, recovery oxygen consumption during PDS during foraging...
dives increased by 44.7% compared with non-foraging dives in Weddell seals (Leptonychotes weddellii) [41]. In our study, some of the turtles (CM2–3, CM5–6, CM9 and CM11) performed feeding bouts (as defined in [27]) before or during the recording period. It was reported that the digesta retention time in juvenile green turtles is approximately 6.5–13.5 days [42], indicating that these turtles were in an absorptive state during the recording period. Halsey et al. [24] used fasted turtles, whereas Enstipp et al. [23] fed their turtles. Thus, the actual energy expenditure of these feeding turtles may have been approximately twofold higher at a maximum than was estimated using the equation of Halsey et al. [24], whereas values for non-feeding turtles may have been approximately 50% lower than was estimated by the equation of Enstipp et al. [23].

Many studies have demonstrated that an external instrument increases the drag and consequently locomotion cost of marine animals (e.g. [28,43–45]). Jones et al. [28] calculated the drag increase of aquatic animals resulting from animalborne instrumentation. Considering this, the equations used in Halsey et al. [24] and Enstipp et al. [23] required data obtained from small archival loggers with which the drag increase was assumed to be less than 5%. In comparison, the drag increase from the attachments used in our study is expected to be 10–20% for turtles CM1–2, 20–30% for turtles CM3–7 and 30–40% for turtles CM8–11, according to Jones et al. [28]. Therefore, the increment in drag from attachment of larger instruments in our study might put an extra load on turtles swimming, resulting in a larger ODBA during a dive in comparison with that during dives without an instrument. This suggests that the oxygen consumption rate estimated in this study was higher owing to the increased drag, although it is not clear that the drag increase is linearly related to the increment in energy expenditure.

(c) Surfacing tactics

The LMM analysis revealed that NB in green turtles was not significantly regulated by water temperature in the present study. Water temperature has significant effects on oxygen consumption and consequently on dive duration in sea turtles [21]. Because there were no substantial fluctuations in water temperature during the experiments in this study (electronic supplementary material, table S2), it would not have had a significant effect on our dive duration results [46].

Respiratory patterns in sea turtles were significantly different among the dive types; the turtles took more breaths after resting dives than after foraging and other dives. Previous studies with green turtles have estimated that the oxygen content of the lungs at the start of a dive is 17.4 vol% [19], and the maximum lung capacity averages 75–121 ml kg\(^{-1}\) [19,47]. Assuming that the oxygen content of the blood and muscles of green turtles is the same as that in loggerhead turtles (Caretta caretta), i.e. 6.7 ml kg\(^{-1}\) [25], the maximum total oxygen store is 19.75–27.75 ml kg\(^{-1}\). Tidal volume was reported to be 24.4–24.62 ml kg\(^{-1}\) [20,48]. Based on the fact that the oxygen content in air is 21 vol%, the amount of oxygen uptake in each inhalation is 5.12–5.17 ml kg\(^{-1}\). Therefore, if turtles almost completely deplete the oxygen stored in their body, they need to take at least 3.8–5.9 breaths to achieve saturation, although NB would increase in nature because the rate of oxygen uptake into the body tissues decreases with cumulative oxygen acquired in the body tissues owing to the difference in the partial pressure of oxygen between body tissues and lungs [49]. Our study showed that the modal NB during resting dives was 5–7, which is a convincing value with regard to ventilation for oxygen replenishment. Moreover, this result indicates that the turtles almost always completed their resting dives using aerobic metabolism because if their dives involved anaerobic metabolism, the NB would need to include additional increments to break down lactate acid. This idea may be supported by the estimates obtained from the equation of Halsey et al. [24] because the total oxygen consumption during most of the resting dives was slightly lower than the range of the maximum oxygen stored in their bodies (figure 5).

During resting dives, NB increased linearly with the total oxygen consumption during the preceding dive. Okuyama et al. [46] suggested two possible reasons for the increase in PDSW with dive duration and activity level, and thus with energy expenditure, during the preceding dive. One is that turtles need time to break down the small amounts of lactate acid that accumulates due to peripheral ischaemia. Another is that small variations in oxygen consumption or intake cause the observed differences during subsequent PDSW, although the amount of oxygen consumed by turtles during dives is almost constant [19,25,26]. Based on the discussion about the frequency distribution of NB in resting dives (see above), the latter is a reliable explanation. Thus, our study indicates that during resting dives, turtles surface with oxygen content in their bodies that is almost depleted, that does not exceed the ADL, and that varies little. They then dive with saturated oxygen content, although the oxygen content in the lungs changes with the intended dive depth [38,39]. This surfacing tactic allows them to dive as long as possible, reducing surfacing effort.

No significant difference was found between the tidal volume of active and that of resting turtles, and no relationship was found between tidal volume and water temperature in either group [21]. During foraging and other dives, NB was lower than that during resting dives, and most counts were fewer than three. Meanwhile, the estimated total oxygen consumption during each foraging and other dives was lower than that during resting dives. Moreover, NB showed a linear relationship with the estimated total oxygen consumption during these dives. From these facts, there are two possible explanations for the surfacing tactics employed in foraging and other dives. One is that turtles replenish their oxygen content to the saturation level before a dive but do not make maximum use of the available amount of oxygen. Consequently, turtles do not need to take as many breaths for replenishment as they do in resting dives. Another explanation is that turtles do not uptake oxygen to the saturation level during foraging and other dives, and they deplete their oxygen before surfacing. However, in other dives, there was wide variation in the amount of oxygen consumed during a dive. This indicates that the former explanation is suitable as a surfacing tactic for other dives because dive duration would be limited within a shorter range by aerobic metabolism if turtles do not uptake oxygen to the saturation level. This is supported by the fact that juvenile green turtles maintained the oxygen content in their blood at high levels during a short period of swimming [20]. However, there is little variation in the relationship between NB and the estimated total oxygen consumption, which might be caused by the oxygen expenditure/uptake in the preceding dive because several air-breathing divers manage their oxygen use and lactate metabolism during multiple cycles and not a single dive cycle.
Turtles sometimes took five to seven breaths during foraging and other dives, as well as resting dives. Therefore, turtles do not always complete dives without depleting the oxygen content in their body. This also suggests that turtles replenish the oxygen that gradually decreased during a sequence of preceding dives. The linear relationship between NB and the estimated total oxygen consumption also indicates that foraging and other dives are almost always completed using aerobic metabolism because there would be no significant linear relationship if anaerobic metabolism were also used to provide locomotory energy. This is consistent with the results of a previous experiment in a tank during which juvenile turtles metabolized aerobically with no or little resort to anaerobiosis during swimming [20].

During foraging dives, turtles fed on seagrass in shallow water (less than 3 m). From the perspective of optimal foraging theory for air-breathing divers [1,2], if the travel cost from the surface to the foraging area is low, then the time spent at the surface should also be low to maximize the time spent in the foraging area. Therefore, turtles in our study would use a dive tactic that consists of continuous dives with a few breaths during each surfacing event to maximize the energy obtained.

Most of the other dives occurred during movement between the feeding ground and the resting place [27]. During this phase, the turtles took breath(s) while swimming. In most cases, only one breath was taken during surfacing. Even though a few breaths were sometimes taken during a single surfacing event, the turtles performed a short-period of shallow dives between breaths (figure 1; electronic supplementary material, movie S3). Swimming at the surface is associated with high drag on animals [50]. In addition, turtles always have to hold their head up when breathing, which would impose further drag. Therefore, this respiratory pattern would be an adaptive behavior to efficiently achieve both respiration and migration. Moreover, surfacing without depleting the body’s oxygen content may also be an adaptive behavior because oxygen depletion requires multiple breaths within a short interval, which may not be efficient.

More than 20 breaths were taken in only a very small proportion of breath events (0.3%: two out of 684). Several possible reasons are advanced for this unusual respiratory behaviour. If this occurs for respiration, it may be in response to anaerobic metabolism owing to the increment in energy expenditure by digestion [36] or some unknown reason. Other reasons involve searching for a resting place and warming [51].

(d) Conclusion

Surfaces are expected to maximize the ratio of submerged to surface time during dives so as to minimize surface time [1,2]. Indeed, northern elephant seals minimize the surface time spent loading oxygen during a foraging trip [13]. In our study, the green turtles also maximized the time submerged during resting dives, which is supported by the confidence of the equation of Halsey et al. [24] and the result of NB during resting dives. If the equation of Enstipp et al. [23] is true, turtles do not expend much of the oxygen stored in their body during dives. In either case, they changed their dive tactic while foraging and in other dives; they surfaced without depleting their estimated stores of oxygen for effective foraging and locomotion. These dichotomous dive tactics were apparently the result of behavioural modifications by turtles depending on the aim of each dive. The dichotomy in the respiratory patterns at the surface may allow researchers to estimate the activity state of sea turtles during boat-based and aerial surveys.

Ethics statement. This study was conducted with the permission of the Okinawa Prefecture (Permission no. 22-3, 23-2, 24-4) for conducting the experiment, and the experimental protocol was approved by Animal Research Committee of Kyoto University (no. 22-4, 24-4).

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