Preliminary result of the relationship between the breathing frequency and dynamic body acceleration (DBA) of the hatchery-reared loggerhead turtle *Carretta carretta*

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ABSTRACT
In this study, the relationship between the breathing frequency and the dynamic body acceleration (DBA) of one hatchery-reared loggerhead turtle *Carretta carretta* was examined using acceleration data loggers. Two acceleration data loggers (M190L-D2GT, W1000-3MPD3GT, Little Leonard, Japan) were attached on the lower-beak and carapace of a hatchery-reared loggerhead turtle, respectively. Breathing was successfully detected from the angle and depth of the beak-attached data logger and DBA, which has been used as an index of activity levels (Wilson et al., 2006), was calculated from the forward acceleration of the carapace-attached logger. There was a positive correlation between the DBA in the previous dive and the breathing frequency; the relationship was exponential. The result suggests that the number of breaths increased exponentially after a more active dive.

KEYWORDS: acceleration data logger, breath, *Carretta carretta*, metabolic

INTRODUCTION

For air-breathing aquatic animals, breathing at the water surface is a limiting factor in all underwater activities such as foraging, resting, mating and predator-avoidance. Therefore, it is essential from the ecological point of view to understand dive-induced breathing patterns in aquatic animals. Dive-induced breathing patterns of sea turtles have been studied in captivity. So far, the relationship between the breathing frequency and the dive duration has been elucidated (Lutcavage and Lutz, 1991). In addition, the difference of breathing frequencies between active dives and resting dives has been understood (Lutz *et al.*, 1989); however, no studies have been conducted on how the breathing frequency changes in accordance with activity levels. The information could provide a novel insight into the estimation of the energy expenditure and metabolic rate of the sea turtles, which can be used as key currencies in behavioral ecology (Krebs and Davies, 1993). The objective of this study was to understand how the breathing frequency changes with activity levels of loggerhead turtles *Carretta carretta*. To achieve the objective, we measured breathing and activity levels of the turtle simultaneously, using acceleration data loggers.

MATERIALS AND METHODS

Sample turtle

One immature hatchery-reared loggerhead turtle was used in this experiment. Standard carapace length and body weight of the turtle were 633 mm and 35.4 kg, respectively. The turtle was reared in Yaeyama Station of the Seikai National Fisheries Research Institute, Fisheries Research Agency.

Attachment of acceleration data loggers and experimental protocol

Two acceleration data loggers (M190L-D2GT and W1000-3MPD3GT; Little Leonardo Co., Tokyo, Japan) were affixed to the lower beak and carapace of the loggerhead turtle respectively, using epoxy putty (Konishi Co., Ltd. Osaka, Japan) and two-component epoxy resin (ITW Industry Co., Ltd. Osaka, Japan). Acceleration data loggers can record depth and temperature at 1 s intervals, and two- or three-axis accelerations at 1/32 s intervals.

We released the logger-attached turtle in the experimental tank (10 m x 10 m x 2.2 m) on 22 April in 2008 and recovered the data loggers in the next day.

Extraction of breathing and activity level from acceleration data

From the acceleration data, we could extract static and dynamic components of the acceleration (Tanaka *et al.*, 2001, Okuyama *et al.*, in press). The static components (or low frequency) of the acceleration represent angles of the body parts; the dynamic components (or high frequency) of the acceleration represent the animal movements such as flipper strokes.

Sea turtles complete a single exhalation and inhalation with each respiration; they extend their neck upward when they breathe at the surface. Therefore, we detected the time of breathing when the turtle was at the surface and angle of the head was upward. In this study, breathing was defined when the head angle was greater than 40^{o} and the swimming depth (which was obtained from the pressure data of carapace-attached logger) was less than 0.2 m (for more details, see Okuyama *et al.*, in press), which was the most appropriate definition to a allow high detection rate and low false detection rate.

Recent studies suggest that dynamic body acceleration (DBA) can serve as an index of activity levels of free-living animals (Wilson *et al.*, 2006, Halsey *et al.*, 2008, Yasuda and Arai, 2009). Therefore, in this study, we calculated the DBA as an index of activity level during each dive. Dynamic components of the forward acceleration from the carapace-attached data-logger were converted into absolute positive units, and the resulting values were averaged during each dive.

For the extractions of these behaviors, we used IGOR Pro ver. 5, IFDL (WaveMatrics, Inc., USA) and Ethographer (Sakamoto *et al.*, 2009).

Data analyses

Diving was defined as the period when no breathing was recorded for over one minute. The number of breaths was then counted in each inter-dive interval and these values were used in regression of the previous dive durations versus the number of breaths. This regression analysis was conducted in order to reaffirm that there is a positive correlation between previous dive durations and number of breaths as is revealed by the previous study using visual observation (Lutcavage and Lutz, 1991).

Breathing frequency was determined as number of breaths divided by the previous dive duration. These values were used in regressions of DBA during the previous dive versus the breathing frequency. Breathing frequency and averaged DBA per dive were used at this time in order to exclude the effect of the previous dive durations on the number of breaths. To understand how the breathing frequency changes with the activity levels, we tested three regression models (linear, exponential and inverse) to estimate the breath frequency from the DBA during the previous dive. The generalized linear model (GLM) and Akaike information criterion (AIC) were used to investigate the effect of the DBA and which regression model was the most predictable.

Statistical analyses were conducted using R 2.8.0 (The R foundation for Statistical Computing, Vienna, Austria) with R library "MASS".

RESULTS AND DISCUSSION

In the analysis investigating the effect of the previous dive duration on the number of breaths, there was a significant positive correlation between these values (Fig. 1; Pearson's correlation test; R=0.62, p<0.01). This result was consistent with the previous study on the relationship between the previous dive durations and the number of breaths conducted by the visual observation (Lutcavage and Lutz, 1991).

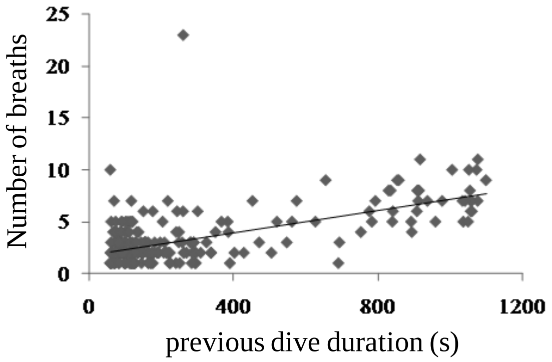

Fig. 1 The relationship between previous dive duration and number of breaths. There was a positive correlation between these values (Pearson's correlation test, R=0.62, p<0.01).

In the analysis investigating the effect of the DBA during the previous dive on the breath frequency, the lowest AIC was obtained for the exponential regression model which included the effect of the DBA (Table 1); the effect of the DBA was also significant (*G*-test, p<0.01). The model indicated that there was a significant positive correlation between the DBA during the previous dive and the breathing frequency (Fig. 2), and that the relationship was exponential. These results suggest that the number of breaths increased exponentially after a more active dive.

Table 1 Model fitting procedure for generalized linear models (GLMs). The best fit model was chosen according to the lowest Akaike information criterion (AIC). k is the number of model parameters.

Parameter	Regression	k	AIC
Null		1	470.1
DBA	Inverse	2	410.6
	Linear	2	400.0
	exponential	2	397.5

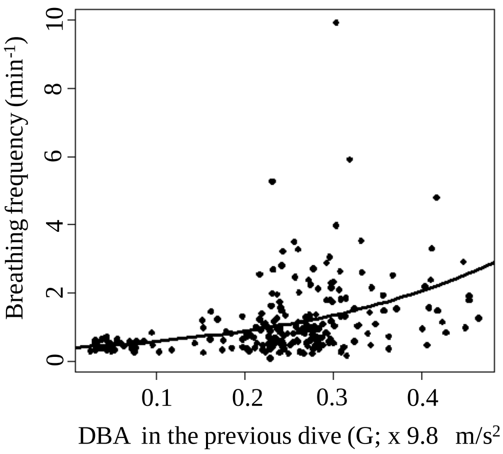

Fig. 2 The relationship between the DBA in the previous dive and the breathing frequency. The line in the figure represents the exponential regression model in GLM.

Understanding the whole mechanism of the relationship between activity levels and oxygen intake is more complex. In addition to the breathing frequency, the tidal volume in each breath and O_2-CO_2 exchange efficiency are related to the oxygen intake (Lutz and Bentley, 1985, Lutcavage *et al.*, 1987, Lutcavage and Lutz, 1991). Therefore, we have to understand how the tidal volume and O_2-CO_2 exchange efficiency change in accordance with the increase of activity levels and breathing frequency, for further elucidating the dive-induced breathing patterns of sea turtles.

Oxygen intake can be measured using a swim chamber with oxygen meter, and Okuyama *et al.*, (in press) suggested the possibility of measurement of tidal volume using the period of the neck-upward motion during the breath using an acceleration data logger. Therefore, simultaneous measurement of activity levels, breathing frequency, tidal volume and oxygen intake would be possible in the swim chamber, clarifying the dive-induced breathing patterns and metabolic rates.

Recently, categorization of underwater behaviors such as foraging, resting, swimming and mating has been conducted using acceleration data (Yoda *et al.*, 2001, Tsuda *et al.*, 2006, Nadimi *et al.*, 2008, Okuyama *et al.*, in press). If we obtain the data of behavioral categories and breathing patterns simultaneously, we could understand which behavior costs and benefits to sea turtle at a given period. The information leads to the elucidation of the time-to-time decision making of sea turtles.

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