1 **Title:**

2 Heart rate and its relationship with activity in free-ranging Cheloniidae sea turtles

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4 **Running title:**

5 Heart rate in free-ranging sea turtles

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21 **Keywords:**

22 Acceleration, Bio-logging, cardiac response, *Chelonia mydas*, Electrocardiogram (ECG), diving

23 physiology

24

25 **Summary statement:**

26 Green sea turtles in free-ranging dive had generally lower heart rate compared to other air-breathing
27 divers and it varied with the amount of exercise. Turtles often showed extreme arrhythmia.

28

29 **Abstract:**

30 The primary oxygen stores in Cheloniidae sea turtles are in the lungs. Therefore, management of
31 blood oxygen transportation to peripheral tissues by cardiovascular adjustments while diving is
32 crucial to maximize benefits from dives. However, heart rate, particularly cardiac response to
33 exercise in free-ranging dives, has rarely been examined for sea turtles. In this study, heart rate and
34 its relationship with the amount of activity were determined in six free-ranging green turtles using
35 bio-logging techniques. Our results demonstrated that resting heart rate took 7–11 h to reduce to
36 steady levels after turtles were released in the tank, indicating that turtles may not present normal
37 physiological rates right after release. After heart rate reduction, resting heart rate of green turtles in
38 free-ranging dives was generally low (10.9 ± 2.5 bpm), but they often presented arrhythmia (4–54
39 bpm) even in resting states. The amount of activity during a dive linearly increased heart rate, but
40 maximum heart rates (39.0–69.8 bpm) were recorded during ventilation at surface. These results
41 indicate that turtles have the capability of cardiac response to increased metabolic demands in their
42 muscles while submerged, and also of cardiovascular adjustment for a rapid renewal of oxygen
43 stores and removal of CO₂ during ventilation. Such well-organized cardiac adjustments may be
44 because of characteristics of Cheloniidae sea turtles such as ectothermy and oxygen storage in lungs
45 while submerged.

46

47 **Introduction**

48 Many air-breathing vertebrates are known to exhibit bradycardia when submerged, which allows
 49 them to regulate their blood oxygen depletion rate, thereby conserving onboard oxygen stores
 50 (Kooyman, 1989; Butler and Jones, 1997). This physiological mechanism enables these animals to
 51 submerge for prolonged periods (Kooyman, 1989; Butler and Jones, 1997). However, a paradoxical
 52 situation is created because underwater activity, such as swimming, should promote an elevation in
 53 heart rate to support the increased demands of the aerobic metabolism if metabolism is to remain
 54 aerobic. Thus, the potential for conflict between diving bradycardia and exercise responses has been
 55 investigated for marine mammals (Davis and Williams, 2012; Noren et al., 2012; Williams et al.,
 56 2015). These studies have revealed that marine mammals modify their level of bradycardia
 57 according to the amount of activity while submerged (Davis and Williams, 2012; Noren et al., 2012;
 58 Williams et al., 2015). Moreover, a recent study revealed that bradycardia was altered not only by
 59 exercise, but also by dive depth and the possibility of lung collapse (Williams et al., 2015).

60 Sea turtles are ectothermic marine animals and have well-adjusted physiological
 61 functions for prolonged dives (reviewed by Lutcavage and Lutz, 1997; Williard, 2013). Their
 62 considerably slow metabolism, for instance, enables turtles to undergo extremely prolonged dives
 63 (e.g., Hochscheid et al., 2005). Moreover, in sea turtles, particularly in Cheloniidae species, the
 64 primary oxygen stores are in the lungs (> 70% of the oxygen present in whole body) rather than in
 65 the blood or tissues (Lutz and Bentley, 1985; Lutcavage and Lutz, 1997). Therefore, the management
 66 of blood oxygen transportation to peripheral tissues (e.g., muscle) while diving by cardiovascular
 67 adjustments is crucial to maximize dive duration and the benefits of underwater activities, such as
 68 feeding, predator avoidance, and mating. Therefore, the cardiac responses of sea turtles to diving
 69 have been of particular interest to comparative biologists (Berkson, 1966; Davenport et al., 1982;
 70 Butler et al., 1984; West et al., 1992; Southwood et al., 1999). However, all of these previous studies,

71 except Southwood et al. (1999), were conducted under experimental conditions in a limited space,
72 and heart rate in free-ranging sea turtles has rarely been examined. Moreover, unlike for marine
73 mammals, knowledge on sea turtle bradycardia while diving remains limited. In this context, the
74 objectives of this study were to examine general aspects of heart rate in free-ranging green sea turtles,
75 *Chelonia mydas* (Linnaeus 1758) and to determine their cardiac response to activity while diving.

76

77 **Materials and methods**

78 *Experimental protocol and instruments*

79 Our experiments were conducted in a tank ($H \times L \times W = 10 \text{ m} \times 10 \text{ m} \times 2.2 \text{ m}$) at the Research
80 Center for Subtropical Fisheries, Seikai National Fisheries Research Institute, Japan Fisheries
81 Research and Education Agency, Japan. Six wild green turtles were captured under the permission of
82 Okinawa Prefecture (No. K30-2) (Table 1).

83 Electrocardiogram (ECG) data of free-ranging green turtles were recorded using an ECG
84 data logger (W400-ECG, Little Leonard Co., Tokyo, Japan, 21 mm $\times$ 109 mm cylindrical logger, 5
85 ms sampling interval, voltage range $\pm 5.9 \text{ mV}$, 60 g, 2 GB memory). Three wires extending from the
86 ECG logger were soldered to two stainless-steel electrodes (positive and negative, 0.9 mm in
87 diameter, 30–35 mm in length) and a 21–23 gauge sterile needle that functions as a ground
88 connection. Length of the two electrodes was modified according to the size of turtles to record clear
89 heart beats. These electrodes were placed in the plastrons under anesthesia. Medetomidine (0.2
90 ml/kg of body weight, Fujita Pharmaceutical Co., Ltd., Tokyo, Japan) was used as a sedative and
91 injected into the base of front flippers. Approximately five minutes after sedative injection,
92 anesthesia was initiated by slow injection of 1-ml diluted water with Propofol (0.01 ml/kg; Mylan
93 Inc., PA, USA) into the cervical vein. Two ECG electrodes were placed through a 1.5-mm hole in the
94 plastron over the heart (Fig. 1). Dental acrylic (Fuji IX GP; GC Co., Ltd., Tokyo, Japan) was used to

95 anchor the electrode and seal the hole. A ground electrode was inserted immediately anterior to the
 96 hindlimb at a depth of 55 mm. Electrode placement required less than 15 min. The ECG data logger
 97 was placed on the carapace using epoxy putty (Cemedine Co., Ltd., Tokyo, Japan). Then, three wires
 98 were fixed on the carapace and plastron and covered using a silylated urethane resin (Konishi Co.,
 99 Ltd., Osaka, Japan) and duct tape (Koyo Kagaku Co., Ltd., Tokyo, Japan) to prevent electrical noise.
 100 After attachment procedures for electrodes and loggers, atipamezole (0.3 ml/kg Mepatia; Fujita
 101 Pharmaceutical Co., Ltd.) was injected into the base of front flippers as a medetomidine antagonist,
 102 to allow the turtle to recover from sedation. Then, each turtle was maintained in the plastic container
 103 ($H \times L \times W = 1 \text{ m} \times 0.7 \text{ m} \times 0.2 \text{ m}$) for ~1–1.5 h before the epoxy putty and resins completely
 104 hardened.

105 In addition to heart rate, depth, temperature, and 3-axis acceleration were simultaneously
 106 recorded using a multi-channel data logger (ORI400-D3GT: 12 mm diameter, 45 mm length, 9 g in
 107 air, memory capacity of 11.5 million data points; or W380-PD3GT: 21 mm diameter, 114 mm length,
 108 59 g in air, memory capacity of 16 million data points; Little Leonardo Co.) placed on the carapace
 109 using epoxy putty (Fig. 1). It recorded depth and temperature at 1 s intervals and 3-axis accelerations
 110 at 1/20 or 1/16 s intervals. The maximum range of the depth sensor was 400 or 380 m, with a
 111 resolution of 0.1 m. The measurement range of the accelerometers was ± 3 or 4 g, with a resolution
 112 of 0.00009 or 0.002 g. Acceleration data were separated into two components: the high frequency
 113 component, which was a dynamic acceleration representing turtle movement (such as swimming),
 114 and the low frequency component, which was gravity acceleration representing the turtle's body
 115 angle (Okuyama et al., 2012). This frequency separation was conducted by filtering analysis (see
 116 Okuyama et al. [2012] for details). Moreover, acceleration data were used to calculate the amount of
 117 activity performed by each turtle, which was given by overall dynamic body acceleration (ODBA;
 118 Wilson et al., 2006). ODBA was used as a proxy of the amount of activity and, consequently,

119 metabolic rate in sea turtles (Halsey et al., 2011; Okuyama et al., 2014). ODBA was calculated from
120 the high frequency component of the 3-axis acceleration data. Furthermore, the number of breaths at
121 the surface after each dive was counted by the head-mounted acceleration data logger (G6a+;
122 dimensions: 40 mm × 28 mm × 17 mm, weight: 19 g in air; measurement range: ± 2 g with a
123 resolution of 0.002 g, Cefas Technology Limited, Suffolk, UK; Fig. 1). Head angle of sea turtles was
124 calculated by the gravity component of acceleration data and depth data and allowed us to count the
125 breathing behavior (Okuyama et al., 2010). Briefly, breathing behavior was defined as occurring
126 when turtles raised their head up with angle $> 30^\circ$ at a depth < 0.15 m (Okuyama et al., 2010).

127 Each turtle with the dataloggers was released into the tank and allowed to swim freely for
128 16.3–21.5 h. Temperature during the experiments ranged from 28.3–30.3°C (Table 1). After the
129 experiments, the turtles were caught and immediately removed from the tank. Then, blood samples
130 were collected from the cervical vein using a syringe (1 ml, Nipro Co., Osaka, Japan) and a needle
131 (20-gage, Terumo Co., Tokyo, Japan). Partial pressures of oxygen and CO₂, besides lactate level in
132 the venous blood, were measured using an i-STAT 1 Analyzer (Abbott Point of Care Inc., IL, USA)
133 to be used as indicators of whether the turtles engaged in anaerobic dives during the experiments.
134 After blood sampling, all electrodes were removed, and the holes were filled with dental acrylic. The
135 turtles were maintained in the rearing tank while the recovery condition of the holes was observed.
136 Two weeks after the experiments, the holes were completely closed, and the turtles were released
137 back into the sea.

138

139 *Extraction of heart rate from ECG data and data analysis*

140 Data of ECG, depth, and acceleration were analyzed using Igor Pro version 6.36 (Wavemetrics, OR,
141 USA). As ECG waveforms and the amplitude of QRS peaks changed in each situation (see the
142 Results section), heart rate was used only to investigate the turtles' cardiac response. The R peak in

143 the QRS complex (the main graphical deflections in an ECG tracing; Fig. 2) was easily detectable,
144 and thus was regarded as one heartbeat.

145 Heart rate was analyzed in two time-scales. One was HR_{dive} , which was calculated by the
146 number of heartbeats during a dive divided by dive duration (min) to investigate the relationship of
147 heart rate between dives and post-dive surface duration. A dive was defined as the period when
148 turtles submerged deeper than 0.5 m and for longer than 30 s; the remaining period was regarded as
149 surface period. The other time-scale was HR_{min} , which was calculated by the number of heartbeats
150 per minute for the analysis of detailed cardiac response. However, when HR_{min} included the surface,
151 pre-surface ascend, and post-surface descend phases, such data were excluded from the analysis to
152 eliminate the effect of tachycardia at surface. Moreover, in some cases, the QRS complex was not
153 identified because of the noise (see the Results section). Therefore, the data of HR_{min} and HR_{dive}
154 including such noise were excluded from the analysis.

155 To investigate resting heart rate, and also to understand the relationship between heart rate
156 and activity level, behavior was divided into two states every minute: resting state and active state.
157 Resting state was defined as the behavior when dynamic acceleration, body angle, and depth were
158 constant throughout a minute, representing a turtle stayed still at the bottom of tank. All other
159 periods were considered to be an active state. Similarly, resting dive was defined as dives in which
160 the turtles were almost in the resting states at the bottom phase of dives. The remaining dives were
161 defined as active dives.

162

163 *Statistical analysis*

164 To determine the baseline of heart rate in green turtles, we analyzed the transition of resting HR_{min} ,
165 because these values appeared to decrease with time elapsed after the release (see the Results
166 section). Thus, HR_{min} data were summarized every hour after the release. Statistical outliers in each

hour-dataset were excluded using the Smirnov-Grubbs test. The remaining non-outlier data were analyzed to determine the significant difference in HR_{min} among hours after the release using multiple comparison Tukey-Kramer test, based on the hypothesis that the baseline of resting heart rate is maintained at a constant level with no significant difference among hours when the heart rate is reduced.

A generalized linear mixed model (GLMM) with a Poisson distribution and a log link function was used to determine the factors affecting heart rate (HR_{min}). Mean ODBA during a minute, body weight of turtles, and mean water temperature were treated as explanatory variables. Individual was treated as a random effect. To determine the effect of heart rate on dive duration, we also investigated the factors regulating dive duration using a GLMM with a Gamma distribution and a log link function. For this model, heart rate (HR_{dive}), mean ODBA, mean water temperature during each dive, and body weight were treated as explanatory variables. We used the ‘lme4’ package in the R v. 3.5.2 (R Development Core Team, 2018) software to run the GLMM analyses.

Results

Extraction of heart rate

A total of 113.8 h of depth, temperature, 3-axis acceleration, and ECG data were obtained from six turtles (Table 1). Most of the ECG data showed a clear QRS complex, but waveform and amplitude of the QRS peaks changed for each situation, presumably because the relative positions of the two electrodes toward the heart slightly changed as a result of the active movements of the turtles (e.g., swimming; Fig. 2). Therefore, in this study, only heart rate was used for the analysis. Furthermore, in some cases, the QRS complex could not be distinguished because of the noise; these data were excluded from the analysis.

Turtles often rested at the bottom of tank, and they swam around the tank during the

191 remaining period. Immediately after turtle release, the resting $HR_{s_{min}}$ were higher than those in other
 192 periods, and then they gradually decreased (Fig. 3). Multiple comparison tests revealed that the
 193 resting $HR_{s_{min}}$ in each hourly data did not significantly differ between all comparisons from 7–11 h
 194 after the release of each turtle (Fig. 3, Table 1). The statistical results of the multiple comparison
 195 tests are shown in Table S1. We therefore regarded periods with non-significant difference in $HR_{s_{min}}$
 196 as the condition in which the heart rates were reducing. To correctly determine the heart rates of sea
 197 turtles under natural conditions, hereafter, only the data from after heart rate reduction were used in
 198 the analyses. Thus, 1,875 minute-datasets including $HR_{s_{min}}$, mean ODBA, and mean water
 199 temperature from six turtles were used in this study (Table 2). Moreover, 347 dive-datasets including
 200 $HR_{s_{dive}}$, dive duration, mean ODBA, and mean water temperature, as well as post-dive surface data
 201 ($HR_{s_{surface}}$, the number of breaths) were also used (Table 3).

203 *Heart rate*

204 Resting HR_{min} was 10.9 ± 2.5 bpm on average ($\pm$ s.d.) (Table 2). However, it was noteworthy that
 205 resting $HR_{s_{min}}$ was not constant even after it reduced; abnormal events of instant, extreme
 206 tachycardia (54 bpm at maximum) sometimes occurred and were detected by the statistical outlier
 207 test (0.0–4.4% of the resting $HR_{s_{min}}$; Table 2, Fig. 4).

208 Active $HR_{s_{min}}$ was 12.7 ± 2.1 bpm on average ($\pm$ s.d.) (Table 2). The GLMM analysis
 209 showed the $HR_{s_{min}}$ significantly increased with mean ODBA (Fig. 5), but had no significant
 210 relationship with body size and water temperature (Table 4).

212 *Cardiac response to the diving behavior*

213 Dive duration was 10.2 ± 2.2 min and 2.9 ± 1.0 min for resting and active dives, respectively. Clear
 214 bradycardia was observed in all dives. Heart rate began to decrease immediately after the start of

215 submergence, and it increased concurrently with the start of ascending to surface (Fig. 4). Thus,
 216 $HR_{s_{dive}}$ was always higher than $HR_{s_{min}}$, because it included such ascending and descending periods
 217 in which heart rates elevated because of tachycardia during surface period. Resting $HR_{s_{dive}}$ was 11.9
 218 ± 2.8 bpm (Table 3). Mean resting $HR_{s_{surface}}$ was 38.4 ± 9.2 bpm and increased 2.2–4.1-fold from
 219 resting $HR_{s_{dive}}$ (Table 3). Meanwhile, mean active $HR_{s_{dive}}$ was 17.6 ± 4.6 bpm, and mean active
 220 $HR_{s_{surface}}$ was 32.7 ± 8.3 bpm (Table 3). Resting $HR_{s_{surface}}$ was significantly higher than active
 221 $HR_{s_{surface}}$ (paired t -test, $N = 6$, $t_5 = 3.0$, $P < 0.05$). Number of breaths during post-dive surface
 222 duration was 1.5 ± 0.5 in resting dives and 1.0 ± 0.1 in active dives (Table 3).

223

224 *Effect of heart rate on dive duration*

225 The GLMM analyses for all dive data, including those on before heart rate reduction, revealed that
 226 dive duration was significantly affected by heart rate, mean ODBA, and body weight of turtles, but
 227 not by water temperature (Table 4). The average ($\pm$ s.d.) partial pressures of oxygen and CO_2 and
 228 lactate level in the venous blood immediately after the experiments were 51.3 ± 9.2 mmHg, $66.3 \pm$
 229 9.4 mmHg, and 1.0 ± 1.2 mmol L^{-1} , respectively.

230

231 **Discussion**

232 This study described the heart rate of green turtles in natural conditions, and the changes in this
 233 feature caused by various factors, such as amount of activity, and air ventilation at surface. Sea
 234 turtles are regarded as ‘surfacers’ rather than ‘divers,’ because they spend most of their time
 235 underwater and surface only briefly for gas exchange (Kooyman, 1989). Therefore, bradycardia is a
 236 usual condition in the life history of sea turtles. The assessment of heart rate under normal condition
 237 in voluntary dives, as performed in this study, is an essential approach to understand the diving
 238 physiology of sea turtles. Our study demonstrated that the heart rate of green turtles was

considerably lower than those of leatherback turtles (Southwood et al., 1999), and marine mammals and birds (Butler and Jones, 1997). On the other hand, the extent of tachycardia at surface in green turtles was wider than that in other animals (e.g., leatherback turtles, 1.4-fold [Southwood et al., 1999]; northern elephant seals *Mirounga angustirostris*, 1.56-fold [Andrews et al., 1997]; and bottlenose dolphin *Tursiops truncatus*, 2.63-fold [Noren et al., 2012]). These well-organized cardiac adjustments may be a result of characteristics of Cheloniidae sea turtles such as being ectotherms with lungs with oxygen stores while submerged. Furthermore, these findings provide essential knowledge for understanding the green turtles' diving strategy as well as their diving physiology.

General perspective of heart rate in green turtles

Heart rate reduction after the animals were released in the tank took 7–11 h. This indicates that the turtles may have taken some time to reach their normal physiologically rates after release, even though they were observed to have settled on the bottom of the tank. Heart rate is known to increase in response to stress in turtles (Cabanac and Bernieri, 2000) and in many other taxa (Tarlow and Blumstein, 2007). Therefore, the stress caused by experimental handling including electrode attachment (although the turtles were under anesthesia) and by being released into a new environment (experimental tank) may have increased heart rate. It took ~7–11 h for the turtle to recover from this stress. This information should be taken into account by researchers when estimating metabolic rates of activity levels in sea turtles (e.g., Halsey et al., 2011) as the metabolic rate may be overestimated because of heart rate elevation resulting from a stress condition. In this study, duration of resting dives before heart rate reduction was shorter than that after heart rate reduction, which demonstrates that high heart rates make dive duration shorter even in resting states without any active metabolism.

Voluntary dives in sea turtles are generally completed with aerobic metabolism (Butler et

al., 1984; Okuyama et al., 2014). The low concentrations of lactate acid in venous blood collected immediately after the experiments supported that the turtles mostly performed aerobic dives in our study (Lutz and Bentley, 1985). Our results demonstrated that heart rate in sea turtles increased linearly with the amount of activity even when submerged with bradycardia. This indicates that sea turtles increase oxygen delivery to active muscles by blood flow during a dive in order to meet metabolic demands, as in marine mammals (Fedak et al., 1988; Williams et al., 1999; Davis and Williams, 2012; Williams et al., 2015). The extent of tachycardia in active states was not higher than that during surface ventilation and during extreme tachycardia in resting state. This indicates that turtles may maintain oxygen delivery to some extent during active swimming. This capability of cardiac adjustment may enable turtles to maintain aerobic metabolism even during active dives.

Previous laboratory studies reported that the mean resting heart rates for juvenile green turtles was around 24 bpm (Butler et al., 1984; West et al., 1992). In terms of scaling, no significant effect of body size was detected in this study, probably because of the small sample size. However, including the data obtained by previous studies (Butler et al., 1984: $N = 5$, body weight = 1.2 kg, water temperature = 28.8°C; West et al., 1992: $N = 10$, body weight = 1.2 kg, water temperature = 28.0°C), the resting heart rate (f_{resting}) decreased with larger body weight (BW), which was expressed by the following power equation using the least square regression ($r^2 = 0.85$, one-way ANOVA, $F_{1,7} = 81.0$, $P < 0.001$. Fig. 6):

$$f_{\text{resting}} = 24.7 BW^{-0.288} \quad (1)$$

Similar physiological parameters such as metabolic rate (Wallace and Jones, 2008) and aerobic dive limit (Hochscheid et al., 2007) were also reported to have a relationship with BW in sea turtles. Mass exponent (-0.288) of power regression of resting heart rate in green turtles are surprisingly similar to those in mammals (-0.25; Stahl, 1967) and in birds (-0.23; Calder, 1968). However, heart rate measured in shallow water chambers by previous studies (35–40 cm in depth;

287 Butler et al., 1984; West et al., 1992) may be a bit higher than the results of our study, conducted in a
288 large tank 2.2 m deep, because dive depth-induced lung collapse reduces heart rate in marine
289 mammals (Williams et al., 2015).

290 Another factor affecting heart rate is ambient water temperature (Davenport et al., 1982);
291 however, no significant effect was detected in this study, presumably because there were no
292 substantial fluctuations in water temperature during the experiments (Table 1). Heart rates of
293 free-ranging leatherback turtles during inter-nesting period in Costa Rica were documented as 17.4
294 bpm on average (Southwood et al., 1999). In that study, leatherbacks mostly conducted V-shaped
295 dives. Such situation is likely similar to the active dives of the current study. Considering the large
296 size difference between the adult leatherback turtles (250–400 kg; Southwood et al., 1999) and
297 sub-adult green turtles (8.5–34.5 kg) in current study, heart rate in leatherback seems to be higher
298 than that in green turtles. This might be because the body temperature of leatherbacks was somewhat
299 higher than the ambient water temperature due to gigantothermy (Paladino et al., 1990).

300

301 *Abnormal cardiac rhythm in resting states*

302 Our study found that abnormal cardiac rhythms including extreme tachycardia generally occurred in
303 green turtles. Irregular cardiac rhythms were also reported in leatherbacks and green turtles
304 (Davenport, 1982, Southwood et al., 1999) and in marine mammals (Williams et al., 2015). In the
305 case of the marine mammals, the cardiac arrhythmias may have occurred as a result of the interplay
306 between sympathetic and parasympathetic drivers for exercise and diving (Williams et al., 2015).
307 This aspect may also be true for sea turtles. However, extreme tachycardia in resting states may also
308 indicate that turtles may adjust the oxygen stored in peripheral tissues (e.g., locomotory muscles),
309 which suffered from anoxia by temporarily increasing blood flow during a dive. Otherwise, it may
310 represent a ‘fight-or-flight’ cardiac response toward some dangerous situation felt by the turtles (i.e.,

311 stress).

312

313 *Cardiac response to diving*

314 Many diving mammals and birds increase their heart rate towards the end of a dive in anticipation of
 315 surfacing (Butler and Jones, 1997), because an anticipatory tachycardia might restore blood flow to
 316 peripheral tissues, flushing out metabolites that may have built up and allowing for a more efficient
 317 removal of metabolic by-products and uptake of oxygen during recovery time at the surface
 318 (Thompson and Fedak, 1993). Our study demonstrated that green turtles also rapidly elevated their
 319 heart rate at the beginning of ascending to surface, as well as leatherback turtles (Southwood et al.,
 320 1999). Tachycardia observed during post-dive surface durations was quite faster than that observed
 321 for leatherback turtles (24.9 bpm, Southwood et al., 1999). Cheloniidae species use their lungs as the
 322 primary oxygen store while diving and have relatively larger lung tidal volumes than those of
 323 deep-dive leatherbacks turtle (Lutcavage and Lutz, 1991). These facts may allow a more rapid
 324 renewal of oxygen stores and removal of CO₂ in Cheloniidae species.

325 Tachycardia during post-dive surface durations in resting dives was faster than in active
 326 dives. Okuyama et al. (2014) suggested that green turtles almost deplete their oxygen stored during
 327 resting dives, and then replenish their oxygen content at surface by taking several breaths (Table 3).
 328 Thus, faster tachycardia may indicate that green turtles make additional adjustment by cardiac
 329 response to achieve rapid replenishment of oxygen store and removal of CO₂, as well as increases
 330 the breath events. As to active dives, meanwhile, green turtles do not deplete their oxygen stores,
 331 followed by only a few breaths for effective locomotion (Table 3, Okuyama et al., 2014). Thus,
 332 partial pressure of remaining oxygen contents in their body may not maximize tachycardia at surface
 333 after active dives.

334

335 **Acknowledgements**

336 We gratefully acknowledge Dr. Y. Kakizoe for prescription of anesthetics and sedatives, and
337 direction for administration of these medicines. The authors thank T. Shimizu for his kind assistance
338 on the experiment. T. Kojima and Y. Makiguchi provided the blood gas analyzer, and useful
339 comments on making the electrodes for ECG monitoring.

340

341 **Competing interests**

342 No competing interests declared

343

344 **Funding**

345 This study was supported by JSPS Grant-in-Aid for Scientific Research (C) (No. 18K05684 to D.S.).

346

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424

425 **Tables**

426 **Table 1.** Summary of physiological characteristics of turtles and experimental conditions.

ID	SCL (cm)	BW (kg)	Duration of experiment (h)	Duration before resting HR _{min} reduction (h)	T _w (°C)
CM1	66.3	34.5	16.3	8	29.5 - 30.3
CM2	58.1	22.5	19.5	10	28.9 - 29.8
CM3	58.1	22.4	19.1	9	29.1 - 29.9
CM4	49.5	15.2	20.2	11	28.3 - 29.4
CM5	48.7	13.2	21.5	10	29.2 - 29.9
CM7	42.3	8.5	17.2	7	28.7 - 29.5

427

428 **Table 2.** Summary of minute-heart rate in each turtle.

Resting HR _{min}						Active HR _{min}				
ID	N	mean ± S.D.				mean ±				
		max	min	Outlier (%)		N	S.D.	max	min	Outlier (%)
CM1	88	8.2 ± 1.3	14	6	2.3	93	14.5 ± 3.5	22	7	0
CM2	205	11.6 ± 2.2	24	7	1.5	47	12.8 ± 2.0	17	8	0
CM3	64	12.4 ± 1.6	17	10	0.0	43	13.7 ± 2.0	19	11	0
CM4	275	7.8 ± 2.2	22	5	4.4	132	8.6 ± 2.1	17	5	6.8
CM5	331	10.6 ± 4.2	54	6	2.1	190	12.6 ± 5.3	37	7	12.6
CM7	277	13.4 ± 3.1	41	9	1.1	163	14.1 ± 3.1	34	10	4.9

429

430 **Table 3.** Summary of dive-heart rate in each turtle.

Resting dive					Active dives			
ID	N	Dive	Post-dive at surface		N	Dive	Post-dive at surface	
		HR _{dive} (bpm)	HR _{surface} (bpm)	N. breaths		HR _{dive} (bpm)	HR _{surface} (bpm)	N. breaths
		(max - min)	(max - min)	(max - min)		(max - min)	(max - min)	(max - min)
CM1	10	9.4 ± 0.7	38.2 ± 7.0	1.6 ± 1.2	95	17.7 ± 3.3	28.4 ± 5.1	1.0 ± 0.2
		(10.2 - 8.2)	(52.0 - 30.0)	(5 - 1)		(30.4 - 10.7)	(42.0 - 12.0)	(2 - 1)

CM 2	7	12.9 ± 2.1 (17.0 - 11.3)	51.1 ± 9.2 (69.8 - 30.7)	1.1 ± 0.4 (3 - 1)	7	23.4 ± 5.7 (31.7 - 16.5)	45.5 ± 8.1 (60.6 - 18.4)	1.0 ± 0.1 (2 - 1)
CM 3	2	14.6 ± 0.5 (14.9 - 14.3)	32.2 ± 4.1 (39.0 - 27.7)	1.0 ± 0.2 (2 - 1)	4	15.4 ± 1.1 (16.9 - 14.3)	35.0 ± 5.7 (44.1 - 28.8)	1.0 ± 0.1 (2 - 1)
CM 4	2 0	8.0 ± 0.5 (9.2 - 7.3)	28.0 ± 8.1 (49.8 - 17.8)	1.4 ± 0.9 (4 - 1)	2 5	11.1 ± 5.0 (32.5 - 7.5)	23.0 ± 5.5 (39.9 - 17.3)	1.0 ± 0.1 (2 - 1)
CM 5	3 1	11.3 ± 1.7 (18.3 - 9.9)	32.9 ± 8.2 (58.8 - 19.6)	1.4 ± 0.7 (3 - 1)	5 3	15.7 ± 4.9 (30.2 - 9.7)	26.5 ± 7.2 (49.0 - 15.7)	1.0 ± 0.2 (2 - 1)
CM 7	3 4	15.0 ± 2.0 (22.6 - 12.9)	47.8 ± 8.4 (69.0 - 35.2)	2.5 ± 1.4 (6 - 1)	5 9	22.3 ± 8.6 (45.2 - 13.8)	38.0 ± 5.2 (50.6 - 27.7)	1.2 ± 0.5 (3 - 1)

431

432 **Table 4.** Summary of the statistics from two generalized linear mixed models' analyses.

Response variable	Distribution	Explanatory variables	Estimate	Std. Error	<i>z</i> or <i>t</i>	<i>P</i>
Heart rate	Poisson	mean ODBA	0.10	0.01	13.06	<0.001
		Body weight	-0.019	0.069	-0.27	0.785
		Temperature	-0.01	0.02	-0.69	0.493
		Intercept	2.43	0.08	30.22	<0.001
Dive duration	Gamma	Heart rate	-0.53	0.04	-14.33	<0.001
		Mean ODBA	-0.28	0.04	-8.00	<0.001
		Body weight	-0.20	0.09	-2.20	<0.05
		Temperature	0.01	0.09	0.07	0.945
		Intercept	1.25	0.05	23.43	<0.001

433

434

435 **Figures and figure legends**

436 **Fig. 1 Illustrations of data logger attachments to a green turtle.** (A) Dorsal view showing the
437 placement of the data loggers and of the electrode leads on the carapace. (B) Ventral view showing
438 the placement of three electrodes and leads on the plastron.

439

440 **Fig. 2. Typical examples of electrocardiogram (ECG) traces showing heart beats in green**
441 **turtles.** (A) Normal ECG trace showing clear a QPR complex. (B) ECG trace at extreme tachycardia.
442 (C) ECG trace when recording condition was not well. Note that the range of the vertical axis is
443 different from those of the other two.

444

445 **Fig. 3 Heart rates in the resting states with elapsed time after release into the tank in each**
446 **turtle.** Circles and vertical bars represent the mean value and standard deviation of heart rate in the
447 resting state in each hour, respectively. Statistical outliers were excluded. Red colors represent heart
448 rate reduction identified using a multiple comparison test.

449

450 **Fig. 4 Typical cardiac responses during a resting dive in a green turtle (CM5).** Blue and red
451 backgrounds represent surface periods and extreme tachycardia period. Black arrows represent
452 breathing event.

453

454 **Fig. 5 Relationships between heart rate and the overall dynamic body acceleration (ODBA) in**
455 **each turtle after heart rate reduction.** Red lines represent the linear regression line calculated
456 using a least-square method.

457

458 **Fig. 6. Heart rate in resting states (bpm) versus body weight (kg) in green turtles.** Circles and

459 verticals bars represent the mean values and standard deviations of heart rates. Blue circles represent
 460 data obtained in this study, whereas green circles represent data from Butler et al. (1984) and West et
 461 al. (1992). Red line represents the heart rate estimated from body weight using Eqn 1 (Heart rate =
 462 $24.7 BW^{-0.288}$).

(A) Dorsal view

(B) Ventral view