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SHORT COMMUNICATION

Effects of a parasympathetic blocker on the heart rate of loggerhead sea turtles during voluntary diving

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ABSTRACT

Diving bradycardia is a reduction in the heart rate mediated by the parasympathetic system during diving. Although diving bradycardia is pronounced in aquatic mammals and birds, the existence of this response in aquatic reptiles, including sea turtles, remains under debate. Using the parasympathetic blocker atropine, we evaluated the involvement of the parasympathetic nervous system in heart rate reduction of loggerhead sea turtles (*Caretta caretta*) during voluntary diving in tanks. The heart rate of the control group dropped by 40–60% from the pre-dive value at the onset of diving; however, administration of atropine significantly inhibited heart rate reduction (P<0.001). Our results indicate that, similar to mammals and birds, the heart rate reduction in sea turtles while diving is primarily mediated by the parasympathetic nervous system. In conclusion, we suggest that diving bradycardia exists not only in aquatic mammals and birds but also in aquatic reptiles.

KEY WORDS: Diving physiology, Diving bradycardia, Heart rate, Atropine, *Caretta caretta*, Parasympathetic nervous system

INTRODUCTION

Air-breathing vertebrates such as mammals and birds exhibit cardiac responses mediated by the autonomic nervous system during diving (Butler and Jones, 1997; Ponganis, 2015). Cardiac responses include diving bradycardia (the reduction in heart rate during diving) mediated by the parasympathetic nervous system and vasoconstriction mediated by the sympathetic nervous system (Butler and Jones, 1997; Ponganis, 2015). This cardiovascular regulation allows diving animals to selectively distribute stored oxygen to oxygen-sensitive tissues while maintaining a consistent blood pressure level. The redistribution of blood flow can regulate the oxygen consumption rate and thus prolong the dive duration (Burggren, 1988; Butler and Jones, 1997; Ponganis, 2015). The cardiac responses of aquatic mammals and birds have been extensively investigated (Butler and Jones, 1997). Field studies in mammals and birds in particular have shown that a profound reduction in heart rate occurs while diving and that the intensity of diving bradycardia could be reflective of diving behaviour, such as dive duration and depth, and the intensity of activity while diving (Meir et al., 2008; McDonald and Ponganis, 2014; Wright et al., 2014; Williams et al., 2015). Furthermore, the contribution of the autonomic nervous system to diving bradycardia has been investigated using pharmacological blockers (Murdaugh et al., 1961; Signore and Jones, 1995; McPhail and Jones, 1999; Elliott et al., 2002). For example, muscarinic blockers such as atropine bind to muscarinic acetylcholine receptors, thereby inhibiting the response to acetylcholine, which is characteristic of the parasympathetic nervous system. Atropine has long been known and used to abolish diving bradycardia in muskrats (Signore and Jones, 1995), harbour seals (Murdaugh et al., 1961) and ducks (McPhail and Jones, 1999); these findings verify that the parasympathetic nervous system is the primary modulator of diving bradycardia.

In contrast, although aquatic reptiles are also air-breathing diving vertebrates, the presence of diving bradycardia in this clade remains under debate. Several studies focused on the autonomic control of cardiac responses in freshwater turtles such as common sliders (*Trachemys scripta*), which are capable of extrapulmonary respiration (Bagatto and Henry, 1999), have described the effects of atropine under forced diving or anoxic conditions for several hours (Hicks and Wang, 1998; Hicks and Farrell, 2000; Overgaard et al., 2002). However, the effects of this compound during voluntary diving have not been investigated in reptiles. Previous studies have shown that heart rate is reduced when voluntary diving in some aquatic reptiles such as green sea turtles (*Chelonia mydas*) (Okuyama et al., 2020) and leatherback sea turtles (*Dermochelys coriacea*) (Southwood et al., 1999), whereas some studies have indicated that this reduction may be the result of a reduction in activity, as observed in common sliders (Krosniunas and Hicks, 2003) and loggerhead sea turtles (*Caretta caretta*) (Williams et al., 2019). For example, in captive loggerhead sea turtles, although heart rates were reduced during resting dives at the bottom of the tank, they were roughly equal to those recorded when the turtles were resting in shallow water with their heads above the surface (Williams et al., 2019). Thus, it remains unclear whether the reduction in the heart rate occurs as a response to diving, a reduction in activity or other unknown factors.

Loggerhead sea turtles are marine reptiles that spend most of their lives at the sea, with up to 26% of their lifetime spent at the surface basking, reproducing and recovering from dives (Lutcavage and Lutz, 1997; Hochscheid et al., 2010). Although they rely almost exclusively on lung breathing and their aquatic oxygen uptake is very limited (Lutz and Bentley, 1985), their diving ability is outstanding among aquatic reptiles. For example, loggerhead sea turtles have been reported to dive for as long as 10 h at a time (Broderick et al., 2007) and at a depth of more than 340 m (Narazaki et al., 2015). Despite their exceptional diving ability, the autonomic nervous control of heart rate while diving has not been investigated in this species.

In this study, we investigated whether the reduction in the heart rate of loggerhead sea turtles during voluntary diving was inhibited after the injection of the muscarinic blocker atropine. The heart rate and behaviour of six captive loggerhead sea turtles were recorded noninvasively using animal-borne recorders. We examined the

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Table 1. Summary of data after injecting saline and atropine in six loggerhead sea turtles

			Saline				Atropine			
Turtle ID	Mass (kg)	Treatment order	Number of dives	Mean dive duration (min)	Mean surface duration (min)	Mean water temperature (°C)	Number of dives	Mean dive duration (min)	Mean surface duration (min)	Mean water temperature (°C)
L2013	38.0	A→S	11	34.1±4.3	5.0±0.9	18.8±0.4	9	41.3±4.1	4.7±2.5	19.3±0.0
L2016	18.5	A→S	3	42.9±5.1	8.9±10.4	18.3±0.2	3	32.8±2.4	4.6±1.7	20.6±0.1
L2023	43.0	S→A	6	35.4±7.3	14.0±7.6	18.8±0.2	14	24.0±4.4	10.3±6.1	18.8±0.3
L2103	34.5	A→S	10	34.4±14.5	7.0±2.2	20.5±0.5	10	29.2±6.9	12.8±12.9	20.1±0.4
L2104	30.5	A→S	9	44.7±6.5	19.0±15.3	21.4±0.0	3	24.5±9.0	23.0±17.2	18.5±0.1
L2109	53.5	S→A	6	34.0±3.8	20.6±13.6	20.5±0.2	8	33.7±4.5	7.7±2.0	21.0±0.1
Grand mean	36.3±11.8		7.5±3.0	37.0±9.1	11.8±10.7		7.8±4.3	30.7±8.1	9.8±8.9	

Means±s.d. are reported. Experiments were conducted in 2020 for L2013, L2016 and L2023, and in 2021 for L2103, L2104 and L2109. For treatment order, A→S indicates that the turtle was first injected with atropine and then with saline, whereas S→A indicates the treatment in the reversed order.

involvement of the parasympathetic nervous system in heart rate reduction in loggerhead sea turtles during voluntary diving, and thus, the existence of diving bradycardia in this species.

MATERIALS AND METHODS

Animals

Six juvenile loggerhead sea turtles [*Caretta caretta* (Linnaeus 1758)] were used in this study. The mean±s.d. body mass of the turtles was 36.3±11.8 kg (18.5–53.5 kg), and the sexes were not determined (Table 1). These turtles were incidentally captured by commercial set nets along the Sanriku coast of Japan during the summers of 2020 and 2021. The study site was a summer-restricted foraging ground for loggerhead sea turtles (Narazaki et al., 2015). Once safely secured from the net, the turtles were promptly transferred to a marine station (International Coastal Research Centre, Atmosphere and Ocean Research Institute, University of Tokyo; 39°2105″N, 141°5604″E). This study was conducted as a part of a 'tag and release' programme, in which loggerhead sea turtles incidentally captured in set nets along the Sanriku coast were handed over to researchers by fishermen. After the turtles were tagged with plastic and metal ID labels, they were kept individually in outdoor water tanks (2×1×1 m, length×width×depth), and the experiments were performed in individual tanks. The turtles were fed squid or fish once every 2 weeks, excluding the day before and the day of the experiments. The amount of feed was determined according to the resting metabolic rate calculated from the ambient water temperature and body mass of loggerhead sea turtles in the Sanriku coastal area (Kinoshita et al., 2018). The turtles were kept at the facility for 1–3 months and were released into the sea near the marine station after the experiments. All experimental procedures were approved by the Animal Ethics Committee of the Atmosphere and Ocean Research Institute of the University of Tokyo (permission no. P18-18 and PH21-4).

Instruments

An electrocardiogram (ECG) was recorded at 250 Hz using an ECG logger (ECG400-DT, Little Leonardo, Tokyo, Japan, cuboid shape: 21 mm wide, 64 mm long, 23 mm high, 60 g mass in air; and ECG400-D3GT, Little Leonardo, cuboid shape: 31 mm wide, 67 mm long, 17 mm high, 61 g mass in air). In addition, the activity of the turtles was recorded using an accelerometer (M190L-D2GT, Little Leonardo; cylindrical shape; 15 mm diameter, 53 mm length, 18 g mass in air). The accelerometer and ECG logger were attached to the turtles along the longitudinal axis of their carapace. The longitudinal acceleration, temperature and depth were recorded at 16, 1 and 1 Hz, respectively.

Instrument attachment

The ECG signals of the turtles were recorded using a previously described non-invasive method that involved attaching two electrode patches to the carapace of the turtles (Sakamoto et al., 2021) with some modifications (Kinoshita et al., 2022). In our study, the electrodes were attached to the anterior and posterior regions of the plastron instead of the carapace. When attaching the electrodes to the plastron, a tyre was placed underneath the turtles to hold them in place. The ECG electrodes were made of conductive adhesive tape (KNZ-ST50 shield cloth tape, Kyowa Harmonet Ltd, Kyoto, Japan) cut into squares (4×5 cm). A lead wire was attached to the ends of the electrodes using the same adhesive tape. Two electrodes were attached to the plastron to measure the voltage difference between the electrodes as ECG signals. One electrode was attached to plastron in the vicinity of the upper left region of the heart as the negative electrode, and the other was attached to plastron in the vicinity of the lower right region of the heart as both the positive and ground electrodes. After attaching the electrodes, a conductive cream (ECG cream, Suzuken, Aichi, Japan) was applied to the surfaces to allow the detection of electrical signals. A portable ECG recorder (Checkme ECG, San-ei Medisys, Kyoto, Japan) was then used to verify that the electrodes detected the ECG signal.

Because seawater intrusion into the electrodes causes electrical noise in the ECG, the water droplets around the electrodes were carefully removed using acetone. Thereafter, the electrodes were waterproofed with waterproof adhesive plasters (BAND-AID Brand Hydroseal XL bandages, Johnson & Johnson, NJ, USA), and the edges of the adhesive plasters were glued using an instant adhesive (Aron Alpha Jelly Extra, Konishi, Osaka, Japan). Waterproof films (FC waterproof free-cut, Hakujuji, Tokyo, Japan) were then pasted over the electrodes. To further ensure that the electrodes were waterproof, they were thinly coated with epoxy (Bond Quick 5, Konishi).

The lead wires of the electrodes were arranged from the plastron along the side of the body to the carapace and fixed with adhesive tape and instant adhesive. These wires were connected to lead wires extending from the ECG logger, and the connection was sealed with a heat-shrinkable tube. Finally, the ECG logger and accelerometer were placed using an instant adhesive on a waterproof adhesive plaster pasted on the carapace. The process of attaching the instruments was completed in approximately 1 h. After the electrodes were attached, the turtles were returned to their tanks and allowed to recover for at least 8 h before conducting the diving experiments.

Diving experiments

The diving experiments consisted of treatment of the six loggerhead sea turtles with a muscarinic antagonist, followed by recording of their ECG and behaviour overnight in the individual tanks. During the dives, the average temperature of the water was temperature of the water averaged 18–21°C (Table 1). The muscarinic antagonist atropine was used to block the parasympathetic nervous system activity. Because atropine dilates the pupil, sunlight may have affected the behaviour and physiological state of the turtles after injection. To prevent the influence of sunlight, the injection was administered after sunset (17:30–18:30 h). All turtles received two treatments: atropine (0.1 mg kg^{-1}) and saline (control). Four turtles (L2013, L2016, L2103 and L2104) were first injected with atropine, and two turtles (L2023 and L2109) were first injected with saline. The effects of the atropine appeared to last for at least one night (Fig. S1). Different treatments were administered at least 1 week apart. Injections were administered into the cervical vein using a syringe (10 or 5 ml) (Terumo, Tokyo, Japan) and a 20-gauge needle (Terumo, Tokyo, Japan). For administration of the treatments, the turtles were temporarily pulled to the edge of the tank. The treatments were performed within 2–6 min. After injection, the turtles were returned to their tanks, and their ECG and behaviour during voluntary diving were recorded overnight. To avoid disturbances, no personnel were present near the tanks during the recording period. The day after the diving experiments, all instruments were removed, and the data were downloaded from the recorders to a computer.

Analysis

The ECG, depth, temperature and longitudinal acceleration were analysed using IGOR Pro version 8.04 (Wavemetrics, Portland, OR, USA) with the Ethographer program package (Sakamoto et al., 2009). After injection, the heart rate seemed to take approximately 40 min to stabilise (Fig. S1). To limit the effects of aerial exposure during injection on the results, data within a 40-min periods after injection were excluded. To limit the effects of daylight disorientation as mentioned in the previous section, data recorded up to 05:00 h the next morning were analysed. In the case of atropine treatment on turtle L2016, the data were analysed only from the time of injection until 23:00 h (ca. 4.5 h) because the ECG logger stopped recording during the measurement. The ECGtoHR program package (Sakamoto et al., 2021) was used to process the ECG data. Noise was removed from the ECG using a bandpass filter, and R waves were detected. The instantaneous heart rates were calculated as the reciprocal of the time interval between two repetitive R waves (RR interval). The heart rate was calculated as the median instantaneous heart rate per minute.

Depth profiles were used to determine when the turtle was submerged. The turtle was considered submerged at a depth greater than 0.2 m. Depth was recorded shallowly because the recorder was attached to the carapace. At the determined depth, the head of the sea turtle was completely submerged, and the turtle was unable to breathe. Because the depth of the tank was 1 m, when the turtles started to move, such as slowly paddling to breathe, they immediately reached the surface. Thus, we calculated the s.d. of the longitudinal acceleration every 10 s to determine the activity of the turtle as either resting or moving. Using these methods, we found that the turtles mostly rested when submerged, with an acceleration s.d. of less than 1 m s^{-2} during this period. Therefore, only when the s.d. of the acceleration was less than 1 m s^{-2} was the turtle regarded as resting; otherwise, it was regarded as moving. Based on the above criteria for depth and longitudinal acceleration, 'diving' was defined as the period in which the turtles were submerged while resting. Because the heart rate tends to change gradually over several minutes, we analysed dives with a duration greater than 10 min. In addition, the period of time between dives where the turtles were at the surface moving for longer than 2 min was defined as 'moving at the surface'. Other behavioural modes such as moving underwater and surface resting only accounted for approximately 5% of the recording period, and the turtles mostly either rested underwater or breathed intermittently between dives. Thus, we analysed 'diving' and 'moving at the surface' as behavioural modes of the turtles.

To reveal the temporal profile of the heart rate during dives, we calculated the heart rate every minute from 2 min before the dive to 2 min after the dive for all dives for each turtle. For each turtle, the heart rate was averaged for each period of diving and moving at the surface. The first and last minutes of each dive were excluded to precisely estimate the mean heart rate during diving, as the recorded values varied largely with activity. Mean values were calculated for each turtle across all its recorded dives, and these means were further averaged within each group to obtain a grand mean. We used linear mixed-effects models to determine the factors affecting heart rate and duration when diving and moving at the surface. The treatment was converted into dummy variables (saline=0, atropine=1) and treated as a fixed effect. We also used two linear mixed-effects models to examine whether behavioural modes affect heart rate within each treatment group. The behavioural modes were converted into dummy variables (moving at the surface=0, diving=1) and treated as a fixed effect. In all models, the water temperature and body mass of turtles were included as fixed effects, and individual turtles were included as a random effect to account for repeated sampling. The coefficients for each term were estimated using maximum likelihood estimation. Differences were considered significant at $P<0.05$. The values are expressed as means±s.d. All statistical analyses were performed using the 'lme4' and 'lmerTest' packages implemented in R version 4.0.0 (https://www.r-project.org/).

RESULTS AND DISCUSSION

We investigated the effects of the muscarinic blocker atropine on the heart rate of loggerhead sea turtles during voluntary diving by measuring the heart rate and behaviour of six captive turtles after the injection of saline and atropine. Fig. 1 shows the heart rate profiles during voluntary diving in each treatment group. At the onset of diving, the heart rates of the control group decreased by approximately 40–60% from the pre-dive value within a few minutes, and this reduction was maintained throughout the dives (Fig. 1). The mean heart rate in the control group during diving was 7.1±2.9 beats min^{-1} (Table 2). In contrast, treatment with atropine significantly increased the mean heart rate during diving to 15.5 ±2.8 beats min^{-1} ($P<0.001$, $t=22.32$, d.f.=85.94; Table 2, Table S1). The heart rate gradually decreased throughout the dives in both treatment groups; however, the heart rates of the atropine-injected group remained higher than those of the control group (Fig. 1). Although the mean heart rate while diving and moving at the surface varied among turtles (Table 2), the inhibition of a profound reduction in the heart rate of sea turtles caused by atropine injection during diving was clear. Our findings suggest that loggerhead sea turtles exhibit diving bradycardia that is mediated by the parasympathetic nervous system. When the turtles re-surfaced after diving, their heart rate returned to a value close to the pre-dive value within a few minutes (Fig. 1). The heart rate while moving at the surface was significantly higher ($P<0.001$, $t=8.06$, d.f.=86.02;

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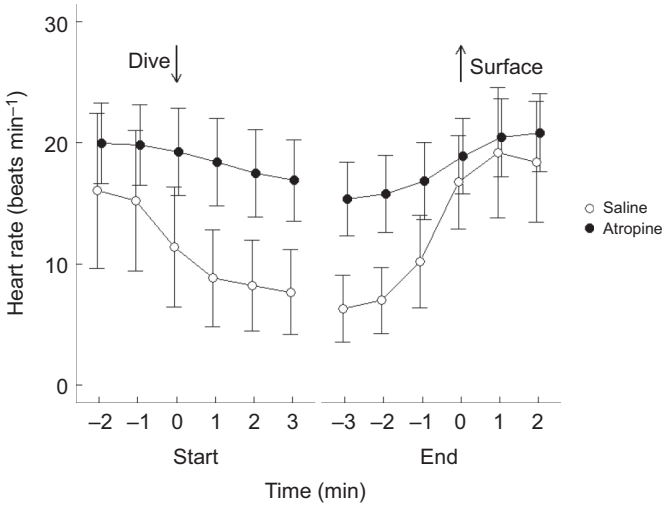

Fig. 1. Heart rate profiles of loggerhead sea turtles before, during and after diving following saline (open) and atropine (filled) injection. Each data point represents the mean (±s.d.) heart rate for all dives of all turtles (*N*=6) in each treatment. The arrows indicate the start and end of diving. The time was set to zero at the start of diving ('start') and then reset to zero at the end of diving ('end').

Table S1) in the atropine-injected group (grand mean, 19.9±2.9 beats min^{-1}) than that in the control group (grand mean, 15.5±4.6 beats min^{-1}; Table 2), indicating that the parasympathetic tone existed once the turtles re-surfaced after submersion. The heart rate in both treatment groups was affected by behavioural modes between diving or moving at the surface (control group, P<0.001, t=−21.03, d.f.=83.16; atropine-injected group, P<0.001, t=−15.31, d.f.=87.27) and water temperature (control group, P<0.01, t=3.12, d.f.=84.38; atropine-injected group, P<0.001, t=8.41, d.f.=93.87). However, the difference in the heart rate while diving and moving at the surface was smaller in the atropine-treated group than in the control group (Fig. 1), reflecting the inhibition of the reduction in the heart rate during diving by atropine administration. Our results show that the muscarinic blocker inhibits the reduction in heart rate during diving (Fig. 1, Table 2; Table S1), consistent with previous studies in mammals (Murdaugh et al., 1961; Signore and Jones, 1995; Elliott et al., 2002) and birds (McPhail and Jones, 1999). Therefore, as in aquatic mammals and birds, the reduction in the heart rate of sea turtles may also be mediated by the activation of the parasympathetic nervous system in response to diving.

As for the reduction in the heart rate of aquatic reptiles during dives, previous studies have suggested that fear, such as that induced by the presence of researchers, may cause the heart rate of aquatic reptiles to decrease as a response of escape (Gaunt and Gans, 1969; Smith et al., 1974). In our study, because ECG was measured non-invasively using a self-contained logger and none of the researchers were allowed near the tanks during the experiments, the heart rate values were unlikely to be affected by these types of disturbances. Alternatively, other studies have suggested that the level of activity during dives affects the heart rate (Southwood et al., 2003; Okuyama et al., 2020); for instance, a reduction in the heart rate may be caused by a lack of activity during dives (Krosniunas and Hicks, 2003; Williams et al., 2019). Under our experimental conditions, the heart rate of the turtles immediately dropped upon diving, and this reduction was inhibited by atropine throughout the dives, as in mammals and birds (Murdaugh et al., 1961; Signore and Jones, 1995; McPhail and Jones, 1999; Elliott et al., 2002). Therefore, we believe that the reduction in heart rate observed in our experimental condition was caused by an increase in parasympathetic tone due to the dive response. However, in free-ranging sea turtles, the intensity of the heart rate reduction and the effect of activity may differ from those in captivity. Free-ranging juvenile loggerhead sea turtles in the western North Pacific actively perform dives, including sporadic dives more than 340 m deep (Narazaki et al., 2015). In marine mammals and birds, the intensity of diving bradycardia can be modulated by dive duration, dive depth and intensity of activity (Meir et al., 2008; McDonald and Ponganis, 2014; Wright et al., 2014; Williams et al., 2015); thus, further investigation on the effect of activity on the heart rate of free-ranging sea turtles would lead to a better understanding of heart rate reduction in aquatic reptiles during diving.

Water temperature had a significant effect on the mean heart rate of turtles while diving (P<0.001, t=4.70, d.f.=90.08) but not while moving at the surface (P=0.085, t=1.74, d.f.=91.10; Table S1). Previous studies have shown that the heart rate increases with water temperature in loggerhead (Hochscheid et al., 2002) and green sea turtles (Southwood et al., 2003). Given that the water temperature range in this study was not wide (18–21°C; Table 1), the effect of water temperature on the heart rate may have differed between the two behavioural modes.

Treatment with atropine did not cause any unusual behaviour in turtles, as determined by depth and longitudinal acceleration (Fig. S2). Although there was some variation in the mean dive and surface duration between individuals and treatments because of the limited number of dives (Table 1), the mean dive duration decreased significantly with the injection of atropine (P<0.05, t=−2.89, d.f.=89.34) and increase in body mass of turtles (P<0.05, t=−2.67, d.f.=13.24; Table S1). In contrast, the mean surface duration did not depend on either treatment (P=0.332, t=−0.98, d.f.=85.71) or body mass (P=0.604, t=0.55, d.f.=5.99; Table S1). A higher body mass

Table 2. Heart rate data of two behavioural modes after injecting saline and atropine in six loggerhead sea turtles

	Heart rate when diving (beats min^{-1})		Heart rate when moving at the surface (beats min^{-1})	
Turtle ID	Saline	Atropine	Saline	Atropine
L2013	7.6±1.7 (11)	15.7±1.5 (9)	19.4±1.9 (11)	19.2±0.6 (9)
L2016	5.0±0.4 (3)	16.9±2.4 (3)	13.9±4.7 (3)	23.7±1.4 (3)
L2023	7.4±0.8 (6)	18.0±2.2 (14)	15.7±1.1 (6)	20.6±1.6 (14)
L2103	11.3±1.5 (10)	15.5±2.0 (10)	19.4±1.4 (10)	22.5±2.2 (10)
L2104	4.5±0.3 (9)	12.5±2.0 (3)	10.7±3.2 (9)	15.8±2.4 (3)
L2109	3.5±0.5 (6)	11.7±0.6 (8)	9.8±1.4 (6)	16.3±1.1 (8)
Grand mean	7.1±2.9 (45)	15.5±2.8 (47)	15.5±4.6 (45)	19.9±2.9 (47)

Means±s.d. are reported. Experiments were conducted in 2020 for L2013, L2016 and L2023, and in 2021 for L2103, L2104 and L2109. The numbers in parentheses indicate the number of dives or times the turtles were moving at the surface.

may affect dive duration because of high volumes of lung oxygen stored or low oxygen consumption rates (Lutcavage and Lutz, 1997). However, as the number of subjects and dives was limited in our study, this assumption could not be confirmed. Some variations were observed in the amplitudes of the ECG waves for each measurement (Fig. S2); however, these variations were attributed to the difference in the magnitude of the recorded voltage owing to the different positions of the electrodes in each instrument attachment. The ECG recordings contained clear R waves and minimal electrical noise. In addition, this electrical noise was mostly removed by the filtering process. Consequently, the heart rate was successfully estimated with high accuracy from the ECG recording period (Fig. S3).

As previous studies have mainly used atropine as a muscarinic blocker (Murdaugh et al., 1961; Signore and Jones, 1995; McPhail and Jones, 1999), we also used atropine to compare our results with those of previous studies. However, atropine acts not only on the muscarinic receptors of cardiac cells, but also on the muscarinic receptors of other cells, such as those of the gastrointestinal tract. Therefore, further studies using a blocker specific to the muscarinic receptors of cardiac cells, such as methoctramine (e.g. Elliott et al., 2002), are needed.

One of the limitations of this study was the lack of investigation of sympathetic nervous control in diving bradycardia. Sympathetic activity may affect reduction in the heart rate in two possible ways: (1) a decrease in sympathetic activity may cause the heart rate to decrease, and (2) sympathetically mediated vasoconstriction can raise blood pressure, thus causing the baroreceptor reflex and possibly reducing the heart rate as a consequence. However, previous research in mammals and birds found that treatment with blockers of the sympathetic system had no effect on diving bradycardia, suggesting that the sympathetic nervous system is not involved in this reflex (Murdaugh et al., 1961; Signore and Jones, 1995; McPhail and Jones, 1999; Elliott et al., 2002). In our results, the reduction in the heart rate of turtles during diving was inhibited by atropine, suggesting that such a reduction primarily occurs owing to the activation of the parasympathetic nervous system. However, underwater activity could modulate the influence of the sympathetic system on heart rate. Additionally, it has been proposed that exercise while diving causes a conflicting response of parasympathetic diving bradycardia and sympathetic exercise tachycardia (increase in heart rate) (Signore and Jones, 1996; Williams et al., 2015). Furthermore, sympathetic vasoconstriction may contribute to optimising diving behaviour, such as by increasing the potential dive duration. Further studies on the effect of the sympathetic nervous system would help determine how the two branches of the autonomic nervous system control cardiac responses to diving and contribute to diving behaviour.

In conclusion, using the parasympathetic blocker atropine, we propose for the first time that diving bradycardia mediated by the parasympathetic nervous system exists not only in endotherms, but also in ectothermic reptiles. Our results suggest that this diving-associated cardiovascular adjustment occurs in a broad range of air-breathing vertebrates. Further studies are required to clarify the effect of activity levels on the intensity of diving bradycardia. In particular, the contribution of the sympathetic nervous system to diving bradycardia warrants further investigation.

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Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: A.S.; Methodology: A.S.; Formal analysis: A.S.; Investigation: A.S., C.K., M.K., T.F.; Data curation: A.S.; Writing - original draft: A.S.; Writing - review & editing: A.S., C.K., M.K., T.F., K.S., K.Q.S.; Visualization: A.S.; Supervision: K.S., K.Q.S.; Funding acquisition: K.S., K.Q.S.

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