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Blue petrel electrocardiograms measured through a dummy egg reveal a slow heart rate during egg incubation

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Abstract

Background Seabirds like penguins and petrels, living in Antarctic and sub-Antarctic regions, often feed hundreds or even thousands of kilometers away from the islands where they breed. They therefore adapted to endure prolonged fasting during egg incubation, enabling their partner to undertake foraging trips that can last up to two weeks. Aside from accumulating and consuming fat reserves, it is unclear whether seabirds have developed further adaptations to extended fasting periods. This lack of knowledge is in part due to their remote nesting location and their extreme sensitivity to manipulation. To overcome this lack of knowledge, we developed a non-invasive device to record the heart rate (HR) of burrow-nesting blue petrels (*Halobaena caerulea*) during egg incubation. For that, we encapsulated a small-size logger in a dummy egg to record electrocardiograms (ECGs) through the featherless incubation patch of the birds.

Results The blue petrels' HR (208 ± 15 beats per min [bpm]; n = 6) that we recorded during egg incubation was slower than the HR predicted by two different allometric functions regressing HR against body mass (242 and 250 bpm). Blue petrels' HR also presented cyclical variation correlated to respiration, resembling the physiological Respiratory Sinus Arrhythmia (RSA) described in humans and other species, and that is mainly modulated by the vagal nerve. Moreover, the basal HR of incubating blue petrels increased about every minute during egg movements that presumably reflect egg turning, important for embryo survival and development. During these events, blue petrels' HR increased up to a maximum of 296 ± 27 bpm for 18 ± 2 s (n = 6). We estimated that those egg movements increased energy expenditure (EE) by 8.4 ± 1.3%, which is approximately 10 times less than the energy increase induced by the disturbance linked with the removal of the dummy egg at the end of the experiment. Interestingly, we noticed that the beginning of HR increase preceded egg movements by 4.3 ± 0.9 s (n = 6), as if birds needed to gradually increase their metabolism to achieve the following action. As well, blue petrels needed 9.1 ± 1.3 s (n = 6) to recover basal levels of HR after the end of egg movements.

Conclusion We recorded for the first time ECGs, HR and RSA in blue petrels in a completely non-invasive way. This allowed us to observe (1) slow basal HR during egg incubation, which could save energy for prolonged fasting and (2) temporal HR increase, possibly necessary to reposition the egg for proper embryonic development.

Keywords Blue petrel, ECG, Heart rate, Egg incubation, Logger, Respiratory sinus arrhythmia, Seabirds

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Background

Most seabirds that breed in the Antarctic and sub-Antarctic regions, such as penguins and petrels, frequently show protracted fasting when incubating their eggs. These birds breed on isolated islands and often feed hundreds or even thousands of kilometers away from their breeding sites [1, 2]. Because of this ecological constraint, one partner warms the egg, fasting, while the other goes feeding at sea. Depending on the ecology of the species, this voluntary fast can last from a few days to more than two weeks, a duration that is often proportional to the birds' size [1, 3, 4]. Birds incubating their eggs rely on their fat reserves to endure extended fasting, which involves the modulation of various metabolic hormones [5, 6] and a significant weight loss [7–11].

Torpor and hibernation are efficient energy-saving mechanisms implemented mainly by some small mammal and avian species when environmental temperatures are low, or food resources become limited [12–17]. These responses often imply a significant decrease in body temperature [18–20] and eventually a concomitant decrease in heart rate (HR) [21]. Similar physiological mechanisms could have evolved in seabirds to endure prolonged fasting during egg incubation and maximize reproductive success. Indeed, heterothermy has already been observed in petrel chicks that endure fasting periods [22, 23]. However, heterothermy during incubation could be incompatible with the need to maintain body temperature high enough for warming the egg [24, 25].

Sleeping may be another solution to preserve energy during incubation. Bonins petrels (*Pterodroma hypoleuca*) for example spend much of their time in the sleeping posture while incubating their egg [7]. Nevertheless, many of the studies on incubation behavior cited above are dated and result from rather invasive practices (e.g. nest modification to observe birds, recording of physiological parameters in birds kept captive in the lab, or heavily equipped with wires in the nest) that could have biased results, due to the lack of adequate technology at the time. Thus, beside the accumulation of fat reserves and the hormonal change regulating its consumption [5, 6], and a decrease of the ratio between energy expenditure (EE) and HR in fasting penguins [34], to our knowledge, in most seabirds it is still unclear whether behavioral and physiological mechanisms involving extended periods of sleep, heterothermy or slow HR, could be activated to support prolonged fasting during egg incubation.

As in other seabirds, burrow-nesting blue petrels (Fig. 1A) incubating their egg need to wait for the return

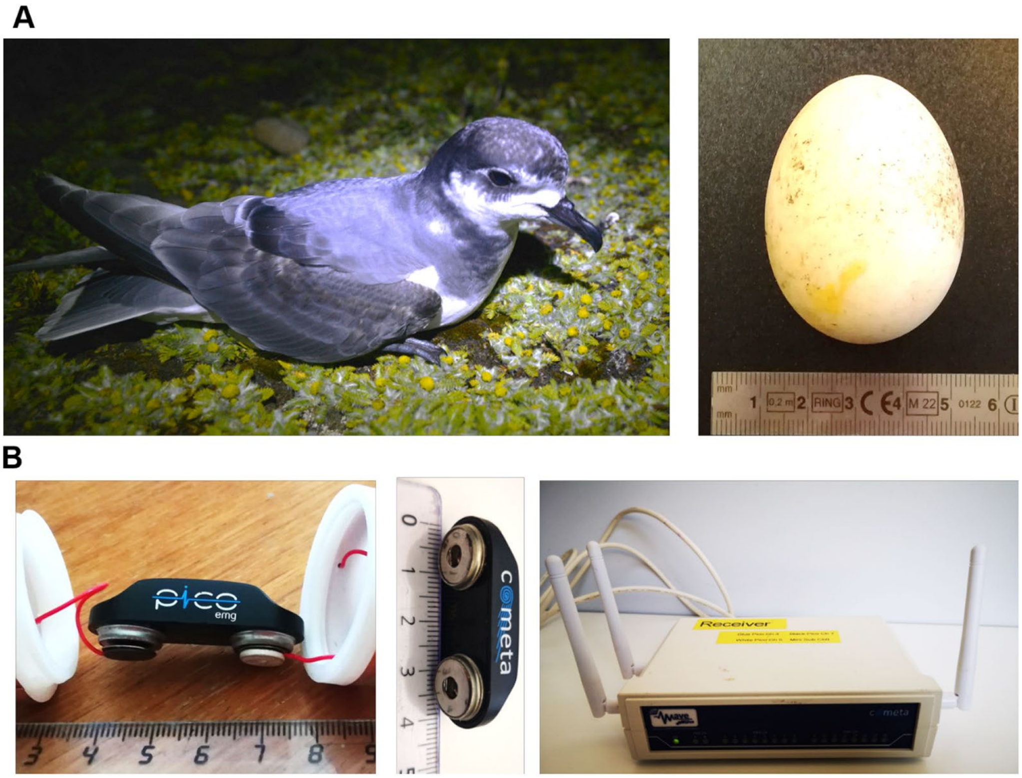

Fig. 1 Study species and equipment. **A** Blue petrel and an abandoned egg of this species. **B** Two halves of the dummy egg attached to the ECG device, ECG device and receiver station for ECG data

of their partner to go feeding. During this period of voluntary fasting, the incubating partner cyclically repositions the egg to ensure proper embryo development [26]. On the other hand, whether these birds activate specific physiological mechanisms to save energy during egg incubation is currently unknown. The underground nesting behavior of blue petrels and other burrowing species, and their adaptation to fasting, are indeed particularly difficult to monitor because birds occupy most of the space available in the burrow, challenging the placement of cameras and other devices for recording behaviors. Furthermore, these animals are cryptic, spend most of their time at sea, and do not survive in captivity, rendering the study of their behaviors and physiology particularly challenging.

HR may be a good proxy to study behavior and physiological parameters such as EE, when properly calibrated with respirometry measurements evaluating oxygen consumption [e.g. 27–29]. Inspired by previous work in which artificial eggs were used to infer HR by measuring bird's arterial pulses, or the sound of heart beating [30–32], we developed a new device to record blue petrel's electrocardiograms (ECGs). We were thus able to precisely follow blue petrels' HR and its oscillation over time by temporarily replacing the egg of an incubating animal with a dummy egg containing an ECG device. Physiological monitoring of incubating birds was therefore performed under completely natural conditions, without the need to handle the animals and surgically implant or attach cumbersome external devices [27, 33–38] that could alter birds physiology and behavior. Our approach has thus the potential to increase the usefulness of HR to study breeding behavior and understand the physiological reactions of birds to environmental perturbations.

Materials and methods

Site and species

Tests were performed in December 2022 on blue petrels (*Halobaena caerulea*) on Verte Island (48°25′–50°00S; 68°27′–70°35′E) in the Kerguelen archipelago (southern Indian Ocean), where we have been monitoring approximately 100 blue petrel nests for the past 20 years. Blue petrels were at half of their egg incubation period which lasts approximately 50 days [4]. Females lay only one egg and both parents incubate it, with incubation shifts lasting 8–12 days. Because of unpredictability in weather and food resources at sea, the return of the non-incubating partner may be delayed, prolonging the duration of the shift for the incubating individual, and by extension its fasting period. When such a delay is excessive, endogenous "signals" suggesting exhaustion of energy reserves and life threat [24], push the incubating individual to desert the egg [39]. Cooling does not preclude egg survival if one of the parents comes back to incubate the egg within 1 to 7 days from the desertion. This adaptation could be related to the burrowing breeding ecology, with the egg protected from the sight of predators [39–41]. However, the embryos from the temporarily abandoned eggs accumulate a developmental delay equal to the number of days during which those eggs were not incubated, delaying the hatching (FB personal observation [41]) and reducing chick survival in the short time window where breeding is possible in the Antarctic summer.

Egg and recording logger

All our monitored burrows are equipped with an inspection hole (10–12 cm diameter) dug 20–25 cm from the incubating chamber, and covered with a flat rock to facilitate the capture of birds for identification.

Using one of the blue petrel's eggs regularly found abandoned during field campaigns as a model (Fig. 1A), we scanned and 3D printed a dummy egg, using PolyLite™ PLA (polylactic acid; Polymaker Changshu, China). The real egg used as a model (height 48.4 mm and diameter 36.4 mm) represents the average size of blue petrels' eggs (FB personal observation). This dummy egg is empty inside and can be screwed off in two halves (Fig. 1B). This allowed us to fit a small and lightweight ECG logger (4 cm of length × 1.4 cm of width × 1 cm of height and 7 g of weight) inside the dummy egg. Using silver conductive paint (RS PRO Conductive Lacquer; RS Components SAS; Beauvais, France) we transformed the two poles of the egg into electrodes that ensured contact with the featherless and highly vascularized skin of the incubating patch of blue petrels. Such electrodes were connected with wires to the ECG logger (Fig. 1B) allowing the recording of one-lead ECGs.

The logger inside the dummy egg was the commercially available Pico EMG (Cometa srl; Milan, Italy; Fig. 1B). This mini device is essentially developed for electromyogram (EMG) recordings. However, when placed close to the thoracic region, it allows to recording ECG traces of high quality with a fixed sampling rate of 2000 Hz. This logger could send ECG signals by Wi-Fi to a receiver station connected to a laptop (Fig. 1B), or work as an autonomous logger using its internal memory. In our setting, the underground nest of blue petrels did not allow signal transmission to the receiver station. Thus, we employed the device as an autonomous logger, recording ECG data on the internal memory. Besides the ECG traces, the Pico EMG is also equipped with an accelerometer that records egg movements on three axes with a rate of 142,8 Hz and a scale of ± 8 g. Data from the logger were exported using the Cometa software EMGandMotionTools and analyzed using LabChart Pro 8 (ADInstruments Ltd; Dunedin, New Zealand).

Protocol

We exchanged the true egg with the dummy one in 8 different nest burrows. The operator (FB) introduced his arm in the burrow's inspection hole with the dummy egg in hand (with bare hands) and gently replaced the true egg with the dummy one, sliding the hand under the petrel. Blue petrels normally have little reaction to this maneuver and do not panic or struggle (although they can sometimes bite), presumably because they evolved without mammalian predators on breeding islands. Once the hand retreated, the animal sat back on the egg. Considering that blue petrel eggs can survive 5–6 days at 5 °C without incubation (see above, [39]), we decided that it was not worth keeping the real egg in an incubator for the 1–3 h that our experiment lasted. We thus kept the egg in a cardboard box filled with cotton balls inside our warmed (12–18 °C) field cabin. After 1–3 h of recording, we exchanged the dummy with the true egg, following the same method, allowing us to download data from the logger. ECG recordings were subdivided into continuous sequences of 20 min to reduce data charging time after data downloading. For most animals, we analyzed ECGs and acceleration data for about 20–40 min (n = 5). In one animal the recording quality was not sufficient to analyze more than 10 min of data. For unknown reasons, data from 2 animals were not exploitable (ECGs quality was not sufficient to analyze HR on a time interval of at least 5 min [42]) and were thus removed from the dataset.

ECG traces

ECG traces plot the variation over time of the difference of electrical potential between two points of contact with the skin, in the thoracic area or at the body extremity (arms and legs). In our experiment, these two points correspond with the silver conductive paint located on the two halves of the dummy egg, which are in direct contact with the featherless skin of the bird's incubation patch. As in humans and other animals where ECGs have been recorded, we were able to recognize main spikes that cross the ECG traces in the positive and negative directions and correspond to the ventricular depolarization of the heart, which then induces ventricular contraction. These spikes reflect the occurrence of a heartbeat and are called R waves. In some ECG traces we were also able to measure smaller spikes that precede R waves and that are characterized by a longer decay. Those smaller spikes are called P waves and correspond to the atrial depolarization inducing atrial contraction. After the R waves, in some ECG traces we were able to record other spikes with an even longer decay, which are commonly called T waves and correspond to the ventricular repolarization that induces ventricular relaxation. R waves were detected automatically using the "ECG analysis" or the "HRV" functions of LabChart Pro 8. Noise related to animal movement during egg movements in some cases reduced the performance of the automatic detection. To overcome this problem, we relied on the possibility to manually add or exclude R waves using the HRV function of LabChart Pro 8. In any case, although we could miss some heartbeats blurred by background noise, this did not significantly affect the different HR measurements that we reported in the manuscript.

Statistics and data analysis

All data were reported as mean ± standard error of the mean. Statistic tests were applied as indicated in the text and figure legends and were computed using GraphPad Prism 9 (GraphPad Software, Boston, Massachusetts, USA). Because for each bird we compared data obtained under different conditions or at different times of the ECG recordings, we used the paired two-tailed T-test when analyzing the difference between 2 groups of data and one-way ANOVA, with Dunnett's multiple comparisons test, when analyzing 3 groups of data. We calculated basal HR during egg incubation by detecting the number of heartbeats during 1 min of ECG recordings, avoiding, when possible, periods in which we observed egg movement events (see below for more details). To obtain such basal HR, we averaged 5 measurements of 1 min randomly taken along the ECG traces of each animal.

At the end of the period of ECG recording, the dummy egg was always found warm, indicating that it was well accepted by the birds during ECG recordings. Accordingly, we were able to obtain a readable signal in most of our tests. Nevertheless, at the beginning of the experiment, just after the egg exchange, the signal was generally noisy, possibly because of repetitive readjustments of the egg and bird positions. These readjustments could be triggered by the movement done by the bird to slide the egg between the dense feathers of the belly and the incubation patch, and/or by the different texture of the dummy egg compared to the real one, or the temperature difference between the two eggs. We thus had to exclude the first 22 ± 8 min (n = 6) from the ECG analyses due to intermittent high noise in the recordings. However, considering that blue petrels could take back a cold egg neglected for up to 5–6 days [39] the temperature difference between the real and the dummy egg should not impact blue petrels' overall behaviors.

HR may be a good proxy for measuring EE changes. In birds, EE is indeed roughly related to HR through a simple linear equation like $Y = mX + c$, where Y is the EE, X is HR and m and c depend on the species considered [28, 29]. Because EE is directly proportional to HR, using the latter we were able to roughly calculate the relative % changes of EE, even without knowing its absolute values.

To that purpose, we counted the number of heartbeats during each egg movement (from the beginning to the end of the event), we transformed these values into beats per minute (bpm) and we measured the % increase versus the basal HR of blue petrels during egg incubation. As well, we also estimated the change of EE caused by disturbance just before the final egg exchange, measuring HR in bpm before returning the real egg and analyzing the % increase versus basal HR during egg incubation. Since egg exchange is a short process, for these data HR was calculated from ECG periods averaging 13 ± 4 s (n = 4), taking one value for each animal into account. The apex of the bell-shaped curve showing HR deviation from basal level, during egg movements, was determined by visual inspection of the HR plot vs. time.

Results

We analyzed ECG data from 6 blue petrel's nests. Body mass, sex and basal HR during egg incubation are reported for each animal in suppl. Table 1. The ECG traces allowed us to clearly distinguish different phases of cardiac depolarization and repolarization, which are the triggers of heart contraction and relaxation, respectively (see methods). In all traces we observed R waves consistent with ventricular depolarizations that reflect the occurrence of heartbeats (see methods, Fig. 2A). In some of these recordings we also identified P and T waves, respectively preceding and following R waves, and corresponding to atrial depolarization and ventricular repolarization (see methods, Fig. 2A). Differences in signal quality were presumably due to the extent of the surface of contact between the electrodes located on the dummy egg and the featherless skin of the birds' incubation patch.

The basal HR of blue petrels during egg incubation was 208 ± 15 bpm with a range going from 165 to 264 bpm (n = 6). In four animals we also recorded HR during the disturbance induced by the experimenter switching eggs at the end of the recordings (Fig. 2B). During disturbance, HR (404 ± 59 bpm; n = 4) was significantly higher than basal HR recorded during egg incubation (*p = 0.0425, t = 3.397, df = 3, by paired two-tailed T-test) showing an increase of 95 ± 32% in the heartbeat's frequency.

Blue petrels, like all birds, show regular egg-repositioning behavior during incubation, vital for embryonic development and hatching success [26]. In between long intervals of no changes in the acceleration trace, we observed spikes triggered by egg movements, which in some cases should reflect active egg repositioning (Fig. 3A). These bouts of egg movement spikes (2.1 ± 0.2 spikes per bout; n = 6) lasted on average 3.8 ± 0.7 s (n = 6). Multiple spikes or longer spiking times are expected to indicate stronger and longer egg movements. Bouts of egg movement spikes occurred on average every 61.9 ± 9.3 s (n = 6) and were absent for 93 ± 2% (n = 6) of the time recorded, suggesting that besides these short

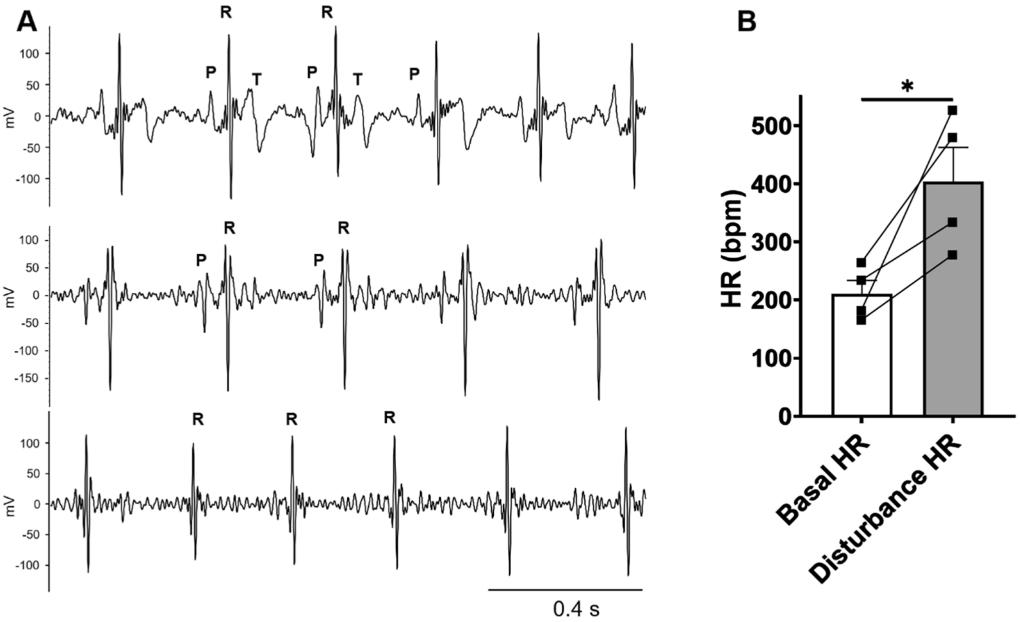

Fig. 2 ECG traces in blue petrels. **A** ECG traces of three blue petrels measured in milliVolts (mV) showing P, R and T waves corresponding to atrial depolarization, ventricular depolarization (heartbeat) and ventricular repolarization, respectively (see methods). Not all recordings had the quality necessary to identify P and T waves, but R waves were always identifiable. **B** Comparison of blue petrels' HR under incubation (basal) and during disturbance before egg substitution (n = 4). *p = 0.0425, t = 3.397, df = 3, by paired two-tailed T-test; n = 4

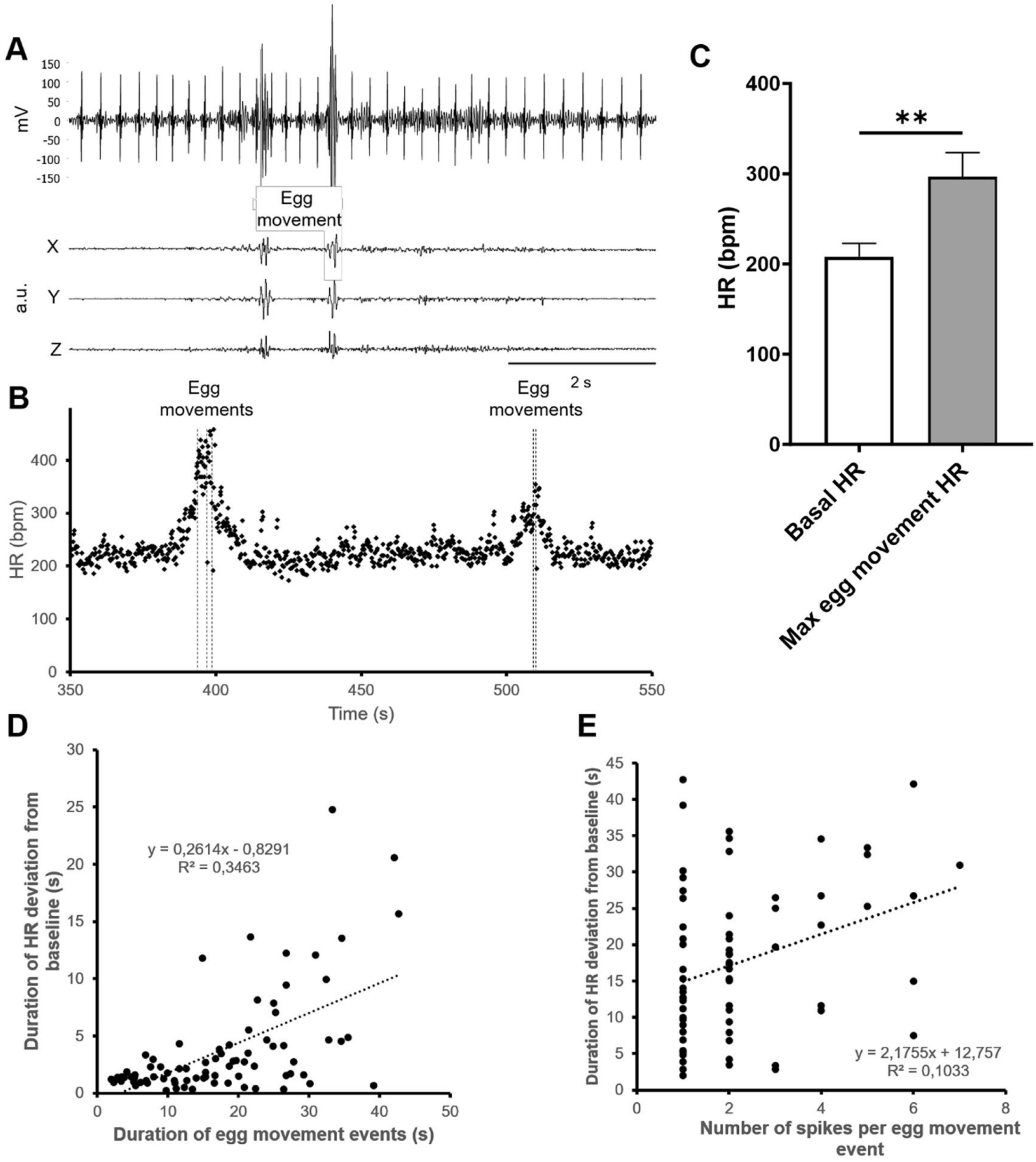

Fig. 3 Correlation between egg movements and HR. **A** Traces of ECG and acceleration recorded in mV and arbitrary units (a.u.), respectively. Note two series of high-intensity spikes presumably induced by egg movements. **B** Plot of HR versus time, showing two peaks of HR and a bell-shaped curve in correspondence with egg movement spikes (dotted lines). **C** Comparison between basal HR during incubation and maximal HR reached during egg movement (**p = 0.0014, t = 6.334, df = 5 by paired two-tailed T-test, n = 6). **D** Correlation between the interval of the egg movement and the interval of the HR bell-shaped curve, i.e. deviation of HR from the baseline ($R^2 = 0.35$, ****p < 0.0001, Spearman's correlation test). **E** Correlation between the interval of HR deviation from basal level and the number of spikes for each egg movement event ($R^2 = 0.10$, *p = 0.0193, Spearman's correlation test)

periods of egg movement, the birds were not moving in their nest burrow.

Compared to basal HR during incubation (208 ± 15 bpm), egg movements gradually increased HR, following a bell-shaped curve that peaked at 296 ± 27 bpm (**p = 0.0014, t = 6.334, df = 5 by paired two-tailed T-test, n = 6, Fig. 3B and C), before returning to its basal level. This deviation of HR lasted on average 18 ± 2 s (n = 6). The apex of the HR bell-shaped curve coincided with the spikes in the acceleration trace in 83 of the 97 egg movement events recorded (n = 6 birds, Fig. 3B). Moreover, the HR increase started before the occurrence of the acceleration spikes (Fig. 3B), preceding egg movement by 4.3 ± 0.9 s ($n = 6$). Also, HR returned to basal levels on average 9.1 ± 1.3 s after the end of the egg movement ($n = 6$), suggesting the existence of phases of preparation and latency surrounding each egg movement.

Relating the total number of heartbeats to egg movements, we estimated that moving the egg induced an 8.4 ± 1.3% increase of EE, compared to basal HR during egg incubation (see methods). Both the duration of egg movements and the number of spikes per event were significantly correlated with the duration of the HR deviation from baseline ($R^2 = 0.35$, $^{****}p < 0.0001$ and $R^2 = 0.10$, $^{*}p = 0.0193$, Spearman's correlation test; Fig. 3D and E, respectively).

We identified bird respiration from the electrical noise that it causes on some ECG traces. In correspondence to respiration we observed cyclical oscillations of HR that resemble the quick variations induced by the physiological mechanism of Respiratory Sinus Arrhythmia (RSA). These HR oscillations have been reported in mammals and other animals and are related to the modulation of HR by the autonomic nervous system [43–46] (Fig. 4A). Minimum and maximum instantaneous HR measured on 10 respiration cycles for each animal (delta between minimum and maximum being 56 ± 8 bpm) were significantly different from the basal HR during egg incubation ($^{***}p = 0.0004$, $F = 44{,}84$ by one-way ANOVA; $^{**}p = 0.0031$ and $^{**}p = 0.0031$ with Dunnett's multiple comparisons test of minimum or maximum versus basal HR during egg incubation; $n = 6$; Fig. 4B). Thus, respiration caused HR oscillations of 27% of the basal HR during egg incubation, suggesting a high vagal modulation under this condition. By visual inspection of the HR plot, in some individuals we observed episodes of strong HR slow down. In one of these birds, this HR slow down coincided with a block of conduction between atria and ventricles that caused a skipped heartbeat (Second-degree atrioventricular block; Fig. 5). These episodes further suggest the presence of a strong vagal tone in blue petrels during egg incubation, which could be part of a regulatory mechanism reducing HR and saving energy during fasting.

Allometric scaling of body mass to HR

To contextualize our HR results within the existing literature, we interpolated HR and body mass previously described in 55 bird species [47–51] to calculate an allometric function that we used to infer the theoretical HR of blue petrels at rest (Fig. 6). For the blue petrel's mass, we used the mean body mass calculated from

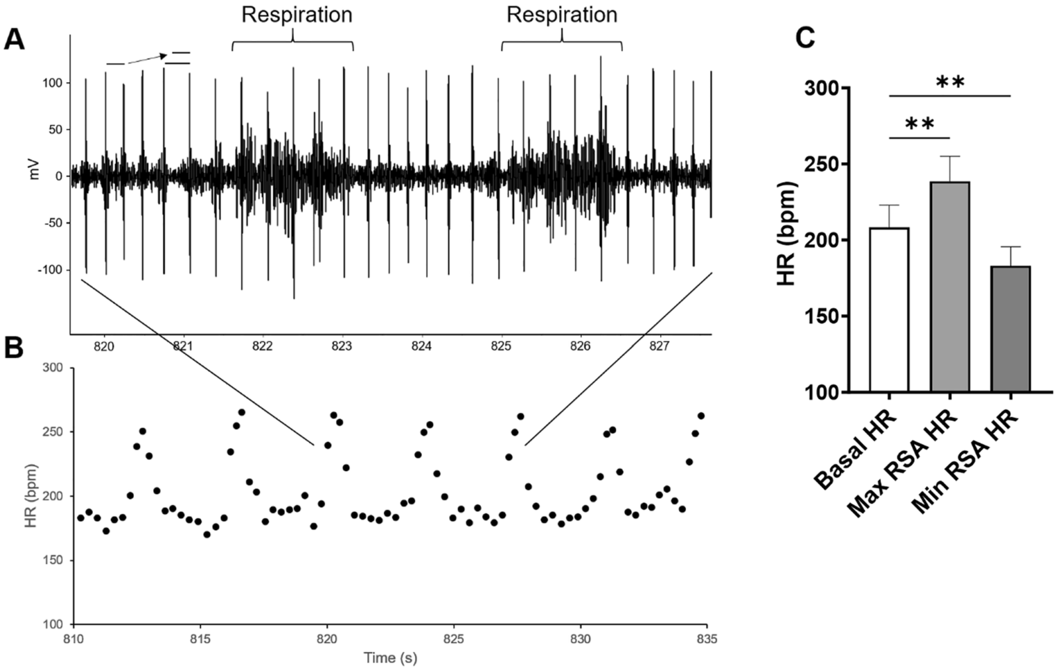

Fig. 4 HR oscillation during blue petrels' respiration. **A** ECG trace showing heartbeats and cyclical noise consistent with respiration. Dashes in the upper left corner compare the second and the fifth RR intervals and highlight the RR difference at successive phases of the respiration cycle. **B** Dot plot of instantaneous HR versus time showing cyclical changes of HR suggestive of RSA. **C** Comparison between basal HR during incubation and minimum and maximum HR during a respiration cycle ($n = 6$). $^{***}p = 0.0004$, $F = 44{,}84$ by one-way ANOVA; $^{**}p = 0.0031$ and $^{**}p = 0.0031$ with Dunnett's multiple comparisons test of minimum or maximum versus basal HR during egg incubation; $n = 6$

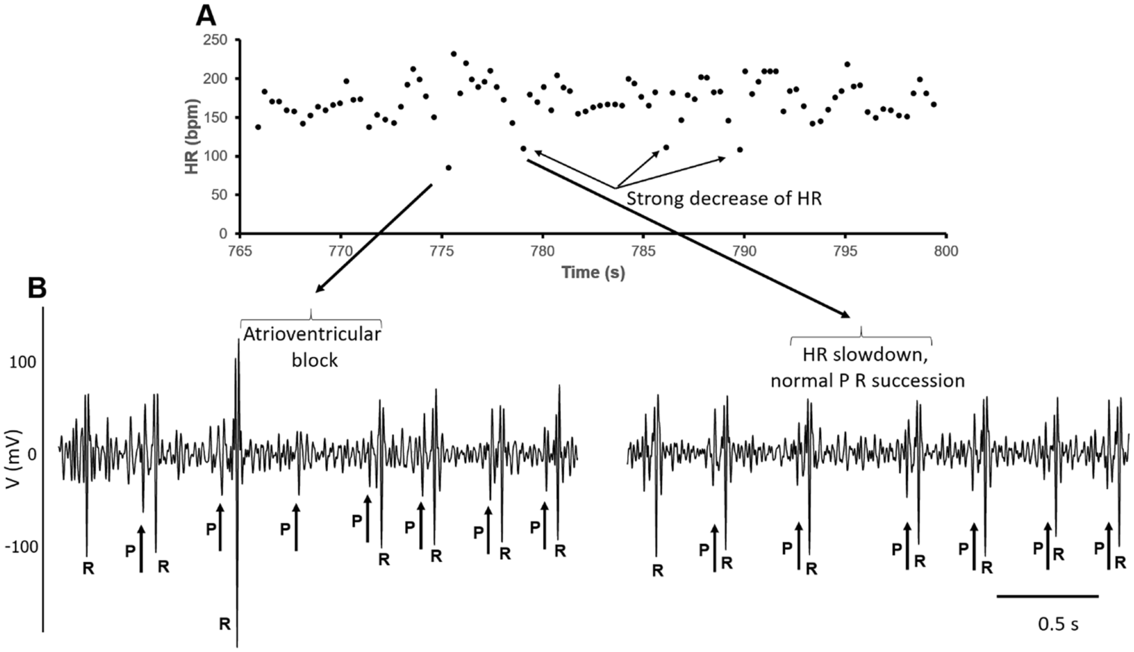

Fig. 5 HR slowdown in relation to respiration. **A** Plot of HR over time, showing cyclical decreases of HR, and in one case a block of conduction between atria and ventricles. **B** left panel: ECG trace showing two consecutive P waves preceding an R wave, which indicates a momentary absence of conduction between atria and ventricles after the first P wave (Atrioventricular block of conduction). **B** right panel: cyclical decreases of HR but with the normal succession between P and R waves. V = voltage

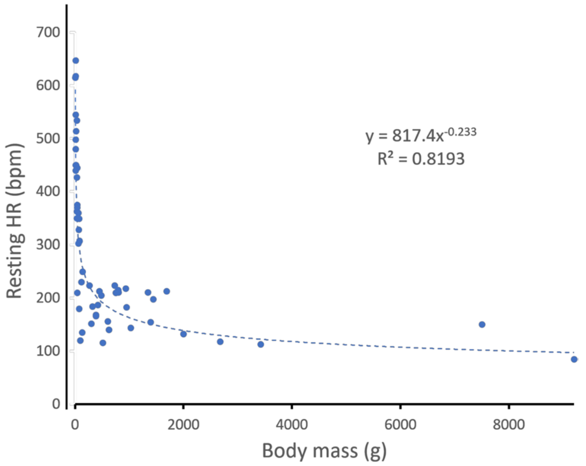

Fig. 6 Allometric curve correlating HR versus body mass of different birds. Allometric curve interpolating HR and body mass with a power function, for data available in the literature for 55 species of birds recorded under rest. Resting conditions mainly were consistent with having the birds quiet, in dark cages and the absence of the experimenter (see supplementary)

20 years of monitoring at Verte island, Kerguelen (185 g, n = 1010). The HR data described in the literature were all recorded on birds maintained at rest, housed in dark cages and the absence of the experimenter (see supplementary material for details of the recording conditions). Thus, in conditions relatively similar to ours, besides the fact that in our measurements the birds were incubating their egg. The allometric function that we obtained ($R^2 = 0.82$, exponent − 0.233) gives a theoretical blue petrel' HR at rest of 242 bpm (Fig. 6), which is higher than the 208 ± 15 bpm that we reported above for petrels incubating their egg. Besides calculating our allometric function, we also investigated whether there were allometric functions available in the literature that relate resting HR with the body mass of birds. We found at least two studies reporting such allometric functions [50, 52]. The function found by Berger et al. [50] resulted in a Blue petrel's hypothetical HR of 250 bpm, which is very similar to our allometric estimation. Concerning the function published by Lindstedt et al. [52], it is not possible to calculate an exact hypothetical HR, because they only provided the exponent of the function. However, this exponent is identical to the one we found with our allometric function (− 0.23 in Lindstedt et al. *vs.* − 0.233 here, see above), suggesting the two allometric functions are comparable.

Considerations about the ECG logger

Although it is developed for EMG, the Cometa Pico EMG logger recorded an excellent ECG signal and has a theoretical recording capacity of 4 h, set by its battery autonomy and memory space. We however encountered some difficulties using the logger in a remote field, preventing us from recording for more than 1–3 h at a time. First, the logger cannot be programmed to start at a specific time but can be started with a remote control. Unfortunately, because blue petrels nest underground, the remote control does not operate correctly, obliging us to start the logger before exchanging the true egg with the dummy one. Second, the sampling rate of 2000 Hz for the biopotential cannot be modified, obliging to acquire more data than what would be necessary for proper ECGs. Third, the downloading time of recorded data is a significant issue because it takes the same amount of time as the recording itself. Thus, the Cometa downloading interface and laptop must be continuously powered and connected while downloading data, which quickly becomes a significant constraint in remote fieldwork. Therefore, future technical improvement will be necessary to obtain longer HR recordings.

Discussion

We analyzed blue petrels' ECGs and behavior during egg incubation, using a logger enclosed in a dummy egg. With this device we were able to record blue petrels' HR and egg movements in a non-invasive way, allowing us to correlate HR with animal behavior during egg incubation.

Basal HR during egg incubation (208 ± 15 bpm) in blue petrels was slower than HR recorded in another burrowing petrel, the Antarctic prion (Pachyptila desolata, reported at 350 to 480 bpm [53]), a species slightly smaller (155 to 180 g 1) that often breeds sympatrically with blue petrels. However, the Antarctic prion's HR was recorded with a more invasive protocol that potentially affected the basal HR [53]. Thus, this protocol could explain why the Antarctic prion's HR is similar to the HR recorded in blue petrels during disturbance caused by egg exchange at the end of the experiment (404 ± 59 bpm). We also observed that the basal HR in blue petrels incubating the dummy egg was about 15% slower than the theoretical resting HR on the ground, estimated with both an allometric power function proposed in the literature (250 bpm [50]) and our allometric function (242 bpm, Fig. 6). This difference suggests that some blue petrels may reduce their metabolism to save energy while incubating their egg.

Our power function calculated with data gathered in the existing literature is approximative and could be biased by the recording conditions. However, these conditions are quite homogeneous across studies and consist of having the birds quiet, in dark cages and the absence of the experimenter (see supplementary materials for details on the recording conditions reported in the literature). Nevertheless, although these conditions are similar to when the birds are in their burrow nest, they do not correspond to the particular state of egg incubation, which could activate specific regulatory mechanisms to decrease HR versus simple resting conditions. Future studies will clarify whether blue petrels have indeed developed specific energy-saving mechanisms involving slower HR, to endure extended fasting while sitting on their egg.

Looking at the plot of HR against time, we observed evident deviations of HR from the basal level, which correspond to periods when the egg was moved (Fig. 3B). Interestingly, these HR increases preceded egg movements by a few seconds, suggesting that blue petrels predict and get ready for movements by mobilizing energy. Similar early increases in HR have been reported in barnacle geese before taking flight [54]. Along the same lines, it took about 9 s for the birds to come back to basal HR levels after the termination of the egg movements, showing a latency that could be necessary to get back to a state of low EE.

EE in birds is directly proportional to HR [28, 29, 55–58]. Thus, based on our HR recordings, we made approximative estimations of how EE changes under different conditions. On these bases, we estimated that the increase in HR induced by egg movements corresponded to a EE raise of 8.4 ± 1.3%, versus basal HR during egg incubation. In comparison, we estimated that the disturbance caused by the perception of the experimenter's arm entering the burrow to return the real egg at the end of the experiment caused a change in HR and EE that was 10 times greater (95 ± 32% increase, versus basal condition) than the effort related to egg movements. This disturbance may be considered a light stress for blue petrels, given that they are not restrained [59], do not move/struggle and only sometimes gently bite like they do for preening. In comparison, birds bite strongly, move, and struggle during a capture-restraint procedure. Repositioning the egg thus entails a negligible energetic cost, albeit still measurable with our approach, which further suggests that blue petrels maintain a slow metabolism during egg incubation. Moreover, the length of egg movements and the number of spikes in the acceleration trace were positively correlated with the length of HR deviation, further indicating a link between egg incubation and HR.

Analyzing the ECG traces, we also observed evident oscillations of HR that correlate with respiration. These oscillations resemble the RSA determined by the autonomic modulation of cardiac activity. In mammals, RSA is thought to ensure a proper match between oxygen ventilation and blood perfusion [44, 60–62] and is generally attributed to quick changes in the vagal tone [47, 63]. The autonomic nervous system activates the vagal nerve during rest and relaxation (rest and digest response), causing a decrease in HR and an increase in

HRV. A higher amplitude between minimum and maximum instantaneous HR increases HRV. Thus, a high delta between the minimum and maximum HR determined by RSA suggests higher vagal tone, which could be part of a mechanism actuated by the birds to reduce metabolism [43–46, 62]. Accordingly, we found that blue petrels' RSA caused a variation of HR equivalent to 27% of basal HR. In one bird we also observed an episodes of atrioventricular blocks, inducing a skipped heartbeat, at the lower HR during the cycle of RSA. A possible explanation for this phenomenon could be that strong vagal tone, during egg incubation, sometimes inhibits conduction through the atrioventricular node, up to generate a momentary block of conduction. Another explanation would be that the specific bird that we recorded had a cardiac pathology causing this kind of conduction block.

Conclusion

Our new non-invasive approach to measure HR gives interesting insights into cardiac activity and, by extension, EE of blue petrels. Indeed, besides cyclical egg movements, the still position and slow HR of some blue petrels suggest that these birds could restrain energy consumption during egg incubation to better resist the prolonged period of fasting while waiting for the other partner to take over incubation.

Moreover, besides recording the behavioral and physiological events occurring while birds incubate their egg, our technique could also be used to investigate the level of stress in response to, among others, predation threats, human disturbance at the colony and environmental changes, opening new possibilities to study the behavior and physiology of seabirds.

Abbreviations

ECGs	Electrocardiograms
HR	Heart rate
RSA	Respiratory sinus arrhythmia
EMG	Electromyogram
bpm	Beats per minute
EE	Energy expenditure

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40317-024-00374-1.

Additional file1.

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Author contributions

AGT and FB designed the study and wrote the manuscript. FB, SC and SB collected the data. SC participated in proofreading the manuscript. AGT analyzed the data and prepared the figures.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

All experimental manipulations cited and performed were approved by French Ethical Committee (n° APAFIS#31453-2021050714441387 v3 on 25/06/2021) after favorable recommendation of the Comité d'Ethique pour L'Expérimentation Animale Languedoc-Roussillon (CEEA-LR), C2EA n°36, and by the Ethical Committee of Réserve Naturelle des Terres Australes et Antarctiques Françaises (arrêté n° 2022-66 du 18/07/2022).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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