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# PRELIMINARY REPORT OF NEW METHOD FOR ECG MEASUREMENT OF EXERCISING BIRDS

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***Abstract:*** To measure the heart rate of diving seabirds, we tested a new method of attaching electrodes and a miniaturized ECG logger with high memory capacity (2 Mbytes) and high frequent sampling (5 ms). A needle type electrode was inserted into subcutaneous tissue of Adélie penguins, and an electrode was implanted under the sternum of hens. Both electrodes were connected to a logger attached to the back of the bird. The mean $\pm$SD of heart rate of penguins for 3 h and of hens for 1 h during a resting period were $74.9 \pm 15.4$ bpm and $245.3 \pm 12.1$ bpm, respectively. The ECG showed electric noise during exercising periods. However, as R peaks were countable in those periods, it was possible to calculate the heart rate during exercising periods from the interval between R peaks. Though the implantation method needs some recovery time for birds after surgery before the ECG measurement, the method reduces the electric noise caused by the locomotor muscle and electrode movement and is suitable to measure the ECG of free-ranging seabirds during dives.

**key words**: ECG, heart rate, data logger, seabirds

## Introduction

Recently free-ranging seabirds have been observed to make extremely long dives which seemed to exceed their aerobic dive limit (ADL) (CROXALL *et al.*, 1991; CROLL *et al.*, 1992; KOOYMAN and KOOYMAN, 1995). The ADL is defined as the longest dive duration performed by using the energy from aerobic metabolism (KOOYMAN, 1989) and is theoretically estimated from the quantity of oxygen stored in the blood, muscle and lungs, and the oxygen consumption rate during diving (summarized in KOOYMAN, 1989). It has been hypothesized that seabirds might decrease their metabolic rate during diving from the fact that the body temperature of seabirds decreased during a series of dives (HANDRICH *et al.*, 1997; BEVAN *et al.*, 1997). This seemed to enable seabirds to make extremely long dives in excess of the calculated ADL. Although the quantification of oxygen consumption of seabird during diving is important for understanding physiological adaptation for long dives (CROXALL *et al.*, 1991; CROLL *et al.*, 1992; KOOYMAN and KOOYMAN, 1995), it is difficult to measure the oxygen consumption of free-ranging seabirds during diving.

One technique for estimating metabolic rate in a field is the double-labeled water (DLW) method. The average metabolic rate during a sampling period is estimated from the turnover rate of the two isotopes ($H_2{}^{18}O$ and $^2H_2O$ or $^3H_2O$). The DLW method sampling period ranges from a few days to several weeks, giving poor temporal resolution (NOLET *et al*., 1992). Many validation studies have been performed comparing the DLW method to direct respirometry (MASMAN and KLAASEN, 1987; SPEAKMAN and RACEY, 1988; GALES, 1989; WEBSTER and WEATHERS, 1989) and summary of these reports has indicated that the average precision of the DLW method is about 12% (SPEAKMAN and RACEY, 1988). BEVAN *et al*. (1995) reported that the metabolic rate measured by the DLW method had less precision during diving or swimming than on land.

An alternative technique for evaluating energy expenditure is to utilize the correlation between heart rate and oxygen uptake (NOLET *et al*., 1992; BEVAN *et al*., 1994). This technique has at least equivalent accuracy to the DLW method and has more detailed time resolution (NOLET *et al*., 1992; BEVAN *et al*., 1994, 1995). However, it is difficult to measure the heart rate of diving seabirds in the field, since the ECG of exercising animals has shown electric noise because of the locomotor muscle and electrode movement. Recently a miniature data logging system has been developed to record the heart rate (WOAKES *et al*., 1995). This device was implanted in the abdominal cavity of seabirds in order to avoid the electric noises, and the heart rate was counted from the voltage between two electrodes. But this method requires surgery twice for implanting and retrieving the devices, and repeated measurement of heart rate of the same bird is impossible.

We carried out the first experiment with Adēlie penguins (*Pygoscelis adeliae*) to measure the ECG using a new miniaturized data logger with high memory capacity and frequent sampling. We used needle type electrodes with Adēlie penguins, but the result included electric noise during exercising. In order to reduce the electric noise during exercising and measure the heart rate in a simple and easy way, we tested a new method of attaching electrodes to hens (*Gallus domesticus*). And we considered the possibility of applying this method to free-ranging seabirds.

## Materials and Methods

ECG logger

ECG loggers were 20 mm in diameter, 90 mm in length, domed-top cylindrical shape, 52 g mass including battery and two lead wires with electrodes were connected to the bottom of the logger (Little Leonard Co. Ltd., Fig. 1a). These loggers were programmed to record the voltage between two electrodes at a range of $\pm 5.8$ mV with $2.9\times10^{-3}$ mV resolution at 5 ms intervals, and can store data in a 2 Mbyte flash memory. The data compression system allows the loggers to record 2–3 times more data than normal. The data were downloaded to a hand-held computer. The lead wire was made of twisted copper coated with vinyl. For penguins, the extremity of the lead wire was ended by a stainless needle, encapsulated into an insulator (Fig. 1a). For hens, the coat was removed on 5 mm at the extremity and the 2 electrode were connected together by a silk thread (Fig. 1b).

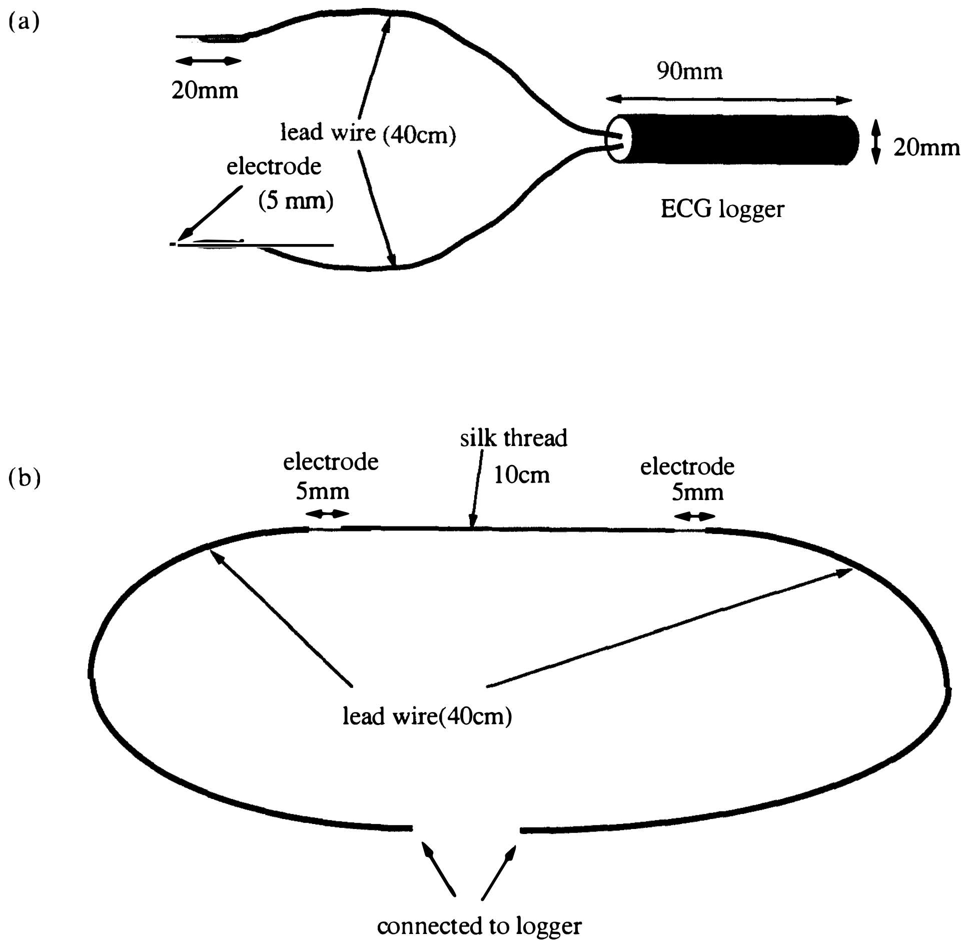


Fig. 1. The ECG logger and the electrodes used for Adélie penguins (a) and hens (b).

Implantation and measurement

ECG was measured for four Adélie penguins breeding at the Hukuro Cove colony, Lützow-Holm Bay (69°12′S, 39°39′E) in austral summer 1996/97 and for two hens in Japan during August to October in 1997 using different ways of attaching the electrodes. For penguins, the sterilized needles were put into subcutaneous tissue. One was fixed above the first rib on the left side of the body and the other was at the middle of the last and eighth rib on the right side. The lead wires were passed under their feathers and fixed by water-proof tape. The loggers were attached to the feathers on the center of back by using water-proof tape. Two non-breeding penguins were kept in the enclosure after the attachment of loggers and observed for 2–3 h and the devices were removed after 6 h. The other two breeding penguins were attached ECG loggers and DT loggers which were recorded depth and ambient temperature every 1 s (14 mm in diameter, 84 mm in length, and 26 g) to monitor behavior of the birds, then the birds were released. When the penguins came back to the colony, they were recaptured and the devices were retrieved.

Implantation surgery was conducted on two hens under anesthesia with 2–3% isoflurane by using the Stephens Anesthetic Machine (Australian Anesthetic Equipment Co. Pty. Ltd.). The sterilized stylet was inserted from the upper side of the clavicle to the abdominal cavity under the sternum. The end of the stylet was pulled out from the incision on the abdominal cavity and tied with sterilized lead wires. Then the stylet was pulled out in the direction of the entrance incision. Each electrode was positioned above and below the heart on the right side and we sutured the incision at the best position by observing with the ECG monitor. The lead wires were passed through the subcutaneous tissue from the position where the lead wires were fixed to the center of back where the logger was attached in the same way as to the penguins. The ECG measurements during resting and exercising periods were conducted one week after the surgery. The lead wires of one hen were removed after the measurement and the bird was dissected one month later in order to examine tissue recovery from surgery damage. The other bird was also dissected to examine the effect of implantation of the electrode for one month.

The data were filtered using a binomial smoothing operation using Igor (WaveMetrics, Inc., USA) on a Macintosh computer. This filtering technique is a Gaussian filter that convolves the data with normalized coefficients derived from Pascal's triangle at a level equal to the smoothing parameter. We set this smoothing parameter to 401. And the difference between these smoothed data and original data was defined as high pass filtered data. The heart rate were counted every 5 min for 30 s by using the high pass filtered data for one bird to examine the change of heart rate according to the behavior. In order to estimate the effect of electric noise, the maximum amplitude of electric noise and the amplitude of ECG during resting were measured over 5 peaks for each individual.

## Results

According to the depth data of the loggers, three Adélie penguins did not dive during the ECG measurement periods (3.6 h after release), and then they started to dive later. When the birds came back to the colony after a foraging trip (92.1 $\pm$ 66.6 h), the electrodes were taken off. The ECG data were obtained for 3.2–3.8 h from five penguins on land. The hens recovered from anesthesia 2–3 h later, and then after one week, the ECGs of the hens were measured for one hour. The hens had looked good in health and normal in their behavior from the measurement to the autopsy. Neither the bird with electrodes removed, nor the bird without removal, showed any infection at the electrode implantation site according to the autopsy performed.

The ECG shows clear peaks and the heart rate can be counted easily from penguins and hens in resting condition (Fig. 2a, b). During the exercising period, however, the ECG included electric noise and detecting all R peaks was difficult (Fig. 2c, d). The heart rate was counted for one penguin for 150 min and one hen for 50 min. During some periods the rate was not countable for penguins because of the large electric noise during exercise. The mean $\pm$ SD of heart rate of penguins and hens during the resting period were 74.9 $\pm$ 15.4 bpm (range 58–130 bpm, $n=24$) and 245.3 $\pm$ 12.1 bpm (range 226–266 bpm, $n=12$), respectively.

The mean amplitudes of two hens in resting period were 0.17 $\pm$ 0.02 mV and 0.39 $\pm$ 0.01 mV and those of penguins were 0.49 $\pm$ 0.03 mV and 0.40 $\pm$ 0.01 mV, while the other two penguins did not exercise during the deployment of the device. The maximum amplitudes of electric noise for hens were 3.8 mV and 4.9 mV, and for penguins were 14.7 mV and 12.1 mV respectively. The amplitudes of electric noise of hens were 12.5–22.6 times larger than those of normal ECG, on the other hand those of penguins were 30.0–

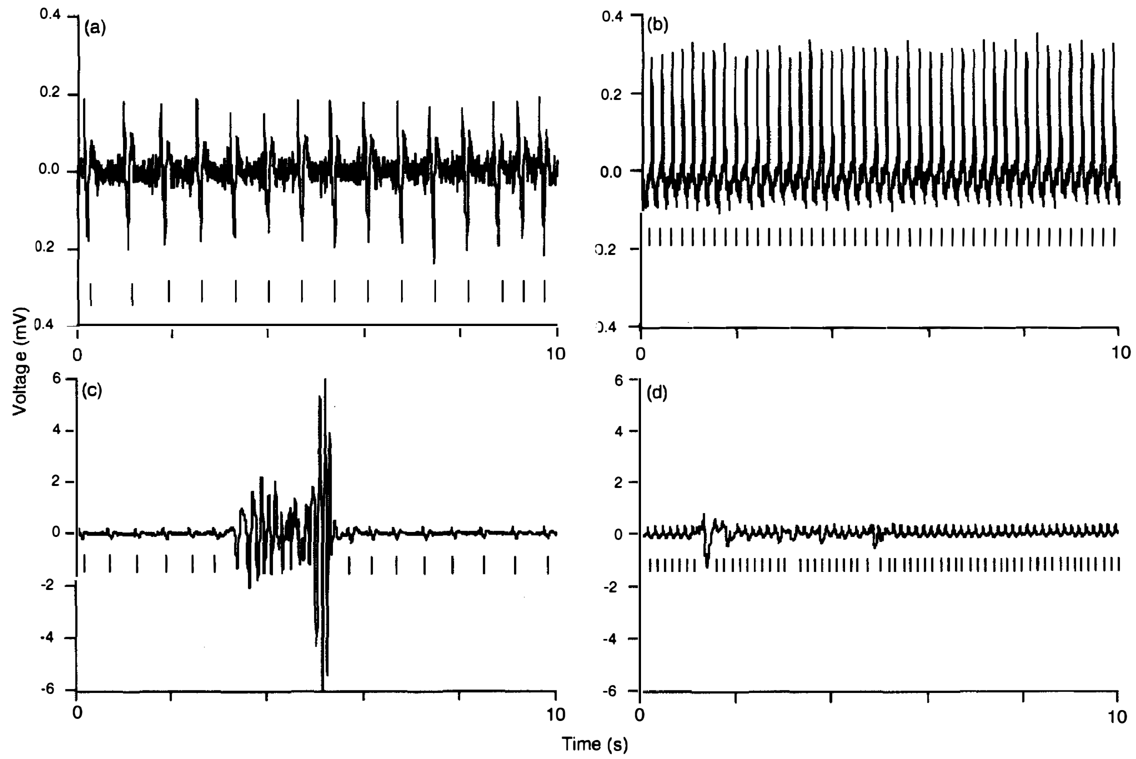

Fig. 2. The ECG during the resting period of Adélie penguin (a) and hen (b), and during the exercising period of Adélie penguin (c) and hen (d). The bars show the peaks counted to calculate the heart rate.

32.8 times larger.

## Discussion

The ECG trace of penguins obtained by needle electrodes and that of hens by implanted electrodes showed clear peaks; the heart rate was easily counted during the resting period (Fig. 2a, b). During the exercising period, there were large electric noises (Fig. 2c, d). However, as the ECG pattern showed normal patterns just after the exercise, the heart rate could be calculated from the interval between the two peaks in most cases. With this method, the heart rate change can be analyzed on a fine time scale. The shape of the ECG also may give useful physiological information, such as the autonomic nerve control concerned with the blood flow. The heart rate data of this study in resting condition are considered to be reliable, as the heart rates of penguins and hens measured in this study were similar to the value for Adélie penguins on land (76–83 bpm, CULIK, 1995) and the normal value of hens (220–340 bpm, KADONO, 1962).

The method for fixing the electrodes was important, since the physical movements of the electrodes caused electric noise on the ECG. Fixing the needles and lead wires was difficult and they were easily taken off by the birds. On the other hand, the implanted electrode was fixed at two positions to the body and the lead wires were passed through the subcutaneous tissue. As a result, those systems were well fixed and never taken off by birds. Moreover, since the electrodes in hens were positioned under the sternum and those in the penguins in the locomotor muscle, the electric noise on ECGs for hens should be less than that for penguins, especially during the exercising period.

In the case of free-ranging seabirds, we have to consider the effect of attachment of the ECG logger and the electrode on the behavior of the birds. Since the mass of loggers was less than 2% of their body mass, this is well within the threshold area (6.8%) of devices with which Jackass penguin (*Spheniscus demersus*) are known to be able to balance their energy budgets at sea (WILSON *et al*., 1986). In this study, however, trip durations of penguins with the ECG loggers attached were longer than those of penguins without loggers (A. KATO unpub. data). The prolonged trip duration might have been caused by the attachment of these devices. Other possible reasons for the long trip duration might be pain from the needle inserted subcutaneously, as there are nerve endings of the sense of pain. Though the implantation method conducted on hens required some recovery time after the surgery before the measurements, the birds looked normal after one week. In this point, the implantation method may disturb the diving behavior of seabirds less than the needle method, though the difference of the damage of the surgery between hens and Adēlie penguins should be examined.

In this study, we failed to measure the ECG of penguins during diving. As the memory capacity is limited and sampling interval is very short, the recording period of the logger is also limited. One solution is to increase the memory size of the logger and the sampling interval can be set as long as possible. Another possibility is to develop an ECG logger with salt water sensor so that the logger starts logging when the bird starts to dive. An advantage of the implantation method is that we can measure the ECG repeatedly with the same birds by changing the device since the logger is externally attached to the bird.

Though the implantation method requires some recovery time, at least several, days for birds after surgery before ECG measurement, this method reduces the surgery for retrieving the device and the electric noise caused by the locomotor muscle and the movement of the electrode. Therefore this method is suitable to measure the ECG of free-ranging seabirds.

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## References

BEVAN, R.M., BOYD, I.L., BUTLER, P.J., REID, K., WOAKES, A.J. and CROXALL, J.P. (1997): Heart rates and abdominal temperatures of free-ranging South Georgian shags, *Phalacrocorax georgianus*. J. Exp. Biol., **200**, 661–675.

BEVAN, R.M., WOAKES, A.J., BUTLER, P.J. and BOYD, I.L. (1994): The use of heart rate to estimate oxygen consumption of free-ranging black-browed albatrosses *Diomedea melanophrys*. J. Exp. Biol., **193**, 119–137.

BEVAN, R.M., SPEAKMAN, J.R. and BUTLER, P.J. (1995): Daily energy expenditure of tufted ducks: A comparison between indirect calorimetry, doubly labeled water and heart rate. Func. Ecol.,

**9**, 40–47.

CROLL, D.A., GASTON, J.A., BURGER, A.E. and KONNOFF, D. (1992): Foraging behavior and physiological adaptation for diving in Thick-billed Murres. Ecology, **73**, 344–356.

CROXALL, J.P., NAITO, Y., KATO, A., ROTHERY, P. and BRIGGES, D.R. (1991): Diving patterns and performance in the Antarctic Blue-eyed Shag *Phalacrocorax atriceps*. J. Zool., London, **225**, 177–199.

CULIK, B. (1995): Energy expenditure of Adêlie penguins. The Penguins: Ecology and Management, ed. by P. DANN *et al*. Sydney, Surrey Beatty, 177–195.

GALES, R. (1989): Validation of the use of tritiated water, doubly-labelled water, and $^{22}$N for estimating food, energy, and water intake in little penguin, *Eudyptula minor*. Physiol. Zool., **62**, 147–169.

HANDRICH, Y., BEVAN, R.M., CHARRASSIN, J.-B., BUTLER, P.J., PÜTZ, K., WOAKES, A.J., LAGE, J. and LE MAHO, Y. (1997): Hypothermia in foraging king penguins. Nature, **388**, 64–67.

KADONO, H. (1962): Physiological studies on the electrocardiogram of the hen III. On the normal value of the electrocardiogram of laying hen. Res. Bull. Fac. Agr. Gifu Univ., **16**, 173–181.

KOOYMAN, G.L. (1989): Diverse Divers. Berlin, Springer, 200 p.

KOOYMAN, G.L. and KOOYMAN, T.G. (1995): Diving behavior of emperor penguins nurturing chicks at Coulman Island, Antarctica. Condor, **97**, 536–549.

MASMAN, D. and KLAASEN, M. (1987): Energy expenditure for free-flight in trained and free-living kestrels *Falco tinnunculus*. Auk, **113**, 253–267.

NOLET, B.A., BUTLER, P.J., MASMAN, D. and WOAKES, A.J. (1992): Estimation of daily energy expenditure from heart rate and doubly labeled water in exercising geese. Physiol. Zool., **65**, 1188–1216.

SPEAKMAN, J.R. and RACEY, P.A. (1988): Validation of the doubly labeled water technique in small insectivorous bats by comparison with indirect calorimetry. Physiol. Zool., **61**, 514–526.

WOAKES, A.J., BUTLER, P.J. and BEVAN, R.M. (1995): Implantable data logging system for heart rate and body temperature: Its application metabolic rates in Antarctic predators. Med. Biol. Eng. Comput., **33**, 145–151.

WEBSTER, M.D. and WEATHERS, W.W. (1989): Validation of the single sample doubly labeled water method. Am. J. Physiol., **256**, R572–R576.

WILSON, R.P., GRANT, W.S. and DUFFY, D.C. (1986): Recording devices on free-ranging marine animals: Does measurement affect foraging performance? Ecology, **67**, 1091–1093.

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