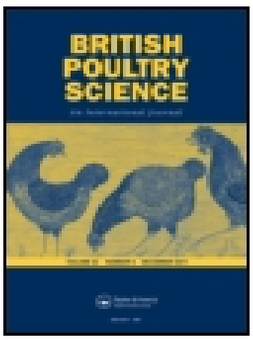

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# Embryonic heart rate measurements during artificial incubation of emu eggs

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**Abstract** 1. Daily changes in embryonic heart rate (HR) of emu were determined non-invasively at 36°C by acoustocardiography (ACG) during the last 30% of artificial incubation (predicted incubation time is 50 d).
2. The pattern of daily changes in mean HR of hatched embryos decreased from about 175 bpm to about 140 bpm towards the end of incubation.
3. The mean HR at 80% of incubation (ca. 170 bpm) was close to the value predicted from an allometric equation reported previously for precocial domesticated birds.
4. ACG could measure embryonic HR even during the external pipping period.
5. If the artificial external pipping procedure is timed correctly after internal pipping, it might aid the embryos in hatching. However, further investigation into this aspect is needed.

## INTRODUCTION

Both the ostrich and the emu (*Dromaius novaehollandiae*) have been domesticated for industry. However, only a few physiological data have been reported for emu embryos and egg incubation (Vleck *et al.*, 1980) and fertile eggs are not always readily available for experimentation. Recently, we were able to borrow emu eggs incubated at a local farm and attempted to measure embryonic heart rate (HR) noninvasively by acoustocardiography in order to investigate the daily changes. For domesticated birds, embryonic HR is allometrically related with egg mass (Tazawa *et al.*, 1991*a*), and in the present report embryonic HR measured in emu was compared with the value predicted from that allometric equation. In addition, on the predicted hatching day of the 1st laid egg, we cracked the eggshell to examine the influence of artificial external pipping (EP) on hatching and the possibility of using acoustocardiography to measure HR during the EP period.

## MATERIALS AND METHODS

### Measurement of acoustocardiogram and heart rate determination

Determination of embryonic viability and HR was made by acoustocardiography in a desk-top still air incubator from day 36 of incubation of the 1st laid eggs until the predicted day of hatching (day 50). The incubator was a wooden thermostatted box 60 × 60 cm wide and 30 cm high, covered with a removable transparent plastic dome. Two round windows, 10 cm across, were cut through the front panel of the chamber and covered with rubber flaps. Water was placed in a tin square plate on the floor of the incubator to supplement humidity. All the eggs were in a horizontal position on metal mesh about 10 cm above the floor and remained there until they hatched or died. Every day, eggs were turned over manually in the morning and evening.

Acoustocardiography is based on the fact that in association with the heartbeat of the embryo inside the eggshell, minute pressure changes occur in the atmosphere surrounding the eggshell, which can be detected with a conventional condenser microphone (Rahn *et al.*, 1990; Wang *et al.*, 1990; Haque *et al.*, 1994; Akiyama *et al.*, 1997, 1999; Tazawa *et al.*, 1998*a*). The cardiogenic signal detected by a microphone was termed as the acoustocardiogram (ACG) (Rahn *et al.*, 1990). In the present study, the microphone was attached and sealed hermetically with Plasticine on to the eggshell for each ACG measurement. It was biased by a 6-volt battery and connected to a Grass polygraph amplifier through the windows of the incubator. The microphone output was monitored in parallel by an oscilloscope. If no ACG signal was detected, the microphone was repositioned on the eggshell until the required signal was obtained. If no signal was detected despite several such trials, the egg was judged infertile, or dead if the ACG had been detected on previous days. Because the microphone was always kept in the same incubator as the eggs, handling and attaching it to the egg were carried out through the windows of the incubator without moving the egg. These procedures minimised disturbances to the eggs and incubation temperature and thus reduced possible influences of egg rotation and temperature change on embryonic HR

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(Vince *et al.*, 1979; Tazawa *et al.*, 1991*b*; Tazawa *et al.*, 1992).

The ACG was recorded on the polygraph chart at a speed of 5 mm/sec. Continuous recordings of no less than 40 s were made every 2 min for at least 10 min. This recording was sectioned every 6 s. A value of HR in beats per min (bpm) for the approximate 6 s period was counted from the number of ACG waves and the time interval between 2 peaks of the 1st and last ACG waves in the period (referred to as $HR_6$). Six to 7 values of $HR_6$ were determined for an approximate 40 s period every 2 min for about 10 min. Finally, 30 to 35 values of $HR_6$ were averaged to give a mean value of HR for an embryo on a given incubation day (referred to as MHR). MHR was an average of at least 400 to 500 heartbeats over about a 10 min period. Measurements were made for 2 eggs concurrently using 2 microphones and 2 recording channels.

### Incubated eggs

Twelve eggs were laid by 2 birds in 1 nest on a kibbutz farm in Israel in early February, 1997. The laying date of the 1st egg was known, although later on the 1st egg could not be distinguished and subsequent eggs were not identified. After the eggs were incubated for several days in the nest, they were collected and transferred to a 36°C incubator in the kibbutz. They were then brought to the laboratory within several hours, weighed to 1 g, and incubated temporarily in a large forced draught incubator set at 36°C. After several hours, each egg was individually transferred to the desk-top still air incubator kept at 36°C and checked by acoustocardiography for signs of life. Live eggs were left for incubation and daily HR measurement. Other eggs were removed and opened to examine if they were infertile or dead. On the date of arrival at the laboratory, embryonic age of the 1st laid egg was 35 d. The total incubation period of the emu was not directly ascertained, but predicted to be 50 d. On day 49 of incubation of the 1st laid egg, the shell over the air cell of all live eggs was cracked with a hammer in an attempt to aid the embryo in hatching (artificial EP). The status of pipping of eggs was then checked visually through a hole made by artificial EP.

## RESULTS

### Acoustocardiogram

Of the 12 eggs, 4 did not show any signal corresponding to the heartbeat on the day of arrival at the laboratory and were opened; three of them were found to be infertile and one had died early in incubation. Experiments were on the remaining 8 live eggs.

Figure 1 shows examples of the ACG in 2 embryos measured on the predicted day of hatching of the 1st laid egg. These eggs had shell fractures and a small hole over the air cell relating to the artificial EP made on the preceding day. The ACG in the upper recording is a monophasic wave signal and a mean of $HR_6$ is 135±2 (SD, $n$=6) bpm, while the ACG in the lower panel comprises sharp compound signals and the mean HR is 158±3 (SD, $n$=6) bpm. The waveforms varied with time even in the same embryos.

### Success of development and developmental patterns of heart rate

The mean egg mass of the emu 15 d prior to artificial EP was 568±11 g (SD, $n$=8). If we assume that water loss during the pre-pipping incubation

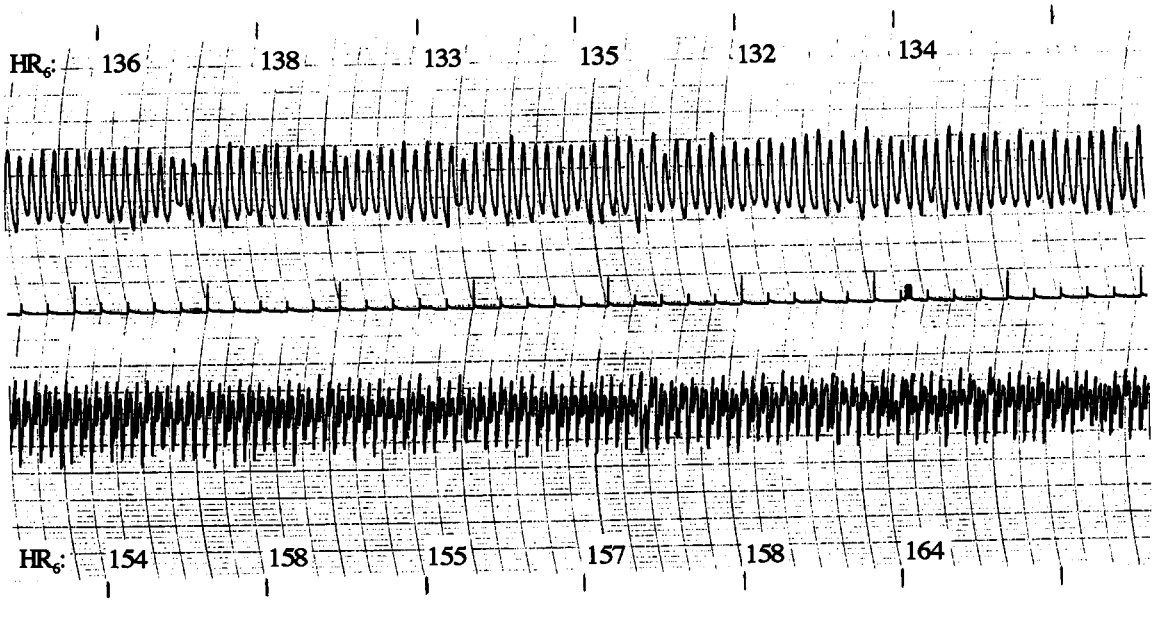


**Figure 1.** *The acoustocardiograms of 2 emu eggs whose eggshell over the air cell was cracked (artificial external pipping) on the previous day. In the centre, short and long time makers are drawn every 1 s and 5 s, respectively. The numerical figures shown every 6 s indicate the heart rate for each approximate 6 s period ($HR_6$).*

period is 13% of the fresh egg mass and occurred linearly with incubation time, the fresh egg mass was estimated to be 625 g.

On day 49 of incubation for the 1st laid egg, none of 5 eggs which were alive had yet externally pipped the eggshell and thus they were all subjected to artificial EP. Through a hole opened by artificial EP it was observed that 3 of them had already pipped the inner shell and chorioallantoic membranes (IP stage). One of these 3 IP embryos, whose ACG could not be detected, occupied the air cell with its head and body and hatched 1 day later (100% of incubation). Its body mass was 440 g (estimated fresh egg mass = 640 g). Two other embryos, whose ACGs were recorded, also pushed their heads into the air cell. One embryo also hatched 1 day later and its body mass was 397 g (estimated fresh egg mass = 620 g). The 3rd whose ACG could be recorded on the day of hatch of the former 2, hatched 1 day later with a body mass of 414 g (estimated fresh egg mass = 643 g). The progress of IP of this embryo was less advanced compared with the former 2 when artificial EP was made. The remaining 2 embryos did not pip the chorioallantoic membrane even 1 day after artificial EP.

Figure 2 presents daily changes (developmental patterns) in MHR. The abscissa indicates normalised incubation days. The day of hatch of the 1st chick was defined as 100% of incubation. Two embryos which died early in the measuring period (at 66% and 77% of incubation) had relatively low HR or temporal bradycardia. Three other embryos which failed at 87% and 95% of incubation showed developmental patterns similar to those of hatched embryos. In Figure 3, the pattern of daily changes in mean HR in 3 hatched embryos is shown together with the HR of ostrich embryos measured in 1996 and 1997. The mean HR decreases from about 175 bpm at 70% to 74% of incubation to 140 bpm at end. The emu HR predicted from the allometric relation is also shown in Figure 3 (circle with dot).

## DISCUSSION

One of the aims of the present study was to clarify whether or not the ACG could be recorded during the pipping period when a pip-hole was made through the eggshell and the egg was opened to the atmosphere. We found that a good ACG signal could be detected by selecting the measuring position on the eggshell (Figure 1). The embryos whose ACGs were presented in Figure 1 did not pip the chorioallantoic membranes even after the artificial EP. This indicates that the blood flow through the chorioallantoic capillaries still remained active in gas exchange while the inside of the egg was opened to the air. In an embryo that hatched on the predicted hatching day, the ACG could not be detected 1 day before hatching. This embryo internally pipped the chorioallantoic membrane and its body occupied the air cell when artificial EP was carried out. It may be inferred that the gas exchange of this embryo took place through the lungs and that the chorioallantoic gas exchange had diminished owing

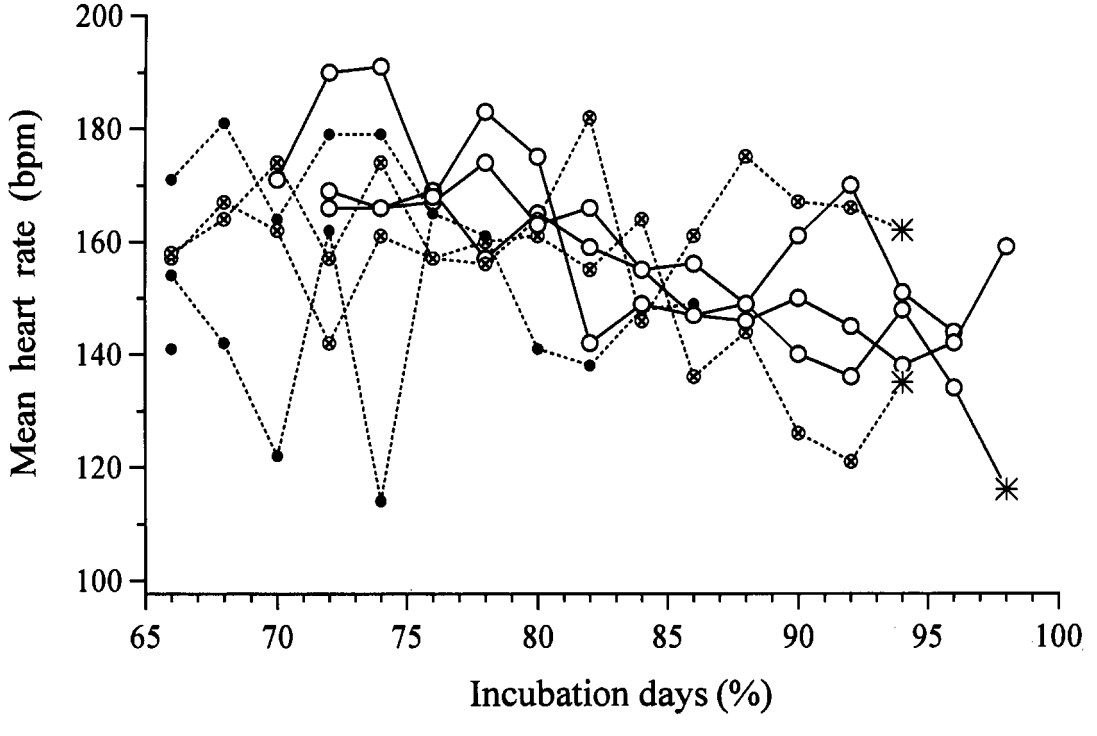


**Figure 2.** *Developmental patterns of mean heart rate plotted against percent incubation days. The hatched embryos are shown by open circles connected by solid lines. Two embryos that died after artificial external pipping are represented by open circles with crosses inside them, connected by dotted lines. These 2 failed eggs were assumed to have died 3 days before the predicted hatching day according to the status of the air cell which was still intact. Embryos that died early in the measuring period are shown by closed circles connected by dotted lines and their 1st day of incubation was assumed to be the same as the other failed eggs. Asterisks show the mean heart rate measured during the artificial external pipping period.*

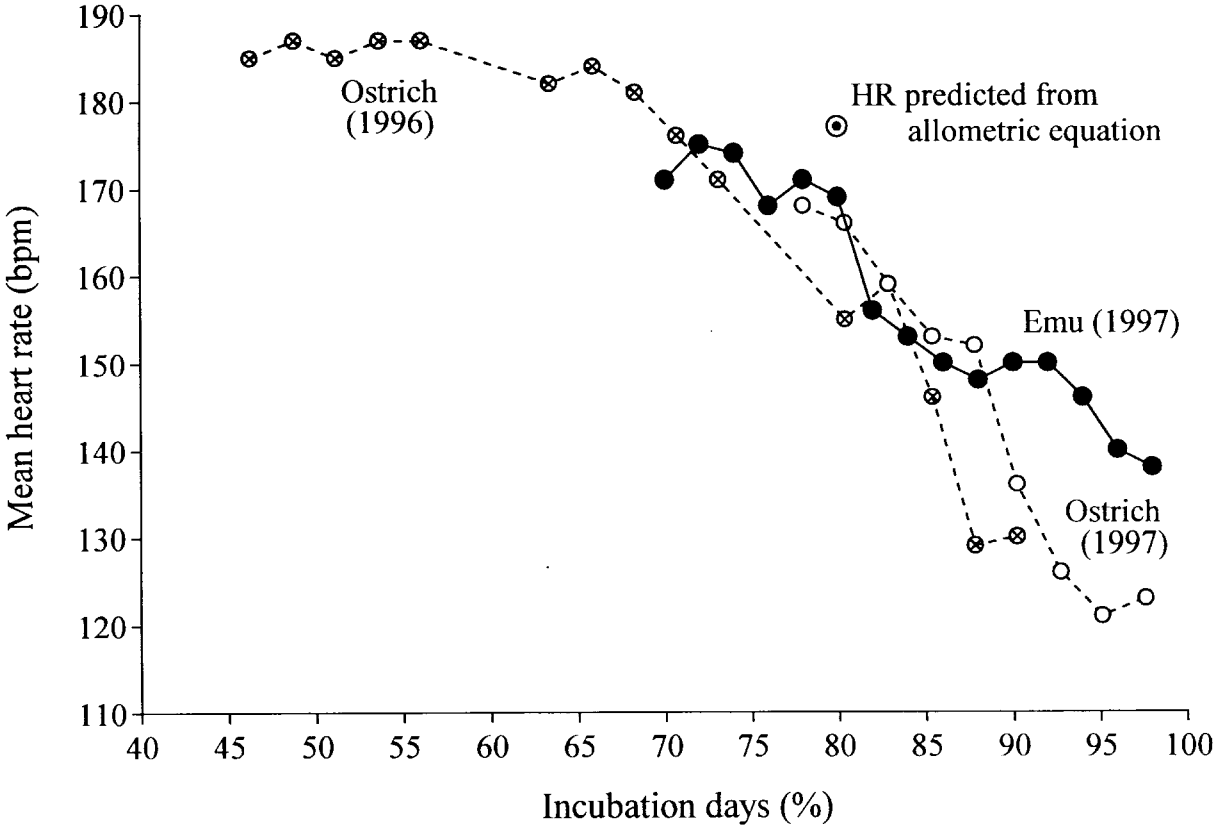


**Figure 3.** *Developmental patterns of mean heart rate in emu embryos of the present study and in ostrich embryos of the previous reports. The ostrich heart rates measured in 1996 (Tazawa* et al., *1998*a*) and 1997 (Tazawa* et al., *1998* b*) are mean values of 12 and 24 eggs, respectively. Emu heart rate at 80% of incubation, predicted from an allometric relation, is shown by the circle with dot inside it.*

to decreasing blood flow through the chorioallantoic capillaries during the IP period. Thus, it may support the proposed mechanism of ACG occurrence originating in pressure oscillations caused by temporal variations in net gas exchange through the chorioallantoic membrane and the eggshell associated with individual heartbeats (Wang *et al.* 1990), in contrast to an alternative suggested mechanism that the ACG of the chick embryo originates at least in part from cyclic changes in heart volume due to heartbeats within the eggshell, which causes pressure changes (Rahn *et al.* 1990). Similarly, because the ACG could be detected even during artificial EP as well as the IP period in another hatched egg, blood flow through the chorioallantoic capillaries of this embryo was probably maintained locally. Thus, if the microphone is placed so that it covers an area of the chorioallantoic membrane through which local blood flow still remains large enough to sustain gas exchange, the microphone is still able to detect the ACG. The changeable waveforms may reflect changes in local blood flow. The ACG is relatively undisturbed by embryonic activities compared with other cardiogenic signals such as the electrocardiogram (Akiyama *et al.*, 1997). The embryonic activities of the emu were strong enough to move the whole egg near the end of incubation. Nevertheless, the ACG could be detected even during such strong egg movements.

Artificial EP was carried out on 5 eggs and 2 of them died 1 day after measurement of ACG. It seems that the EP procedure was mistimed for these 2 eggs: it took place before the IP and may have cause embryonic death. However, if artificial EP was timed correctly after the IP, it might aid the embryos in hatching because the 3 embryos which had pipped internally hatched 1 to 2 days after cracking the eggshell. But, more study is needed together with a reliable method for identifying the IP. A condenser measuring system could detect breathing activities in perinatal chick embryos (Akiyama *et al.*, 1999) and this may be used to determine time of the artificial EP.

MHR varied daily in up-and-down fashion in some embryos (Figure 2). In avian embryos, HR becomes variable towards the end of incubation; it has been shown that HR for a period of few seconds (for example, $HR_6$ in this study) may vary widely during a given measurement period (10 min in this study) with embryonic development, not only in precocial domesticated birds but also in semi-precocial seabirds and altricial birds (Tazawa *et al.*, 1991*a*, *b*, 1994; Tazawa and Whittow, 1994). The ostrich also shows increasing variability in embryonic HR during the last stages of incubation (Tazawa *et al.*, 1998*a*, *b*). Thus MHR determined over a 10 min period does not necessarily represent the mean value of HR on a given day late in incubation for a given embryo. However, it is not always practical to measure HR for a prolonged period if the aim is to determine the developmental patterns of HR in

many embryos incubated at the same time. We used a 10-min measurement for individual embryos as was done previously in other species of birds (Tazawa *et al.*, 1991*a*, *b*, 1994; Tazawa and Whittow, 1994; Tazawa *et al.*, 1998*a*, *b*). The averaged pattern of daily changes in HR indicated that HR decreased steadily from about 175 bpm to about 140 bpm during the last 30% of incubation (Figure 3). The ostrich also shows decreasing pattern of HR towards the end of incubation (Tazawa *et al.*, 1998*a*, *b*). The developmental pattern of HR in both species overlapped during 70% to 90% of incubation, but differed during the last 10% of incubation. The oxygen consumption of both emu and ostrich embryos also decreased towards the end of incubation (Hoyt *et al.*, 1978; Vleck *et al.*, 1980; Ar, 1996). Incubation was longer and the decrease in $O_2$ consumption smaller in the emu than in the ostrich. Because the percentage decrease in HR during the last stages of incubation was lower in the emu compared with the ostrich, the decrease in HR may be related to a decrease in $O_2$ consumption.

In 6 species of precocial domesticated birds whose average egg mass ranged from about 11 g in Japanese quail to about 160 g in geese, the embryonic HR at 80% of incubation and at a temperature of 38°C can be expressed by an allometric equation (Tazawa *et al.*, 1991*a*);

$$HR = 429 \cdot Mass^{-0.118}$$

The HR calculated from the allometric equation is 200 bpm for the mean egg mass (634 g for the 3 hatched embryos) at 38°C. This value is calculated to be 176 bpm at 36°C assuming that temperature coefficient of HR is 2 as in other avian embryos (Tazawa *et al.* 1991*b*, 1992). The HR measured at 36°C in the present study was about 170 bpm at 80% of incubation, which is close to the predicted value.

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