ALLOMETRIC RELATIONSHIPS BETWEEN EMBRYONIC HEART RATE AND FRESH EGG MASS IN BIRDS

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Summary

Previously, we have measured daily changes (developmental patterns) in embryonic heart rate (f_H) in altricial and semi-altricial (ASA) birds (range of mean fresh egg mass approximately 1–20 g), semi-precocial seabirds (egg mass approximately 38–288 g) and precocial birds (egg mass approximately 6–1400 g). An allometric relationship between embryonic f_H at 80 % of incubation duration (ID) and fresh egg mass (M) has been derived for six species of precocial bird (f_H at 80 % ID=$429M^{-0.118}$). In the present study, additional measurements of embryonic f_H in three ASA species, the barn owl *Tyto alba*, the cattle egret *Bubulcus ibis* and the lanner falcon *Falco biarmicus*, were made to extend the egg mass range (20–41 g), and the allometric relationships of embryonic f_H for these ASA birds and the precocial and semi-precocial (PSP) groups were investigated from published data. The developmental patterns of embryonic f_H in three relatively large ASA species did not show a significant increase prior to the pipping period, unlike those in small ASA birds, but tended to be constant, with a subsequent increase during pipping.

The allometric relationship derived for ASA birds was f_H at 80 % ID=$371M^{-0.121}$ (r=–0.846, P<0.001, N=20) and that for PSP birds was f_H at 80 % ID=$433M^{-0.121}$ (r=–0.963, P<0.001, N=13). The slopes were parallel, but f_H of ASA embryos was low compared with that of PSP embryos with the same egg mass. In ASA birds, embryonic f_H was maximal during the pipping (perinatal) period, and the maximum f_H (f_{Hmax}) was significantly related to fresh egg mass: f_{Hmax}=$440M^{-0.127}$ (r=–0.840, P<0.001, N=20). The allometric relationships for f_H at 80 % ID in PSP and f_{Hmax} in ASA embryos were statistically identical. Accordingly, embryonic f_H at 80 % ID in PSP birds and f_{Hmax} during pipping in ASA birds can be expressed by a single allometric equation: f_H=$437M^{-0.123}$ (r=–0.948, P<0.001, N=33).

Key words: altricial, semi-altricial, bird, precocial, semi-precocial, allometry, developmental pattern, embryo, heart rate, prenatal, perinatal.

Introduction

In chickens *Gallus domesticus*, the body mass of the embryo increases in a geometric fashion until growth rate slows down during the last stages of prenatal development. The geometric increase in embryo wet mass until day 18 of incubation is well described by a fourth-power function of incubation time (Tazawa and Hou, 1997). During the same period of incubation, the embryonic heart rate (f_H) changes, but in a different way; f_H increases rapidly after the heart commences beating and reaches an asymptote early in the second week of incubation. During the last half of prenatal development, the daily change in f_H becomes small (Cain et al., 1967; Van Mierop and Bertuch, 1967; Girard, 1973; Laughlin et al., 1976; Tazawa et al., 1991a; Howe et al., 1995; Akiyama et al., 1999). Thus, developmental patterns of embryonic f_H in chickens are not directly related to the increase in body mass of the embryo and cannot be expressed as a function of body mass. Furthermore, in other avian species whose developmental

patterns of embryonic f_H have been studied, changes in f_H with embryonic age were not related to the increase in embryonic mass (Tazawa et al., 1991a; Tazawa et al., 1991b; Tazawa et al., 1994; Tazawa et al., 1998a; Tazawa et al., 1998b; Tazawa et al., 2000; Burggren et al., 1994; Tazawa and Whittow, 1994; Pearson et al., 1998; Pearson et al., 1999; Pearson and Tazawa, 1999a; Pearson and Tazawa, 1999b). Consequently, within a species, changes in embryonic f_H during development are unrelated to increases in body mass.

However, among species of birds, it was reported that the embryonic f_H at a given percentage of incubation duration (ID) and mean f_H during incubation were related to fresh egg mass; i.e. embryonic f_H was allometrically related to egg mass (Tazawa et al., 1991a; Ar and Tazawa, 1999). An allometric equation for f_H at 80 % ID was derived for six species of domesticated precocial birds, whose egg mass ranged from 11 g in the Japanese quail *Coturnix coturnix japonica* to 160 g

in the goose *Anser cygnoides* (Tazawa et al., 1991a). This equation effectively predicted the embryonic f_H at 80 % ID in ostrich *Struthio camelus* and emu *Dromaius novaehollandiae* (Tazawa et al., 1998b; Tazawa et al., 2000).

Recently, the embryonic f_H of king quail *Coturnix chinensis*, which is the smallest measured precocial bird (Pearson et al., 1998), and that of several small altricial and semi-altricial birds was determined (Pearson et al., 1999; Pearson and Tazawa, 1999a; Pearson and Tazawa, 1999b). To date, embryonic f_H data for nine precocial bird species (egg mass range from 6 g for the king quail to 1400 g for the ostrich), three semi-precocial seabirds (from 38 g for the brown noddy *Anous stolidus*, 57 g for wedge-tailed shearwater *Puffinus pacificus* and 288 g for laysan albatross *Diomedea immutabilis*) and 17 altricial and semi-altricial birds (from 0.96 g for the zebra finch *Taeniopygia guttata* to 20.5 g for the crow *Corvus corone*) are available. To extend the egg mass range of combined altricial and semi-altricial (referred to as ASA) birds beyond 20 g, additional measurements of embryonic f_H are reported for three species in the present study. This study was designed to allow derivation of better allometric equations of embryonic f_H for the combined precocial and semi-precocial (referred to as PSP) embryos and ASA embryos on the basis of a larger f_H data set.

Materials and methods

Measurements of embryonic heart rate

Collection and incubation of eggs

Eggs of the altricial barn owl *Tyto alba* (N=13), semi-altricial cattle egret *Bubulcus ibis* (N=14) and lanner falcon *Falco biarmicus* (N=4), laid at the I. Meir Segal's Garden for Zoological Research of Tel Aviv University in February and March 1997, were used for measurements of developmental patterns of embryonic f_H.

Two clutches of barn owl eggs and four clutches of cattle egret eggs were collected from artificial breeding nests and natural breeding nests, respectively, and transferred to a desk-top still-air incubator in the laboratory. In addition, a clutch (N=4) of a lanner falcon that had been incubated at 36±0.5 °C in a small forced-draught incubator with an automatic turning device as part of a breeding program in the zoo were measured. These eggs and the incubator were brought to the laboratory for f_H measurements. The laying dates of some of the eggs were not known.

The length (L, in cm) and maximum breadth (B, in cm) of each egg were measured with a micrometer to 0.01 cm. The fresh egg mass (M, in g) was calculated using the following equation (Hoyt, 1979),

$$M = 0.548LB^2. \qquad (1)$$

The still-air incubator was the same as that used in previous experiments on emu eggs (Tazawa et al., 2000), and the temperature was kept at 36 °C. It was used not only for incubating the barn owl and cattle egret eggs but also for measuring the f_H of all eggs including those of the lanner falcon. The incubator was a wooden box 60 cm by 60 cm wide and 30 cm high, covered with a removable transparent plastic dome. Two 10 cm diameter round windows opened through the front panel of the chamber and were covered with rubber flaps. Water was placed in a plate on the floor of the incubator to maintain humidity at 50–60 %. Eggs were placed horizontally on metal mesh approximately 5 cm above the floor. Barn owl and cattle egret eggs were kept in the same incubator until they hatched or died, and were turned daily manually by 180 ° in the morning and evening. Lanner falcon eggs were transferred within 10 s from their incubator to the still-air incubator on each day of measurement and returned to their incubator after the measurements. Measurements were started at least 2 h after egg transfer and egg turning.

Acoustocardiography and determination of mean heart rate

Heart rates were determined by acoustocardiography, as in previous studies (Akiyama et al., 1997; Tazawa et al., 1998a; Tazawa et al., 1998b; Tazawa et al., 2000). The acoustocardiogram (ACG) was detected by a condenser microphone attached and sealed hermetically with Plasticine to the eggshell. It was biased by a 6 V 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; it was judged to be dead if the ACG had been detected on previous days. The microphone was kept in the same incubator as the eggs; handling of the microphone and attaching it to the egg were achieved through the windows of the incubator. These procedures minimized disturbance to the eggs and incubation temperature and thus any influences of egg handling and temperature changes on embryonic f_H (Vince et al., 1979; Tazawa et al., 1991b; Tazawa et al., 1992).

The ACG was recorded on the polygraph chart at a speed of 5 mm s^{-1}. Continuous recordings of no less than 40 s were made every 2 min for at least 10 min. These recordings were sectioned into 6 s segments. f_H (in beats min^{-1}) for an approximate 6 s period was counted from the number of ACG waves and the time between two peaks of the first and last ACG waves in that period (referred to as f_{H6}). Between six and seven values of f_{H6} were determined for each approximately 40 s period. Finally, the 30–35 values of f_{H6} thus obtained were averaged to give a mean f_H for an embryo on a given incubation day (referred to as f_{Hmean}). Thus, f_{Hmean} was an average of at least 500–900 heartbeats over a period of approximately 10 min.

Allometric relationships between embryonic heart rate and fresh egg mass

Collection of embryonic heart rate data

We have previously measured developmental patterns of embryonic f_H in precocial birds (Tazawa et al., 1991a; Tazawa et al., 1998a; Tazawa et al., 1998b; Tazawa et al., 2000; Pearson et al., 1996; Pearson et al., 1998), semi-precocial

seabirds (Tazawa et al., 1991b; Tazawa and Whittow, 1994) and altricial and semi-altricial birds (Burggren et al., 1994; Tazawa et al., 1994; Pearson and Tazawa, 1999a; Pearson and Tazawa, 1999b; Pearson et al., 1999) at an incubation temperature of 36–38 °C, depending upon species. We took embryonic f_H data on a given incubation day from these previous measurements. Those measured at a temperature (T in °C) differing from 38 °C were converted to the value at 38 °C using the following equation:

$$f_H(38\,^\circ\mathrm{C}) = f_H(T\,^\circ\mathrm{C})\mathrm{e}^{[0.0639(38-T)]}, \tag{2}$$

where the temperature coefficient (Q_{10}) of f_H was assumed to be 2 (Tazawa et al., 1991b; Tazawa et al., 1992; Ono et al., 1994).

Regression analysis and statistical analyses

Embryonic f_H data from previous studies together with the present results were plotted against individual egg masses on log/log coordinates, and the relationship was expressed by a power-function regression equation using the least-squares method. The correlation coefficient of the allometric equation was examined for its significance by *t*-test. The 95 % confidence intervals of the regression slope and the *y* intercept were determined to test for statistical differences between two allometric equations. Regressions were judged not to be significantly different if both slopes and *y* intercepts overlapped in their 95 % confidence intervals. Unless stated otherwise, results are presented as means ± S.D.

Results

Developmental patterns of embryonic heart rate

Two barn owl clutches were collected and incubated for f_H measurement, one clutch contained six eggs, of which three eggs hatched at different times prior to the end of the experimental period (10–28 March). f_H data measured for the first three hatched eggs are represented by open circles in Fig. 1A. The second clutch had seven eggs; the two eggs that hatched prior to the end of the experimental period are shown by filled circles in Fig. 1A. The mean fresh mass of the five eggs whose developmental patterns of f_H are shown in Fig. 1A was 20.1±0.6 g.

Individual clutches of the cattle egret contained 3–4 eggs. Four eggs of one clutch all died during the experimental period, as did two other eggs from other nests. Eight eggs from three

clutches hatched prior to the end of the experimental period. The developmental patterns of f_H in these eight eggs are shown in Fig. 1B. The mean fresh mass of these eight eggs was 27.3±3.3 g.

Two eggs of the lanner falcon died and the remaining two eggs pipped the eggshell externally during the experimental period. These living eggs were returned to the zoo together with the incubator after the f_H measurement at the end of
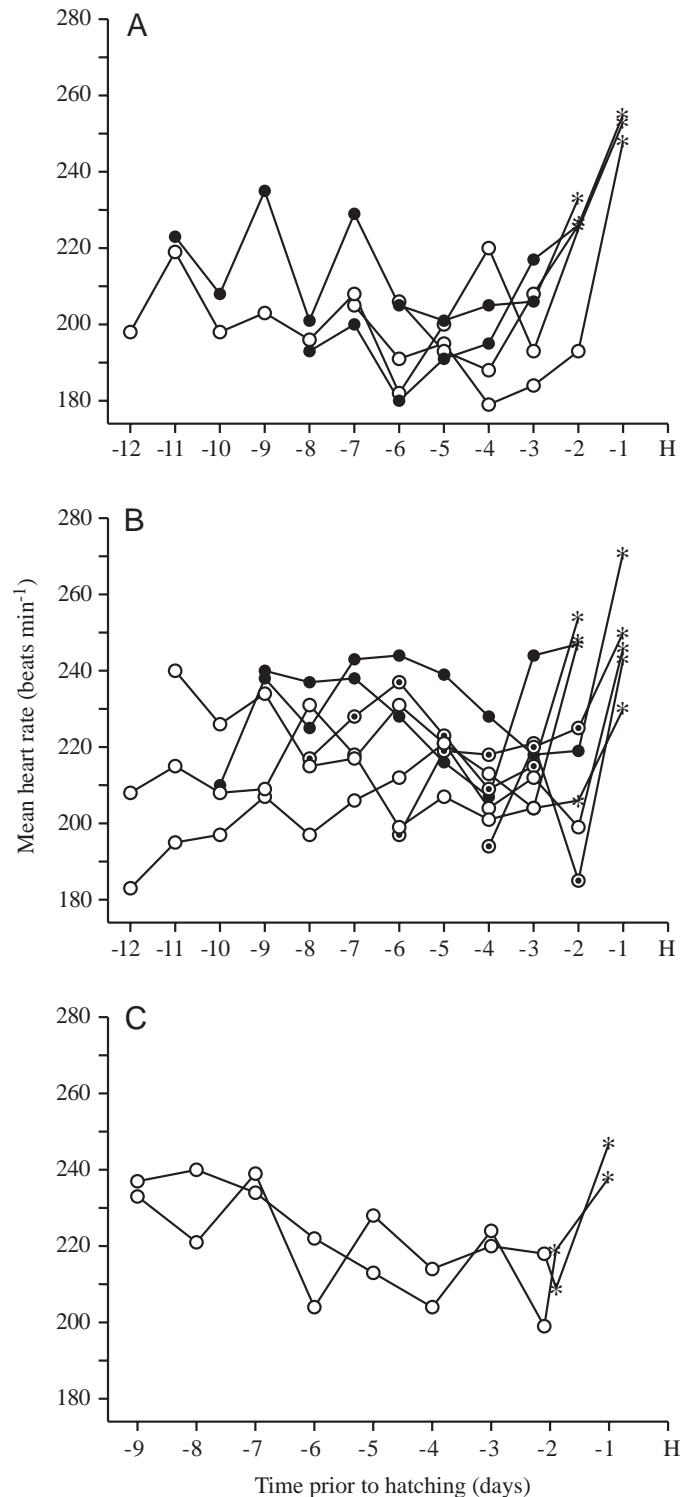

Fig. 1. (A) Developmental patterns of embryonic heart rate in barn owl eggs that hatched. The abscissa shows time prior to hatching (H). Asterisks indicate the occurrence of external pipping. Open and filled symbols indicate eggs from the two different clutches. (B) Developmental patterns of embryonic heart rate in three clutches of cattle egret eggs that hatched. The abscissa shows time prior to hatching (H). Asterisks indicate the occurrence of external pipping. Siblings of the same clutch are shown by the same symbol. (C) Developmental patterns of embryonic heart rate in two lanner falcon eggs that hatched. Two days prior to hatching (H), heart rate measurements were made before (open circles) and during (asterisks) external pipping.

Table 1. *Heart rate at 80% of incubation duration and maximum heart rate during prenatal development together with fresh egg mass in nine species of precocial bird*

	Egg mass (g)	N	f_H at 80% ID (beats min^{-1})	$f_{H\text{max}}$ (beats min^{-1})	ID (days)
[2]King quail *Coturnix chinensis*	6.0±0.4	14	341±8 (81%)	341±8 (81%)	16
[1]Japanese quail *Coturnix coturnix japonica*	10.7±0.7	8	319±8 (82%)	326±7 (76%)	17
[1]Chicken *Gallus domesticus*	64.9±2.5	17	287±9 (81%)	287±9 (81%)	21
[1]Duck *Anas platyrhynchos*	79.0±2.5	7	247±15 (82%)	258±11 (61%)	28
[1]Turkey *Meleagris gallopavo*	82.9±2.6	9	246±10 (79%)	248±10 (75%)	28
[1]Peafowl *Pavo cristatus*	111.3±9.3	7	262±12 (79%)	267±9 (86%)	28
[1]Goose *Anser cygnoides*	158.3±11.3	9	224±8 (80%)	248±10 (60%)	30
[5]Emu *Dromaius novaehollandiae*	634±9	3	192±7 (80%)	199±11 (72%)	50
[3]Ostrich 1 *Struthio camelus*	1331±154 1328±163	12 12	173±14 (81%)	208±9 (55%)	42
[4]Ostrich 2	1395±199	24	185±12 (81%)		

Values were determined at 38 °C or converted to that at 38 °C using equation 2 (emu and ostrich).

If f_H was not measured at 80%, the value closest to 80% of ID was used; the percentage of ID at which f_H was measured is shown in parentheses.

N, number of eggs; ID, incubation duration; f_H, heart rate; $f_{H\text{max}}$, maximum heart rate.

[1]Tazawa et al., 1991b; [2]Pearson et al., 1998; [3]Tazawa et al., 1998b; [4]Tazawa et al., 1998a; [5]Tazawa et al., 2000.

For ostrich, values measured in two different breeding seasons are given.

For $f_{H\text{max}}$, the value in parentheses is the percentage ID at which $f_{H\text{max}}$ was recorded.

Values are means ± S.D.

experimental period to prevent any further disturbance during hatching. Fig. 1C shows the developmental patterns of f_H until the second day of external pipping. Fresh egg masses were 40.8 and 41.6 g.

Allometric relationships

Precocial birds

In addition to a previous study, which determined the allometric relationship between embryonic f_H and egg mass in six species of precocial birds (Tazawa et al., 1991a), the developmental patterns of embryonic f_H in three further precocial species have been measured: king quail, emu and ostrich (Pearson et al., 1998; Tazawa et al., 1998a; Tazawa et al., 1998b; Tazawa et al., 2000). In ostrich eggs, f_H measurements were made during two breeding seasons (Tazawa et al., 1998a; Tazawa et al., 1998b). Egg masses and f_H data are summarized in Table 1. We have recalculated the allometric relationship adding data for these three species (four data points including two separate points for ostrich). Fig. 2 presents this relationship between f_H at 80% of incubation duration (f_H at 80% ID) and fresh egg mass on logarithmic axes.

The allometric equation for this extended data set is:

$$f_H \text{ at } 80\% \text{ ID} = 434M^{-0.122} \ (r=-0.976, P<0.001, N=10). \quad (3)$$

The 95% confidence interval of the slope ranges from −0.100 to −0.145, and that of the y intercept ranges from 388 to 486.

Table 1 also shows the prenatal maximum f_H ($f_{H\text{max}}$) with the percentage ID at which max f_H was recorded. $f_{H\text{max}}$ is correlated with egg mass as:

$$f_{H\text{max}} = 413M^{-0.102} \ (r=-0.966, P<0.001, N=9). \quad (4)$$

The 95% confidence interval of the slope ranges from −0.078 to −0.126, and that of the y intercept ranges from 367 to 464.

Semi-precocial birds

Developmental patterns of embryonic f_H in semi-precocial birds have been reported previously for three Hawaiian seabirds: brown noddy, wedge-tailed shearwater and laysan albatross (Tazawa et al., 1991a; Tazawa and Whittow, 1994). The patterns showed a plateau during at least the last 20% ID. The pipping period was longer than that in precocial birds; the pipping sequence was also different in the laysan albatross.
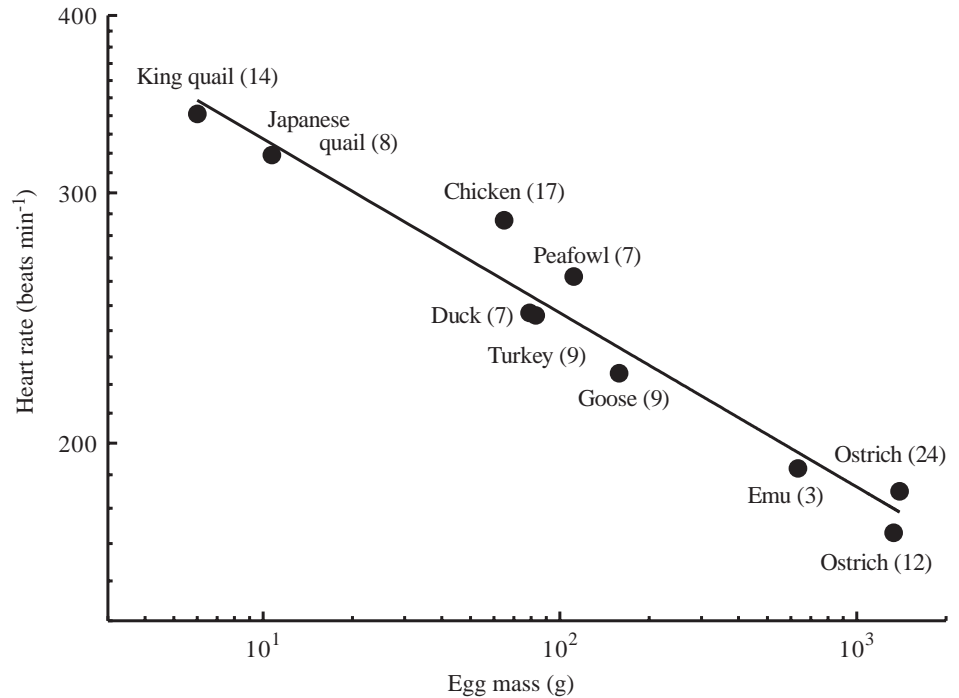
Fig. 2. Mean heart rate at 80 % of incubation duration in nine species of precocial bird plotted as a function of fresh egg mass on logarithmic axes. The number of eggs is indicated in parentheses. For ostrich, values were measured in two breeding seasons and they are shown as two separate points.

Because of the small number of determinations of f_H at 80 % ID, the f_H prior to internal or external pipping ($f_{H_{pre-IP}}$) is presented in Table 2 together with that obtained at the first star fracture of the eggshell. The correlation between f_H and egg mass was not significant.

Altricial and semi-altricial birds

Fig. 3 shows embryonic f_H at 80 % ID of 20 altricial and semi-altricial bird species plotted against their fresh egg masses. The embryonic f_H of the barn owl, cattle egret and lanner falcon determined in the present study after conversion to 38 °C are also shown. The relationship between f_H at 80 % ID of the embryos of 20 altricial and semi-altricial species and their egg masses is expressed by:

$$f_H \text{ at } 80\% \text{ ID} = 371M^{-0.121} \; (r=-0.846, P<0.001, N=20). \quad (5)$$

The 95 % confidence interval of the slope ranges from −0.083 to −0.158, and that of the y intercept ranges from 344 to 401.

The data of f_H at 80 % ID used for calculation of the above allometric equation and plotted in Fig. 3 are presented in Table 3, which also summarizes f_H during internal pipping (IP) and/or external pipping (EP). Values were not obtained for several species during either internal or external pipping. For these species, the perinatal maximum f_H (i.e. $f_{H_{max}}$) was either $f_{H_{IP}}$ or $f_{H_{EP}}$. In the remaining species whose $f_{H_{IP}}$ and $f_{H_{EP}}$ were both measured, $f_{H_{max}}$ was $f_{H_{EP}}$ except in the house martin *Delichon urbica*. $f_{H_{max}}$ was correlated with egg mass as follows:

$$f_{H_{max}} = 440M^{-0.127} \; (r=-0.840, P<0.001, N=20). \quad (6)$$

The 95 % confidence interval of the slope ranges from

Table 2. *Heart rate prior to internal pipping and at the first star fracture of the eggshell in three species of semi-precocial seabird*

	Egg mass (g)	N	$f_{H_{pre-IP}}$ (beats min^{-1})	f_H at first star fracture of the shell (beats min^{-1})	ID (days)
[1]Brown noddy *Anous stolidus*	37.9±2.2	8	298±7	303±13	35
[2]Wedge-tailed shearwater *Puffinus pacificus*	57.2±2.3	11	244±10	252±11	52
[2]Laysan albatross *Diomedea immutabilis*	288±18	5	232±15	233±15	65

$f_{H_{pre-IP}}$, heart rate prior to internal pipping; f_H, heart rate; N, number of eggs; ID, incubation duration.
[1]Tazawa et al., 1991b; [2]Tazawa and Whittow, 1994.
Values were converted from 36 to 38 °C using equation 2.
Values are means ± S.D.

Table 3. *Heart rate at 80% of incubation duration and during internal pipping and external pipping in 20 species of altricial and semi-altricial bird*

	Egg mass (g)	N	f_H at 80% ID (beats min^{-1})	f_{HIP} (beats min^{-1})	f_{HEP} (beats min^{-1})	ID (days)
[2]Zebra finch *Taeniopygia guttata*	0.96±0.13	8	335±10	376±20	405±12	14
[2]Bengalese finch *Lonchura striata* var. *domestica*	1.10±0.12	5	404±36	409±25	448±35	15
[2]Marsh tit *Parus palustris*	1.39±0.04	5	363±17	409±19	–	14
[1]Bank swallow *Riparia riparia*	1.42±0.10	18	298±12	–	352±16	14
[2]Great tit *Parus major*	1.59±0.14	11	348±11	432±13	495±14	14
[2]Varied tit *Parus varius*	1.69±0.01	3	356±7	434±11	–	14
[2]Tree sparrow *Passer montanus*	2.09±0.07	8	335±13	411±32	–	12
[2]Budgerigar *Melopsittacus undulatus*	2.19±0.19	8	314±14	339±15	364±12	18
[2]House martin *Delichon urbica*	2.25±0.04	3	357±7	369±8	367±11	15
[2]Japanese bunting *Emberiza spodocephala*	2.56±0.09	2	370±5	426±1	–	13
[2]Red-cheeked starling *Sturnus philippensis*	4.14±0.01	2	358±1	409±5	–	14
[2]Cockatiel *Nymphicus hollandicus*	5.08±0.18	7	300±8	318±25	344±19	20
[2]Brown-eared bulbul *Hypsipetes amaurotis*	6.4±0.5	3	333±7	402±8	–	16
[1]Domestic pigeon *Columba domestica*	17.1±1.0	12	247±17	–	276±13	18
[1]Fantail pigeon *Columba domestica*	19.7±2.4	6	267±10	–	293±6	18
[1]Homing pigeon *Columba domestica*	19.8±1.2	8	230±16	–	273±4	18
[3]Crow *Corvus corone*	20.5±2.2	8	297±11	348±35	366±22	20
[4]Barn owl *Tyto alba*	20.1±0.6	5	219±11	–	276±13	30
[4]Cattle egret *Bubulcus ibis*	27.5±3.3	8	251±8	–	283±12	23
[4]Lanner falcon *Falco biarmicus*	41.2±0.4	2	242±9	–	276±6	33

f_H, heart rate; f_{HIP}, heart rate during internal pipping; f_{HEP}, heart rate during external pipping; N, number of eggs; ID, incubation duration.
[1]Tazawa et al., 1994; [2]Pearson et al., 1999; [3]Pearson and Tazawa, 1999a; [4]present study.
Values were measured at 38°C or converted to 38°C using equation 2 (crow).
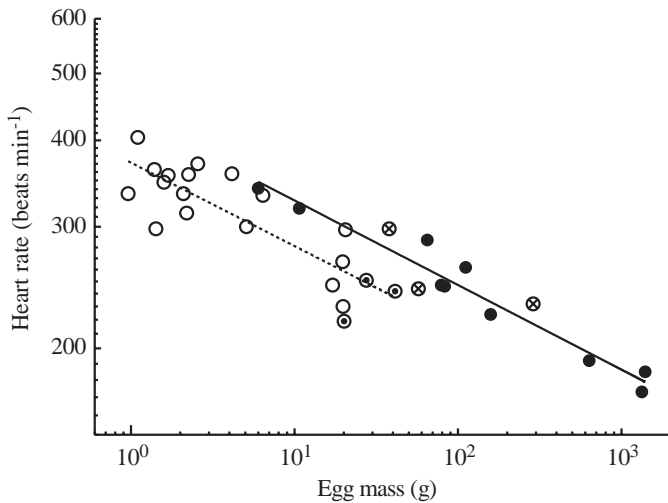Values are means ± S.D.

Fig. 3. Mean heart rate at 80 % of incubation duration in 20 altricial and semi-altricial species plotted as a function of their fresh egg mass (open circles). The three species whose heart rates were measured for the first time in the present study are indicated by the open circles with a dot inside. The broken line is the least-squares regression line (see equation 5). Filled circles and the solid line show the heart rate at 80 % incubation duration in the precocial birds. Heart rates prior to pipping in three semi-precocial seabird species are shown by circles with crosses inside.
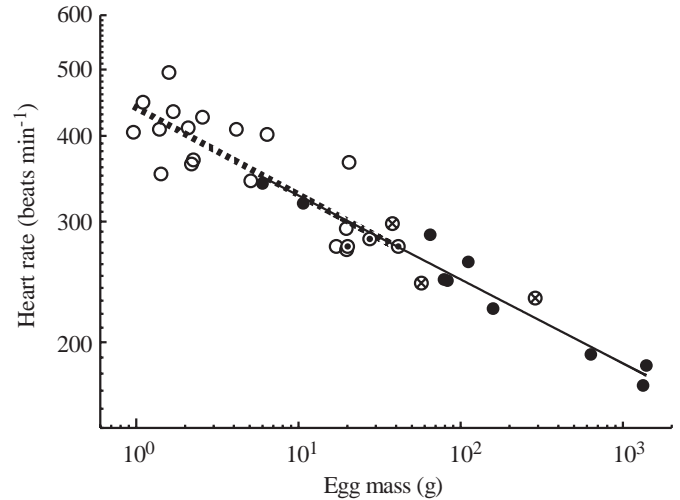
Fig. 4. Maximum heart rate during internal and external pipping (the perinatal period) in 20 species of altricial and semi-altricial bird (open circles and open circles with a dot inside) and heart rate at 80 % of incubation duration in embryos of precocial (filled circles) and semi-precocial (open circles with a cross) species as a function of their fresh egg mass. The broken line is the regression line for perinatal maximum heart rate in the embryos of 20 altricial and semi-altricial avian species. The solid line is the regression line drawn for heart rate at 80 % of incubation duration in the embryos of 12 precocial and semi-precocial species. Note that the regression lines overlap. For equations, see text.

−0.086 to −0.167, and that of the *y* intercept ranges from 405 to 477.

The *y* intercepts of f_H at 80 % ID (equation 5) and $f_{H_{max}}$ (equation 6) are significantly different since their 95 % confidence intervals do not overlap. However, the 95 % confidence intervals of the slopes overlap and thus are not statistically different.

Comparison among all species

Because $f_{H_{pre-IP}}$ of the three semi-precocial seabirds may be considered to represent f_H at 80 % ID, these values were combined with f_H at 80 % ID of precocial birds to define an allometric equation for 12 precocial and semi-precocial (PSP) bird species (including two data points for ostrich because values were measured in two breeding seasons):

$$f_H \text{ at } 80\% \text{ ID/pre-IP} = 433M^{-0.121} \ (r=-0.963, P<0.001, N=13).$$
$$(7)$$

The 95 % confidence interval of the slope ranges from −0.099 to −0.144, and that of the *y* intercept ranges from 388 to 484.

The allometric relationships between embryonic f_H at 80 % ID of 12 PSP bird species (equation 7) and f_H at 80 % ID of nine precocial bird species and egg mass (equation 3) were not significantly different. In addition, the allometric relationship between f_H at 80 % ID and egg mass of 12 PSP bird species (equation 7) was not significantly different from that obtained for $f_{H_{max}}$ in 20 ASA birds (equation 6).

Fig. 4 shows $f_{H_{max}}$ of 20 ASA bird species and f_H at 80 % ID of 12 PSP bird species plotted against individual egg masses. The allometric equation for the combined f_H data

($f_{H_{max}}$ of the embryos of 20 ASA species and f_H at 80 % ID of the embryos of 12 PSP species) is:

$$f_H = 437M^{-0.123} \ (r=-0.948, P<0.001, N=33).$$
$$(8)$$

The 95 % confidence interval of the slope ranges from −0.108 to −0.139, and that of the *y* intercept ranges from 415 to 461.

Discussion
Measurement of developmental patterns of embryonic heart rate

Critical remarks

When using the acoustocardiographic technique, the cardiogenic signal is detected by a microphone attached hermetically using Plasticine to the eggshell. The Plasticine may occlude some pores in the eggshell and impede gas exchange through those pores. Experimentally covering one-quarter to half of the eggshell with epoxy cement caused hypoxia and hypercapnia in growing chick embryos (Tazawa et al., 1971, 1988; Okuda and Tazawa, 1988). Experimental acute hypoxia decreased embryonic f_H (Laughlin, 1978; Tazawa, 1981). In chicken eggs, however, partial covering of an area similar to that in the present experiment (approximately $2\,cm^2$) with tape and epoxy resin did not influence blood gas properties (Tazawa et al., 1980). However, because most of the eggs in the present study are smaller than chicken eggs, there is a possibility that the f_H measured was reduced by impeded gas exchange, which could cause embryonic death during the

experimental period. The death of all four siblings in one clutch of cattle egret is likely to be due not only to the experimental procedures but also to some congenital reason.

Recent improvement in the acoustocardiographic technique has made it possible to determine the instantaneous f_H of chick embryos throughout the last half of the incubation period (Akiyama et al., 1997; Akiyama et al., 1999). This prolonged measurement of f_H indicated that ultradian rhythms of f_H with a 40–90 min period and an oscillatory magnitude of up to 50 beats min^{-1} occurred late in incubation (Akiyama et al., 1999). In addition, measurements of allantoic arterial blood pressure or electrocardiograms in chicken eggs showed that embryonic f_H during the last stages of incubation fluctuated markedly with both short (in seconds and minutes) and long (in hours) periods (Höchel et al., 1998; Tazawa et al., 1999; Moriya et al., 2000). Thus, it is possible that the mean f_H obtained over a 10 min period (f_{Hmean}) does not represent the true mean embryonic f_H for a given incubation day. At present, it is not practical to measure instantaneous f_H of many embryos continuously throughout incubation. Thus, we consider f_{Hmean} as used here to be an approximation suitable for investigating the developmental patterns of f_H.

Differences in developmental patterns of embryonic heart rate

The mean egg masses of barn owl, cattle egret and lanner falcon (20, 28 and 41 g, respectively) extend the egg mass range studied previously in ASA birds. The developmental patterns of f_H during late incubation in these relatively large ASA eggs were similar to those in the three semi-precocial seabirds studied to date whose egg masses were approximately 38, 57 and 288 g (Tazawa et al., 1991b; Tazawa and Whittow, 1994). In both groups, there was a relatively constant f_H during the last stages of prenatal development; a small increase coincident with the initiation of external pipping followed by a large increase as external pipping became established. In contrast, the embryonic f_H in small ASA eggs, such as swallow and sparrow, showed a marked increase during the last stages of the prenatal period and a further increase at the initiation of external pipping (Burggren et al., 1994; Tazawa et al., 1994; Pearson et al., 1999; Pearson and Tazawa, 1999b). A different pattern was found for the embryonic f_H in large precocial eggs such as ostrich, emu and goose, in which f_H decreased during the last 30 % of incubation. The developmental pattern of mean f_H in eggs of small precocial birds such as king quail (approximately 6 g) and Japanese quail (approximately 11 g) showed a maximum f_H at 80 % ID (Table 1; Pearson et al., 1998; Tazawa et al., 1991a). Intermediate-sized precocial avian eggs whose f_H was measured showed intermediate patterns. These differences may indicate that the changes in f_H towards the end of the incubation may be related more to egg mass than to the degree of development at hatching (Ar and Tazawa, 1999).

Allometric relationships
Comparison with previously obtained relationships

Tazawa et al. (1991a) derived an allometric relationship between embryonic f_H and fresh egg mass for six species of domesticated precocial birds. Mean egg mass ranged from 10.7 g (Japanese quail) to approximately 158 g (goose). The embryonic f_H in Japanese quail, chicken (mean egg mass approximately 65 g) and turkey (approximately 83 g) increased at 60–70 % ID, with a subsequent decrease towards the end of the pre-pipping period followed by an increase during external pipping. The f_H of duck (approximately 79 g) and goose gradually decreased after 60 % ID, while that of peafowl (approximately 111 g) steadily increased embryonic f_H towards external pipping. f_H during external pipping increased in turkey, but showed no particular trend in duck and peafowl. Because of the variety observed in developmental patterns during the last stages of incubation and the initiation of internal pipping at 90 % ID in some ducks and geese, the allometric relationship between f_{HEP} and egg mass was not significant. Although the allometric relationships between embryonic f_H and egg mass at 60, 70 and 80 % ID were all significant, the power of the allometric equation at 60 % ID (i.e. −0.080) was small compared with those for later periods because the mean f_H of the six species at this stage varied over a narrow range. At 80 % ID, the differences in mean f_H were greater, yielding the allometric equation (Tazawa et al., 1991a):

$$f_H \text{ at } 80\,\% \text{ ID} = 429M^{-0.118} \ (r=0.881, P<0.001, N=6). \quad (9)$$

The 95 % confidence interval of the slope ranged from −0.030 to −0.205, and that of the y intercept ranged from 295 to 624.

The allometric equation of f_H at 80 % ID derived here from nine species of precocial birds including king quail, emu and ostrich (equation 3) was not significantly different from equation 9, yielding almost identical predictions for f_H.

Comparison of allometric relationships between PSP and ASA embryos

The developmental patterns of embryonic f_H towards the end of the prenatal period in PSP birds tend to change from an increasing to a decreasing trend as egg mass increases. Thus, the stage of incubation at which embryos show the maximum f_H during the prenatal period (i.e. f_{Hmax}) shifts to an earlier stage of incubation as eggs increase initial mass (Table 1). Consequently, the age for f_{Hmax} was different among species, and the slope of the allometric equation becomes less steep (equation 4). The correlation between prenatal f_{Hmax} and egg mass was marginally less good than that of f_H at 80 % ID (equation 3).

In several altricial species, external pipping occurred before f_H during internal pipping (f_{HIP}) had been measured. In contrast, perinatal embryos of a few altricial species (i.e. embryos that had pipped internally the chorioallantoic membrane and inner shell membrane) hatched before f_{HEP} could be measured. In all cases of ASA embryos, the maximum f_H (i.e. f_{Hmax}) was recorded during the pipping period and had a significant allometric relationship with a slope of −0.127 and y intercept of 440 (equation 6). This allometric relationship was not statistically different from that of embryonic f_H at 80 % ID in PSP birds (equation 7). At 80 % ID, the rate of oxygen

consumption ($\dot{M}_{O_2}$) of precocial embryos reached a plateau defined by a break in the previously exponential increase (Hoyt et al., 1978; Vleck et al., 1979; Vleck et al., 1980; Høiby et al., 1983; Tazawa and Rahn, 1987; Kuroda et al., 1990; Prinzinger and Dietz, 1995; Haque et al., 1996; Pearson et al., 1996). In large precocial embryos, this plateau in $\dot{M}_{O_2}$ becomes a decline. The plateau or decline in $\dot{M}_{O_2}$ is either less pronounced or nonexistent in ASA embryos (Prinzinger and Dietz, 1995). Since f_H and $\dot{M}_{O_2}$ during the last stages of incubation may be considered to be correlated, it appears that the f_H of PSP embryos at approximately 80 % ID represents an equivalent stage of development to that of pipping in ASA embryos (i.e. $f_{H_{max}}$). Thus, $f_{H_{max}}$ in ASA embryos and f_H at 80 % ID in PSP embryos can be represented by a single allometric equation (equation 8). We suggest that, for the same fresh egg mass, the relative incubation stage of approximately 80 % in PSP embryos is comparable with that of ASA birds during hatching in terms of f_H, $\dot{M}_{O_2}$, growth rate and perhaps the development of thermoregulation (Whittow and Tazawa, 1991).

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