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DEVELOPMENTAL PATTERNS OF HEART RATE IN ALTRICIAL AVIAN EMBRYOS AND HATCHLINGS

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Summary

The aims of this study were to determine the patterns of development of heart rate (f_H) in altricial avian embryos and hatchlings, and then to examine how f_H is regulated to meet metabolic requirements in altricial embryos. Embryonic mean heart rate (f_H) in 12 altricial species (Passeriformes and Psittaciformes) increased during pre-pipping incubation in all species except the cockatiel (*Nymphicus hollandicus*), in which f_H tended to decrease prior to pipping. The rate of increase in f_H tripled during the pipping phase in all species, and f_H was significantly higher during the pipping period and in hatchlings than during pre-pipping development. The O_2 pulse (O_2 consumed per cardiac beat) of altricial embryos increased in direct proportion to embryo mass ($\log_e/\log_e$ base), although f_H was often low prior to pipping, implying that stroke volume increases in the second half of incubation. We conclude that f_H contributes more than other factors towards supplying the metabolic demands of the embryo during the middle of incubation and the final pipping phase, but less during the intervening period of late incubation.

Key words: altricial, embryo, hatchling, mean heart rate, oxygen pulse, bird.

Introduction

Tazawa et al. (1991) have shown that the development patterns of embryonic heart rate (f_H) in many domesticated precocial species are very variable with respect to incubation age among species prior to pipping. However, embryonic f_H at the end of incubation is closely related to fresh egg mass in a reciprocal relationship, which reflects the fact that embryos from smaller eggs have higher metabolic rates (Paganelli and Rahn, 1984). There are only two published accounts of the development of embryonic f_H in two altricial avian species (Tazawa et al., 1994; Burggren et al., 1995). In the domestic pigeon (*Columba domestica*), mean heart rate (f_H) was initially stable, followed by a period of gradual increase in f_H; f_H increased continuously in the bank swallow (*Riparia riparia*). However, the largest increases in f_H occurred in both species from shortly before the time embryos were confirmed to be pipped. In the absence of studies of other altricial species, it is uncertain whether altricial embryos have a characteristic pattern of development of f_H. Furthermore, we know of only one study that has measured hatchling f_H in an altricial species, that of the house wren (*Troglodytes aedon*) (Odum, 1941). Thus, there is a need for investigations of the development of f_H in altricial embryos and hatchlings.

Recent studies suggest that all hatchling development modes have a common developmental pattern of metabolism (Prinzinger and Dietz 1995; Prinzinger et al., 1995). The major difference is that the largest increase in the rate of oxygen consumption occurs significantly later in the incubation period, and oxygen consumption plateaus for a very brief period in altricial embryos in comparison with precocial embryos, on the basis of normalised incubation time.

It is of great interest to know how cardiac output is adjusted to meet the changing metabolic requirements of the growing embryo. In the chicken (*Gallus domesticus*), the amount of O_2 delivered to embryonic tissues per cardiac beat, termed the 'O_2 pulse', increases linearly throughout the last half of incubation (Haque et al., 1996), while the f_H of chick embryos, according to many studies, is stable or decreases between mid-incubation and pipping (see Tazawa and Hou, 1997). This implies that stroke volume increases significantly throughout the last half of incubation and makes an important contribution to cardiac output, which increases in parallel with embryo mass (Tazawa and Hou, 1997).

The first aim of the present study was to determine the developmental patterns of f_H in altricial embryos and hatchlings of 10 passerine species and two parrot species, ranging in fresh egg mass from 0.96 to 6.40 g. f_H shortly before pipping is allometrically related to fresh egg mass in precocial embryos with an exponent of –0.11 (Tazawa et al., 1991). This is correlated with higher mass-specific metabolic rates in smaller embryos (Paganelli and Rahn, 1984). Therefore, we hypothesise that f_H in altricial species will increase during development in order to attain the higher f_H predicted by the allometric trend for precocial embryos, but within a shorter incubation period than for precocial species. Our second aim

was to use the $\bar{f}_H$ development patterns of altricial embryos to suggest how f_H contributes to changes in cardiac output during the last half of incubation. We calculated the embryonic O_2 pulse ($\mu l\, O_2\, beat^{-1}$) for four altricial species examined for $\bar{f}_H$ in this study.

Materials and methods

Egg collection and incubation

Eggs from the following species were collected under permit from the Japanese Department of Environment during the spring/summer of 1997 and spring of 1998, from the surrounds of Muroran and Noboribetsu Cities; great tit (*Parus major* Temminck and Schlegel), marsh tit (*Parus palustris* Stejneger), varied tit (*Parus varius* Temminck and Schlegel), tree sparrow (*Passer montanus* Stejneger), house martin (*Delichon urbica* Bonaparte), Japanese bunting (*Emberiza spodocephala* Temminck), red-cheeked myna (*Sturnus philippensis* Forster) and brown-eared bulbul (*Hypsipetes amaurotis* Temminck). In addition, we obtained eggs from zebra finch (*Taeniopygia guttata* Reichenbach), Bengalese finch (*Lonchura striata* var. *domestica* Sykes), budgerigar (*Melopsittacus undulatus* Gould) and cockatiel (*Nymphicus hollandicus* Wagler) from breeding adults maintained in colonies in a laboratory.

Eggs were brought to the laboratory and incubated in a small still-air incubator (Zenkei table top model 40, Japan) at 38±0.5 °C. Relative humidity (RH) was controlled by vents so that eggs achieved approximately 15 % mass loss during incubation (RH 55–65 %). All f_H measurements were made in a larger still-air incubator (Sakura IF-B3, Tokyo) at 38±0.2 °C. Nests (wild and domestic species) were inspected every 2–3 days to determine egg-laying dates.

Embryonic ballistocardiogram

The audiocartridge measuring system described previously in many studies of precocial avian embryos was used to measure the ballistic movements attributable to embryonic f_H (Suzuki et al., 1989; Tazawa et al., 1989, 1991; Pearson et al., 1998). The ballistocardiogram signal was amplified (Bioelectric amplifier type 4124, NEC San-ei) between 85 and 95 dB, then high- and low-pass-filtered between 4 and 30 Hz to remove baseline instability and high-frequency noises. Ballistocardiogram signals of embryos were then measured after a 40 min period to allow for thermal equilibration and to minimise the possible influences of recent handling on f_H.

Hatchling $\bar{f}_H$: measurements

The $\bar{f}_H$ of hatchlings was measured non-invasively using a flexible piezo-electric polyvinylidene fluoride (PVDF) film described in detail by Tazawa et al. (1993) with the difference that hatchlings were unrestrained during measurements in the present study. Instead, the PVDF film was taped to the inner surface of a Petri dish, and all sides of the film were blocked in with pieces of polystyrene foam to dimensions of approximately 25 mm×22 mm. A hatchling was then placed onto the surface of the film, but was prevented from moving off the film by the foam. The output signal was amplified by 54–60 dB and bandpass-filtered between 5 and 24 Hz using the same system as for embryonic measurements. The dish with PVDF film was placed inside the same incubator as for embryonic measurements under darkened conditions. Chicks were placed individually on the dish with the film soon after hatching (in most cases within 2 h), and after 20 min the digitised signal was recorded and processed as for embryonic signals (detailed below).

f_H calculations and statistics

Amplified ballistocardiogram and piezo-electric film signals were digitised using a 12-bit A/D converter with an input of ±5 V every 5 ms and stored on a personal computer. f_H measurements were recorded for 2 min periods, repeated over five consecutive runs for individual embryos and hatchlings during periods when the f_H signal was least disturbed by activity and external noises, which were evident as large deflections on the oscilloscope display (approximately 10–15 min). Calculations of $\bar{f}_H$ were the same as in a recent study (Pearson et al., 1998). In brief, the recorded data files were processed by computer using Burg's algorithm (maximum entropy method), which divided the 2 min data file into 5 s periods and calculated the power spectrum density of each interval individually, which we defined as the 'f_{H5}'. This procedure was necessary for accuracy since the peaks attributable to cardiac contractions were of similar amplitude to peaks of non-cardiac origin and to background noise in younger embryos and were therefore not always self-evident. f_H was determined for undisturbed intervals only, which was always at least 50 % or more of the total 5 s intervals of the five runs (10 min total). The mean (± S.D.) of all f_{H5} values for all embryos of the same species was determined and is referred to as the daily $\bar{f}_H$ ($\bar{f}_{Hsp}$) of that species. $\bar{f}_{Hsp}$ values for each species were analysed for significant changes ($P<0.05$) between late incubation and in the hatchling using one-sample *t*-tests. Linear regressions between pre-pipping $\bar{f}_H$ and age were fitted by the method of least squares. The significance of differences in mean values of fresh egg mass and $\bar{f}_H$ among *Parus* species was examined using analysis of variance (ANOVA). Relationships between $\log_e$-transformed O_2 pulse and embryo mass data were fitted with second- or third-order polynomial regressions using SYSTAT (Wilkinson, 1990), which explained more variation in the O_2 pulse than linear regressions in all species examined.

Results

Relationships between $\bar{f}_H$ and incubation time

$\bar{f}_{Hsp}$ values in 12 species are presented in relation to incubation age in Fig. 1. $\bar{f}_{Hsp}$ increased with incubation age in all species except the cockatiel (species 9), which tended to decrease until shortly before the pipping period (regression slope=–0.85; $t_{1,4708}$=–6.419; $P<0.0001$) (Fig. 1). With regard to the three tit species, $\bar{f}_{Hsp}$ values at 80 % of incubation (day 12), maximal pipping $\bar{f}_{Hsp}$ (day 14) and hatchling $\bar{f}_H$ were

significantly different between species (one-sample *t*-test; $P<0.001$ for all combinations), and mean fresh egg mass was significantly different (ANOVA: $F_{2,17}=1.844$, $P<0.005$). However, the differences in $f_{H_{sp}}$ values between species were not consistent with respect to age, and interspecific variability in tit f_H was similar to intraspecific variability in the other species. Subsequently, the $f_{H_{sp}}$ data for tit embryos and hatchlings were pooled for comparisons and to determine the common $f_{H_{sp}}$ pattern (Fig. 1, species 3). The $f_{H_{sp}}$ values of pipped embryos (including both internal and external pipping) and hatchlings of the 12 species were higher than the $f_{H_{sp}}$ values at the end of the pre-pipping phase (defined as 80 % of incubation) (Table 1). Embryos were compared for significant changes in $f_{H_{sp}}$ between pre-pipping, maximal f_H during the pipping phase and hatchling f_H (day 0) using *t*-tests for species with four or more individuals. Without exception, the $f_{H_{sp}}$ of pipped embryos and hatchlings was significantly higher than that of pre-pipped embryos in all species (all *t*-tests; $P<0.001$). The maximum $f_{H_{sp}}$ of pipped embryos was significantly lower than hatchling $f_{H_{sp}}$ in Bengalese finches and budgerigars, but significantly higher in tits, and did not differ significantly in zebra finches and tree sparrows (Table 1).

The $f_{H_{sp}}$ of pipped embryos and hatchlings exceeded 400 beats min^{-1} in passerines, but was 340–380 beats min^{-1} on average in the parrot species (Fig. 1; Table 1). Incubation periods varied between 12 days for the tree sparrow and 20 days for the cockatiel. In all species, embryonic $f_{H_{sp}}$ increased to a maximum between 90 and 97 % of normalised incubation time (Fig. 1).

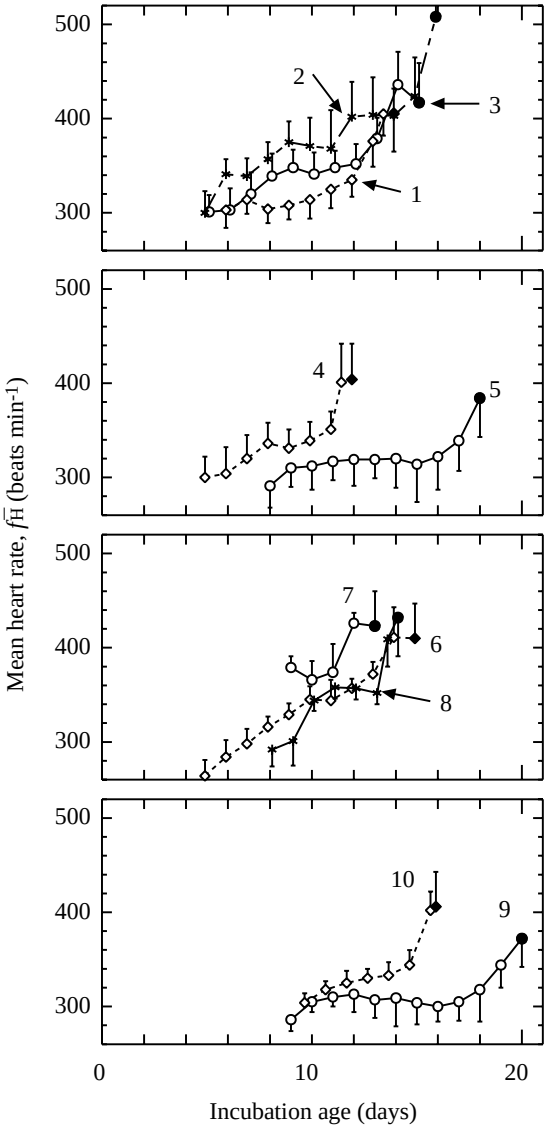

Fig. 1. The development of mean heart rate (f_H, beats min^{-1}) with respect to incubation age (days) in embryos and hatchlings of 12 altricial species of bird. Numerals indicate species identification as listed in Table 1, and error bars are one standard deviation (one-tailed for clarity). Embryos and hatchlings of (1) zebra finch (*Taeniopygia guttata* *N*=8), (2) Bengalese finch (*Lonchura striata* var. *domestica* *N*=5), (3) tits (*Parus major*, *Parus varius* and *Parus palustris* *N*=23), (4) tree sparrow (*Passer montanus* *N*=11), (5) budgerigar (*Melopsittacus undulatus* *N*=13), (6) house martin (*Delichon urbica* *N*=5), (7) Japanese bunting (*Emberiza spodocephala* *N*=2), (8) red-cheeked myna (*Sturnus philippensis* *N*=2), (9) cockatiel (*Nymphicus hollandicus* *N*=9) and (10) brown-eared bulbul (*Hypsipetes amaurotis* *N*=3) were examined. Open symbols are for embryos; filled symbols are for hatchlings.

O_2 pulse of embryos

The O_2 pulse (µl O_2 $beat^{-1}$) of embryos of four altricial species was determined using published mean rates of oxygen consumption (ml O_2 day^{-1}) and $f_{H_{sp}}$ for each day of incubation. Rates of oxygen consumption were obtained from the following sources: cockatiel (Pearson, 1995), budgerigar (Bucher, 1983), tits (*Parus major* in Prinzinger et al., 1995)

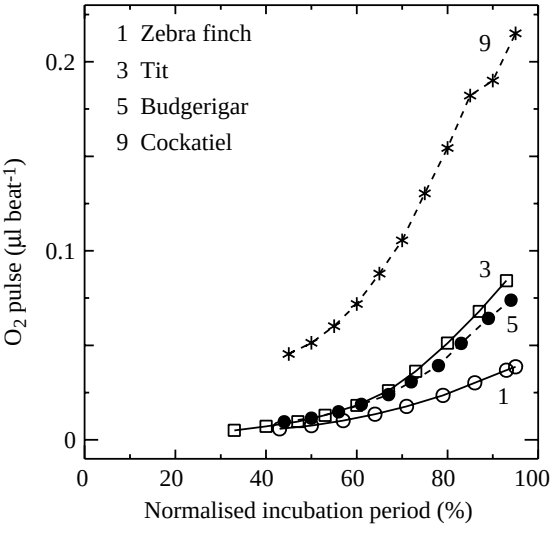

Fig. 2. Relationships between calculated O_2 pulse (µl O_2 $beat^{-1}$) and normalised incubation period (%) for embryos up to the pipping phase of incubation. Values were calculated from the mean heart rate of each species and the published rate of O_2 consumption for each day of incubation (see text).

Table 1. *Mean heart rates of 12 species of altricial embryos during late incubation and in hatchlings*

Species	Mean egg mass	Age (days)	Mean heart rate (beats min^{-1})			*N*
			Pre-pipping	Pipping	Hatchling	
1 Zebra finch	0.96	11	335±18	405±23	406±41	(8, 6, 4)
2 Bengalese finch	1.10	12	404±48	424±41	508±43	(5, 5, 4)
3 Tit spp.	1.55	12	352±21	436±35	417±42	(19, 17, 15)
4 Tree sparrow	2.09	9.5	335±20	401±41	404±38	(8, 7, 6)
5 Budgerigar	2.19	14.5	314±36	350±30	377±40	(8, 5, 6)
6 House martin	2.25	12	357	411	410	(3, 2, 1)
7 Japanese bunting	2.56	10.5	370	426	423	(2, 2, 2)
8 Red-cheeked myna	4.14	11	358	409	432	(2, 2, 2)
9 Cockatiel	5.08	16	300±16	344±24	372	(7, 4, 3)
10 Brown-eared bulbul	6.40	13	333	402	406	(3, 3, 3)

Pre-pipping mean heart rate ($\bar{f}_H$) is defined as $\bar{f}_H$ at 80 % of incubation; maximal $\bar{f}_H$ during pipping and $\bar{f}_H$ of hatchlings on day 0 (mean ± S.D.) are also given.

The number of embryos and hatchlings at each age is indicated by *N*. Species, mean fresh egg mass and age at pre-pipping are also indicated.

Values for tit are the mean value for three *Parus* species (see text).

Two-sample *t*-test (*N*>3): pre-pipping *versus* pipping/hatchling $\bar{f}_H$, $P<0.001$ in species 1–5, 9; pipping *versus* hatchling $\bar{f}_H$, $P<0.001$ in species 2, 3 and 5; not significant in species 1 and 4.

and zebra finch (Vleck et al., 1980). No correction was made for differences in incubation temperature (36–38 °C) between the metabolic studies and the present study because calculated Q_{10} values for preliminary measurements of embryonic oxygen consumption of cockatiel embryos over this temperature range were approximately 1 (Pearson, 1995).

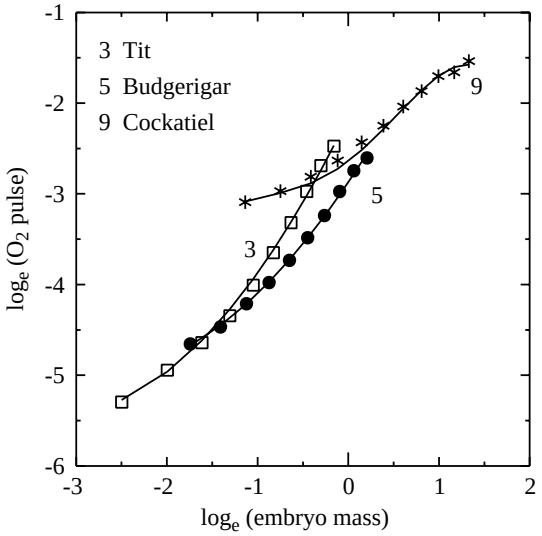

Fig. 3. Relationships between calculated O_2 pulse (μl O_2 beat^{-1}) and mean embryo mass (g). The O_2 pulse data are the daily means calculated for each species in Fig. 2. Mean embryo mass ($\bar{M}_{emb}$) on each day of incubation was estimated from parabolic growth functions fitted using the least-squares method (see text). Lines represent significant polynomial regressions fitted between means for variables on each day of incubation ($P<0.001$) for tit [$\log_e(O_2\ \text{pulse}) = -2.153+2.030(\log_e\bar{M}_{emb})+0.313(\log_e\bar{M}_{emb})^2$, $r^2=0.998$]; budgerigar [$\log_e(O_2\ \text{pulse}) = -2.872+1.483(\log_e\bar{M}_{emb})+0.258(\log_e\bar{M}_{emb})^2$, $r^2=0.999$] and cockatiel [$\log_e(O_2\ \text{pulse}) = -2.620+0.877(\log_e\bar{M}_{emb})+0.203(\log_e\bar{M}_{emb})^2-0.189(\log_e\bar{M}_{emb})^3$, $r^2=0.998$].

However, the rates of oxygen consumption were corrected for differences in mean fresh egg mass between studies. The O_2 pulse increased exponentially in all species from early incubation to a maximum at pipping (Fig. 2). The O_2 pulse of cockatiel embryos increased rapidly after mid-incubation, with only a slight slowing in the rate of increase during the early pipping period. In zebra finch, tit and budgerigar embryos, the O_2 pulse increased from similar values initially, but diverged after mid-incubation. Growth functions for the same species, except the zebra finch, were determined by fitting parabolic functions to wet embryo mass (g) data in relation to incubation age (days) (cockatiel, Pearson, 1995; budgerigar and tit, Pearson and Tazawa, 1999). Calculated O_2 pulses on each day of incubation are presented in relation to mean embryo mass (predicted from growth curves) (Fig. 3). Polynomial regressions for these relationships ($\log_e/\log_e$-transformed) were highly significant for three species. The slope of the fitted relationships was lowest at small embryo masses in three species, but also decreased again shortly before internal pipping in the cockatiel.

Discussion

Development of f_H *in embryos*

Mean heart rate ($\bar{f}_H$) development patterns of the altricial embryos in the present study (Fig. 1) and that of Tazawa et al. (1994) appear to be characterised by two phases in development. In the present study during the first pre-pipping phase (mid-incubation to pre-pipping), the rate of change in $\bar{f}_H$ was greatest and positive in Bengalese finches and negative in the cockatiel. In the final pipping phase of development, the rate of change in $\bar{f}_H$ increased threefold to pipping-hatchling $\bar{f}_{Hsp}$ levels from pre-pipping values in all species (Fig. 1). In precocial embryos, the $\bar{f}_{Hsp}$ of some species (e.g. *Anas platyrhynchos*, *Meleagris gallopavo* and *Pavo cristatus*) does

not increase during the pipping phase (Tazawa et al., 1991). However, we consider that altricial embryos are more likely to increase $\bar{f}_{H_{sp}}$ towards hatching since smaller species attain higher hatchling f_H values within shorter incubation periods.

Pipping and $\bar{f}_H$ *increases*

The $\bar{f}_H$ for all altricial species examined showed significant increases during the pipping phase (Table 1). However, the period for which $\bar{f}_H$ was sustained at maximal pipping levels varied among species. We consider that such differences reflect variability among species in the timing of pipping and hatching events, although a causal relationship is not implied. Rather, such variability is likely to be the result of phylogenetic constraints acting upon the growth and developmental rates of individual species. Internal pipping was confirmed (frequent candling of the translucent eggshells) in embryos of tit species, finches, budgerigar, cockatiel, house martin and Japanese bunting between 1 and 2 days prior to hatching, although external pipping was not always seen. In contrast, embryos of the tree sparrow, red-cheeked myna and brown-eared bulbul internally pipped and then hatched within half a day in a very rapid eruption. We do not think hatching was accelerated as a result of handling during preparation for measurements since all species were treated similarly. Rather, the rapid hatching may reflect the thin shells of the latter species, whereas all the other species were observed systematically breaking the shell by thrusting their beak against the widening arc of the cracked shell and slowly rotating around a substantial proportion of the perimeter before emerging as much as a day later. Thin shells have higher water vapour conductances to allow optimal evaporative water loss (approximately 15 % of fresh egg mass) within a shorter than expected incubation period on the basis of egg mass (Ar and Rahn, 1978). Thus, shorter pipping to hatching intervals may result secondarily from the rapid embryonic growth rates and shorter incubation periods of the latter species.

Physiological significance of f_H *changes*

We suggest that the development of $\bar{f}_H$ in embryos of altricial species is only partly responsible for meeting the metabolic requirements of the embryos. Cardiac output, the product of f_H and stroke volume, and the arteriovenous blood oxygen content difference determine embryonic metabolic rate. We assume that the arteriovenous blood oxygen content difference changes during development do not differ among birds. Tazawa and Hou's (1997) calculations for chicken embryos indicate that cardiac output increases as a power function of incubation age and probably increases in parallel with embryo mass during the second half of incubation. Comparisons of the development patterns of $\bar{f}_H$ and metabolic rate (as the O_2 pulse) of embryos suggest that the importance of contributions of f_H to cardiac output change during incubation. Pearson and Tazawa (1999) also suggest that the relationship between f_H and embryo mass is complex and changes during incubation. $\bar{f}_H$ is linearly related to embryo mass in the tit, budgerigar and two species of crow (*Corvus corone* and *Corvus macrorhynchos*) during early incubation at least, but for a period that varies among species, and is independent of embryo mass during the remainder of incubation.

At any given stage of incubation, the altricial embryo is a smaller fraction of the hatchling mass than is the precocial embryo (Hoyt, 1987). Absolute growth rates in the precocial embryo increase after 60 % of incubation, only after the chorioallantoic membrane grows to cover the inner shell membrane completely (Dietz et al., 1998). Bucher and Barnhart (1984) used both candling and a ratio of 'effective' G_{O_2}/'total' G_{O_2} (where G_{O_2} is the oxygen conductance of the eggshell and membranes) to estimate when chorioallantoic membrane growth in a parrot species, the lovebird (*Agapornis roseicollis*), was completed. They report that the chorioallantoic membrane is not completely developed until the pipping period in that altricial species. Our own observations, made by candling incubated eggs every day during this study, suggest that chorioallantoic membrane growth was probably not complete until 1–2 days prior to internal pipping in all species. These observations are also supported by internal inspection of eggs that were opened for a study of embryonic growth (Pearson and Tazawa, 1999). Absolute growth rates of altricial embryos increase most after 80 % of incubation (Hoyt, 1987; Pearson and Tazawa, 1999), coincident with the increases in metabolic rate that occur later than in precocial embryos (Vleck et al., 1980; Hoyt, 1987; Pearson, 1995).

The calculated O_2 pulse of altricial embryos increased approximately exponentially, with the greatest absolute increases occurring from approximately mid-incubation (Fig. 2), but $\bar{f}_H$ remained low until the pipping phase of incubation. This implies that the contribution of f_H to cardiac output during the late pre-pipping phase is less important than during the middle of incubation. The polynomial regressions fitted between O_2 pulse and wet embryo mass ($\log_e/\log_e$ base) are sigmoidal in shape, indicating that increases in O_2 pulse with development are smallest at very low embryo masses in all species examined and are possibly constrained during the final pipping phase in the cockatiel (Fig. 3). The O_2 pulse has not been determined for early incubation, but is probably independent of embryo mass before 30 % of incubation because the rate of O_2 consumption is very low (Vleck et al., 1980; Prinzinger and Dietz, 1995; Pearson, 1995; Prinzinger et al., 1995). A high f_H is very important for the circulation of nutrients to the early fish embryo (Mirkovic and Rombough, 1998). We speculate that the contribution of f_H to supplying both the O_2 demands and the nutrient supplies of the avian embryo may prove to be more significant than other factors (stroke volume and blood O_2 capacity) during the first half of incubation since $\bar{f}_H$ is directly related to embryo mass during much of this early period (Pearson and Tazawa, 1999). Aside from increasing cardiac output, altricial embryos may also be able to meet the higher metabolic demands of late incubation by other blood and circulatory adjustments (e.g. increased haematocrit, haemoglobin O_2-affinity and circulation rate), but this remains to be investigated.

In conclusion, we find that the development patterns of $\bar{f}_H$ in altricial embryos are variable between species during the pre-pipping phase of development, even when differences in incubation period are taken into account by normalisation. However, of the 12 species examined here, all species except the cockatiel exhibited a pattern of increasing $\bar{f}_H$ during the pre-pipping phase. Furthermore, embryos of all 12 species increased $\bar{f}_H$ most during the pipping phase and maintained high $\bar{f}_H$ levels after hatching.

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References

Ar, A. and Rahn, H. (1978). Interdependence of gas conductance, shell thickness and functional pore area. *Condor* **76**, 153–158.

Bucher, T. L. (1983). Parrot eggs, embryos and nestlings: Patterns and energetics of growth and development. *Physiol. Zool*. **56**, 465–483.

Bucher, T. L. and Barnhart, M. C. (1984). Varied egg gas conductance, air cell gas tensions and development in *Agapornis roseicollis*. *Respir. Physiol*. **55**, 277–289.

Burggren, W. W., Tazawa, H. and Thompson, D. (1995). Intraspecific variability in avian embryonic heart rate: potential genetic and maternal environmental influences. *Israel J. Zool.* **40**, 351–362.

Dietz, M. W., van Kampen, M., van Griensven, M. J. M. and van Mourik, S. (1998). Daily energy budgets of avian embryos: the paradox of the plateau phase in egg metabolic rate. *Physiol. Zool*. **71**, 147–156.

Haque, M. A., Pearson, J. T., Hou, P-C. L. and Tazawa, H. (1996). Effects of pre-incubation storage on embryonic functions and growth. *Respir. Physiol.* **103**, 89–98.

Hoyt, D. F. (1987). A new model of avian embryonic metabolism. *J. Exp. Zool.* **(Suppl. 1)**, 127–138.

Mirkovic, T. and Rombough, P. (1998). The effect of body mass and temperature on the heart rate, stroke volume and cardiac output of larvae of the rainbow trout, *Oncorhynchus mykiss*. *Physiol. Zool*. **71**, 191–197.

Odum, E. P. (1941). Variations in the heart rate of birds. *Ecol. Monogr*. **11**, 299–326.

Paganelli, C. V. and Rahn, H. (1984). Adult and embryonic metabolism in birds and the role of shell conductance. In *Respiration and Metabolism of Embryonic Vertebrates* (ed. R. S. Seymour), pp. 193–204. Dordrecht, Boston, London: Dr W. Junk Publishers.

Pearson, J. T. (1995). A comparative study of the energetics of avian reproduction. PhD dissertation, University of Adelaide.

Pearson, J. T. and Tazawa, H. (1999). Development of cardiac rhythms in altricial avian embryos. *J. Comp. Biochem. Physiol.* A (in press).

Pearson, J. T., Tsudzuki, M., Nakane, Y., Akiyama, R. and Tazawa, H. (1998). Development of heart rate in the precocial king quail, *Coturnix chinensis*. *J. Exp. Biol*. **201**, 931–941.

Prinzinger, R. and Dietz, V. (1995). Qualitative course of oxygen consumption in altricial and precocial birds. *Respir. Physiol*. **100**, 289–294.

Prinzinger, R., Schmidt, M. and Dietz, V. (1995). Embryogeny of oxygen consumption in 13 altricial and precocial birds. *Respir. Physiol*. **100**, 283–287.

Suzuki, Y., Musashi, H. and Tazawa, H. (1989). Noninvasive heart rate monitoring system for avian embryos based on ballistocardiogram. *Med. Biol. Eng. Comput.* **27**, 399–404.

Tazawa, H., Hashimoto, Y., Takami, M., Yufu, Y. and Whittow, G. C. (1993). Simple, noninvasive system for measuring the heart rate of avian embryos and hatchlings by means of a piezoelectric film. *Med. Biol. Eng. Comput.* **31**, 129–134.

Tazawa, H., Hiraguchi, T., Kuroda, O., Tullett, S. G. and Deeming, D. C. (1991). Embryonic heart rate during development of domesticated birds. *Physiol. Zool.* **64**, 1002–1022.

Tazawa, H. and Hou, P.-C. L. (1997). Avian cardiovascular development. In *Development of Cardiovascular Systems* (ed. W. W. Burggren and B. B. Keller), pp. 193–210. Cambridge: Cambridge University Press.

Tazawa, H., Suzuki, Y. and Musashi, H. (1989). Simultaneous acquisition of ECG, BCG and blood pressure from chick embryos in the egg. *J. Appl. Physiol.* **67**, 478–483.

Tazawa, H., Watanabe, W. and Burggren, W. W. (1994). Embryonic heart rate in altricial birds, the pigeon (*Columba domestica*) and the bank swallow (*Riparia riparia*). *Physiol. Zool.* **40**, 1448–1460.

Vleck, C. M., Vleck, D. and Hoyt, D. F. (1980). Patterns of metabolism and growth in avian embryos. *Am. Zool*. **20**, 405–416.

Wilkinson, L. (1990). *SYSTAT: the System for Statistics*. Evanston, IL: SYSTAT. 638pp.