Development and regulation of heart rate in embryos and hatchlings of gulls (Larus schistisagus and Larus crassirostris) in relation to growth

Accepted: 18 May 2000

Abstract We compared the developmental patterns of mean heart rate in Larus crassirostris and L. schistisagus embryos and chicks with other avian species of different hatching developmental modes. We proposed that, since mean heart rate is inversely related to fresh egg mass in all birds, larger species reached a higher fraction of their hatching mean heart rate by the end of the early phase of incubation and that heart rate contributions to supplying the increasing metabolic demands during mid and late incubation phases were less important than in smaller avian species. Mean heart rate was essentially independent of age throughout the mid-incubation phase (33% of normalised incubation until pipping), but increased with time during early (L. schistisagus only investigated) and late-incubation phases in both species. The O₂ pulse of L. schistisagus embryos and chicks increased linearly with yolk-free body mass (log-log) after the early-phase of incubation until shortly before pipping, but was independent of body mass in the periods before and after. We conclude that a high heart rate in this first period is probably more important for increasing the circulation of nutrients to the embryo at a stage when extra-embryonic circulation to the yolk sac is limited by the size of the growing area vaculosa. Furthermore, large increases in mean heart rate during the late-incubation phase are probably important for increasing the cardiac output to hatching levels with onset of endothermy. However, mean heart rate is stable over the mid-incubation while O₂ pulse increases, suggesting that increases in stroke volume and other circulatory adjustments may be entirely responsible for the largest increases in O₂ transport during incubation of large avian species.

Key words Avian · Larus · Growth · Heart rate · O₂ pulse

Abbreviations ACG acoustocardiogram · ECG electrocardiogram · HR heart rate · MHR mean heart rate · Q₁₀ temperature coefficient · T_ambient ambient temperature

Introduction

The developmental patterns of mean heart rate (MHR) have now been investigated for a modest sample of avian species across the whole range of fresh egg masses and for all hatching developmental types (Tazawa et al. 1991a, 1991b, 1994, 1998; Tazawa and Whittow 1994; Pearson et al. 1998; Ar and Tazawa 1999; Pearson et al. 1999; Pearson and Tazawa 1999a, 1999b). The MHR developmental patterns of individual species vary considerably, and appear to be related to incubation period, egg mass and developmental type. Nevertheless, in common with all species towards the end of incubation, the MHR of all embryos is related to fresh egg mass with an exponent of ~0.11 (Tazawa et al. 1991a; Ar and Tazawa 1999), whereas during the first half of incubation MHR is often a low and variable fraction of the pipped embryo or hatching value (Ar and Tazawa 1999; Pearson and Tazawa 1999b).

According to the Fick principle, O₂ consumption is the product of heart rate (HR), stroke volume and the blood O₂ content difference between arterial and venous blood. In adult birds, cardiac output is primarily adjusted by changes in HR, as HR and O₂ consumption are related to body mass to the power of ~0.25, and stroke volume and the arterio-venous blood O₂ content difference are both directly proportional to body mass (Calder 1968). If the HR of a growing bird is directly
related to its O₂ consumption, then the O₂ delivered to its tissues per cardiac beat (O₂ pulse) should be constant during development; however, this is not the case. In the precocial chicken *Gallus domesticus* and semi-precocial seabirds (Diomedea immutabilis and Puffinus pacificus) the O₂ pulse increased during late incubation (Tazawa and Whittow 1994; Haque et al. 1996). Furthermore, O₂ pulse increased exponentially during incubation in four small altricial species with the greatest absolute increases in O₂ pulse occurring before the greatest absolute increases in embryo mass (about 80% of incubation) from around mid-incubation (Pearson et al. 1999). The relationships between O₂ pulse, MHR and embryo mass in small altricial species indicate that cardiac output is likely to increase with development to supply the increasing metabolic demands, but the role of HR in O₂ transport changes over three defined phases (early, mid and late) of incubation (Pearson et al. 1999; Pearson and Tazawa 1999b). During early incubation, from when a cardiac rhythm first develops to about 40% of normalised incubation, MHR increases rapidly in direct proportion to embryo mass in altricial species, but is independent of embryo mass in both mid and late phases (Pearson and Tazawa 1999b). We therefore proposed that a high HR in this first period is probably more important than stroke volume for increasing cardiac output, and thereby increasing the circulation of nutrients to the embryo (as the O₂ demand is small) at a stage when extra-embryonic circulation to the yolk sac is limited by the size of the growing area vaculosa (Pearson et al. 1999). Since MHR is inversely related to fresh egg mass in all birds, and preliminary investigations suggest that larger species tend to reach a higher fraction of their hatching MHR by the end of the early phase of incubation, the importance of HR in the transportation of O₂ during mid and late incubation phases decreases in larger avian species (Tazawa et al. 1991a; Ar and Tazawa 1999; Pearson and Tazawa 1999a, b). Our aim was to confirm whether the same relationships between MHR, O₂ pulse and embryo mass in small species apply to larger avian species.

**Materials and methods**

**Egg collection and incubation**

Eggs from the slaty-backed gull *Larus schistisagus* and black-tailed gull *L. crassirostris* were collected under permit during the months of May and June from Daikoku Island (City of Muroran) and cliffs at Shimamaki Village in Hokkaido, Japan, in the years 1997–1999 and 1998–1999, respectively. At the time of collection, eggs varied from freshly laid to 2 weeks old, except during the 1999 collection when eggs were determined to be fresh (candled for signs of an embryo and air cell). These latter eggs were used in the growth studies (detailed below), and all other eggs were incubated and HR measured every day until hatching. Eggs were transported to the laboratory in Muroran in a single container without any supplemental heat, which took 40 min (Daikoku Island site) to 3 h (Shimamaki). Eggs were then measured for length (L) and maximum breadth (B), weighed and incubated in one of two still incubators at 36 ± 0.5 °C and 40% relative humidity. Eggs with embryos < 2 weeks old were automatically turned every 1.5 h. Fresh mass (± 0.1 g) was estimated for older embryos at the time of collection using a linear regression based on egg size determined from known fresh eggs (both species combined: mass (g) = 17.291 + 0.0061[L²]; r² = 0.976. n = 19, F = 701.38, P < 0.0001). As no single egg was incubated for the entire incubation period in the laboratory, we assume incubation periods of 27 days for *L. schistisagus* (27 days in *L. argentatus* of similar egg mass; Drent 1970) and 25 days for *L. crassirostris* (Kiyosu 1947).

**Heart rate detection and experimental plan**

Embryonic HR was determined by either the non-invasive acoustocardiogram (ACG) method or the semi-invasive electrocardiogram (ECG) method for embryos < 40% (*L. schistisagus* only) or >85% of incubation and chicks (day 0–2, where 0 denotes day of hatch), as detailed in Pearson and Tazawa (1999a). ACG signals were detected as pulsatile pressure waves using a small condenser microphone glued to the outer surface of the intact eggshell (Rahn et al. 1990; Akiyama et al. 1997). Thus, the methods differ in that the ECG recordings of pipped embryos and chicks were of sequential time intervals between adjacent R waves of the QRS complex (filtered 25–200 Hz) that exceeded the user-determined threshold voltage set in the computer, whereas all ACG and ECG recordings of wave signals (filtered between 1 Hz and 20 Hz) from pre-pipping embryos were sampled at 100 Hz, as the QRS complex was not well defined in young *L. schistisagus* embryos (5–25 days). In all experiments, the HR signals of individual embryos and chicks were recorded for a minimum of 30 min (every day in the case of embryos not sacrificed for growth determinations). MHR was determined by power spectral analysis of whole 30-min recordings from reconstructed signals as described in detail in Pearson and Tazawa (1999a, 1999b) for ACG and the ECG of young embryos. The recordings of instantaneous HR from ECG of pipped-embryos and chicks were averaged over the 30-min period to determine comparable MHR values. As HR changed most dramatically and frequently during late incubation, longer measurements of instantaneous HR (12–16 h) were recorded and then averaged over 10-s intervals (defined as MHRₐ) to examine long-term changes in HR baseline during late incubation. Recordings of *Larus* embryo and chick HR were made in two separate darkened incubators at 36 °C and, when time permitted, at 38 °C after 30-min equilibration periods, respectively.

**Breathing frequency calculations**

ACG signals are dominated by ventilatory movements after pipping (Akiyama et al. 1997, 1999a). Therefore, mean breathing frequency was identified as a spectral peak using the MHR calculation procedure (see above). Furthermore, since respiratory sinus arrhythmia (prolonged heart interbeat interval during inspiration) was also evident as oscillatory fluctuations in instantaneous HR, we employed a fast-Fourier transform to determine breathing frequency from ECG recordings. Instantaneous HR data of 15–30 min duration (ECG measurements of pipped embryos and chicks) were divided into 512 point time series intervals using a rectangular window, the power spectra of each interval determined after normalisation, then averaged over all intervals to improve spectra resolution as described previously (Pearson et al. 1998; Moriya et al. 1999). Breathing frequency was also confirmed by random visual observation of chicks in separate measurements (incubator outer door open while inner glass door was closed) for 30-s periods.

**Body and heart mass determinations**

Some of the *L. schistisagus* embryos were measured for MHR and then sacrificed after chilling eggs at 9 °C to stop development. Embryos were removed and extra-embryonic tissues dissected away